Validation of an Agilent GC-MS System With a Polyarc-FID

Validering av et Agilent GC-MS-system med Polyarc-FID

Bachelor Thesis

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Preface

This thesis marks the completion of my bachelor's degree of chemical engineering at the Norwegian University of Science and Technology. The work has been done in collaboration with the employer Chiron AS, where the task was to validate their newly installed Agilent GC-MS instrument with a Polyarc-FID. Because of the situation with the SARS-CoV-2 pandemic, all of the practical work had to be done by the staff at Chiron. Evaluation of all the results was done by the writer through TeamViewer.

From Chiron I would like to express gratitude to my wonderful supervisor Stine Rapp for being adaptable in the situation, giving me the opportunity to contribute and for helpful guidance. Further I want to acknowledge Eskil Hermansson for excellent teamwork and guidance with the results. Also, I wish to thank Marie Zoch for preparing samples for me. From NTNU, thanks to my supervisor Eirik Sundby for feedback and help with the thesis. Last, but not least, I want to thank friends and family for motivating, cheering and supporting me through the semester.

Trondheim, May 20, 2021

Solfrid Margrete Rydså

Abstract

Validation of an Agilent 8890 gas chromatograph (GC) with a mass spectrometer (MS), a Polyarc reactor and flame ionization detector (FID) was performed for the employer, to try to achieve an accredited method for GC-MS/Polyarc-FID detection according to ISO 17025. The validation was carried out by evaluating chromatographic purity, accuracy, precision, instrumental repeatability, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness.

For evaluation of chromatographic purity, repeatability and robustness, a series of samples was prepared containing four different purities of the active ingredient 1-fluorododecane, spiked with the two impurities n-dodecane and n-pentadecane. The certified reference material (CRM) caffeine pharmaceutical secondary standard was used as an external standard. For results and determined purities, single values, the average, variance, standard deviation (SD), percentage relative SD (%RSD) and the measurement uncertainty was reported. Evaluation of accuracy, precision, linearity and LOD/LOQ were carried out by using four CRM solutions of benzo[a]pyrene, naphthalene, PCB 52 and caffeine. The difference in concentration from target was reported, as well as %RSD. Linearity was evaluated based on the correlation coefficient from graphic representations of the results.

The instrument was successfully validated for purity analysis. It fulfilled the set criteria for chromatographic purity, linearity, LOD, robustness and measurement uncertainty. There were problems regarding concentration verification of the CRM solutions, as well as the instrumental repeatability, where the criteria was not fulfilled. The repeatability problem was solved at the end of the project by the instrument vendor. Further work must be performed to achieve a complete validation that includes concentration verification.

Sammendrag

Validering av en Agilent 8890 gasskromatograf (GC) med massespektrometer (MS), en polyarc-reaktor og flammeionisasjonsdetektor (FID) ble utført for oppdragsgiver, for å forsøke å oppnå en akkreditert metode for GC-MS/polyarc-FID-deteksjon i henhold til ISO 17025. Valideringen ble utført ved å evaluere de ulike valideringsparameterne kromatografisk renhet, nøyaktighet, presisjon, instrumentell repeterbarhet, linearitet, deteksjons- og kvantifiseringsgrense, og robusthet.

Vurdering av kromatografisk renhet, repeterbarhet og robusthet ble utført med prøver bestående av fire ulike renheter av den aktive ingrediensen 1-fluordodekan, tilsatt to urenheter i form av n-dodekan og n-pentadekan. Et sertifisert referansemateriale av koffein ble bruk som ekstern standard. For resultater og bestemt renhet ble enkelt- og gjennomsnittsverdier, varians, standardavvik, prosentvis relativt standardavvik og måleusikkerhet rapportert. Evaluering av nøyaktighet, presisjon, linearitet, og grenser for deteksjon og kvantifisering ble utført ved hjelp av fire sertifiserte referanseløsninger av benzo[a]pyren, naftalen, PCB 52 og koffein. For nøyaktighet og presisjon ble differanse fra kjent konsentrasjon bestemt, og prosent relativt standardavvik ble rapportert. Linearitet ble evaluert basert på korrelasjonskoeffisient fra grafiske resultater.

Instrumentet ble vellykket validert for renhetsanalyse. Det oppfylte de satte kriteriene for kromatografisk renhet, linearitet, deteksjonsgrense, robusthet og måleusikkerhet. Det oppsto problemer med bestemmelse av referanseløsningenes konsentrasjon, i tillegg til repeterbarhet, hvor kriteriene ikke ble oppfylt. Problemet med repeterbarhet ble på slutten av prosjektet løst av instrumentleverandøren. Videre arbeid kreves for å oppnå en fullstendig validering som inkluderer konsentrasjonsverifisering.

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1 Introduction

Chiron AS has over 30 years of experience with research, and are a leading company regarding chemical analysis and production of reference materials within petrochemicals, food, environmental, forensic and pharmaceutical chemistry [1]. Chiron delivers reference materials specifically designed and developed to satisfy the need of individual customers, and has a portfolio containing over 15 000 unique products [1, 2]. These are manufactured in accordance to the ISO 17034 standard, which contains requirements for the competence of reference material producers [3]. ISO is the International Organization for Standardization [4]. Reference materials can be categorized as either certified reference materials (CRM) or just reference materials (RM) [5]. CRM requires a Certificate of Analysis (CoA), that provides values for uncertainty and traceability. RM is a more generic term, and covers materials that are sufficient homogenous, stable, and fit for the intended use.

In November 2018, Chiron received the ISO 17025 accreditation, and in November 2020 the ISO 17034. These accreditations cover LC-UV for chromatographic purity assessment. A new GC-MS instrument with a Polyarc-FID was installed in January 2021. The task for this thesis was to validate the instrument and develop procedures in order to obtain an accredited method for GC-MS/Polyarc-FID detection according to ISO 17025. The Polyarc reactor system is a relatively new technology, and should, in combination with the flame ionization detector (FID), give analysts a better capability of determining purity and quantification, faster and with lower costs [6].

For validation of chromatographic purity, a series of test samples will be prepared, containing the proposed "active ingredient" (AI) 1-fluorododecane. These samples will be spiked with two "impurities", n-dodecane and n-pentadecane, at the same amount. In addition, a series of CRM solutions ordered from Merck/Supelco and Teknolab/Restek will be used for concentration verification. The evaluation will be done according to ISO 17025 and the criteria given in the validation protocol set by Chiron AS for purity assessment, given in Appendix C. Hereinafter Chiron will be referred to as the employer.

The thesis will initially give an introduction to chromatographic theory, where the most important parts of the GC instrument will be presented. The Polyarc reactor system will be explained in more detail here. In addition, the concept of method validation and applied parameters used for this validation will be explained. Further, detailed descriptions of the materials and methods used will be provided. Last, all of the results from the instrumental tests and calculations done based on the these will be presented, discussed and summarized in a conclusion.

2 Theory

Chromatography is an analytical method used for separating components in a sample using a column, based on their various physical and chemical properties [7, 8]. The sample is injected into the column via manual or automatic injection, where it travels together with a mobile phase, in direct contact with a stationary phase. Due to the components difference in boiling point and/or affinity to the stationary phase, they will elute from the column with different retention times. The retention time defines the time range from sample introduction to elution from the column [8]. At the eluting point one or multiple detectors will produce a signal which can be interpreted in a chromatogram.

2.1 Gas Chromatography

Gas chromatography (GC) is a powerful method that can analyze and separate quite complex analytes, given that the components are stable at high temperatures and have sufficient volatility [7, 8]. In GC the mobile phase is gas, the driving force is gas pressure (flow), and the stationary phase can be both solids and liquid films. Today the most used columns are open tubular, with a non-volatile liquid stationary phase coated as a thin film on the inner wall of the capillary. This method is called gas-liquid chromatography and is based on partition. Multiple detectors can be combined for quantification and qualification in the same analysis [7]. In this project the GC-instrument combines the mass spectrometer (MS) and a flame ionization detector (FID). The FID is in addition combined with a new technology called Polyarc, which functions as a catalyst [9]. A flow chart of the GC-MS system with the Polyarc-FID is illustrated in Figure 2-1.

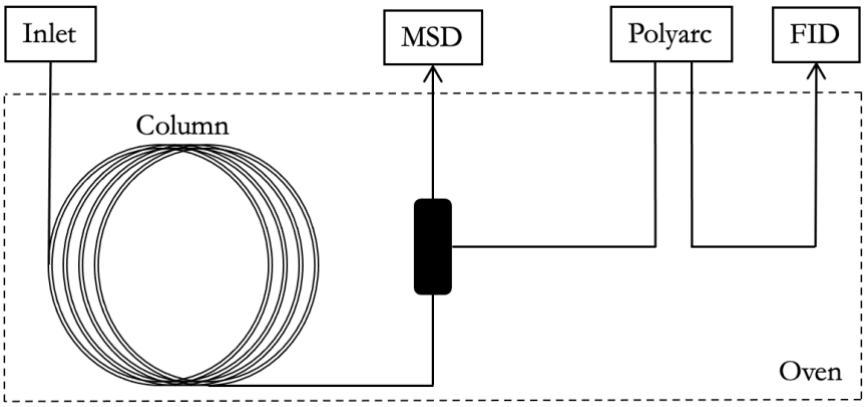

Figure 2-1 Schematic view of a GC-MS with a Polyarc-FID, inspired by Jones at ARC [10].

In GC the mobile phase is also called the carrier gas [8]. The gas does not directly interact with the sample but carries it through the column. It has a few important requirements: it has to be inert, of high purity and with no traces of oxygen, water or hydrocarbons. Usually is consists of either helium, nitrogen or hydrogen. Although it is the most expensive, helium is usually chosen because of good efficiency at higher flow rates, and less safety measures required compared to hydrogen.

2.1.1 Injection

There are different injections systems used in GC, depending on the type of column used. In capillary columns the most applied technique is split/splitless injection [8]. The split/splitless injector, illustrated in in Figure 2-2, can be operated in both split and splitless mode. This allows either part of the sample to be injected into the column, which provides efficiency and sharper peaks, or all of the sample, which are suitable for trace analyses. The amount applied to the column is called the split ratio, which is given in milliliter per minute. The split can be given as for example 1:20, which means 1 mL/min of the sample is transferred to the column, while 20 mL/min goes out through the split vent. Inside the injector the sample travels through a liner, which consists of a glass tube that usually is partly filled with glass or quartz wool.

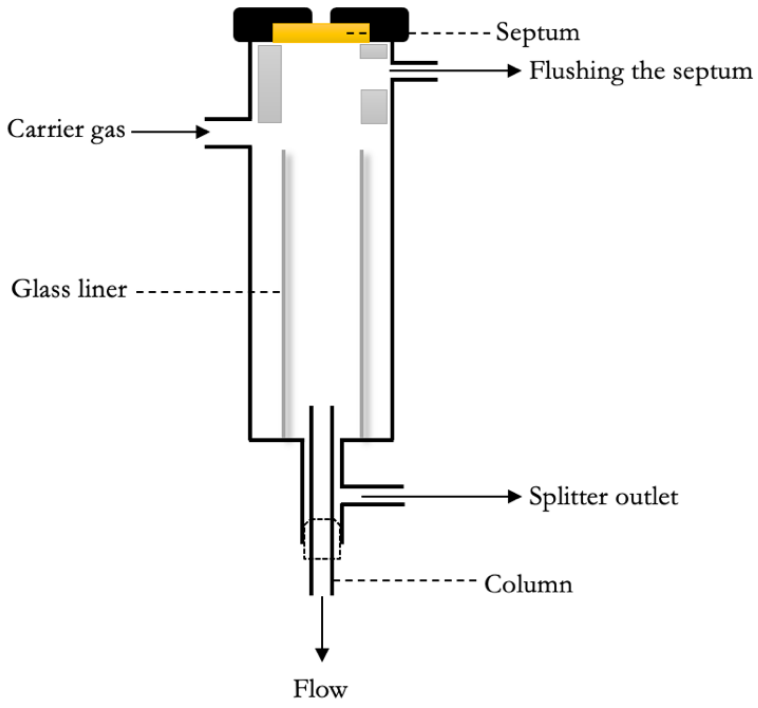

Figure 2-2 Illustration of a split/splitless injector, inspired by Lundanes et al. [8].

2.1.2 Column

The most used column in GC today is the wall-coated open tubular column (WCOT), illustrated in Figure 2-3 [8]. The WCOT capillary column usually has an inner diameter (ID) from 0.1-0.5 mm and length ranging from 10-100 m. The stationary phase is coated as a thin film on the inside of the column wall, where the film thickness is usually between 0.1-0.5 μm. The WCOT capillary column provides a high efficiency with a low plate number. The column is placed inside the column oven, which provides the correct temperature for the analysis - either a constant (isothermal) temperature or a programmed temperature increase (gradient) [7].

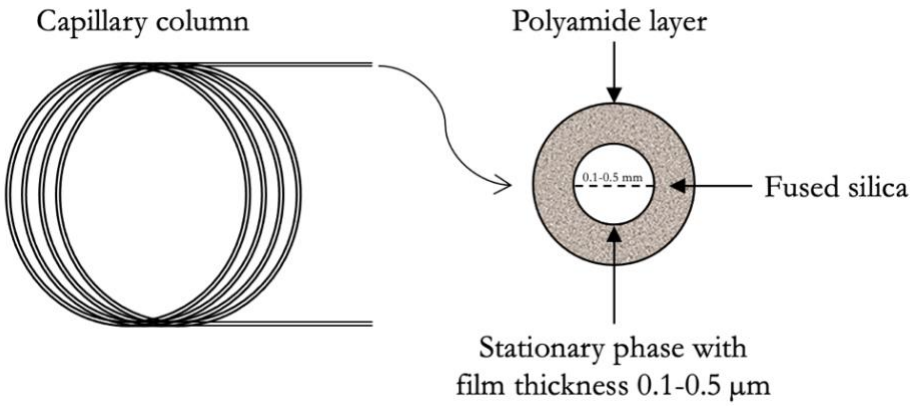

Figure 2-3 Illustration of a WCOT fused silica capillary column, inspired by Lundanes et al. [8].

2.1.3 Detection

Mass Spectrometry Detector

The mass spectrometer (MS) is a universal detector that can be used for both qualitative and quantitative determinations [8]. Mainly it consists of an ionization unit, a mass/charge analyzer and an ion detector. It is commonly used for the identification of compounds and has a mass range that covers all molecular masses that can be analyzed with GC. In GC-MS systems today the most used mass analyzer is the quadrupole, see Figure 2-4. When the ions fragmented by the ionization unit reaches the quadrupole, they enter an oscillating electric field between the four rods [7, 8]. The voltage is adjusted so that a stable path is created [11]. This allows ions of a certain mass/charge (m/z) ratio to travel through the oscillating field and reach the detector (the ion transducer). The quadrupole can in addition be combined with a mass spectral library for easier

identification of compounds [7, 8]. It should be noted that not all searches in the library will show the correct results. Parameters such as the retention time and other selective detectors should be used in addition for evaluation of the compound.

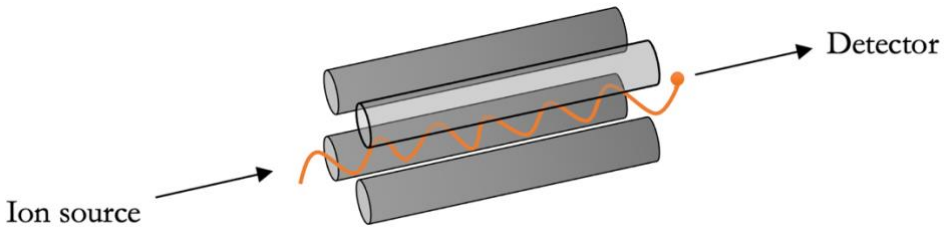

Figure 2-4 Illustration of the quadrupole, inspired by Lundanes et al. [8].

Flame Ionization Detector

The flame ionization detector (FID), illustrated in Figure 2-5, is an almost universal and destructive detector which is mostly used for organic compounds [8]. It consists of a heated block (300-350°C), inlets for H_2, carrier gas and air, a flame and a collector electrode. Ions generated in the flame will produce a current proportional to the amount of compound burned. It detects all hydrocarbons well, except CH_4 and HCOOH, and has a minimum detectable amount on-column from 0.01 – 0.1 ng, depending on the columns efficiency.

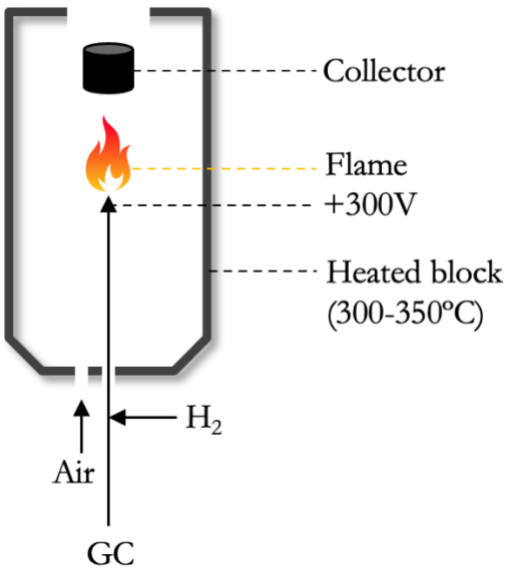

Figure 2-5 Schematic view of the flame ionization detector, inspired by Lundanes et al. [8].

Polyarc Reactor

The Polyarc system is a new technology developed by the Activated Research Company (ARC). In Figure 2-6 a schematic view of the Polyarc is presented. The Polyarc reactor transforms the FID to an universal carbon detector, and functions as a catalyst to optimize GC-FID analyses [9]. Using hydrogen gas and air it converts all organic molecules to methane, water and non-carbonaceous by-products. This gives the FID a more uniform response and removes the need for individual calibration of reference standards, which is both time-saving and cost-effective [6]. In addition, it gives an increased signal and better sensitivity of analytes compared to conventional GC-FID analyses [6, 12], see Figure 2-7. Thus, the combination of the FID with a Polyarc should lead to improved productivity, efficiency and detectability for laboratories.

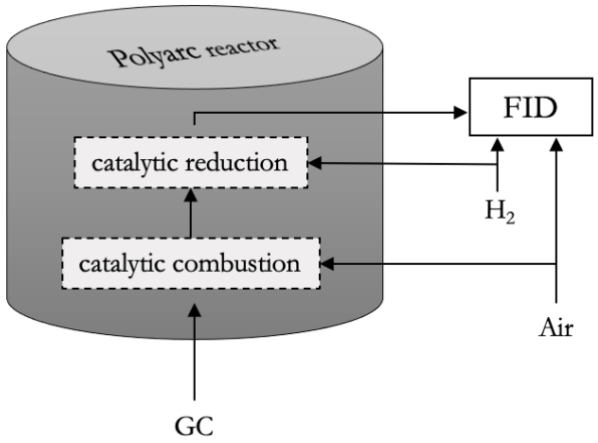

Figure 2-6 Schematic view of the Polyarc reactor, inspired by Jones at ARC [10].

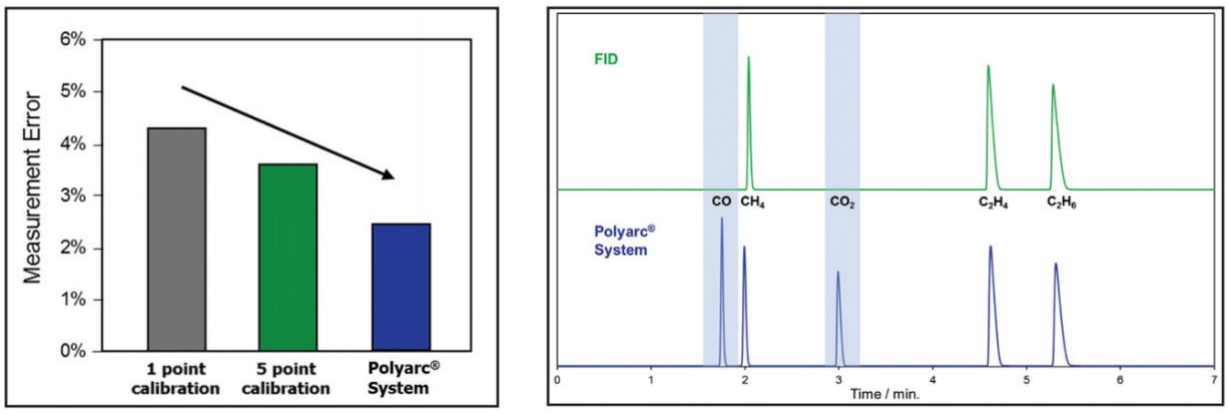

Figure 2-7 Illustrations from the Polyarc product brochure, which claims lower measurement error and improved accuracy, precision and sensitivity [6].

The conversion of carbon-containing analytes occurs in a two-step reaction. The analytes eluting from the column undergoes a complete combustion in an oxidation chamber, followed by complete reduction in a methanation chamber [12]. The reactions are presented in Equation 2.1 (combustion) and Equation 2.2 (reduction). Since all organic substances are converted to methane, the response per carbon atom should be identical for all compounds [6]. Because of the universal response, the response factor (RF) that usually varies between analytes should always be close to unity, see Equation 2.3.

$$C_xH_yO_z + nO_2 \xrightarrow[450℃]{} x\,CO_2 + \frac{y}{2}H_2O \tag{2.1}$$

$$x\,CO_2 + 4x\,H_2 \xrightarrow[450℃]{} x\,CH_4 + 2x\,H_2O \tag{2.2}$$

$$RF = 1 = \frac{\text{mol C / area}}{\text{mol C}_{std} \text{ / area}_{std}} \tag{2.3}$$

Calculations of the mass concentration of the analyte, with an external or an internal standard, can be done by the help of Equation 2.4 [13]. Alternatively, a calibration curve can be created in the GC software with respect to the molecular weight of the standard and number of carbons. Preferably a well-known standard reference should be used, that works well for the current analysis parameters.

$$c_A = c_S \left(\frac{area_A}{area_S}\right)\left(\frac{mw_A}{mw_S}\right)\left(\frac{\#C_S}{\#C_A}\right) \tag{2.4}$$

c_A: Mass conc. of the analyte (µg/mL)
c_S: Mass conc. of the standard (µg/mL)
$Area_A$: Integrated peak area of the analyte
$Area_S$: Integrated peak area of the standard
Mw_A: Molecular weight of the analyte
Mw_S: Molecular weight of the standard
$\#C_S$: Number of C atoms per molecule of the standard
$\#C_A$: Number of C atoms per molecule of the analyte

2.2 Method Validation

The term validation means to verify and document that an analytical method is suitable for the purpose, and is performed to make sure the method can produce accurate analyses [7, 14]. There are various parameters that can be assessed, depending on the method, instrument and laboratory, to ensure that the instrument is "fit for purpose". Key parameters that are usually considered include purity, selectivity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, range and robustness. Methods for quantification should normally assess all of these parameters [7]. The following chapters will address the parameters used for the validation of the 8890/5977B GC-MS/Polyarc-FID from Agilent.

2.2.1 Chromatographic Purity

One of the main uses of GC is evaluating sample purity [7]. The simplest method for evaluation of purity, and relative quantification of impurities, is normalizing peak areas as shown in Equation 2.5 [8].

$$\text{area\%} = \frac{\text{peak area}}{\text{total area}} \cdot 100\% \tag{2.5}$$

Many of the following validation parameters are included in evaluation of purity. In that context, values such as the average, variance, standard deviation (SD), percentage relative SD (%RSD), the uncertainty (u) and expanded uncertainty (U) should be reported [7, 14]. The value u is the standard deviation of the mean, while the expanded uncertainty U multiplies u by a coverage factor k [15]. A value of k = 2 is accepted for a confidence level at 95%. RSD as a percentage value can be calculated as presented in Equation 2.6. The uncertainties u and U are calculated as presented in Equation 2.7 and 2.8.

$$\%\text{RSD} = \frac{\sigma}{\bar{x}} \cdot 100\% \tag{2.6}$$

σ: Standard deviation

$\bar{x}$: Average value

$$u = \frac{\sigma}{\sqrt{N}} \tag{2.7}$$

u: Measurement uncertainty

N: Number of values

$$U = u \cdot k \tag{2.8}$$

U: Expanded measurement uncertainty

k: Coverage factor, 1.96 ≈ 2 for CI 95% [15]

The criteria set by the employer for evaluating chromatographic purity are as following:

- Generally, purity ± 0.5% from target purity.
- 99% target purity: %RSD < 0.5 for AI and %RSD < 50 for each impurity.
- 97% target purity: %RSD < 0.5 for AI and %RSD < 20 for each impurity.
- 95% target purity: %RSD < 0.5 for AI and %RSD < 10 for each impurity.
- 90% target purity: %RSD < 0.5 for AI and %RSD < 10 for each impurity.

2.2.2 Accuracy and Precision

Level of accuracy indicates how close the experimental data match a conventional or accepted "true value" [7]. Techniques to measure accuracy contains use of reference samples, standard addition or a different analytical method to show similarity in the experimental data. Precision on the other hand covers the concept of consistency. Every analytical process will have some level of systematic error of unknown cause. Usually, precision is measured by the help of standard deviation. The concept of intermediate precision includes a couple factors that should be considered, such as variation of analysts and days [14]. For the intermediate precision test performed in this validation, two different persons are performing the analysis, over three different days. The variation is reported as %RSD. The criteria set by the employer for this validation is a value of %RSD < 3% for evaluation of precision, and a value of ± 5% from the target value for accuracy.

External Standard

To evaluate samples used for precision tests, an external or internal reference can be used [8]. In this project the pharmaceutical secondary standard caffeine is used as an external standard. By plotting area results from the reference samples against the known concentrations, a calibration curve is created, illustrated in Figure 2-8 below.

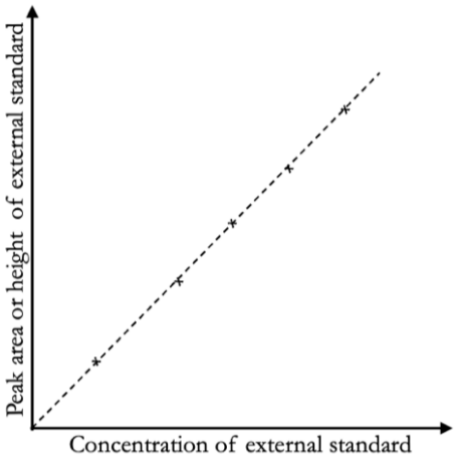

Figure 2-8 Calibration curve for an external standard.

2.2.3 Repeatability

Instrumental repeatability is quite closely related to precision [7]. To evaluate repeatability, multiple injections should be performed from a single test solution. The sample should be prepared by only one analyst and performed within the shortest possible time, and at least six injections should be performed to avoid a large uncertainty [14]. A repeatability test in combination with intermediate precision gives a good indication of the methods performance. The criteria set by the employer for the GC-MS/FID instrumental repeatability are values of %RSD < 1% for AI and %RSD < 50 for the respective impurities.

2.2.4 Linearity and Range

Analytical response from a procedure should in most cases be in direct proportion to the concentration of analyte in the sample, in a linear relationship [7, 14]. To examine linearity, analysis with increasing concentration of the analyte is done and plotted as theoretical concentration vs. response. From this, a correlation coefficient can be calculated, where a value of $R^2 \geq 0.999$ is generally accepted [7]. For this validation a

value of $R^2 > 0.995$ is considered acceptable. Evaluation of range is usually done by looking at a range from a lower concentration and up to a set maximum concentration [8]. For example, from the limit if detection, and up to the highest concentration used for other tests such as accuracy, precision and linearity. The criterion for this validation, set by the employer, is that the instrument detects a content of AI from 100% to 0.1%.

2.2.5 LOD and LOQ

LOD and LOQ are closely related concepts [7]. LOD covers peaks in the chromatogram that can be distinguished from the baseline noise. The peak itself cannot be quantified but can indicate whether a component is present in the sample. LOQ on the other hand covers the lowest concentration of a component that can be quantified. These are often based on the signal-to-noise ratio (S/N), illustrated in Figure 2-9. The most common definition, and criteria set for this validation, is the amount of analyte that results in a ratio of $S/N \geq 3$ for LOD and $S/N \geq 10$ for LOQ [7]. In addition, criteria for both limits are set by the employer to $\leq 0.1\%$ for impurities for chromatographic purity.

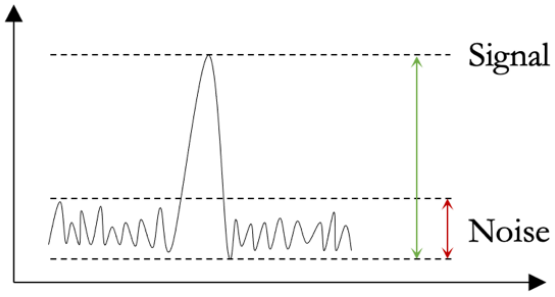

Figure 2-9 Illustration of the definitions signal and noise.

2.2.6 Robustness

To measure robustness of an analytical method, the system can be tested by its ability to perform with acceptable variations when certain parameters are changed [14]. In GC, parameters such as gas flow, heating rate, split ratio, column type, sample preparation, injection, column or detection temperature can be evaluated. In this validation of the GC-MS system with the FID-Polyarc, robustness is measured by modifications of two different injector temperatures, and two temperature gradients for the column. A difference in chromatographic purity lower than 0.2%, compared to the original method, are considered acceptable according to the employer.

3 Materials and Methods

3.1 Reagents and Equipment

Reagents

Helium, 5.0 Detector quality, Linde (p/n 100349)

Hydrogen, from generator at Chiron

Isooctane, J.T. Baker (p/n 9335-22)

Methanol, ChromAR HPLC, Macron Fine Chemicals (p/n 6795-25)

1-Fluorododecane (98%), Prod. no. 8239.12, batch no. 6761

n-Dodecane (99,3%), Prod. no. 1132.12, batch no. 6908

n-Pentadecane (99%), Prod. no. 1135.15, batch no. 2197

Benzo[a]pyrene in acetone (1000 μg/mL), CRM, Merck/Supelco, prod. no. CRM40071 (Appendix E.1)

Naphthalene in methanol (200 μg/mL), CRM, Merck/Supelco, prod. no. CRM48641 (Appendix E.2)

PCB 52 in ACN (50 μg/mL), 5mL ampoule, CRM, Teknolab/Restek, prod. no. R33257 (Appendix E.3)

Caffeine Pharmaceutical Secondary Standard (99,96%), CRM, Merck/Supelco, prod. no. PHR1009, batch no. LRAC4115 (Appendix E.4)

Equipment

Agilent 8890 GC with Split/Splitless and FID

Polyarc Reactor System, Activated Research Company (ARC)

Agilent 5977B MSD

Agilent 7693 Automatic Liquid Sampler (ALS)

Agilent Ultra Inert Inlet Liner 5190-2295, 78.5 mm, 870 μL

Agilent J&W HP-5ms Ultra Inert GC Column, 30 m x 250 μm x 0.25 μm

Precision Zero Air 1500 Gas Generator, Peak Scientific

Precision 100 Hydrogen Generator, Peak Scientific

Precision Air Compressor, Peak Scientific

Figure 3-1 presents an overview of the 8890/5977B GC/MSD, mounted by Matriks AS. The MSD is located on the left side of the GC column oven, directly below the autosampler, and the FID and Polyarc are built into the top of the GC. In addition to the main instrument, it consists of an air and H_2 generator from Peak Scientific, a vacuum pump for MSD, and an air compressor from Peak Scientific. The helium gas supply is located below on the first floor. Figure 3-2 shows a close-up of the autosampler.

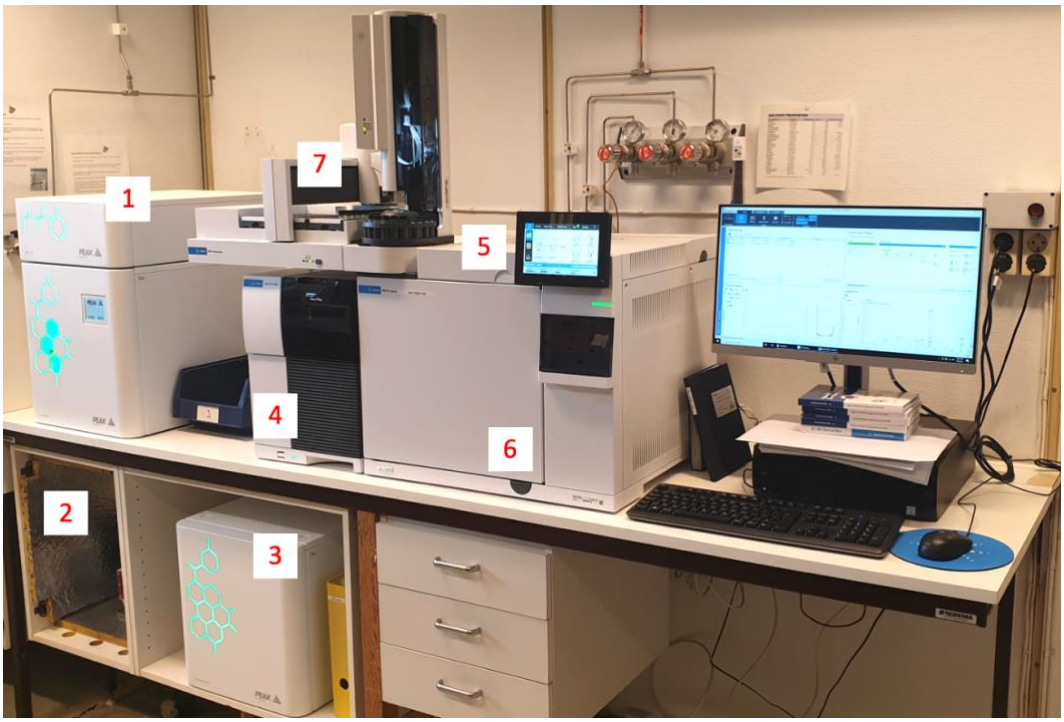

Figure 3-1 Overview of the GC. (1) Air and H_2 generators, (2) vacuum pump for MSD, (3) air compressor, (4) MSD, (5) Polyarc/FID, (6) column oven, (7) autosampler. Photo: Stine Rapp.

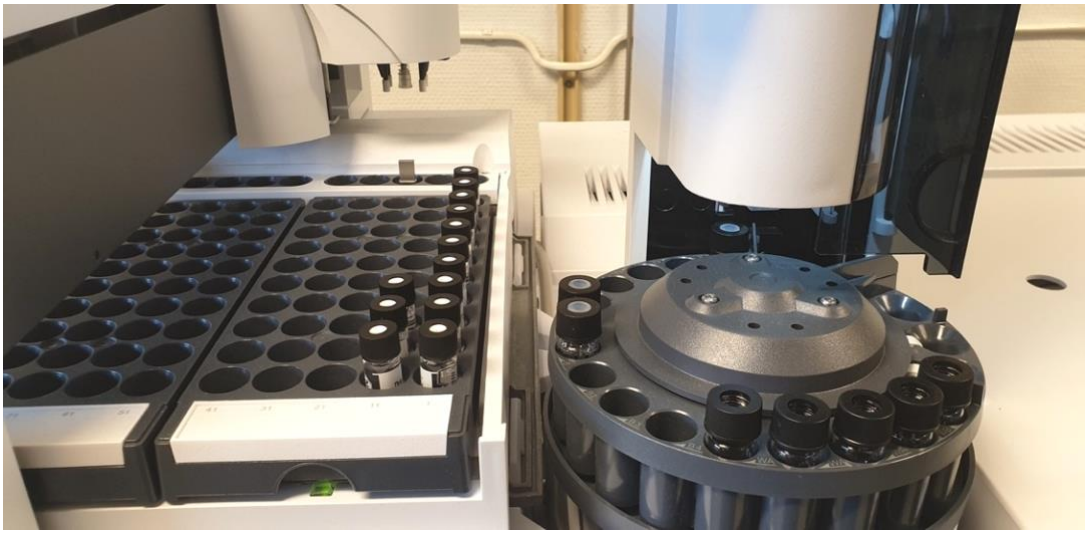

Figure 3-2 The 7693A autosampler, with the ability to load 150 samples. Photo: Stine Rapp.

3.2 Preparation of Samples

Sample preparation was carried out according to the validation protocol, given in Appendix D. Four samples with different purity of the proposed active ingredient (AI) 1-fluorododecane and the two impurities, n-dodecane and n-pentadecane, were made for evaluation of chromatographic purity, repeatability and robustness. The desired purity percentages and an overview of samples are given in Table 3-1. Sample A1 with 90% purity was prepared with 1-fluorododecane (98%, 90 mg,), n-dodecane (99.3%, 5 mg) and n-pentadecane (99%, 5 mg). Isooctane (100 mL) was added as solvent to achieve a total concentration of substances at 1000 µg/mL. The sample was diluted x3 for sample A2, and x10 for sample A3. The same method was used to prepare sample B, C and D. Details of the preparations and actual concentrations are given in Appendix D, Table 7-1 and Table 7-2.

Table 3-1 Overview of percentage AI and impurities in the purity test samples.

Sample	AI	Impurity 1	Impurity 2	Dilution
A1 / A2 / A3	1-Fluorododecane (90%)	n-Dodecane (5.0%)	n-Pentadecane (5.0%)	x1/x3/x10
B1 / B2 / B3	1-Fluorododecane (95%)	n-Dodecane (2.5%)	n-Pentadecane (2.5%)	x1/x3/x10
C1 / C2 / C3	1-Fluorododecane (97%)	n-Dodecane (1.5%)	n-Pentadecane (1.5%)	x1/x3/x10
D1 / D2 / D3	1-Fluorododecane (99%)	n-Dodecane (0.5%)	n-Pentadecane (0.5%)	x1/x3/x10

Four certified standard material (CRM) solutions were ordered for evaluation of intermediate precision and concentration verification:

- IPA: Benzo[a]pyrene (1000 µg/mL) in acetone
- IPB: Naphthalene (200 µg/mL) in methanol
- IPC: Polychlorinated biphenyl (PCB) 52 (50 µg/mL) in acetonitrile
- Caffeine Pharmaceutical Secondary Standard (99,96%)

The samples IPA, IPB and IPC were used as delivered. The external standard caffeine (99.96%) was dissolved in methanol to obtain corresponding concentrations (50, 200 and 1000 μg/mL). See Appendix E for the Certificates of Analysis, and Appendix D for sample preparations of caffeine in Table 7-3.

Caffeine (99.96%) for the linearity/range test was prepared at sixteen different concentrations ranging from 2000 μg/mL to 0.06 μg/mL, see Table 3-2. Methanol was used as solvent. See Appendix D for details, with actual concentrations in Table 7-4.

Table 3-2 Overview of the caffeine samples used for linearity and range.

Sample	Concentration [μg/mL]
A	2000
B	1000
C	500,0
D	250,0
E	125,0
F	62,50
G	31,25
H	15,63
I	7,81
J	3,91
K	1,95
L	0,98
M	0,49
N	0,24
O	0,12
P	0,06

3.3 Experimental Procedures

A summary of the equipment and method used for validating the GC-MS/Polyarc-FID is given in Table 3-3. The method was based on setups previously used for GC analyses at the employer. The full acquisition method report is given in Appendix B.

Table 3-3 Description of the standard acquisition method used for validation.

Parameter	Description
Test method	Gas chromatography
Equipment	Agilent 8890/5977B GC/MSD with a Polyarc-FID and autosampler 7693A
Liner	Ultra Inert Inlet Liner 5190-2295, 78.5 mm, 870 µL
Column	J&W HP-5ms Ultra Inert GC Column, dimensions: 30 m x 250 µm x 0.25 µm
Injection volume	1 µL
Split	1:20
Flow	1.2 mL/min
Gradient	20 °C (hold 4 min) to 325 °C @ 20 °C/min (hold 10 min)
Detection	FID at 250 °C and MSD from start mass (m/z) 50 to 800

3.3.1 Purity and Repeatability

All of the 12 test samples A1-D3 were injected 3 times each using the given acquisition method to evaluate chromatographic purity. Sample A1 (AI 90%, undiluted) was injected an additional 7, to a total of 10 times consecutively, to evaluate instrumental repeatability. The external reference caffeine (200 µg/mL) was injected 3 times. Evaluation of the chromatograms were generally done by automatic integration of peak areas. Integration events for purity analysis was set to values as following for FID/MS TIC SCAN:

	Undiluted (A1-D1):	Diluted (A2-D3):
• Slope sensitivity:	2/5000.00000	2/5000.00000
• Peak width:	0.00100/0.00100	0.00100/0.00100
• Area reject:	0.05000/10000.0	0.050000/2000.0
• Height reject:	0.50000/50000.0	0.050000/3000.0
• Shoulders mode:	Off/Drop	Off/Drop

Area and peak rejects were set higher for the undiluted samples to avoid integration of irrelevant impurities and baseline noise. Integration was, for all analyses, generally off for FID from 0.001 to 5.000 mins to avoid integration of the solvent top. Average, variance, SD, %RSD, u and U were calculated using excel.

3.3.2 Intermediate Precision

For intermediate precision tests, three samples were analyzed on three different days. New samples were taken for each of the three days, and the tests were performed by two analysts. The CRM-solutions IPA (1000 μg/mL), IPB (200 μg/mL) and IPC (50 μg/mL) were injected six times each day for concentration verification. Caffeine (1000, 200 and 50 μg/mL) was injected six times each day as well, as an external standard reference. Integration events for analysis was set to values as following for FID/MS TIC SCAN:

- Slope sensitivity: 2/5000.00000
- Peak width: 0.00100/0.01000
- Area reject: 0.10000/100000.0
- Height reject: 1.00000/20000.0
- Shoulders mode: Off/Drop

Determinations of concentration was done by creating a calibrating curve in OpenLAB software with respect to the external standard reference caffeine. Calculations of the carbon concentration are given in Appendix A. The concentration of the external standard was reported as μg C pr. mL. Averages, variance, SD, %RSD, u and U were calculated using excel. Accuracy was calculated as the percentage difference from target.

3.3.3 Linearity, Range and LOD

The sixteen samples A-P (2000-0.06 μg/mL) of caffeine were injected once. Integration events for analysis was set to values as following for FID/MS TIC SCAN:

	Sample A-I (high conc.):	Sample J-P (low conc.):
• Slope sensitivity:	5/5000.00000	2/5000.00000
• Peak width:	0.00200/0.01000	0.00100/0.00100
• Area reject:	1.00000/50000.0	0.050000/1000.0
• Height reject:	0.10000/50000.0	0.010000/1200.0
• Shoulders mode:	Off/Drop	Off/Drop

The peak width, area and height rejects were set higher for the more concentrated samples, to avoid integration of irrelevant impurities and baseline noise. The area and height results were plotted against the theoretical concentration. Samples M (0.49 µg/mL) and L (0.98 µg/mL) from linearity test and sample D3 (99% purity, diluted x10) was used for determination of LOD. Values of S/N ratio was calculated by and retrieved from OpenLAB software.

3.3.4 Robustness

Sample D1 (AI 99%, undiluted) was injected 3 times with: two different temperatures for the injector and two different temperature gradients for the column. When analyzing with extra methods for the injector, the original method was used for the column, and vice versa. The temperature for the injector was changed from the original at 250°C to 245°C for modified method 1, and to 255°C for method 2. Integration events was set to the same as for accuracy/precision. Table 3-4 presents the changes ± 5°C performed for the modified methods 1 and 2 for the column. The percentage difference in results compared to the original methods was calculated using excel. Integration events was set to the same as accuracy/precision method.

Table 3-4 Description of extra methods for the gradient temperatures for the column.

	Rate [°C/min]	**Value [°C]**	**Hold time [min]**	**Run time [min]**
Original method	-	50	4	4
	20	325	10	27,75
Extra method 1	-	45	4	4
	15	320	10	32,33
Extra method 2	-	55	4	4
	25	330	10	25

4 Results

All of the analysis reports from the validation can be retrieved from and are stored at the employer. Examples of analysis reports for individual injections are given in Appendix H.

4.1 Validation Summary

A summary of the validation results is given in Table 4-1. The main results are presented, as well as whether or not they fulfilled the criteria for %RSD, accuracy, R^2, S/N and U. Detailed information of the results for each parameter are given in the following chapters.

Table 4-1 Results from the validation, with characteristics, criteria, test results and remarks.

	Criteria	**Results**			**Remarks**
		Test result	***Acceptable***	***Not acceptable***	
Purity 90%	%RSD < 0.5 for AI %RSD < 10 for each impurity	0.24% (AI) 1.76% (Imp. 1) 1.55% (Imp. 2)			All criteria fulfilled when normalized chromatograms were used for evaluation. %RSD is here reported from the average of purity for all dilutions. Accuracy is reported as diff. purity% from target % for all dilutions.
	Accuracy: ± 0.5% from target purity.	0.05% (AI) 0.03% (Imp. 1) -0.07% (Imp. 2)	☒	☐	
Purity 95%	%RSD < 0.5 for AI %RSD < 10 for each impurity	0.03% (AI) 0.97% (Imp. 1) 1.40% (Imp. 2)			
	Accuracy: ± 0.5% from target purity.	-0.40% (AI) 0.05% (Imp. 1) 0.34% (Imp. 2)	☒	☐	
Purity 97%	%RSD < 0.5 for AI %RSD < 20 for each impurity	0.14% (AI) 8.25% (Imp. 1) 4.86% (Imp. 2)			
	Accuracy: ± 0.5% from target purity.	-0.29% (AI) 0.17% (Imp. 1) 0.12% (Imp. 2)	☒	☐	
Purity 99%					
	%RSD < 0.5 for AI %RSD < 50 for each impurity	0.12% (AI) 7.06% (Imp. 1) 14.15% (Imp. 2)			
	Accuracy: ± 0.5% from target purity.	-0.12% (AI) -0.02% (Imp. 1) 0.14% (Imp. 2)	☒	☐	

Table 4-1 Continued.

Repeatability	%RSD < 1% for AI %RSD < 50% for impurities	5.10% (AI 95%, FID) 5.30% (AI 90%, FID) < 50% (all imps., FID) 4.01% (AI 90%, MS) 2.70% (AI 95%, MS) < 50% (all imps., MS)	☐	☒	
Precision	%RSD < 3%	5.40% (IPA) 2.60% (IPB) 3.97% (IPC)	☐	☒	Average values for 3 days.
	Accuracy: ± 5% from target value.	-24.85% (IPA) 28.12% (IPB) -16.13% (IPC)	☐	☒	
Linearity	$R^2_{FID} > 0.995$	Polyarc-FID: $R^2 = 1$ (A[a], 13p) $R^2 = 0.9992$ (A, 7p) $R^2 = 0.9991$ (H[b], 13p) $R^2 = 0.9986$ (H, 7p)	☒	☐	Criteria fulfilled when looking at both full range (13 points), as well as a smaller range (7 points).
Range	From 100% of AI to 0.1% AI	2000 – 0.49 µg/mL	☒	☐	
Robustness	Purity < 0.2% difference compared to original method.	Original method: -0.01% (AI) -0.08% (Imp. 1) 0.09% (Imp 2) Column extra temp. 1: 0.05% (AI) -0.12% (Imp. 1) 0.07% (Imp. 2) Column extra temp. 2: 0.11% (AI) -0.16% (Imp. 1) 0.05% (Imp. 2) Injector extra temp. 1: 0.11% (AI) 0.14% (Imp. 1) 0.03% (Imp. 2) Injector extra temp. 2: 0.09% (A) 0.13% (Imp. 1) 0.04% (Imp. 2)	☒	☐	Test were performed over three days by two analysts. Normalized chromatograms were used for evaluation. Original methods are analyzed on a different day. Percentages are given as diff. purity % from target.
LOD	≤ 0.1% for impurities (S/N ≥ 3)	≤ 0.06% S/N ≥ 3.2	☒	☐	Evaluated from sample L, M & D3.
LOQ	≤ 0.1% for impurities (S/N ≥ 10)		☐	☒	Not evaluated.
Uncertainty	U < 0.5 for chromatographic purity.	0.04 (90% AI) 0.01 (95% AI) 0.03 (97% AI) 0.02 (99% AI)	☒	☐	Measurement uncertainty for purity analyses set to U < 0.05.

[a]) A = peak area
[b]) B = peak height

4.2 Chromatographic Purity

Table 4-2 to Table 4-5 in the following pages presents all of the results from tests of chromatographic purity. Respectively as normalized chromatograms for samples with 90%, 95%, 97% and 99% purity, given with dilutions x3 and x10. Variance, SD, %RSD, u and U are given for each dilution, the average, and all dilutions. Generally, all of the results for chromatographic purity had values that fulfilled the criteria of %RSD, when looking at both AI and impurities in diluted and undiluted samples. The difference in purity % from target were generally lower than 0.5%. Results from MS detector expressed as response factors are given in Appendix G.

4.2.1 Measurement Uncertainty

Measurement uncertainty was determined for purity analysis with Polyarc-FID. When looking at AI, the measurement uncertainty was calculated to values of 0.04, 0.01, 0.03 and 0.02 for undiluted samples with respectively 90%, 95%, 97% and 99% purity. Uncertainty for purity analyses could be set to $U < 0.05$. If diluted samples were included, the highest uncertainty observed for all dilutions and purities were $U = 0.13$ for AI of sample with 90% purity. The measurement uncertainty for all purities and dilutions could be set to $U < 0.15$.

Table 4-2 Normalized chromatograms for sample A with 90% purity.

Purity%, 90% x1			
	AI	Imp 1	Imp 2
Inj 1	84,79	6,26	8,95
Inj 2	84,84	6,26	8,90
Inj 3	84,74	6,29	8,96
Average	84,79	6,27	8,94
Variance	0,0014	0,0002	0,0008
SD	0,04	0,02	0,03
%RSD	0,04	0,25	0,31
u	0,02	0,01	0,02
U	0,04	0,02	0,03

Purity%, 90% x3			
	AI	Imp 1	Imp 2
Inj 1	85,26	6,07	8,68
Inj 2	85,23	6,03	8,74
Inj 3	85,18	6,08	8,74
Average	85,22	6,06	8,72
Variance	0,0010	0,0005	0,0009
SD	0,03	0,02	0,03
%RSD	0,04	0,37	0,34
u	0,02	0,01	0,02
U	0,04	0,03	0,03

Purity%, 90% x10			
	AI	Imp 1	Imp 2
Inj 1	84,82	6,15	9,04
Inj 2	84,97	6,02	9,01
Inj 3	84,92	6,06	9,03
Average	84,90	6,08	9,02
Variance	0,0041	0,0028	0,0002
SD	0,06	0,05	0,01
%RSD	0,08	0,87	0,14
u	0,04	0,03	0,01
U	0,07	0,06	0,01

Purity 90% x1			
Purity %	84,79	6,27	8,94
Target%	85,02	6,16	8,82
Diff. purity %	0,23	-0,11	-0,12

Purity 90% x3			
Purity %	85,22	6,06	8,72
Target%	85,02	6,16	8,82
Diff. purity %	-0,20	0,10	0,10

Purity 90% x10			
Purity %	84,90	6,08	9,02
Target%	85,02	6,16	8,82
Diff. purity %	0,12	0,08	-0,20

Average purity, 90%			
	AI	Imp 1	Imp 2
Dil. x1	84,79	6,27	8,94
Dil. x3	85,22	6,06	8,72
Dil. x10	84,90	6,08	9,02
Average	84,97	6,13	8,89
Variance	0,0336	0,0092	0,0164
SD	0,18	0,10	0,13
%RSD	0,22	1,57	1,44
u	0,11	0,06	0,07
U	0,21	0,11	0,15

Purity for all dilutions, 90%			
	AI	Imp 1	Imp 2
Average	84,97	6,13	8,89
Variance	0,0402	0,0117	0,0191
SD	0,20	0,11	0,14
%RSD	0,24	1,76	1,55
u	0,07	0,04	0,05
U	0,13	0,07	0,09
95% CI	0,13	0,07	0,09

Purity for all dilutions, 90%			
	AI	Imp 1	Imp 2
Purity %	84,97	6,13	8,89
Target%	85,02	6,16	8,82
Diff. purity %	0,05	0,03	-0,07

Table 4-3 Normalized chromatograms for sample B with 95% purity.

Purity%, 95% x1			
	AI	Imp 1	Imp 2
Inj 1	94,28	2,47	3,26
Inj 2	94,25	2,48	3,26
Inj 3	94,28	2,47	3,25
Average	94,27	2,47	3,26
Variance	0,0002	0,0001	0,0000
SD	0,01	0,01	0,01
%RSD	0,01	0,29	0,19
u	0,01	0,00	0,00
U	0,01	0,01	0,01

Purity %, 95% x3			
	AI	Imp 1	Imp 2
Inj 1	94,29	2,48	3,22
Inj 2	94,31	2,50	3,19
Inj 3	94,29	2,51	3,20
Average	94,30	2,50	3,20
Variance	0,0001	0,0002	0,0002
SD	0,01	0,01	0,01
%RSD	0,01	0,54	0,41
u	0,01	0,01	0,01
U	0,01	0,02	0,02

Purity %, 95% x10			
	AI	Imp 1	Imp 2
Inj 1	94,20	2,45	3,35
Inj 2	94,30	2,53	3,26
Inj 3	94,27	2,47	3,26
Average	94,26	2,48	3,29
Variance	0,0018	0,0010	0,0017
SD	0,04	0,03	0,04
%RSD	0,04	1,28	1,26
u	0,02	0,02	0,02
U	0,05	0,04	0,05

Purity 95% x1			
Purity %	94,27	2,47	3,26
Target%	93,87	2,54	3,59
Diff. purity %	-0,40	0,07	0,33

Purity 95% x3			
Purity %	94,30	2,50	3,20
Target%	93,87	2,54	3,59
Diff. purity %	-0,43	0,04	0,39

Purity 95% x10			
Purity %	94,26	2,48	3,29
Target%	93,87	2,54	3,59
Diff. purity %	-0,39	0,06	0,30

Average purity, 95%			
	AI	Imp 1	Imp 2
Dil. x1	94,27	2,47	3,26
Dil. x3	94,30	2,50	3,20
Dil. x10	94,26	2,48	3,29
Average	94,27	2,49	3,25
Variance	0,0003	0,0001	0,0012
SD	0,02	0,01	0,03
%RSD	0,02	0,40	1,06
u	0,01	0,01	0,02
U	0,02	0,01	0,04

Purity for all dilutions, 95%			
	AI	Imp 1	Imp 2
Average	94,27	2,49	3,25
Variance	0,0011	0,0006	0,0021
SD	0,03	0,02	0,05
%RSD	0,03	0,97	1,40
u	0,01	0,01	0,02
U	0,02	0,02	0,03
95% CI	0,02	0,02	0,03

Purity for all dilutions, 95%			
	AI	Imp 1	Imp 2
Purity %	94,27	2,49	3,25
Target %	93,87	2,54	3,59
Diff. purity %	-0,40	0,05	0,34

Table 4-4 Normalized chromatograms for sample C with 97% purity.

Purity %, 97% x1			
	AI	Imp 1	Imp 2
Inj 1	96,56	1,57	1,87
Inj 2	96,51	1,59	1,90
Inj 3	96,49	1,61	1,90
Average	96,52	1,59	1,89
Variance	0,0009	0,0002	0,0002
SD	0,03	0,01	0,02
%RSD	0,03	0,90	0,84
u	0,02	0,01	0,01
U	0,03	0,02	0,02

Purity %, 97% x3			
	AI	Imp 1	Imp 2
Inj 1	96,74	1,50	1,76
Inj 2	96,61	1,51	1,88
Inj 3	96,63	1,62	1,75
Average	96,66	1,54	1,80
Variance	0,0035	0,0032	0,0036
SD	0,06	0,06	0,06
%RSD	0,06	3,64	3,31
u	0,03	0,03	0,03
U	0,07	0,06	0,07

Purity%, 97% x10			
	AI	Imp 1	Imp 2
Inj 1	96,92	1,29	1,78
Inj 2	96,58	1,38	2,04
Inj 3	96,75	1,35	1,91
Average	96,75	1,34	1,91
Variance	0,0200	0,0013	0,0113
SD	0,14	0,04	0,11
%RSD	0,15	2,64	5,56
u	0,08	0,02	0,06
U	0,16	0,04	0,12

Purity 97% x1			
Purity %	96,52	1,59	1,89
Target%	96,35	1,66	1,99
Diff. purity %	-0,17	0,07	0,10

Purity 97% x3			
Purity %	96,66	1,54	1,80
Target%	96,35	1,66	1,99
Diff. purity %	-0,31	0,12	0,19

Purity 97% x10			
Purity %	96,75	1,34	1,91
Target%	96,35	1,66	1,99
Diff. purity %	-0,40	0,32	0,08

Average purity, 97%			
	AI	Imp 1	Imp 2
Dil. x1	96,52	1,59	1,89
Dil. x3	96,66	1,54	1,80
Dil. x10	96,75	1,34	1,91
Average	96,64	1,49	1,87
Variance	0,0087	0,0119	0,0023
SD	0,09	0,11	0,05
%RSD	0,10	7,31	2,55
u	0,05	0,06	0,03
U	0,11	0,13	0,06

Purity for all dilutions, 97%			
	AI	Imp 1	Imp 2
Average	96,64	1,49	1,87
Variance	0,0190	0,0151	0,0082
SD	0,14	0,12	0,09
%RSD	0,14	8,25	4,86
u	0,05	0,04	0,03
U	0,09	0,08	0,06
95% CI	0,09	0,08	0,06

Purity for all dilutions, 97%			
	AI	Imp 1	Imp 2
Purity %	96,64	1,49	1,87
Target%	96,35	1,66	1,99
Diff. purity %	-0,29	0,17	0,12

Table 4-5 Normalized chromatograms for sample D with 99% purity.

Purity %, 99% x1			
	AI	Imp 1	Imp 2
Inj 1	98,35	1,12	0,53
Inj 2	98,33	1,13	0,54
Inj 3	98,30	1,15	0,55
Average	98,33	1,13	0,54
Variance	0,0003	0,0001	0,0000
SD	0,02	0,01	0,01
%RSD	0,02	1,02	1,29
u	0,01	0,01	0,00
U	0,02	0,01	0,01

Purity %, 99% x3			
	AI	Imp 1	Imp 2
Inj 1	98,39	1,03	0,58
Inj 2	98,52	1,08	0,40
Inj 3	98,39	1,09	0,52
Average	98,43	1,07	0,50
Variance	0,0039	0,0006	0,0056
SD	0,06	0,03	0,08
%RSD	0,06	2,38	14,99
u	0,04	0,01	0,04
U	0,07	0,03	0,09

Purity %, 99% x10			
	AI	Imp 1	Imp 2
Inj 1	98,44	1,14	0,43
Inj 2	98,58	0,95	0,47
Inj 3	98,65	0,96	0,40
Average	98,56	1,01	0,43
Variance	0,0077	0,0075	0,0008
SD	0,09	0,09	0,03
%RSD	0,09	8,56	6,76
u	0,05	0,05	0,02
U	0,10	0,10	0,03

Purity 99% x1			
Purity %	98,33	1,13	0,54
Target%	98,32	1,05	0,63
Diff. purity %	-0,01	-0,08	0,09

Purity 99% x3			
Purity %	98,43	1,07	0,50
Target%	98,32	1,05	0,63
Diff. purity %	-0,11	-0,02	0,13

Purity 99% x10			
Purity %	98,56	1,01	0,43
Target%	98,32	1,05	0,63
Diff. purity %	-0,24	0,04	0,20

Average purity, 99%			
	AI	Imp 1	Imp 2
Dil. x1	98,33	1,13	0,54
Dil. x3	98,43	1,07	0,50
Dil. x10	98,56	1,01	0,43
Average	98,44	1,07	0,49
Variance	0,0087	0,0023	0,0021
SD	0,09	0,05	0,05
%RSD	0,09	4,49	9,36
u	0,05	0,03	0,03
U	0,11	0,06	0,05

Purity for all dilutions, 99%			
	AI	Imp 1	Imp 2
Average	98,44	1,07	0,49
Variance	0,0143	0,0057	0,0048
SD	0,12	0,08	0,07
%RSD	0,12	7,06	14,15
u	0,04	0,03	0,02
U	0,08	0,05	0,05
95% CI	0,08	0,05	0,05

Purity for all dilutions, 99%			
	AI	Imp 1	Imp 2
Purity %	98,44	1,07	0,49
Target%	98,32	1,05	0,63
Diff. purity %	-0,12	-0,02	0,14

Figure 4-1 to Figure 4-5 presents examples of chromatograms for a blank sample containing the solvent isooctane, and undiluted samples A1 with 90% purity, B1 with 95% purity, C1 with 97% purity and D1 with 99% purity. The blank sample did in not contain any interfering components.

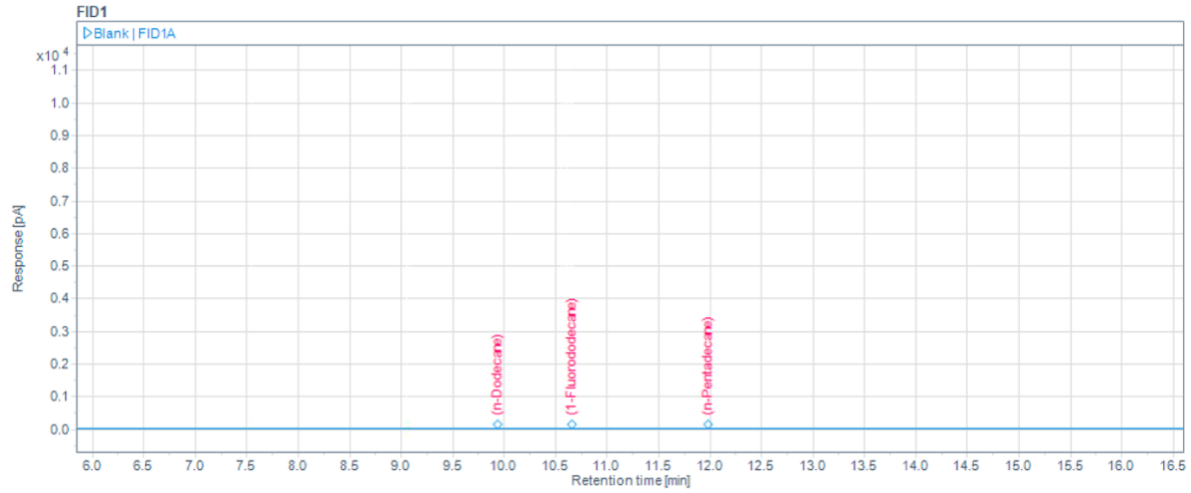

Figure 4-1 Chromatogram for blank sample of isooctane.

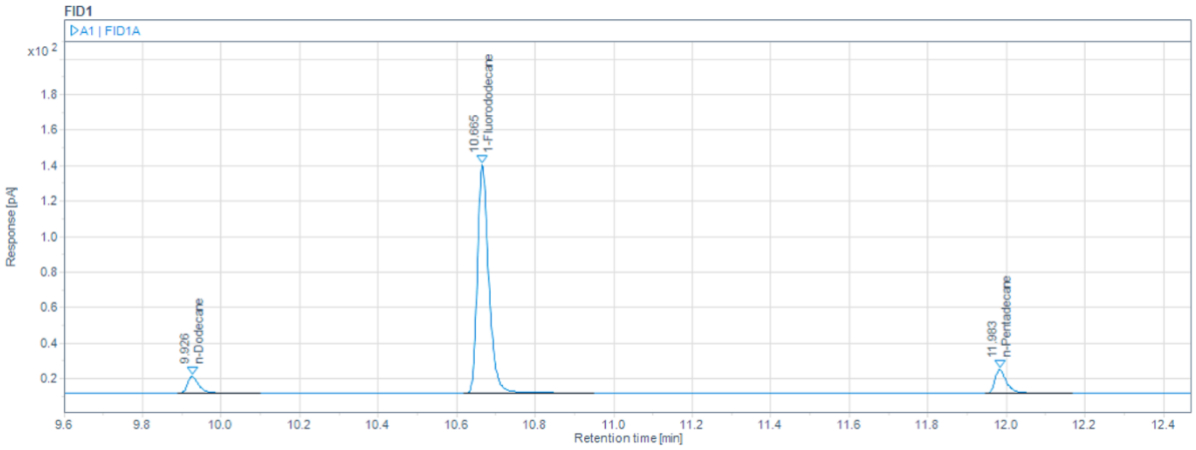

Figure 4-2 Chromatogram for sample A1 with 90% purity.

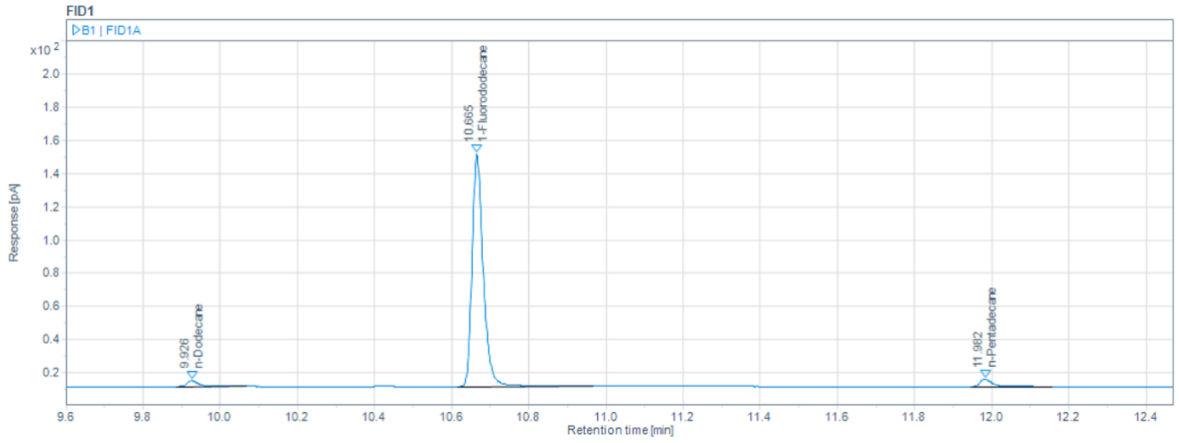

Figure 4-3 Chromatogram for sample B1 with 95% purity.

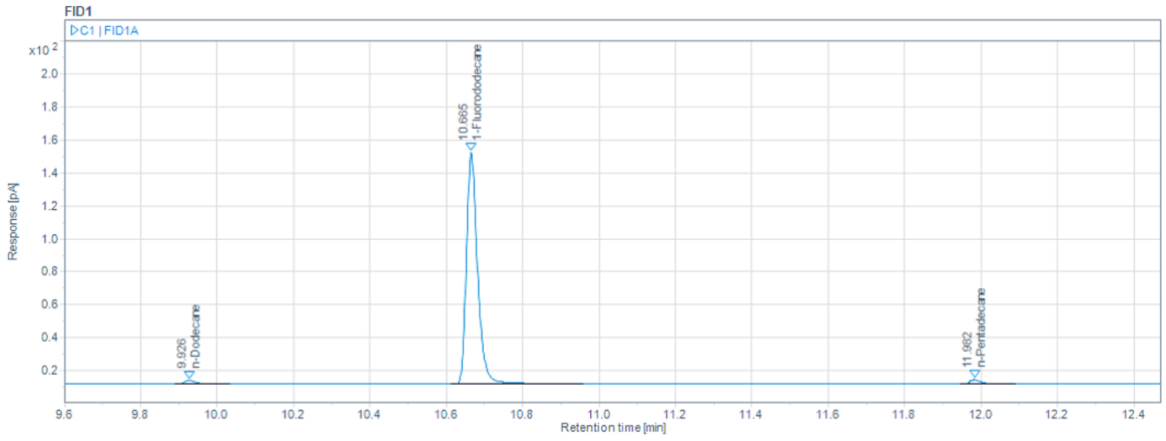

Figure 4-4 Chromatogram for sample C1 with 97% purity.

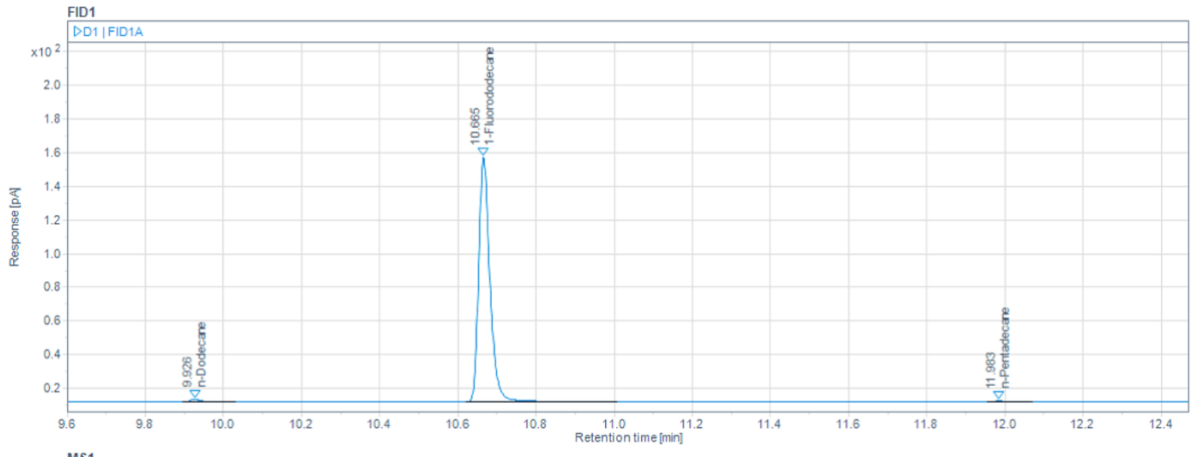

Figure 4-5 Chromatogram for sample D1 with 99% purity.

4.3 Intermediate Precision

4.3.1 Concentration Verification

Table 4-6, Table 4-7 and Table 4-8 in the following pages presents results for the concentration verification of respectively benzo[a]pyrene (IPA, 1000 μg/mL), naphthalene (IPB, 200 μg/mL) and PCB 52 (IPC, 50 μg/mL). Results are given as carbon concentrations (μg C/mL) for each of the three days, with the average, variance, SD, %RSD, u and U. Accuracy results for each day are given as difference from the target concentration. In addition, there is a summary of both concentration and accuracy for the average over three days. All of the results for accuracy showed differences from target value were greater than the criteria ± 5%, which is considered not acceptable. The results vary from a difference at 14.02% (IPC day 3) up to 32.20% (IPB day 3). Values for %RSD were generally lower than the criteria of < 3%, except for IPA day 3 (%RSD = 4.59%) and IPC day 1 (%RSD = 4.19).

Table 4-6 Results from concentration verification of CRM IPA.

CRM IPA	Day 1
	Conc
Inj 1	755,91
Inj 2	765,25
Inj 3	749,24
Inj 4	745,02
Inj 5	754,82
Inj 6	721,10
Average	748,56
Variance	1,90E+02
SD	13,78
%RSD	1,84
u	5,62
U	11,25

Accuracy	
Target conc	1000
Conc day 1	748,56
Diff. Conc	-251,44
Diff. %	-25,14

CRM IPA	Day 2
	Conc
Inj 1	695,74
Inj 2	741,97
Inj 3	695,69
Inj 4	712,73
Inj 5	719,39
Inj 6	724,90
Average	715,07
Variance	2,66E+02
SD	16,30
%RSD	2,28
u	6,65
U	13,31

Accuracy	
Target conc	1000
Conc day 2	715,07
Diff. Conc	-284,93
Diff. %	-28,49

CRM IPA	Day 3
	Conc
Inj 1	712,89
Inj 2	817,15
Inj 3	801,32
Inj 4	803,39
Inj 5	820,31
Inj 6	790,41
Average	790,91
Variance	1,32E+03
SD	36,30
%RSD	4,59
u	14,82
U	29,64

Accuracy	
Target conc	1000
Conc day 3	790,91
Diff. Conc	-209,09
Diff. %	-20,91

Conc. summary for 3 days	
Average	751,51
Variance	1645
SD	40,56
%RSD	5,40
u	9,56
U	19,12
95 % CI	18,74

Accuracy summary for 3 days	
Target conc	1000
Conc summary	751,51
Diff. Conc	-248,49
Diff. %	-24,85

Table 4-7 Results from concentration verification of CRM IPB.

CRM IPB	Day 1
	Conc.
Inj 1	252,21
Inj 2	250,83
Inj 3	248,24
Inj 4	248,75
Inj 5	248,20
Inj 6	251,06
Average	249,88
Variance	2,42E+00
SD	1,56
%RSD	0,62
u	0,63
U	1,27

Accuracy	
Target conc.	200
Conc. day 1	249,88
Diff. conc	49,88
Diff. %	24,94

CRM IPB	Day 2
	Conc.
Inj 1	257,61
Inj 2	257,32
Inj 3	252,05
Inj 4	254,56
Inj 5	256,16
Inj 6	249,05
Average	254,46
Variance	9,34E+00
SD	3,06
%RSD	1,20
u	1,25
U	2,50

Accuracy	
Target conc.	200
Conc. day 2	254,46
Diff. conc	54,46
Diff. %	27,23

CRM IPB	Day 3
	Conc.
Inj 1	263,62
Inj 2	267,20
Inj 3	260,63
Inj 4	264,74
Inj 5	264,96
Inj 6	265,26
Average	264,40
Variance	3,96E+00
SD	1,99
%RSD	0,75
u	0,81
U	1,63

Accuracy	
Target conc.	200
Conc. day 3	264,40
Diff. conc.	64,40
Diff. %	32,20

Conc. summary for 3 days	
Average	256,25
Variance	44
SD	6,67
%RSD	2,60
u	1,57
U	3,14
95% CI	3,08

Accuracy summary for 3 days	
Target conc.	200
Conc. summary	256,25
Diff. conc.	56,25
Diff. %	28,12

Table 4-8 Results from concentration verification of CRM IPC.

CRM IPC	Day 1
	Conc.
Inj 1	39,73
Inj 2	43,29
Inj 3	43,91
Inj 4	40,38
Inj 5	44,11
Inj 6	43,79
Average	42,54
Variance	3,17E+00
SD	1,78
%RSD	4,19
u	0,73
U	1,45

Accuracy	
Target conc.	50
Conc. day 1	42,54
Diff. conc.	-7,47
Diff. %	-14,93

CRM IPC	Day 2
	Conc.
Inj 1	39,50
Inj 2	40,17
Inj 3	40,53
Inj 4	40,51
Inj 5	40,21
Inj 6	40,70
Average	40,27
Variance	1,54E-01
SD	0,39
%RSD	0,98
u	0,16
U	0,32

Accuracy	
Target conc.	50
Conc. day 2	40,27
Diff. conc.	-9,73
Diff. %	-19,46

CRM IPC	Day 3
	Conc.
Inj 1	42,74
Inj 2	43,82
Inj 3	43,16
Inj 4	42,17
Inj 5	42,79
Inj 6	43,27
Average	42,99
Variance	2,64E-01
SD	0,51
%RSD	1,20
u	0,21
U	0,42

Accuracy	
Target conc.	50
Conc. day 3	42,99
Diff. conc.	-7,01
Diff. %	-14,02

Conc. summary for 3 days	
Average	41,93
Variance	3
SD	1,66
%RSD	3,97
u	0,39
U	0,78
95% CI	0,77

Accuracy summary for 3 days	
Target conc.	50
Conc. summary	41,93
Diff. conc.	-8,07
Diff. %	-16,13

4.4 Instrumental Repeatability

In Table 4-9 and Table 4-10 results from repeatability tests of Polyarc-FID are presented, respectively for sample A with 90% purity and sample B with 95% purity. In Table 4-11 and Table 4-12 results from MSD are given. Results for 90% purity were automatically integrated, while results for 95% purity were manually integrated. The results for both purities did not fulfill the criterion of %RSD < 1% for AI, with values of 5.30% and 5.10% for FID, and 4.01% and 2.70% for MS.

Table 4-9 Repeatability results for sample A with 90% purity from Polyarc-FID.

Repeatability for sample 90% X1

GC/Polyarc-FID

	Area AI	Area Imp 1	Area Imp 2	Purity %
Inj 1	260,98	19,25	27,56	84,79
Inj 2	237,24	17,52	24,89	84,84
Inj 3	237,96	17,67	25,17	84,74
Inj 4	261,63	19,28	27,78	84,75
Inj 5	265,82	19,72	27,98	84,78
Inj 6	266,92	19,73	28,19	84,78
Inj 7	237,88	17,49	25,13	84,81
Inj 8	237,64	17,52	24,90	84,85
Inj 9	264,34	19,39	27,79	84,86
Inj 10	264,51	19,59	27,92	84,77
Average	245,39	18,15	25,87	84,79
Variance	1,7E+02	9,3E-01	2,0E+00	1,4E-03
SD	13,02	0,97	1,41	0,04
%RSD	5,30	5,32	5,44	0,04
u	4,12	0,31	0,44	0,01
U	8,23	0,61	0,89	0,02
			Target	85,02
			Diff	-0,23

Table 4-10 Repeatability results for sample B with 95% purity from Polyarc-FID.

Repeatability for sample 95% X1

GC/Polyarc-FID

	Area AI	Area Imp 1	Area Imp 2	Purity %
Inj 1	246,98	6,46	8,53	94,28
Inj 2	271,87	7,17	9,42	94,25
Inj 3	273,25	7,17	9,42	94,28
Inj 4	248,12	6,53	8,55	94,27
Inj 5	276,36	7,23	9,68	94,23
Inj 6	245,75	6,46	8,50	94,27
Inj 7	274,09	7,20	9,52	94,25
Inj 8	276,19	7,21	9,69	94,24
Inj 9	248,28	6,48	8,59	94,28
Inj 10	275,95	7,28	9,60	94,23
Average	264,03	6,93	9,12	94,27
Variance	1,8E+02	1,3E-01	2,5E-01	3,1E-04
SD	13,47	0,36	0,50	0,02
%RSD	5,10	5,17	5,53	0,02
u	4,26	0,11	0,16	0,01
U	8,52	0,23	0,32	0,01
			Target	96,35
			Diff	-2,08

Table 4-11 Repeatability results for sample A with 90% purity from MS detector.

Repeatability for sample 95% X1

GC/MS

	Area AI	Area Imp 1	Area Imp 2	Purity %
Inj 1	13522659,14	245082,26	386734,37	95,54
Inj 2	14190883,42	287264,30	454821,14	95,03
Inj 3	14258642,73	291941,17	460670,70	94,99
Inj 4	13595446,65	258268,31	402546,94	95,36
Inj 5	14352477,12	297709,47	471876,98	94,91
Inj 6	13597443,41	256542,05	397790,73	95,41
Inj 7	14426219,25	295046,55	469825,01	94,96
Inj 8	14430993,76	299494,83	478711,18	94,88
Inj 9	13734339,12	262874,08	409751,40	95,33
Inj 10	14498229,00	301220,47	482484,69	94,87
Average	13990728,43	274762,58	434075,40	95,18
Variance	1,4E+11	4,1E+08	1,3E+09	5,7E-02
SD	378217,28	20245,38	35734,49	0,24
%RSD	2,70	7,37	8,23	0,25
u	119602,80	6402,15	11300,24	0,08
U	239205,61	12804,30	22600,48	0,15
			Target	96,35
			Diff	-1,17

Table 4-12 Repeatability results for sample B with 95% purity from MS detector.

Repeatability for sample 90% X1

GC/MS

	Area AI	Area Imp 1	Area Imp 2	Purity %
Inj 1	11266848,6	941538,4	1751486,3	80,71
Inj 2	10501459,0	847776,0	1568179,3	81,30
Inj 3	10629764,8	860658,2	1600338,8	81,20
Inj 4	11332407,7	964446,2	1793430,9	80,43
Inj 5	11516227,0	982642,6	1832743,6	80,36
Inj 6	11523387,6	986680,7	1832947,3	80,34
Inj 7	10669827,9	873398,0	1630466,5	80,99
Inj 8	10687785,6	878253,6	1637683,0	80,95
Inj 9	11574595,8	996710,8	1855164,1	80,23
Inj 10	11640761,3	997665,9	1860124,9	80,29
Average	10799357,5	883324,2	1640001,4	81,07
Variance	1,9E+11	3,4E+09	1,2E+10	1,5E-01
SD	432965,31	58047,00	109212,61	0,38
%RSD	4,01	6,57	6,66	0,47
u	136915,65	18356,07	34536,06	0,12
U	273831,30	36712,15	69072,12	0,24
			Target	85,02
			Diff	-3,95

4.4.1 Mass Identification MSD

Table 4-13 presents repeatability of mass identification of AI for sample with 90% and 95% purity. Results are given as m/z values closest to the M_W of 1-fluorododecane at 188.32 Da. The MSD shows stable results, with no difference in m/z for the injections. Figure 4-6 shows an example of one of the mass spectrums used for evaluation.

Table 4-13 Repeatability of mass identification (m/z) for GC/MS.

Repeatability for sample 90% and 95%	**X1**
GC/MS	
	AI 90% purity [m/z]	AI 95% purity [m/z]
Inj 1	188,1	188,1
Inj 2	188,1	188,1
Inj 3	188,1	188,1
Inj 4	188,1	188,1
Inj 5	188,1	188,1
Inj 6	188,1	188,1
Inj 7	188,1	188,1
Inj 8	188,1	188,1
Inj 9	188,1	188,1
Inj 10	188,1	188,1
Average	188,1	188,1
Variance	0,00	0,00
SD	0,00	0,00
%RSD	0,00	0,00
u	0,00	0,00
U	0,00	0,00

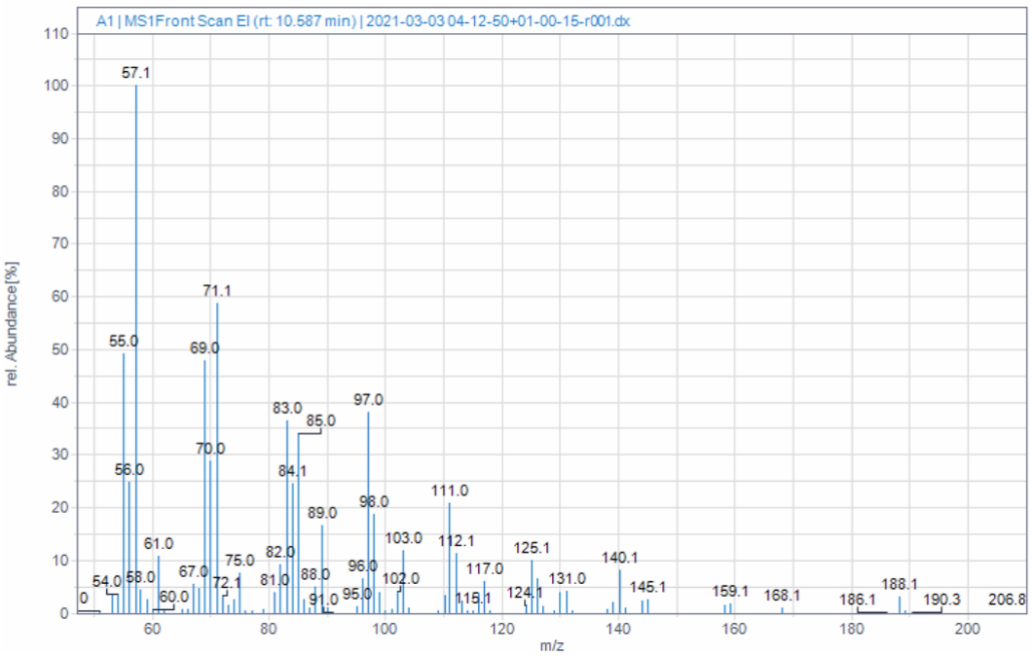

Figure 4-6 Mass spectrum of AI from sample A1 (90% purity).

4.4.2 Retention Times FID

Retention times from GC-FID through three different days are given in Table 4-14, Table 4-15 and Table 4-16, respectively for IPA, IPB and IPC. The retention times proved to be stable both for injections and day variation, with all values of %RSD < 0.06.

Table 4-14 Results from testing intermediate precision of retention times for IPA.

CRM IPA	Day 1	Day 2	Day 3	Ret. time for 3 days	
	Ret. time	Ret. time	Ret. time	Average	18,69
Inj 1	18,69	18,68	18,68	Variance	0
Inj 2	18,69	18,68	18,68	SD	0,00
Inj 3	18,69	18,68	18,68	%RSD	0,02
Inj 4	18,69	18,68	18,68	u	0,00
Inj 5	18,69	18,68	18,68	U	0,00
Inj 6	18,69	18,68	18,68	95% CI	0,00
Average	18,69	18,68	18,68		
Variance	1,14E-06	1,14E-06	2,22E-07		
SD	1,07E-03	1,07E-03	4,71E-04		
%RSD	5,71E-03	5,71E-03	2,52E-03		
u	4,36E-04	4,36E-04	1,92E-04		
U	8,71E-04	8,71E-04	3,85E-04		

Table 4-15 Results from testing intermediate precision of retention times for IPB.

CRM IPB	Day 1	Day 2	Day 3	Ret. time for 3 days	
	Ret. time	Ret. time	Ret. time	Average	9,94
Inj 1	9,94	9,94	9,94	Variance	0
Inj 2	9,94	9,94	9,94	SD	0,00
Inj 3	9,94	9,94	9,94	%RSD	0,04
Inj 4	9,94	9,94	9,94	u	0,00
Inj 5	9,94	9,94	9,94	U	0,00
Inj 6	9,94	9,94	9,94	95% CI	0,00
Average	9,94	9,94	9,94		
Variance	0,00	0,00	1,39E-07		
SD	0,00	0,00	3,73E-04		
%RSD	0,00	0,00	3,75E-03		
u	0,00	0,00	1,52E-04		
U	0,00	0,00	3,04E-04		

Table 4-16 Results from testing intermediate precision of retention times for IPC.

CRM IPC	Day 1	Day 2	Day 3	Ret. time for 3 days	
	Ret. time	Ret. time	Ret. time	Average	14,61
Inj 1	14,62	14,62	14,61	Variance	0
Inj 2	14,62	14,62	14,61	SD	0,00
Inj 3	14,62	14,62	14,61	%RSD	0,03
Inj 4	14,62	14,62	14,61	u	0,00
Inj 5	14,62	14,62	14,61	U	0,00
Inj 6	14,62	14,62	14,61	95% CI	0,00
Average	14,62	14,62	14,61		
Variance	2,22E-07	5,56E-07	4,72E-07		
SD	4,71E-04	7,45E-04	6,87E-04		
%RSD	3,22E-03	5,10E-03	4,70E-03		
u	1,92E-04	3,04E-04	2,81E-04		
U	3,85E-04	6,09E-04	5,61E-04		

4.5 Linearity and Range

In Table 4-17 the results from the linearity and range test performed with the caffeine standard are given. All of the results from Polyarc-FID shows a correlation coefficient greater than the criterion of $R^2 > 0.995$. The results from MS shows values lower than 0.995. Peak area for sample concentration at 1000 μg/mL proved to be approximately 300. At 100 μg/mL (0.1% of 1000 μg/mL) the peak area was approximately 0.25. The range criteria set to from 100% to 0.1% of AI was fulfilled.

Table 4-17 Results from linearity tests for both GC-MS and GC-FID/Polyarc.

Sample	Conc. range [μg/mL]	Peak	Detector	R^2
M – A	0,49 – 2000	Area	Polyarc-FID	1
M – G	0,49 – 31,25	Area	Polyarc-FID	0,9992
M – A	0,49 – 2000	Height	Polyarc-FID	0,9991
M – G	0,49 – 31,25	Height	Polyarc-FID	0,9986
M – A	0,49 – 2000	Area	MS	0,9438
M – G	0,49 – 31,25	Area	MS	0,9777

From Figure 4-7 to Figure 4-12 below, graphic presentations of the linearity results are shown in the same order as tabulated above. Graphs are given for both the full range of 13 points detected by the instrument (0.49 – 2000 μg/mL) and a smaller range of seven points (0.49 – 31.25 μg/mL). All of the peak area and height results used for the plotting of graphs are given in Appendix F, Table 7-5 (FID areas), Table 7-6 (FID heights) and Table 7-7 (MS areas).

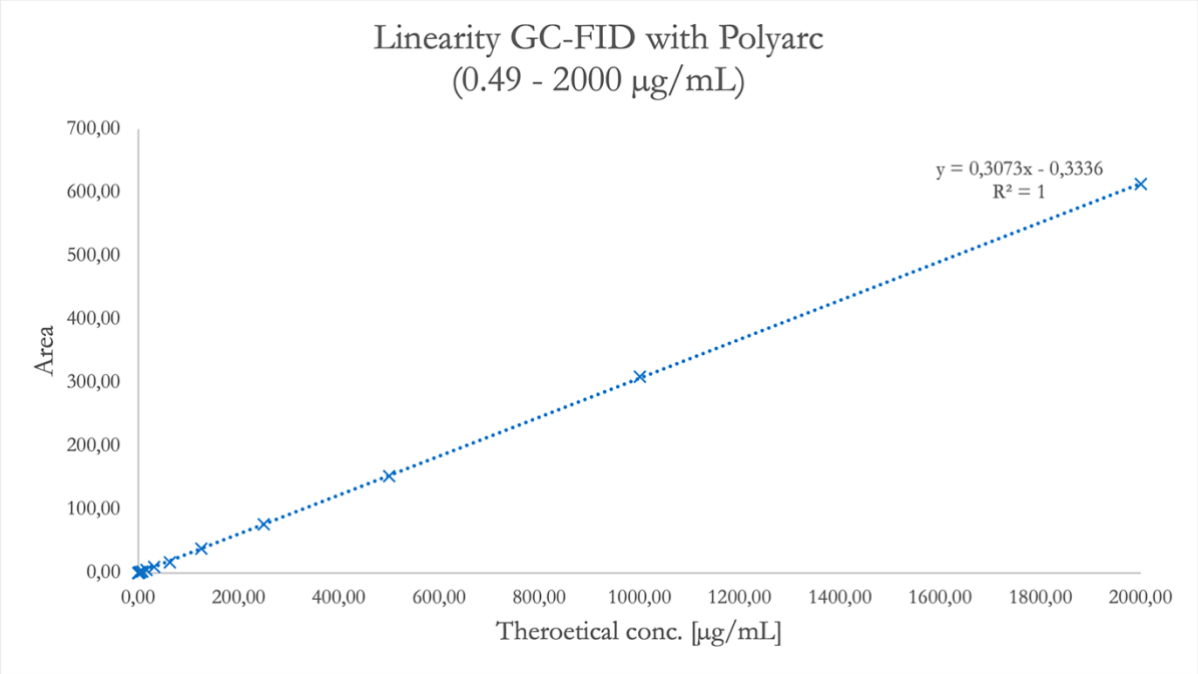

Figure 4-7 Linearity results of caffeine for peak areas from GC/Polyarc-FID.

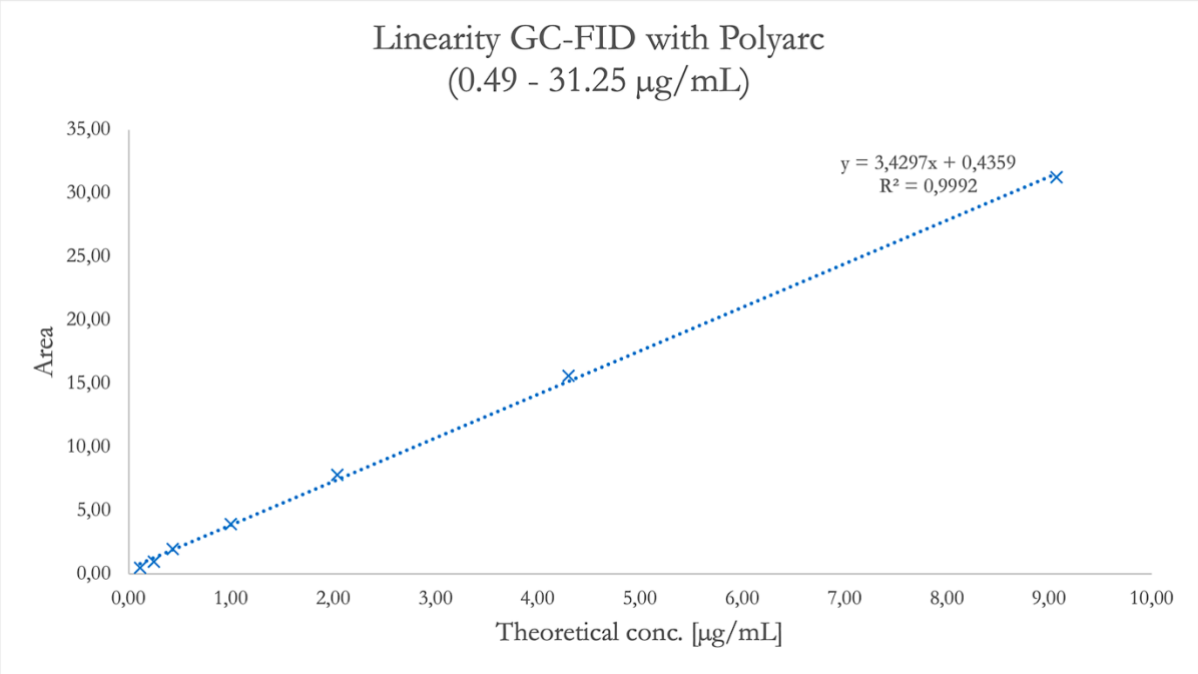

Figure 4-8 Linearity results of caffeine for peak areas of a smaller range for the Polyarc-FID.

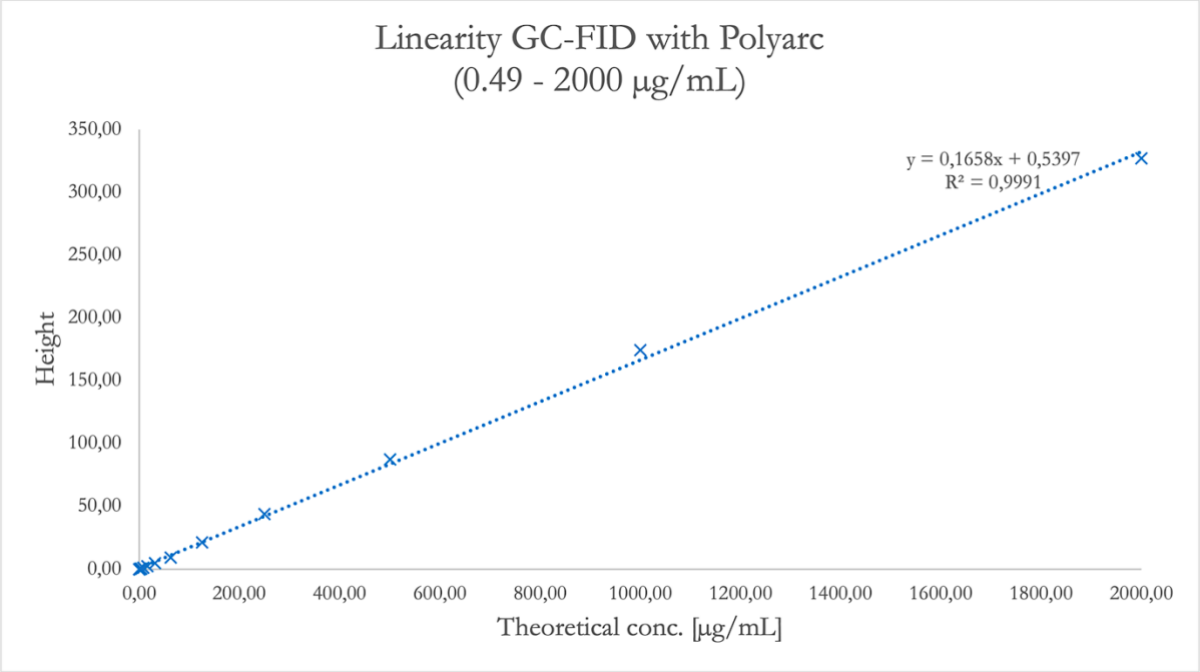

Figure 4-9 Linearity results of caffeine for peak heights from GC/Polyarc-FID.

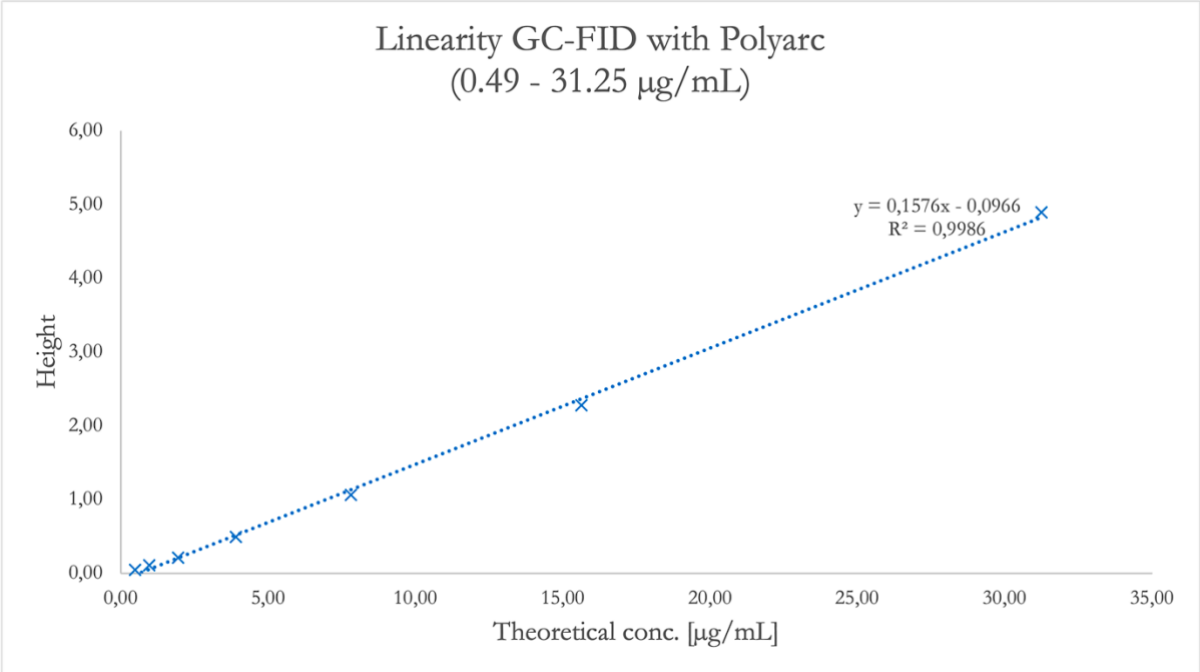

Figure 4-10 Linearity results of caffeine for peak heights of a smaller range for the Polyarc-FID.

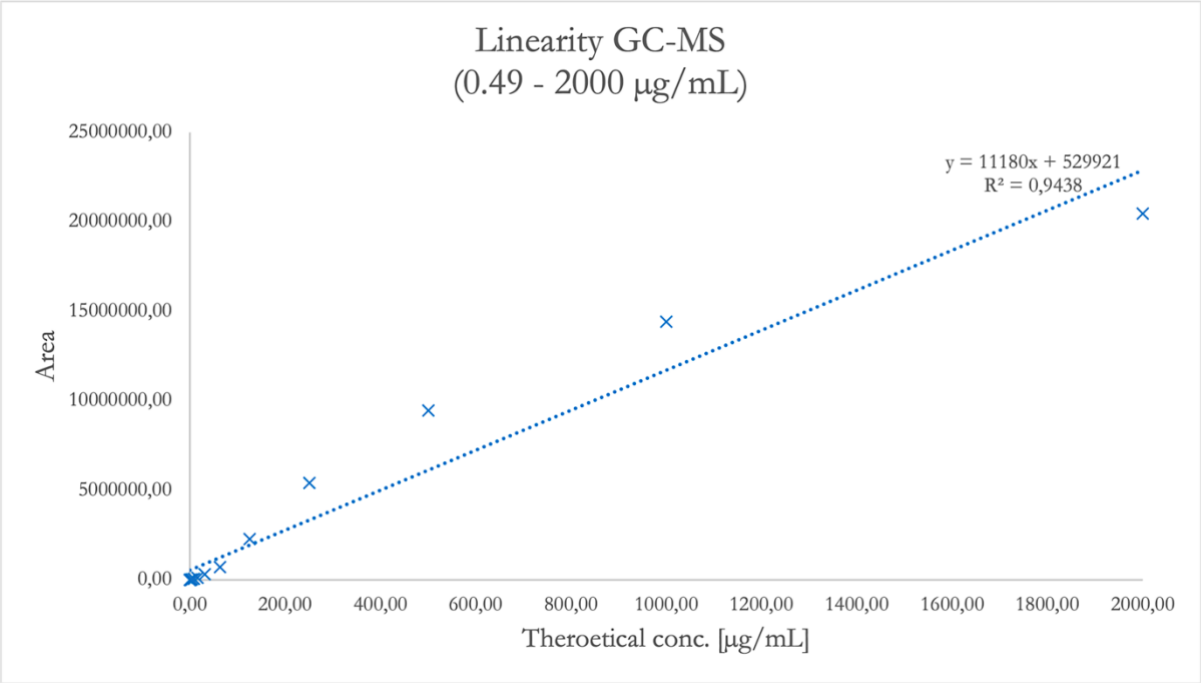

Figure 4-11 Linearity results of caffeine for peak areas from GC/MS.

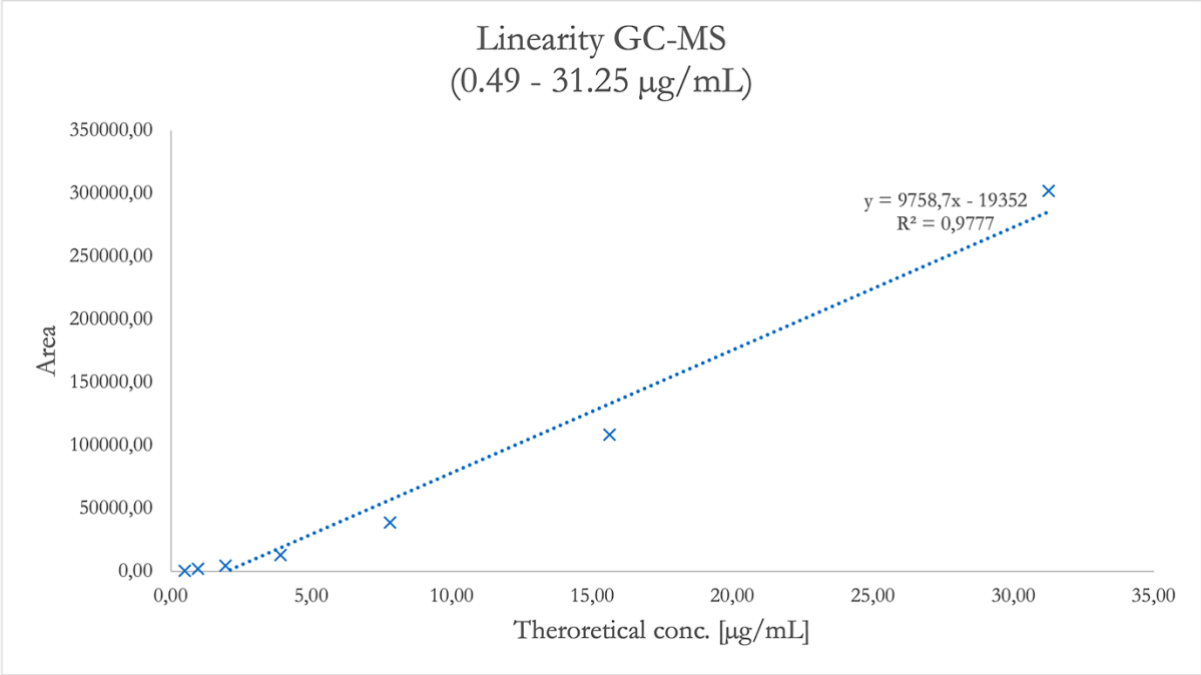

Figure 4-12 Linearity results of caffeine for peak areas of a smaller range for the MS.

4.6 LOD

In Table 4-18 the results from evaluation of LOD are given. The S/N ratio, concentration and corresponding purity % are presented for the three samples L and M from the linearity tests, and sample D3 (99% purity, dilution x10) from purity tests. The results fulfilled the criteria set to ≤ 0.1% for impurities an S/N ≥ 3 for LOD, with corresponding purity at 0.06% and the lowest value of S/N = 3.2 (n-pentadecane). Figure 4-13, Figure 4-14, and Figure 4-15 on the following page presents chromatograms for the samples used for evaluating LOD.

Table 4-18 The S/N ratio for sample L, M, and D3, concentrations and corresponding purity %.

Sample	Component	S/N	Conc. [µg/mL]	Corresponds to purity % at sample conc. 1000 µg/mL
L	Caffeine	10,5	0,98	-
M	Caffeine	5,2	0,49	-
D3 (Inj. 1)	1-Fluorododecane	1059,0	98,78	9,88%
D3 (Inj. 2)	1-Fluorododecane	1033,4	98,78	9,88%
D3 (Inj. 3)	1-Fluorododecane	1024,8	98,78	9,88%
D3 (Inj. 1)	n-Dodecane	9,2	1,06	0,11%
D3 (Inj. 2)	n-Dodecane	8,4	1,06	0,11%
D3 (Inj. 3)	n-Dodecane	8,8	1,06	0,11%
D3 (Inj. 1)	n-Pentadecane	5,0	0,63	0,06%
D3 (Inj. 2)	n-Pentadecane	4,7	0,63	0,06%
D3 (Inj. 3)	n-Pentadecane	3,2	0,63	0,06%

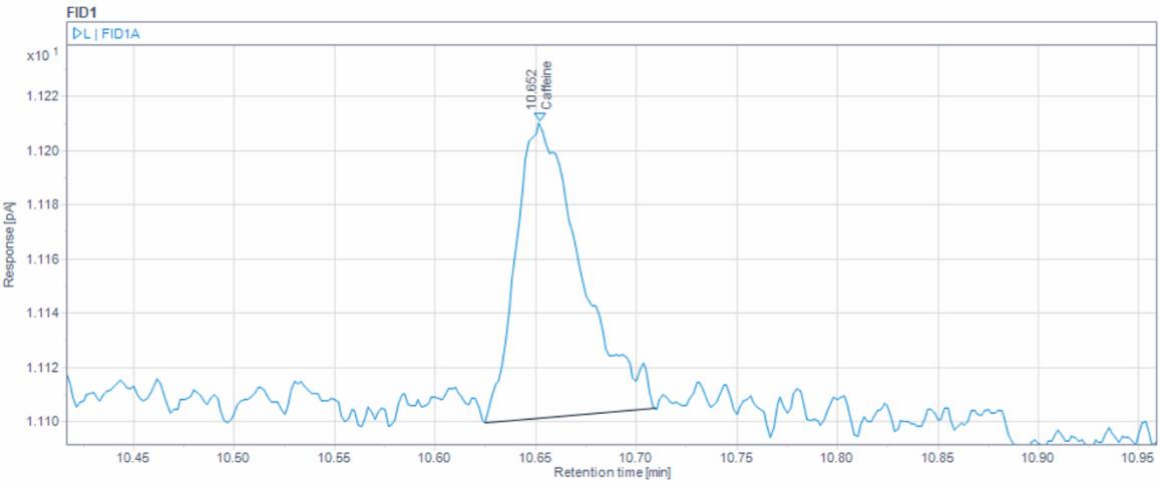

Figure 4-13 Chromatogram for sample L (0,98 μg/mL) from linearity test. S/N = 10.5.

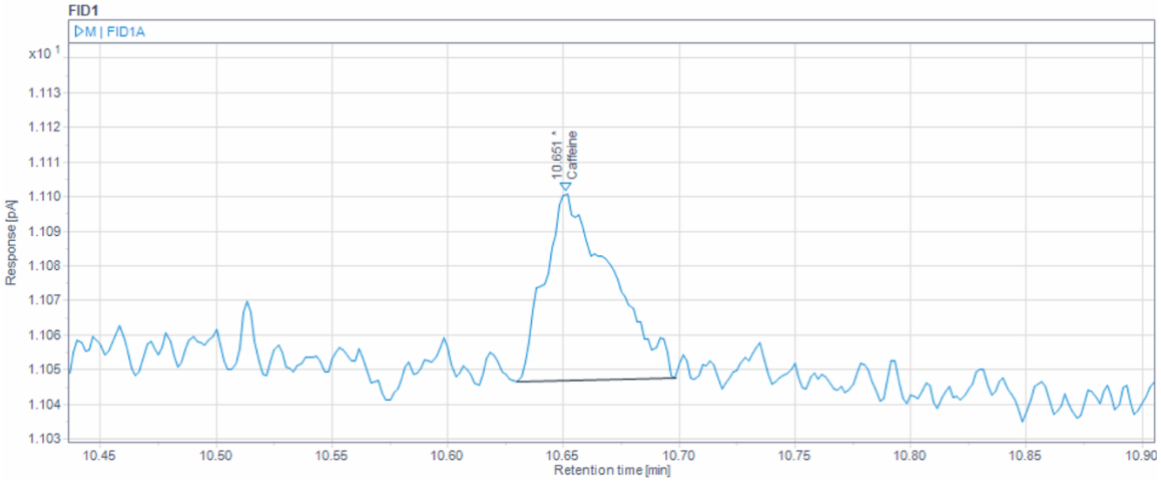

Figure 4-14 Chromatogram for sample M (0,49 μg/mL) from linearity test. S/N = 5.2.

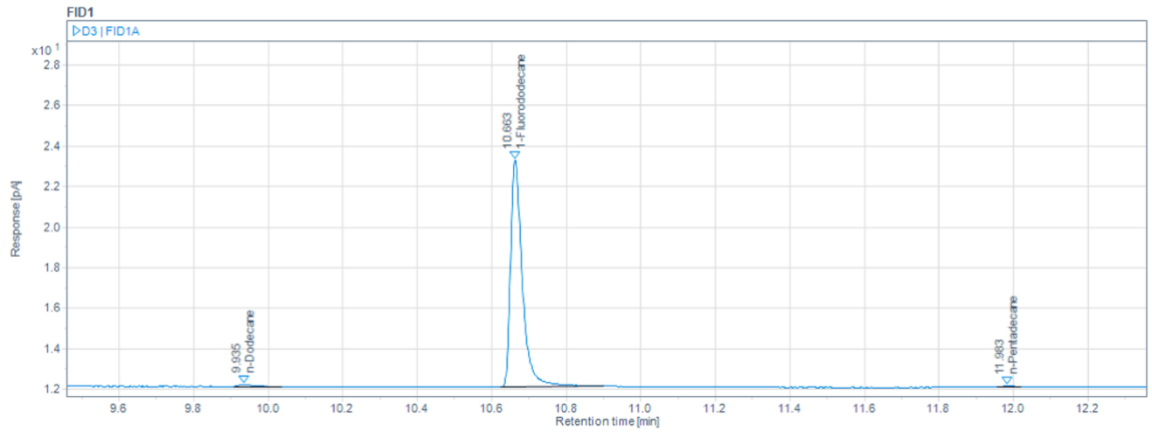

Figure 4-15 Chromatogram for sample D3 with 99% purity (diluted x10).

4.7 Robustness

In Table 4-19 results for the robustness tests with changes for the temperature gradient are given. The results showed no significant changes in purity, with differences from target for AI at 0.05% from target for extra method 1, and 0.11% for method 2. Values for %RSD were within the criteria for 99% purity, with values of %RSD < 0.03 for AI.

Table 4-19 Normalized chromatograms from robustness tests with column temperature gradients. Results for the original method were analyzed on a different day.

Purity %, 99% x1, Column temp. Grad. Acc. Org. method

	AI	Imp 1	Imp 2
Inj 1	98,35	1,12	0,53
Inj 2	98,33	1,13	0,54
Inj 3	98,30	1,15	0,55
Average	98,33	1,13	0,54
Variance	0,0003	0,0001	0,00
SD	0,02	0,01	0,01
%RSD	0,02	1,02	1,29
u	0,01	0,01	0,00
U	0,02	0,01	0,01

Purity %, 99% x1, Column temp. Grad. Acc. Extra meth. 1

	AI	Imp 1	Imp 2
Inj 1	98,29	1,16	0,56
Inj 2	98,25	1,19	0,56
Inj 3	98,26	1,16	0,57
Average	98,27	1,17	0,56
Variance	0,0002	0,0002	0,00
SD	0,01	0,01	0,01
%RSD	0,02	1,14	1,25
u	0,01	0,01	0,00
U	0,02	0,02	0,01

Purity %, 99% x1, Column temp. Grad. Acc. Extra meth. 2

	AI	Imp 1	Imp 2
Inj 1	98,18	1,25	0,58
Inj 2	98,22	1,19	0,59
Inj 3	98,23	1,18	0,58
Average	98,21	1,21	0,58
Variance	0,0005	0,0008	0,00
SD	0,02	0,03	0,01
%RSD	0,02	2,28	0,99
u	0,01	0,02	0,00
U	0,03	0,03	0,01

Purity %, 99% x1, Column temp. Grad. Acc. Org. method

Purity %	98,33	1,13	0,54
Target%	98,32	1,05	0,63
Diff purity %	-0,01	-0,08	0,09

Purity %, 99% x1, Column temp. Grad. Acc. Extra meth. 1

Purity %	98,27	1,17	0,56
Target%	98,32	1,05	0,63
Diff purity %	0,05	-0,12	0,07

Purity %, 99% x1, Column temp. Grad. Acc. Extra meth. 2

Purity%	98,21	1,21	0,58
Target%	98,32	1,05	0,63
Diff purity %	0,11	-0,16	0,05

Average purity, 99%

	AI	Imp 1	Imp 2
Original	98,33	1,13	0,54
Extra meth. 1	98,27	1,17	0,56
Extra meth. 2	98,21	1,21	0,58
Average	98,27	1,17	0,56
Variance	0,0024	0,0009	0,0003
SD	0,05	0,03	0,02
%RSD	0,05	2,61	3,07
u	0,03	0,02	0,01
U	0,06	0,04	0,02

Purity for all column temp. methods, 99%

Average	98,27	1,17	0,56
Variance	0,0031	0,0014	0,0004
SD	0,06	0,04	0,02
%RSD	0,06	3,25	3,49
u	0,02	0,01	0,01
U	0,04	0,03	0,01
95% CI	0,04	0,02	0,01

Purity for all column temp. methods, 99%

Purity %	98,27	1,17	0,56
Target%	98,32	1,05	0,63
Diff purity %	0,05	-0,12	0,07

Chromatograms in Figure 4-16, Figure 4-17 and Figure 4-18 presents the visual difference for the column temperature gradients, respectively between the original method, extra method 1 and extra method 2. The retention times various to some degree with the methods, but no other significant differences were noticed.

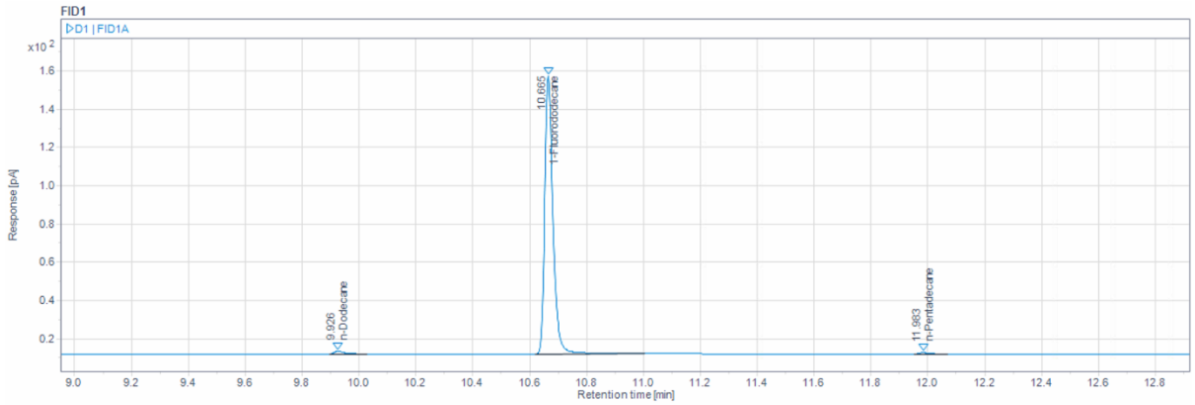

Figure 4-16 Chromatogram for sample D1 with 99% purity, original column gradient method.

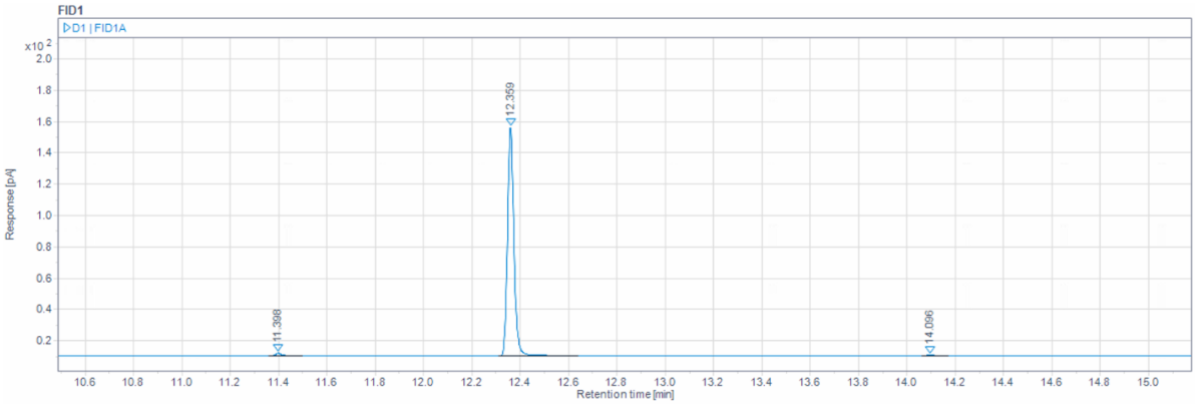

Figure 4-17 Chromatogram for sample D1 with 99% purity, extra column gradient method 1.

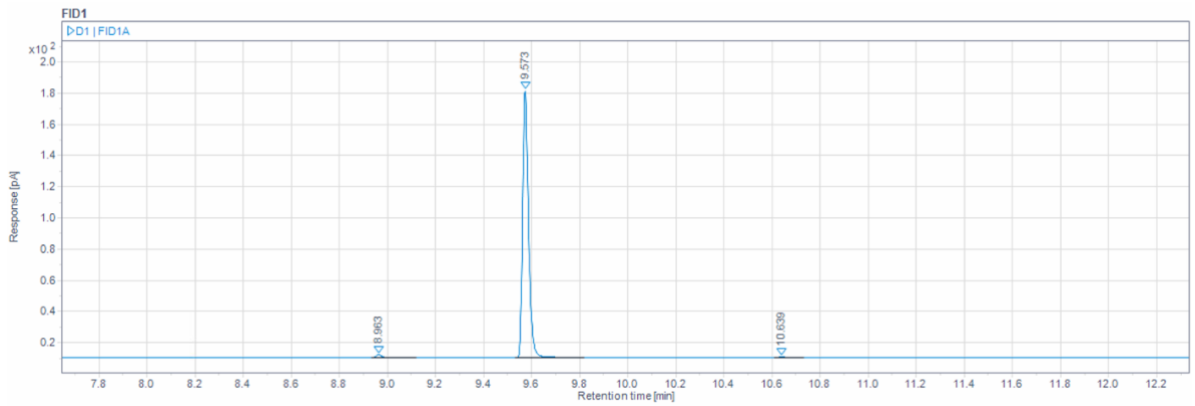

Figure 4-18 Chromatogram for sample D1 with 99% purity, extra column gradient method 2.

In Table 4-20 results for the robustness tests with changes for the injector temperature are given. The results showed no significant changes in purity, with differences from target for AI at 0.11% from target for extra method 1, and 0.09% for method 2. Values for %RSD were within the criteria for 99% purity, with values of %RSD < 0.03 for AI.

Table 4-20 Normalized chromatograms from robustness tests with temperatures for the injector. Results for the original method were analyzed on a different day.

Purity %, 99% x1, Injector temp. Acc. Org. method

	AI	Imp 1	Imp 2
Inj 1	98,35	1,12	0,53
Inj 2	98,33	1,13	0,54
Inj 3	98,30	1,15	0,55
Average	98,33	1,13	0,54
Variance	0,0003	0,0001	0,0000
SD	0,02	0,01	0,01
%RSD	0,02	1,02	1,29
u	0,01	0,01	0,00
U	0,02	0,01	0,01

Purity %, 99% x1, Injector temp. Acc. Extra meth. 1

	AI	Imp 1	Imp 2
Inj 1	98,20	1,19	0,61
Inj 2	98,22	1,19	0,59
Inj 3	98,23	1,18	0,59
Average	98,21	1,19	0,60
Variance	0,0002	0,0000	0,0001
SD	0,02	0,01	0,01
%RSD	0,02	0,50	1,63
u	0,01	0,00	0,01
U	0,02	0,01	0,01

Purity %, 99% x1, Injector temp. Acc. Extra meth. 2

	AI	Imp 1	Imp 2
Inj 1	98,24	1,17	0,59
Inj 2	98,23	1,19	0,59
Inj 3	98,22	1,18	0,60
Average	98,23	1,18	0,59
Variance	0,0000	0,0000	0,0000
SD	0,01	0,01	0,00
%RSD	0,01	0,52	0,69
u	0,00	0,00	0,00
U	0,01	0,01	0,00

Purity %, 99% x1, Injector temp. Acc. Org. method

Purity %	98,33	1,13	0,54
Target %	98,32	1,05	0,63
Diff purity %	-0,01	-0,08	0,09

Purity %, 99% x1, Injector temp. Acc. Extra meth. 1

Purity %	98,21	1,19	0,60
Target%	98,32	1,05	0,63
Diff purity %	0,11	-0,14	0,03

Purity %, 99% x1, Injector temp. Acc. Extra meth. 2

Purity %	98,23	1,18	0,59
Target%	98,32	1,05	0,63
Diff purity %	0,09	-0,13	0,04

Average purity, 99%

	AI	Imp 1	Imp 2
Original meth.	98,33	1,13	0,54
Extra meth. 1	98,21	1,19	0,60
Extra meth. 2	98,23	1,18	0,59
Average	98,26	1,17	0,58
Variance	0,0025	0,0006	0,0007
SD	0,05	0,02	0,03
%RSD	0,05	2,06	4,44
u	0,03	0,01	0,01
U	0,06	0,03	0,03

Purity for all injector temp. methods, 99%

Average	98,26	1,17	0,58
Variance	0,0030	0,0007	0,0008
SD	0,06	0,03	0,03
%RSD	0,06	2,32	4,90
u	0,02	0,01	0,01
U	0,04	0,02	0,02
95% CI	0,04	0,02	0,02

Purity for all injector temp. methods, 99%

Purity %	98,26	1,17	0,58
Target%	98,32	1,05	0,63
Diff purity %	0,06	-0,12	0,05

Chromatograms in Figure 4-19, Figure 4-20 and Figure 4-21 presents a visualization for the different temperatures for the injector, respectively between the original method, extra method 1 and extra method 2. The differences were not significant between the methods, with retention times relatively identical (note that the axes are not identical).

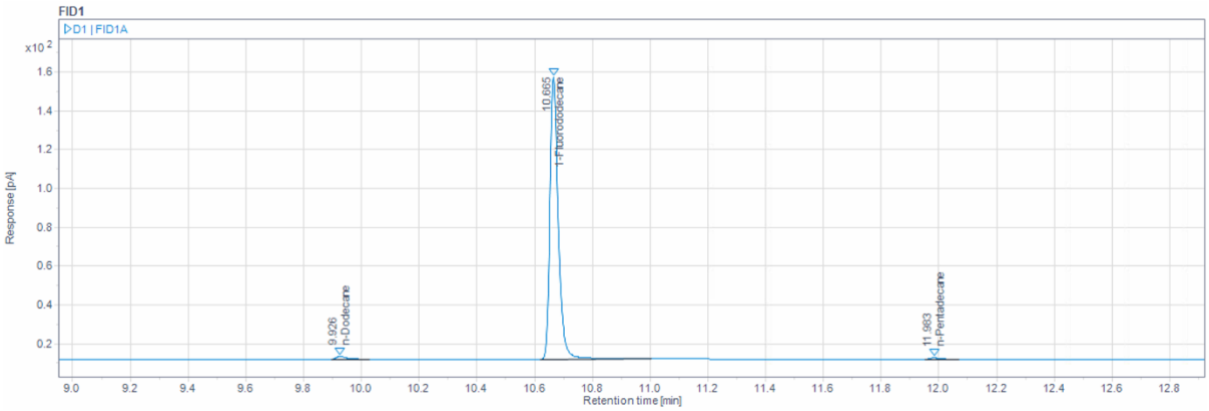

Figure 4-19 Chromatogram for sample D1 with 99% purity, original injector temp. (250°C).

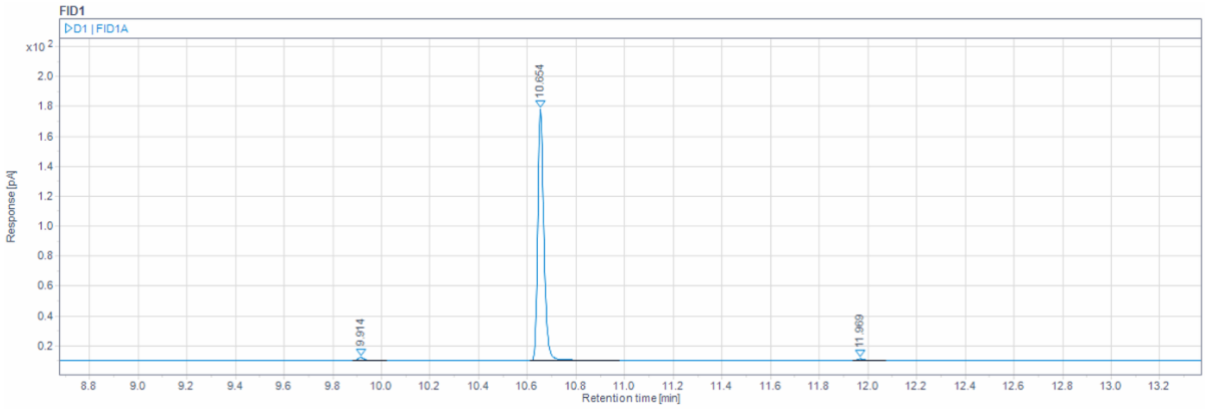

Figure 4-20 Chromatogram for sample D1 with 99% purity, extra injector temp. 1 (245°C).

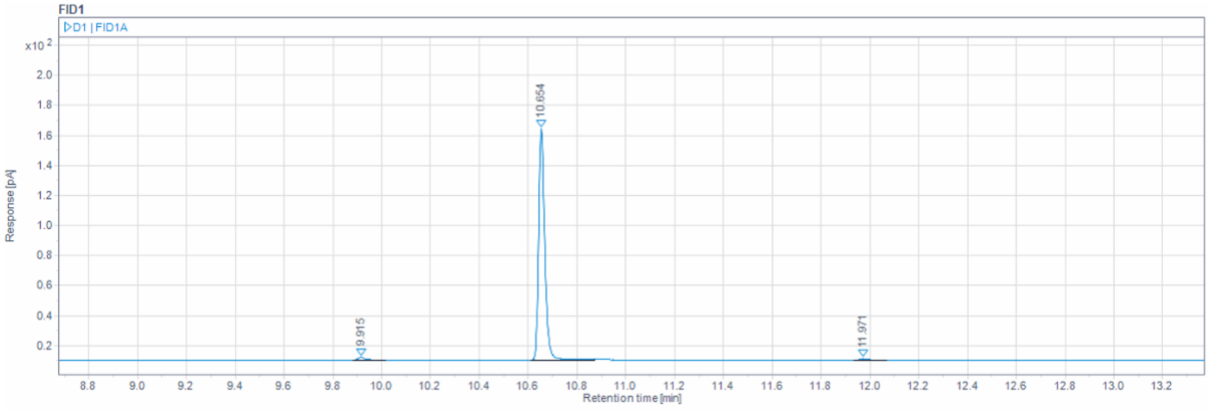

Figure 4-21 Chromatogram for sample D1 with 99% purity, extra injector temp. 2 (255°C).

5 Discussion

5.1 Purity Analysis With Polyarc-FID

The Agilent 8890/5977B GC/MS with the combination of FID and Polyarc reactor proved to an accurate method of analyzing sample purity for various analyzes [10]. Variations of impurity content from 1% to 10% did not affect the methods ability to analyze purity according to the criteria set by the employer. In addition to the originally prepared samples at 1000 µg/mL, there were performed analysis with samples diluted x3 and x10. The variations in concentration did not affect the determination of purity.

Sample B with 95% purity had to be re-analyzed because of a difference from purity target up to 0.86%. After the second test was performed, the results fulfilled the criteria of ± 0.5%, with all differences < 0.44%. Because the evaluation was based on automatic integration, this may have been avoided by manually integrating all of the injections for the undiluted and diluted samples. The rest of the tests fulfilled the criteria after the first run, which indicates that automatic integration still is an adequate method of evaluating results.

Comparison of chromatograms for samples analyzed with FID only versus Polyarc-FID shows a higher signal with narrower and taller peaks, see Figure 5-1. This indicates that the Polyarc provides analyses with higher efficiency and better detection [6]. The peaks are in addition observed with greater signal differences for the compounds, which proves that it potentially can be an improved method of analyzing purity.

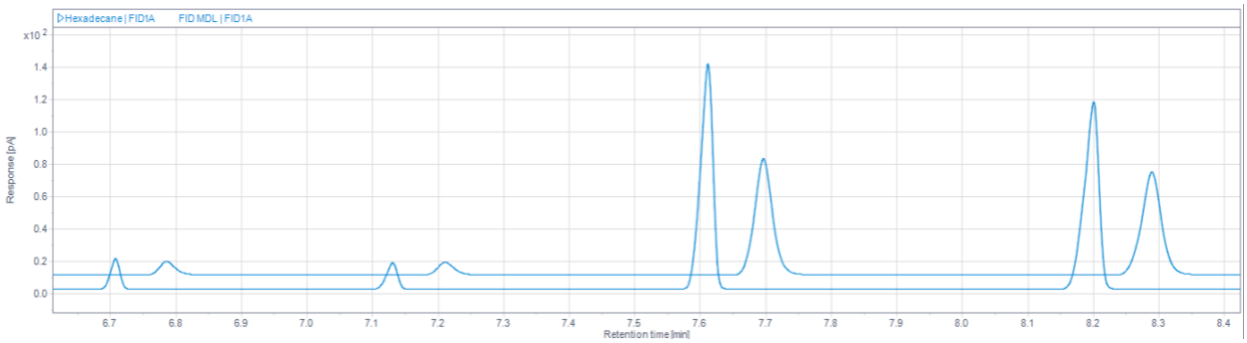

Figure 5-1 Chromatogram of a checkout sample analyzed with and without a Polyarc, where the tallest and narrowest peaks are results from Polyarc-FID.

5.2 Purity Analysis With MSD

In general, the MSD was intended for compound identifications. Considering the results from MS expressed as response factors (from Table 7-8 to Table 7-11 in Appendix G), they prove that the detector is not suitable for purity analysis with this analytical method. The variations of the response factor values were too great for accurate analysis of both the active ingredient and impurities [14]. The unsuitableness is supported by the results for linearity, which shows a more non-linearly relationship between theoretical concentration and area results. When looking at the peak shapes from MS, there are generally not enough data points recommended for quantification using peak areas [7]. The peak maximum is not recorded in addition to the fact that the top seems wider at the bottom, exemplified in Figure 5-2. This is mass spectrometer dependent, and could possibly be mitigated by changes in the scan speed [16].

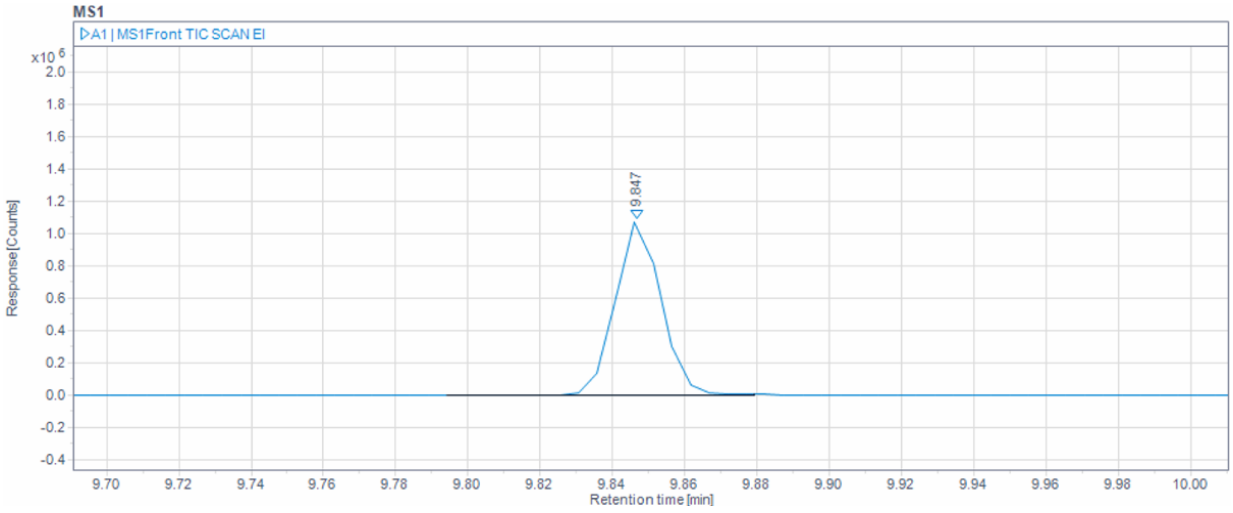

Figure 5-2 Chromatogram from MSD of impurity n-dodecane.

5.3 Linearity and Range

The GC/FID with the Polyarc performed well regarding linearity and range tests. Results for peak areas over the total range of 13 points (0.49 - 2000 µg/mL) gave the most linear relationship, with the highest possible correlation coefficient of $R^2 = 1$. When looking at a smaller range of seven points (0.49 - 31.25 µg/mL), the criterion set by the employer ($R^2 > 0.995$) was still met with a value of $R^2 = 0.9992$. When looking at peak height, the results showed a bit lower degree of linearity. They were still satisfactory, with $R^2 = 9991$

for the full range, and $R^2 = 0.9986$ for the smaller range. Results for linearity from the MS detector gave a poorer linear relationship, as expected for GC-MS analyses [17].

LOD was successfully evaluated from impurity of the sample with 99% purity and the two linearity samples with the lowest theoretical concentrations detectable by the instrument (0.49 and 0.98 μg/mL). The lowest concentrated impurity detected was n-pentadecane. The lowest S/N ratio from the three injections showed a value of 3.2, which is considered acceptable for LOD [7]. The LOQ was not established because of problems with concentration verification, which is discussed further in section 5.4.

5.4 Intermediate Precision

Unfortunately, the results for intermediate precision were not satisfactory. The results did not meet the criteria of %RSD < 3% for precision and ± 5% for accuracy set by the employer. There were challenges regarding calculating the concentration of the CRM solutions. When using the calibration curve created in OpenLAB from the external reference caffeine, neither benzo[a]pyrene (IPA), naphthalene (IPB) or PCB 52 (IPC) were near their true concentrations. The greatest difference was observed when analyzing naphthalene on day 3, with a difference from target purity at 32.20%. Generally, the difference regarding all of the CRMs was greater than 14%, which is not satisfactory. Some of the differences can be explained by the fact that calculations were done with regard to the number of carbons. PCB 52 consists largely of carbon and hydrogen, but also four chlorine atoms. Because of this, the difference from the known concentration at 50 μg/mL will naturally vary before recalculations. Benzo[a]pyrene and naphthalene, on the other hand, consists exclusively of carbons and hydrogens. The difference in concentration was thus unexpectedly large.

When attempting to calculate the mass concentrations manually using Equation 2.4 provided from ARC [13] (see examples in Appendix A) the results were too low for benzo[a]pyrene (1000 μg/mL), with a concentration at 777.23 μg/mL. The discrimination may be explained partly because of the high boiling point at 495°C [18], and that the injector temperature was set significantly lower at 250°C. There were also discussions with the instrument vendor about the type of inlet liner used (no. 5190-2295), which possibly contributed to the problem. This liner has a relatively large area of glass-wool,

which may have filtered, or "captured", too much of the sample. For naphthalene (200 μg/mL) and PCB 52 (50 μg/mL) the results were too high, with concentrations at respectively 303.85 μg/mL and 98.97 μg/mL. Whether the cause of discrepancy was due to the calculations or the method was never determined. For the writer, the pandemic situation was an additional challenge. A personal participation at the employer's laboratory would probably have provided a greater insight to the analyzes, and the causes to why the results were not satisfactory.

5.5 Instrumental Repeatability

The instrument did not perform as expected regarding repeatability of 10 injections for a single sample performed consecutively. The initial injections performed with sample A1 with 90% purity was automatically integrated when evaluating peak areas. Results from FID/Polyarc gave values of %RSD greater than 5% for AI, which were not considered satisfactory according to the criteria of %RSD < 1% set by the employer. The test was reanalyzed with sample B1 with 95% purity, and this time the peak areas were manually integrated. This however did not prove any significant changes regarding %RSD for the injections. The MSD showed some of the same tendencies, which might indicate that the problem could be within the injection or the acquisition method, and not regarding the performance of the detectors or the Polyarc.

The problems with repeatability were solved directly before submission of this thesis, by the help of the instrument vendor. The problem was mainly due to the syringe and the settings in the automatic liquid sampler. There were some bubble formations during drawing of the sample, which was the source of the uneven results. The syringe was replaced with one containing Teflon, the draw-speed was lowered to 150 and the viscosity delay was set to 2s. The liner was replaced with a universal mid-frit one (no. 5190-5105), which has a smaller filter area that contains of glass-frit instead of glass-wool.

5.6 Robustness

The instrument proved to be robust enough to handle temperature changes of internal parameters for both for the injector and gradient for the column. Tests was performed with a change ± 5°C for both the injector and all temperature parameters for the column

gradient. Differences in purity were not significant, with values lower than 0.2% difference compared to the original method. Intermediate precision tests could have been an additional confirmation of robustness according to external parameters [14]. Unfortunately, those results were not satisfactory enough to be evaluated and discussed in connection with robustness.

5.7 Measurement Uncertainty

Measurement uncertainty for purity analyses with Polyarc-FID was set to $U < 0.05$, based on results from AI of undiluted samples with purity percentages 90-99%. The result was sufficiently small [14] and well within the criterion of $U < 0.5$ set by the employer. Comparing the result with others from conventional GC-FID, purity results of CRM solutions used for drugs in sport analysis gave values of expanded uncertainty $U = 0.09$ [19]. Determinations of free glycerol in biodiesel using GC-FID got a value of $U = 0.16$ [20]. This indicates, to some degree, that the Polyarc successfully contributes to lowering the measurement error [6]. When looking at all dilutions, measurement uncertainty for all purities and dilutions was set to $U < 0.15$. A slightly greater value, but still sufficient for fulfilling the criterion of $U < 0.5$ for chromatographic purity.

5.8 Further Work

Due to the time limits of this project, in addition to the pandemic situation, the writer was not able to participate in solving the problems with concentration verification and repeatability. The cause of the concentration errors was not identified, but as mentioned, it was discussed whether the errors may originate from the type of liner used for the analysis. Ultra-Inert Inlet Liner 5190-5105 was installed for the purpose of additional tests in due time. Regarding the Polyarc and supposed uniform response, it may be more appropriate to calibrate with separate reference standards for different product groups by size and/or chemical properties.

6 Conclusion

In this thesis, an Agilent 8890/5977B GC-MS system with a Polyarc-FID has been successfully validated for purity assessment in various analyses. The instrument was tested for performance regarding chromatographic purity, accuracy, precision, repeatability, linearity, range, LOD/LOQ and robustness. The combination of GC-FID with the Polyarc reactor proved to be an accurate and simple method for analyzing purity. It provides better detection than conventional GC-FID. When evaluating normalized chromatograms, the results from GC-FID/Polyarc fulfilled all of the criteria for purity analyses, at respectively 90%, 95%, 97% and 99% purity. Measurement uncertainty for purity analyses was set to $U < 0.05$. For all purities and dilutions, the uncertainty was set to $U < 0.15$

The instrument performed well regarding robustness and was able to provide accurate purity results when varying internal method parameters. The temperature gradient for the column and injector temperature was tested ± 5°C from the original method. Differences in measured purity were not significant between the varied methods, with values lower than the set criteria of difference in purity < 0.2%. The FID/Polyarc proved to perform well regarding linearity, with all tests within acceptable correlation coefficients of $R^2 > 0.995$. The range criteria from 0.1% of AI to 100% of AI was fulfilled, as well as the criteria for LOD at ≤ 0.1% for impurities with a S/N ratio ≥ 3.

The instrument did not fulfill the criteria of repeatability, with values of %RSD greater than 1% for the active ingredient 1-fluorododecane. At the end of this project, the problem was solved by the instrument vendor by changing the syringe and settings of the automatic liquid sampler. Concentration verification analysis did not meet the criteria set for accuracy as difference in target purity ± 5%, or precision as %RSD < 3%. There were problems regarding area results for all of the CRM solutions used for intermediate precision, probably due to the type of liner used for the analysis. Further work must be done to meet the criteria and obtain a complete validation including concentration verification.

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7 Appendices

A Calculations for the Polyarc

Carbon concentration of the external standard reference used for creating a calibration curve in OpenLAB are given from Equation A.1-A.4. Calculations are done with respect to number of carbons, and therefore given as μg C/mL.

$$f = \frac{Mw_{\#C}}{Mw_{Caffeine}} = \frac{8\ C \cdot 12.01\ Da}{194.19\ Da} = 0.495\ C \tag{A.1}$$

$$c_{std\_C\ (1000)} = c_{std} \cdot f = 993.09\ \frac{\mu g}{mL} \cdot 0.495\ C = 491.58\ \frac{\mu g\ C}{mL} \tag{A.2}$$

$$c_{std\_C\ (200)} = 201.01\ \frac{\mu g}{mL} \cdot 0.495\ C = 99.50\ \frac{\mu g\ C}{mL} \tag{A.3}$$

$$c_{std\_C\ (50)} = 51.09\ \frac{\mu g}{mL} \cdot 0.495\ C = 25.29\ \frac{\mu g\ C}{mL} \tag{A.4}$$

Examples of manually calculated concentrations of CRM solutions according to peak areas from GC-FID/Polyarc (injection 1, day 1). Areas are average values from six injections.

$$c_{Benzo[a]pyrene\ (IPA)} = 993.09\ \mu g/mL\ \left(\frac{157.611}{105.316}\right)\left(\frac{252.131\ Da}{194.19\ Da}\right)\left(\frac{8}{20}\right) = 771.86\ \mu g/mL \tag{2.4}$$

$$c_{Naphthalene\ (IPB)} = 201.01\ \mu g/mL\ \left(\frac{51.477}{17.891}\right)\left(\frac{128.171\ Da}{194.19\ Da}\right)\left(\frac{8}{10}\right) = 305.39\ \mu g/mL \tag{2.4}$$

$$c_{PCB\ 52\ (IPC)} = 51.09\ \mu g/mL\ \left(\frac{7.146}{3.619}\right)\left(\frac{291.988\ Da}{194.19\ Da}\right)\left(\frac{8}{12}\right) = 101.12\ \mu g/mL \tag{2.4}$$

B Acquisition Method Report

Acquisition Method Report

Agilent OpenLAB

Method Information

Last Saved As:	D:\CDSProjects\8890\Methods\Validation\Standard.amx
Modified:	2021-03-01 15:44:55+01:00
Modifier:	SYSTEM
Created:	2021-02-08 09:55:25+01:00
Creator:	SYSTEM
Description:	
Version:	2021-0301-1444-55094
Method Status:	Generic

Method Properties

Instrument Technique:	Gas Chromatography

Schema version

Schema version:	2.3

GC

Module Display Name:	Agilent 8890
Module Type:	GC
Order:	1

GC Summary

Run Time:	27.75 min
Post Run Time:	0 min

Oven

Equilibration Time:	0.5 min
Max Temperature:	350 °C
Maximum Temperature Override:	Disabled
Slow Fan:	Disabled

Temperature

Setpoint:	On
(Initial):	50 °C
Hold Time:	4 min
Post Run:	70 °C

Program

Row ID	Rate (°C/min)	Value (°C)	Hold Time (min)
1	20	325	10

ALS

ALS Errors:	Pause for user interaction

Front Injector

Syringe Size:	10 µL
Injection Volume:	1 µL
Solvent A Washes (PreInj):	3
Solvent A Washes (PostInj):	3
Solvent A Volume:	8 µL
Solvent B Washes (PreInj):	3
Solvent B Washes (PostInj):	3
Solvent B Volume:	8 µL
Sample Washes:	1
Sample Wash Volume:	8 µL
Sample Pumps:	6
Dwell Time (PreInj):	0 min
Dwell Time (PostInj):	0 min
Solvent Wash Draw Speed:	300 µL/min
Solvent Wash Dispense Speed:	6000 µL/min
Sample Wash Draw Speed:	300 µL/min
Sample Wash Dispense Speed:	6000 µL/min
Injection Dispense Speed:	6000 µL/min
Viscosity Delay:	0 sec
Sample Depth:	Disabled
Injection Type:	Standard
L1 Airgap:	0 µL
Solvent Wash Mode:	A, B

Sample Overlap

Mode:	Sample overlap is not enabled

Front SS Inlet He

Mode:	Split
Heater:	On 250 °C
Pressure:	On 16.108 psi
Total Flow:	On 28.2 mL/min
Septum Purge Flow:	On 3 mL/min
Pre-Run Flow Test:	Off
Gas Saver:	On 40 mL/min after 2 min
Split Ratio:	20 :1
Split Flow:	24 mL/min
Liner:	A Liner has not been selected.

Thermal Aux 2 (User Configurable)

Temperature

Setpoint:	On
(Initial):	450 °C

Column

Column Outlet Pressure:	0 psi

Column #1

Column Information:	Agilent 19091S-433UI: 0321561H
Description:	HP-5MS UI
Temperature Range:	-60 °C—325 °C (350 °C)
Dimensions:	30 m x 250 µm x 0.25 µm (Uncalibrated)
Column lock:	Locked
In:	Front SS Inlet He
Out:	PSD 2
(Initial):	50 °C
Pressure:	16.108 psi
Flow:	1.2 mL/min
Average Velocity:	25.811 cm/sec
Holdup Time:	1.9371 min
Control Mode:	Constant Flow

Flow

Setpoint:	On
(Initial):	1.2 mL/min
Post Run:	0.57353 mL/min

Column #2

Column Information:	Agilent
Description:	MS Restrictor
Temperature Range:	-60 °C—400 °C (400 °C)
Dimensions:	1.44 m x 150 µm x 0 µm (Uncalibrated)
Column lock:	Unlocked
In:	PSD 2 He
Out:	MSD
(Initial):	50 °C
Pressure:	4 psi
Flow:	1.9048 mL/min
Average Velocity:	229.58 cm/sec
Holdup Time:	0.010454 min
Control Mode:	Constant Pressure

Pressure

Setpoint:	On
(Initial):	4 psi
Post Run:	10 psi

Column #3

Description:	FID Resistrictor
Temperature Range:	-60 °C—400 °C (400 °C)
Dimensions:	0.53 m x 150 µm x 0 µm (Uncalibrated)
Column lock:	Unlocked
In:	PSD 2 He
Out:	Front Detector FID
(Initial):	50 °C
Pressure:	4 psi
Flow:	1.9776 mL/min
Average Velocity:	177.09 cm/sec
Holdup Time:	0.0049879 min
Control Mode:	Constant Pressure

Pressure

Setpoint:	On
(Initial):	4 psi
Post Run:	10 psi

Front Detector FID

Makeup:	He
Heater:	On 350 °C
H2 Flow:	On 1.5 mL/min
Air Flow:	On 250 mL/min
Makeup Flow:	On 20 mL/min
Carrier Gas Flow Correction:	Constant Makeup and Fuel Flow
Flame:	On
Blank Evaluation Setpoints:	
Perform Blank Evaluation Test:	Off
Initial Baseline Minimum:	2 pA
Initial Baseline Maximum:	20 pA
Initial Baseline Noise:	0.3 pA
Final Baseline Minimum:	2 pA
Final Baseline Maximum:	40 pA
Final Baseline Noise:	0.6 pA
Total Peak Area:	100 pA*sec
Maximum Peak Height:	3 pA
Time Window Start:	0 min
Time Window End:	999.99 min

Detector Evaluation

Perform Detector Evaluation Test:	On
Signal Selected:	No Signal Selected
Checkout Sample:	None

MSD Transfer Line

Temperature

Setpoint:	On
(Initial):	300 °C

Aux EPC 1,2,3

Aux EPC 1 Air

Pressure

Setpoint:	On
(Initial):	21 psi

Aux EPC 2 H2

Pressure

Setpoint:	On
(Initial):	21 psi

Aux EPC 3 N2

Pressure

Setpoint:	Off
(Initial):	10 psi

PSD 2

PSD Purge:	On 5 mL/min
PSD 2 He:	Supplies Column 2

Signals

Signal #1: Front Signal

Description:	Front Signal
Details:	Front Signal (FID)
Save:	On
Data Rate:	10 Hz
Dual Injection Assignment:	Back Sample

Signal #2:

Description:	None

Signal #3:

Description:	None

Signal #4:

Description:	None

Signal #5:

Description:	None

Signal #6:

Description:	None

Signal #7:

Description:	None

Signal #8:

Description:	None

GC/MS

Module Display Name:	Agilent 5977 MSD
Module Type:	GC/MS
Order:	1

Acquisition Method

Tune File:	etune
Ion Source:	EI
Source Temperature:	230 °C
Quad Temperature:	150 °C
Fixed Electron Energy:	70 eV
Acquisition Type:	Scan
Stop Time:	10 min
Solvent Delay:	6 min
Trace Ion Detection:	Off
Gain Factor:	0.5
EM Saver:	Off
EM Saver Limit:	N/A

Scan Time Segments

Scan Time Segments

Row ID	Time	Start Mass	End Mass	Threshold
1	6	50	500	150

Row ID	Scan Speed
1	1,562 [N=2]

Real-Time Plots

Total Ion:	Enabled
BPC:	Disabled

C Validation Protocol

Document "Validation protocol for chromatographic purity determination and concentration verification by GC/MS/polyarcFID (GC1600-04)", ID 1024 - EQS

Validation protocol for chromatographic purity determination and concentration verification by GC/MS/polyarcFID (GC1600-04)

Document administrator: Stine Rapp	Valid from: 16.02.2021	Revision: 2.0
Approved by: Maren Grøndahl	Scheduled for revision: 16.02.2022	ID: 1024

1 Objective

Validation protocol for the method used regarding quality analysis of Reference Material (RM), manufactured and/or distributed by Chiron AS by using GC/MS/FID incl Polyarc (GC1600-04).

Results must demonstrate that the analysis meet the pre-determined acceptance criteria under normal conditions and can be considered as Fit for Purpose.

2 Scope

Validation of the method for quality control by using GC/MS/FID incl Polyarc (GC1600-04). Analysis of Reference Material (RM), manufactured and/or distributed by Chiron AS.

Tests will include concentration determination for the three chosen products. Tests will include purity analysis of three main substances; (AI) and their impurities used in this validation schedule. Concentration dependent response will also be illustrated. Repeatability for the MS (response and mass) and FID/Polyarc. Retention time stability will be studied. Intermediate precision will be analyzed over three days by using CRM products. Accuracy for these CRM products will also be analyzed.

3 Procedure

3.1 Validation approach

Validation Approach

Prospective	x
Concurrent	
Retrospective	
Re-validation	
Ph. Eur. Verification	

3.2 Testing facilities

Chiron AS

Stiklestadveien 1

7041 TRONDHEIM

3.3 Responsibilities

First analyst: Stine Rapp

Second analyst: Hana Spickova

QC Manager: Jon E. Johansen

QA: Maren Grøndahl

3.4 Equipment

According to method 11062.

3.5 Instrument Instruction

See document 11059.

3.6 Analytical Method

See document 11062.

3.7 Chromatographic purity

Prepare 4 samples with four different purities for AI (Active Ingredient) and two different impurities. Preparation is described in excel sheet. Concentration of the AI is approx. 1000 µg/mL. Dilute the samples A, B, C and D x3 and x10. Prepare external reference solutions, Caffeine CRM, and calculate concentration. Inject each sample and dilution three times. Calculate concentration with GC software for Polyarc. Evaluate normalized chromatograms as well from the injections above.

3.7.1 Test samples

Active Ingredient (AI): 1-Fluorododecane, prod. no. 8239.12, batch no. 6761
Impurity 1: n-Dodecane, prod. no. 1132.12, batch no. 6908
Impurity 2: n-Pentadecane, prod. no. 1135.15, batch no. 2197

- Sample A: Product, 1-Fluorododecane (AI), Spiked with 2 impurities at level 0.5% each
- Sample B: Product, 1-Fluorododecane (AI), Spiked with 2 impurities at level 1.5% each
- Sample C: Product, 1-Fluorododecane (AI), Spiked with 2 impurities at level 2.5% each
- Sample D: Product, 1-Fluorododecane (AI), Spiked with 2 impurities at level 5.0% each

3.7.2 Criteria Chromatographic Purity

- AI should have %RSD < 0,5 at 99% target purity. %RSD < 50 for each impurity
- AI should have %RSD < 0,5 at 97% target purity. %RSD < 20 for each impurity
- AI should have %RSD < 0,5 at 95% target purity. %RSD < 10 for each impurity
- AI should have %RSD < 0,5 at 90% target purity. %RSD < 10 for each impurity

Report single values, average, variance, SD, %RSD, u and U.

Report purity and difference from target value expressed as %.

Criteria: Purity ±0.5% from target purity

3.8 Instrumental Repeatability

Inject sample A undiluted 10 times consecutively on the instrument to evaluate the repeatability of the instrument. Report results for AI as well as for both impurities.

Criteria: RSD should be < 1% for AI and RSD should be < 50% for respective impurity

3.9 Intermediate Precision

Three samples should be analyzed on three days by two analysts for concentration verification. Perform six injections of each sample each day. Accuracy will be noted as well.

Report single values, average, variance, SD, %RSD, u and U. Report accuracy as difference from target value expressed as %.

Criteria %RSD: <3%
Criteria Accuracy: ±5% from target value.

3.9.1 Samples for concentration verification.

- Sample IPA: Benzo[a]pyrene solution 1000 µg/mL in ACN, CRM, Merck/Supelco, prod. no. CRM40071
- Sample IPB: Naphthalene solution 200 µg/mL in methanol, CRM, Merck/Supelco, prod. no. CRM48641
- Sample IPC: PCB 52 solution 50 µg/mL in ACN 5mL/ampoule, CRM, Teknolab/Restek, prod. no. R33257
- Ref substance: Caffein Pharmaceutical Secondary Standard, 99.96 %, CRM, Merck/Supelco, prod. no. PHR1009, bath no. LRAC4115

3.10 Linearity/Range

Linearity range in reference solution will be tested from approx. 0.1 µg/mL to approx. 1000 µg/mL.

Criteria linearity: R2 > 0.995
Criteria range: From 0.1% of AI to 100% AI.

3.11 Robustness

Inject sample A x1 with two different temperature gradients for the column. Inject sample A x1 with two different temperatures for the injector. Inject external standard and calculate purity for the sample. Perform all injections x3.

Criteria: Chromatographic purity < 0.2% difference compared to original method.

3.12 LOD/LOQ

Evaluate the sample used for linearity test and sample A1 diluted x10.

Criteria for LOD is set to ≤ 0.1% for impurities (S/N ≥3) for chromatographic purity.
Criteria for LOQ is set to ≤ 0.1% for impurities (S/N ≥10) for chromatographic purity.

3.13 Measurement uncertainty

The measurement uncertainty will be calculated.

Criteria for chromatographic purity: U <0.5

3.14 Time schedule

Practical work: 6 weeks

Report: One week after performance, preferably

4 Reporting

Validation report will contain and follow the headlines mentioned above.

5 Storage

Validation protocol, validation report and raw data are stored for minimum ten years (documents 10284, 10636) or as long as they are in use.

Electronic location: F:\Quality Control\Internal Quality Control\Validation\UHPLC
Hard copy: In a binder located at QD's office.

6 References

Method Validation in Pharmaceutical Analysis. A guide to best practice by J. Ermer and J.H.McB. Miller

General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017)

General requirements for the competence of reference material producers (ISO 17034:2016)

7 Associated documents

- Approval of Test Results (document 10784)
- Assuring the quality of test results (document 10657)
- Calculations_GCMSFID_Polyarc_Fit for purpose_20210208_eh
- Calculation sheet and summary of results (document 10750)
- Chromatographic Purity determination by GCMS (Screening) (document 10926)
- Estimation of uncertainty of measurement (document 10651)
- GCMS (GC1600-02) Method (document 10293)
- General Guidelines for working in the Laboratory (document 10422)
- Handling Events (document 10282)
- Instrument instruction for GCMS with polyarc FID (GC1600-04) (document 11059)
- Laboratory responsibilities (document 10285)
- Method for chromatographic purity and identity by GC/MS with polyarc FID (document 11062)
- Procedure for keeping lab journals and appendixes (document 10407)
- Quality records (document 10636)
- Staff training plan – GC (document 10670)
- Technical records (document 10284)
- Use of GCMS polyarc FID with OpenLAB software (document 11060)

8 Document History

Version No.	Version date	Description of change	Author name
0	N/A	Draft version	Eskil Hermansson
1.0	16.02.2021	Initial release	Stine Rapp
2.0	16.02.2021	Updated format	Stine Rapp

D Preparation of Samples

Table 7-1 presents details for the preparation of samples A-D. Isooctane was used as solvent. A2-D2 were diluted x3, and A3-D3 were diluted x10, as given in Table 7-2.

Table 7-1 Details for preparations of sample A1-D1.

90 % Sample A1

Purity factor	Density		Subst. (theor.) [mg]	Subst. (pract.) [mg]	Subst. sample [mL]	Solvent [mL]	Solvent [g]	*Conc (w/v)* [µg/mL]	Target [%]
0,98	0,807	1-Fluorododecane (AI); 8239.12 b6761	90	94,170	0,117	105,718	73,146	*873,09*	85,02
0,993	0,75	n-Dodecane (Imp. 1); 1132.12 b6908	5	6,720	0,009			*63,26*	6,16
0,99	0,769	n-Pentadecane (Imp. 2); 1135.15 b2197	5	9,660	0,013			*90,60*	8,82
			100	110,55	0,138			*1026,95*	

95 % Sample B1

Purity factor	Density		Subst. (theor.) [mg]	Subst. (pract.) [mg]	Subst. sample [mL]	Solvent [mL]	Solvent [g]	*Conc (w/v)* [µg/mL]	Target [%]
0,98	0,807	1-Fluorododecane (AI); 8239.12 b6761	95	99,070	0,123	102,919	71,210	*943,48*	93,87
0,993	0,75	n-Dodecane (Imp. 1); 1132.12 b6908	2,5	2,635	0,004			*25,55*	2,54
0,99	0,769	n-Pentadecane (Imp. 2); 1135.15 b2197	2,5	3,735	0,005			*36,06*	3,59
			100	105,44	0,131			*1005,09*	

97 % Sample C1

Purity factor	Density		Subst. (theor.) [mg]	Subst. (pract.) [mg]	Subst. sample [mL]	Solvent [mL]	Solvent [g]	*Conc (w/v)* [µg/mL]	Target [%]
0,98	0,807	1-Fluorododecane (AI); 8239.12 b6761	97	97,215	0,120	99,350	68,740	*959,07*	96,35
0,993	0,75	n-Dodecane (Imp. 1); 1132.12 b6908	1,5	1,640	0,002			*16,52*	1,66
0,99	0,769	n-Pentadecane (Imp. 2); 1135.15 b2197	1,5	1,975	0,003			*19,80*	1,99
			100	100,83	0,125			*995,39*	

99 % Sample D1

Purity factor	Density		Subst. (theor.) [mg]	Subst. (pract.) [mg]	Subst. sample [mL]	Solvent [mL]	Solvent [g]	*Conc (w/v)* [µg/mL]	Target [%]
0,98	0,807	1-Fluorododecane (AI); 8239.12 b6761	99	105,310	0,130	104,488	72,295	*987,84*	98,32
0,993	0,75	n-Dodecane (Imp. 1); 1132.12 b6908	0,5	1,100	0,001			*10,59*	1,05
0,99	0,769	n-Pentadecane (Imp. 2); 1135.15 b2197	0,5	0,650	0,001			*6,29*	0,63
			100	107,06	0,133			*1004,72*	

Isooctane was used as solvent (solution no. 2016-101)

Table 7-2 Details for preparation of diluted samples A2-D2 and A3-D3.

90 % Sample, Dil. X3 = Sample A2

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*291,03*
Imp 1	2000	4000	*21,09*
Imp 2			*30,20*

90 % Sample, Dil. X10 = Sample A3

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*87,31*
Imp 1	1000	9000	*6,33*
Imp 2			*9,06*

95 % Sample, Dil. X3 = Sample B2

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*314,49*
Imp 1	2000	4000	*8,52*
Imp 2			*12,02*

95% Sample, Dil. X10 = Sample B3

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*94,35*
Imp 1	1000	9000	*2,56*
Imp 2			*3,61*

97 % Sample, Dil. X3 = Sample C2

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*319,69*
Imp 1	2000	4000	*5,51*
Imp 2			*6,60*

97 % Sample, Dil. X10 = Sample C3

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*95,91*
Imp 1	1000	9000	*1,65*
Imp 2			*1,98*

99 % Sample, Dil. X3 = Sample D2

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*329,28*
Imp 1	2000	4000	*3,53*
Imp 2			*2,10*

99 % Sample, Dil. X10 = Sample D3

	V1 [µL]	V2 [µL]	*Conc (w/v) [µg/mL]*
AI			*98,78*
Imp 1	1000	9000	*1,06*
Imp 2			*0,63*

Isooctane was used as solvent (solution no. 2010-010)

Table 7-3 shows preparations of the external standard caffeine at three different concentrations, 1000, 200 and 50 μg/mL. Methanol was used as solvent. Table 7-4 shows the preparation of the 16 samples A-P for the linearity/range tests. Concentration at 0,06 μg/mL corresponds to impurity 0,1% when AI is 100 μg/mL.

Table 7-3 Preparation of the external standard caffeine solutions.

Caffeine, CRM/RM-93		Substance [mg]	mL	c [μg/mL]	K-sol. V1 [μL]	Solvent V2 [μL]	c [μg/mL]	200-sol. V3 [μL]	Solvent V4 [μL]	c [μg/mL]
Purity f.: 0,9996	Wanted conc.			1000			200			50
Solvent: methanol		5	5	999,6	1000	3998	200	1000	3000	50
Density: 0,7923	Actual [mg]	8,35	6659,11	993,09	810,27	3192,82	201,01	818,04	2400,71	51,09

Methanol was used as solvent (solution no. 2020-100)

Table 7-4 Preparation of samples A-P for the linearity/range test.

Linearity/Range	Sample name	m (AI) [mg]	V (AI) [μL]	m (IO) [mg]	V (IO) [μL]	Conc. [μg/mL]	Wanted conc. [μg/mL]
	A	7,025		2432,525	3515,718	1998,17	2000
	B	1395,305	2016,628	1383,805	2000,007	1003,22	1000
	C	1383,305	1999,285	1363,725	1970,986	505,18	500,0
	D	1382,610	1998,280	1364,145	1971,593	254,29	250,0
	E	1386,350	2003,686	1382,140	1997,601	127,34	125,0
	F	1387,920	2005,955	1363,640	1970,863	64,23	62,50
	G	1381,220	1996,271	1381,220	1996,271	32,12	31,25
	H	1388,250	2006,432	1352,730	1955,095	16,27	15,63
	I	1385,785	2002,869	1355,400	1958,954	8,22	7,81
	J	1389,895	2008,809	1361,560	1967,857	4,15	3,91
	K	1386,810	2004,350	1343,875	1942,297	2,11	1,95
	L	1381,145	1996,163	1365,015	1972,850	1,06	0,98
	M	1380,295	1994,934	1363,025	1969,974	0,53	0,49
	N	1381,130	1996,141	1365,175	1973,081	0,27	0,24
	O	1382,600	1998,266	1369,380	1979,159	0,13	0,12
Density (IO): 0,6919	P	1373,765	1985,496	1366,655	1975,220	0,07	0,06

Isooctane was used as solvent (solution no. 2010-010)

E Certificates of Analysis

E.1 Benzo[a]pyrene Solution

Certificate of Analysis

Benzo[a]pyrene solution

Certified Reference Material

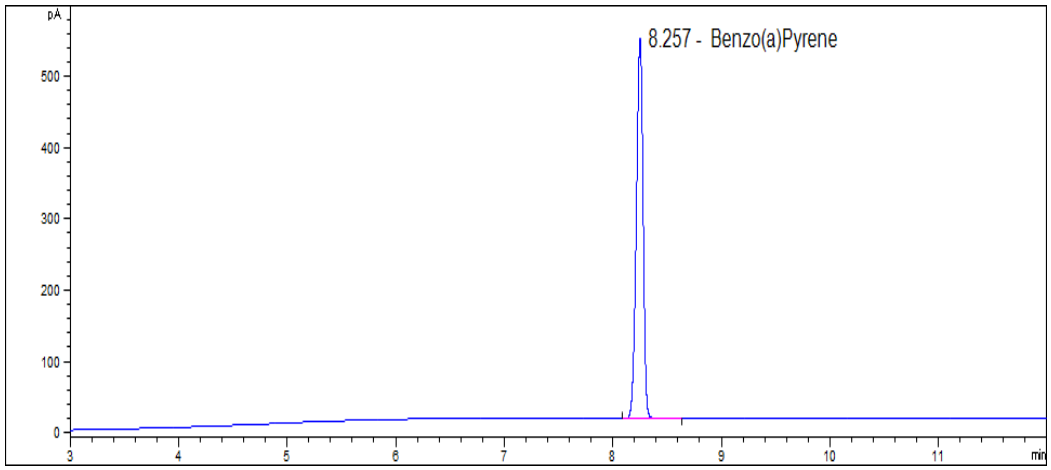
Description

Product ID CRM40071
Lot **LRAC7104**
Expiration Date October 2023
Manufacturing Date October 2020
Storage Conditions Refrigerate
Solvent/Matrix ACETONE

Certified Values

Analyte	*Certified Value* [1,4]	*Units*	*Raw Material Purity,%*	*Elution order*	*Raw Material Lot*	*CAS*
BENZO(A)PYRENE	1000 ± 63	µg/mL	100.0	1	SLBV8459	50-32-8

Informational Values



Additional Information:

Analytical Method Parameters:
Column: SPB-5, 30 m × 0.53 mm I.D., 1.5 µm film thickness
Carrier Gas: H2, Flow: 3.0 mL/min
Inlet Temperature: 250 °C, Injection Volume: 1 µL
Injection Mode: Split (Split Ratio: 10:1)
Temperature Program: 260 °C (Hold 1min) @ 10 °C/min to 300 °C (Hold 7 min)
Detector: FID, Detector Temperature: 310 °C

SIGMA-ALDRICH®
2931 Soldier Springs Rd. Laramie, Wyoming 82070 USA
800-325-5832
TechService@milliporesigma.com www.sigma-aldrich.com

Description

Lot **LRAC7104**
Expiration Date October 2023
Manufacturing Date October 2020
Storage Conditions Refrigerate
Solvent/Matrix ACETONE

1 **Metrological traceability:** Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. The balance used to weigh raw materials is accurate to +/-0.0001 g and calibrated regularly using mass standards traceable to NIST. All dilutions were performed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.
4 Ucrm - Uncertainty values in this document are expressed as Expanded Uncertainty (Ucrm) corresponding to the 95% confidence interval. Ucrm is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies. The mathematical representation of the Ucrm calculation is as follows:

$$u_{CRM} = \sqrt{u_{char}^2 + u_{\text{hom}\ ogeneity}^2 + u_{stability}^2}$$

k: Coverage factor derived from a t-distribution table, based on the degrees of freedom of the data set. Assume 2.0 for a **Confidence interval = 95%**

6 Analytical Value- For QC verification of the certified value only- not to be used in calculations. Represents the analytical data obtained by comparison to a standard as analyzed by the method described in the CoA or another acceptable method. The result may differ from the certified value and UCRM based on method uncertainty as well as the uncertainty associated with the standard used for comparison.

Traceability: The standard was manufactured under an ISO/IEC 17025:2017 certified quality system. The balance used to weigh raw materials is accurate to +/- 0.0001g and calibrated regularly using mass standards traceable to NIST. All dilutions were preformed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.

Homogeneity: Homogeneity was assessed in accordance with ISO 17034:2016. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared using a one-way analysis of variance approach as described by TNI EL-V3-2009 Appendix A.2. See Instructions for minimum sub-sample size.

Expiration is at end of month given on certificate and label.

MSDS reports for components comprising greater than 1.0% of the solution or 0.1% for components known to be carcinogens are available upon request.

THIS PRODUCT WAS DESIGNED, PRODUCED AND VERIFIED FOR ACCURACY AND STABILITY IN ACCORDANCE WITH **ISO/IEC 17025:2017 (ANAB Cert AT-1467)** and **ISO 17034:2016 (ANAB Cert AR-1470).**

Andy Ommen - QC Manager

Mark Pooler - QA Supervisor

Certification Date November 05, 2020
Version 0-1152020

E.2 Naphthalene Solution

Certificate of Analysis

NAPHTHALENE SOLUTION,1X1ML,200UG/ML,METHANOL

Certified Reference Material

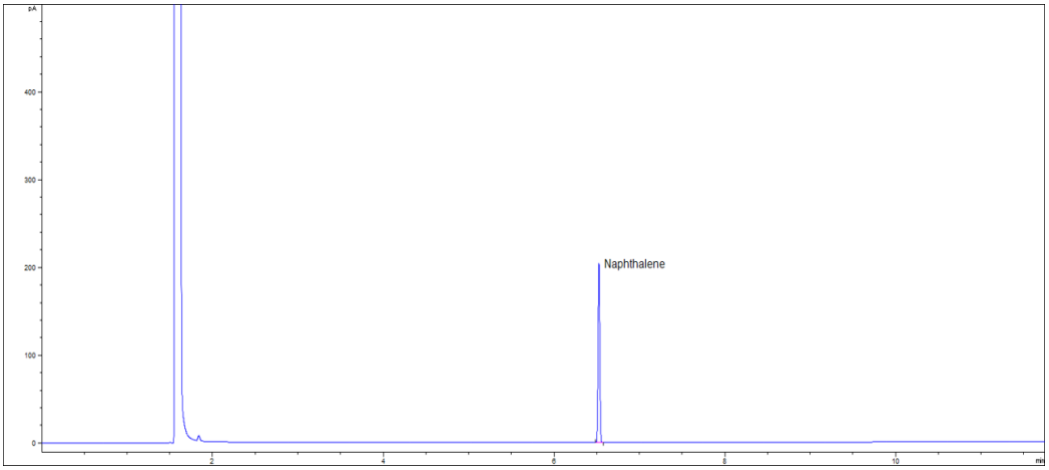
Description

Product ID CRM48641
Lot **LRAC3486**
Expiration Date July 2022
Manufacturing Date July 2019
Storage Conditions Room Temperature
Solvent/Matrix METHANOL

Certified Values

Analyte	*Certified Value* [1,4]	*Units*	*Raw Material Purity,%*	*Analytical Value* [6]	*Elution order*	*Raw Material Lot*	*CAS*
NAPHTHALENE	200 ± 6	µg/mL	100.0	195	1	01112017-5	91-20-3

Informational Values



Additional Information:
Analytical Method Parameters:

Column: SPB-5, 30 m x 0.53 mm x 1.5 µm df
75°C (1 min) to 250°C (2 min) at 20°C/min
Detector: FID, 320°C
Injection Volume: 0.5 µL
Split 5:1

ANAB ACCREDITED REFERENCE MATERIAL PRODUCER
ANAB ACCREDITED TESTING LABORATORY

SIGMA-ALDRICH®
2931 Soldier Springs Rd. Laramie, Wyoming 82070 USA
307-742-5452
rtctechgroup@sial.com www.sigma-aldrich.com

Description

Lot	LRAC3486
Expiration Date	July 2022
Manufacturing Date	July 2019
Storage Conditions	Room Temperature
Solvent/ Matrix	METHANOL

1 Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. The balance used to weigh raw materials is accurate to +/-0.0001 g and calibrated regularly using mass standards traceable to NIST. All dilutions were performed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.
4 Ucrm - Uncertainty values in this document are expressed as Expanded Uncertainty (Ucrm) corresponding to the 95% confidence interval. Ucrm is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transpot). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies. The mathematical representation of the Ucrm calculation is as follows:

$$u_{CRM} = \sqrt{u_{char}^2 + u_{hom\ ogeneity}^2 + u_{stability}^2}$$

Coverage factor derived from a t-distribution table, based on the degrees of freedom of the data set. Assume 2.0 for a Confidence interval = 95%

6 Analytical Value- For QC verification of the certified value only- not to be used in calculations. Represents the analytical data obtained by comparison to a standard as analyzed by the method described in the CoA or another acceptable method. The result may differ from the certified value and UCRM based on method uncertainty as well as the uncertainty associated with the standard used for comparison.

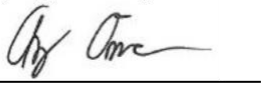
Traceability: The standard was manufactured under an ISO/IEC 17025:2017 certified quality system. The balance used to weigh raw materials is accurate to +/- 0.0001g and calibrated regularly using mass standards traceable to NIST. All dilutions were preformed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.

Homogeneity: Homogeneity was assessed in accordance with ISO 17034:2016. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared using a one-way analysis of variance approach as described by TNI EL-V3-2009 Appendix A.2. See Instructions for minimum sub-sample size.

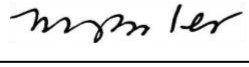
Expiration is at end of month given on certificate and label.

THIS PRODUCT WAS DESIGNED, PRODUCED AND VERIFIED FOR ACCURACY AND STABILITY IN ACCORDANCE WITH **ISO/IEC 17025:2017 (ANAB Cert AT-1467)** and **ISO 17034:2016 (ANAB Cert AR-1470).**

MSDS reports for components comprising greater than 1.0% of the solution or 0.1% for components known to be carcinogens are available upon request.



Andy Ommen - QC Manager



Mark Pooler - QA Supervisor

Certification Date August 13, 2019
Version 0-8132019

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

Page 2 of 2

E.3 PCB 52 Solution

CRM/RM-103

RESTEK® CERTIFIED REFERENCE MATERIAL

110 Benner Circle
Bellefonte, PA 16823-8812
Tel: (800)356-1688
Fax: (814)353-1309

www.restek.com

Certificate of Analysis

FOR LABORATORY USE ONLY-READ SDS PRIOR TO USE.

This Reference Material is intended for Laboratory Use Only as a standard for the qualitative and/or quantitative determination of the analyte(s) listed.

Catalog No. : 33257 **Lot No.:** A0116257

Description : PCB 52 Stock Solution
50µg/mL, Acetonitrile,5mL/ampul

Container Size : 5 mL **Pkg Amt:** > 5 mL

Expiration Date : April 30, 2022 **Storage:** 10°C or colder

Handling: This product contains PCBs.

CERTIFIED VALUES

Elution Order	Compound	Grav. Conc. (weight/volume)	Expanded Uncertainty (95% C.L.; K=2)		
1	2,2',5,5'-Tetrachlorobiphenyl (BZ#52)	50.0 µg/mL	+/- 0.3544	µg/mL	Gravimetric
	CAS # 35693-99-3 (Lot 090210KS)		+/- 1.5965	µg/mL	Unstressed
	Purity 98%		+/- 2.0789	µg/mL	Stressed

Solvent: Acetonitrile
CAS # 75-05-8
Purity 99%

Column:
30m x .25mm x .2um
Rtx-CLP II (cat.# 11323)

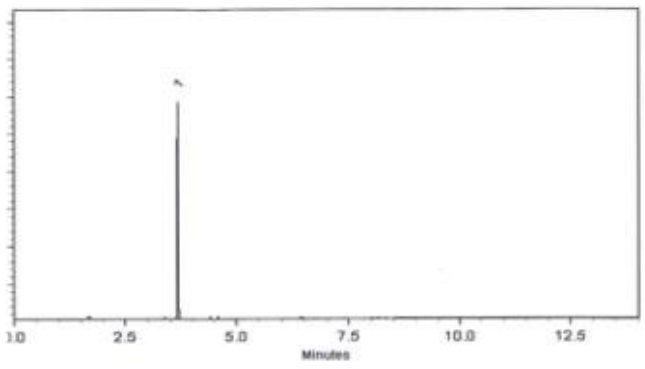
Carrier Gas:
helium-constant pressure 20 psi.

Temp. Program:
200°C to 300°C
@ 25°C/min. (hold 10 min.)

Inj. Temp:
250°C

Det. Temp:
300°C

Det. Type:
ECD



This chromatogram represents a general set of testing conditions chosen for product acceptance. For optimal results in your lab, conditions should be adjusted for your specific instrument, method, and application.

Brandon Cook - Mix Technician Date Mixed: 03-Jan-2016 Balance: 1128353505

Jennifer L. Pollino - QC Analyst Date Passed: 06-Jan-2016

Manufactured under Restek's ISO 9001:2008 Registered Quality System Certificate #FM 80397

General Certified Reference Material Notes

Expiration Notes:

- Expiration date valid for unopened ampul stored in compliance with the recommended conditions.
- Uncertainty, concentration, and expiration of the CRM are based on the unopened product being stored according to the recommended condition found in the storage field.

Purity Notes:

- Purity and/or chemical identity are determined by one or more of the following techniques: GC/FID, HPLC, GC/μECD, GC/MS, LC/MS, RI, and/or melting point.
- Compounds with a listed purity of less than 99% have been weight corrected to compensate for impurities and/or salts. A correction factor is used to calculate the amount of compound necessary to achieve the desired concentration of the parent compound in solution.
- Purity of isomeric compounds is reported as the sum of the isomers.
- Purity values are rounded to the nearest whole number.

Certified Uncertainty Value Notes:

- The uncertainties are determined in accordance with ISO Guides 34 and 35. The certified combined stressed uncertainty value (includes gravimetric uncertainty, homogeneity between-ampul uncertainty, storage stability uncertainty and shipping stability uncertainty and were combined using the following formula:

$$U_{combined\ stressed} = k\sqrt{U^2_{gravimetric} + U^2_{homogeneity} + U^2_{storage\ stability} + U^2_{shipping\ stability}}$$

  k is a coverage factor of 2, which gives a level of confidence of approximately 95%.
- It is important to note that the shipping stability uncertainty was obtained under temperature extremes for specific time intervals; therefore, the certified combined stressed uncertainty value should only be applied to the product if it was stored at non-standard temperature conditions up to and including 7 days. Contact Restek Technical Service at www.restek.com/Contact-Us for use recommendations if your shipment was in-transit for more than 7 days at non-standard temperature conditions.
- Apply the certified combined unstressed uncertainty value if the product was received under standard shipping conditions. Apply the certified combined stressed uncertainty value if the product was received under non-standard conditions as specified below.

Label Conditions	Standard Conditions	Non-Standard Conditions
25°C Nominal (Room Temperature)	< 60°C	≥ 60°C up to 7 days
10°C or colder (Refrigerate)	< 40°C	≥ 40°C up to 7 days
0°C or colder (Freezer)	< 25°C	≥ 25°C up to 7 days

- Separate (not combined) uncertainty values for gravimetric uncertainty are also displayed on the certificate, if needed, separate homogeneity between-ampul uncertainty, storage stability uncertainty and shipping stability uncertainty values are available by contacting Restek Technical Service at www.restek.com/Contact-Us.
- The packaged amount is the minimum sample size for which uncertainty is valid. The ampules are over-filled to ensure that the minimum packaged amount can be sufficiently transferred.

Manufacturing Notes:

- Concentration is based upon gravimetric preparation using either a balance whose calibration has been verified daily using NIST traceable weights, and/or dilutions with Class A glassware.

Handling Notes:

- Samples should be transferred into deactivated vials for handling and storage. Restek supplies deactivated vials along with most standards packed in 2 mL ampules. Due to space constraints, Restek does not supply vials for larger volume ampules. Restek sells DMDCS for the purpose of glassware deactivation as catalog number 31861, which includes complete instructions. Restek will also deactivate larger volume vials from our inventory as a custom ordered item. Contact your Restek sales or customer service representative for details.
- If any undissolved material is visible inside the ampul, sonicate the unopened ampul until the material is completely dissolved.

E.4 Caffeine Pharmaceutical Secondary Standard

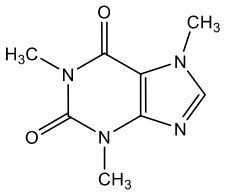
Supelco®

www.sigmaaldrich.com

Certificate of Analysis – Certified Reference Material

CAFFEINE

Product no.:	PHR1009-1G
Lot no.:	LRAC4115
Description of CRM:	White Powder
Expiry date:	30 November 2023
Storage:	Room Temperature/Protect from Light
Certificate version:	LRAC4115.1 (*Note: Certificates may be updated due to Pharmacopeial Lot Changes or the availability of new data. Check our website at: www.sigma-aldrich.com for the most current version.)*
Chemical formula:	$C_8H_{10}N_4O_2$
Molecular mass:	194.2
CAS No.:	58-08-02



Analyte	Certified Purity ± associated uncertainty *U*, *U*=*k* · *u* (*k*=) (Mass Balance/ basis)
CAFFEINE	**99.96 % Ucrm = ± 0.7 %, k = 2.0 (Mass Balance/as is basis)**

Metrological traceability:	Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. Additional traceability to Primary Standards is established through comparative assay determinations. See "Details on metrological traceability" on page 2.
Measurement method:	Where applicable, the certified value is based on a purity determination by mass balance. See "Certification process details" on page 3.
Intended use:	Intended for R&D and Analytical Use only. Not for drug, household or other uses.
Minimum sample size:	20 mg
Instructions for handling and correct use:	Do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.
Health and safety information:	All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.
Accreditation:	Sigma-Aldrich RTC is accredited by the US accreditation authority ANAB as a registered reference material producer AR-1470 in accordance with ISO 17034.
Certificate issue date:	04 February 2020

ANAB ACCREDITED REFERENCE MATERIAL

ISO 17034
AR-1470

[Andy Ommen; Quality Control]

[Mark Pooler; Quality Assurance]

Sigma-Aldrich RTC, 2931 Soldier Springs Rd. Laramie, WY 82070, USA;
Tel. 1 307-742-5452; Fax 1 307-855-831-9211; www.sigmaaldrich.com
Sigma-Aldrich RTC is a subsidiary of Merck KGaA, Darmstadt, Germany.

Packaging:	1 g in amber vial
Details on metrological traceability:	This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error. Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances. Further traceability to a corresponding Primary Standard may be achieved through a direct comparison assay. Where a Primary Standard is available, the assay value will be included in the specified section of the COA.
Associated uncertainty:	Uncertainty values in this document are expressed as Expanded Uncertainty (U_{CRM}) corresponding to the 95% confidence interval. U_{CRM} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a t-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

Traceability Assay:
Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD (as is basis)

ASSAY VALUE	**vs. USP LOT**
99.3 %	**R04330** Labeled Content = 0.998 mg/mg

ASSAY vs. EP CRS (as is basis)

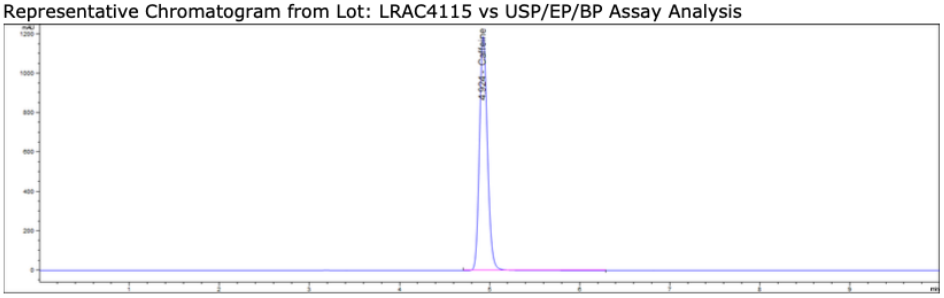
ASSAY VALUE	**vs. EP BATCH**
98.0 %	**4.0** Labeled Content = 99.9 % $C_8H_{10}N_4O_2$

ASSAY vs. BP CRS (as is basis)

ASSAY VALUE	**vs. BP BATCH**
99.5 %	**3271** Labeled Content = 99.9 % of $C_8H_{10}N_4O_2$

Method: HPLC (ref.: Caffeine, Current Compendial Monographs)
Column: Ascentis Express C18, 150 mm x 4.6 mm x 5 µm
Mobile Phase: 0.82 g/L Sodium Acetate (pH to 4.5 w/glacial acetic acid), THF, Acetonitrile
Mobile Phase Ratio: 95.5:2:2.5
Flow Rate: 0.8 mL/min
Column Temperature: 30 °C
Injection: 10 µL
Detector: 275 nm

Representative Chromatogram from Lot: LRAC4115 vs USP/EP/BP Assay Analysis



Certification process details:

The certified purity is determined by mass balance and calculated as

$$\%\ Purity = \left(\frac{(100 - TCI)}{100} * \frac{(100 - LOD)}{100} * \frac{(100 - H2O)}{100} * \frac{(100 - ROI)}{100} * \frac{(100 - RS)}{100}\right) * 100\%$$

- TCI = Total Chromatographic Impurities
- LOD = Loss on Drying
- H_2O = Water content determined by Karl Fischer analysis
- ROI = Residue on Ignition
- RS = Residual Solvents

Methods for impurity determination may be added or deleted as required. The following techniques are applied:

CHROMATOGRAPHIC IMPURITY ANALYSIS

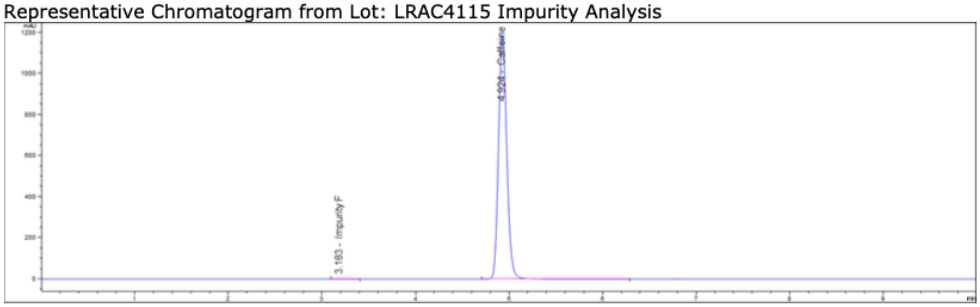
METHOD: HPLC (ref.: Caffeine, Current Compendial Monographs)
See HPLC Assay for Method Parameters

Impurities Detected:

Impurity F: 0.03 %

Total Impurities: **0.03 %**

Representative Chromatogram from Lot: LRAC4115 Impurity Analysis



RESIDUAL SOLVENTS
Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)
Column: SPB-624
Carrier gas: He
Flow: 1.2 mL/min
Split Ratio: 1:5
Injection/Temperature: 1 mL/220 °C
Temperature Program: 40 °C for 5 min, 8 °C/min to 200 °C, hold 5 min

Solvents Detected: **None**

LOSS ON DRYING/VOLATILES
Method: 80 ºC for 4 hours (ref.: Current Compendial Monographs)
Mean of three measurements, Loss = **0.006 %**

RESIDUE ANALYSIS
Method: Sulfated Ash (ref.: Current Compendial Monographs)
Sample Size: ~ 100 mg
Mean of three measurements, Residue = **0.004 %**

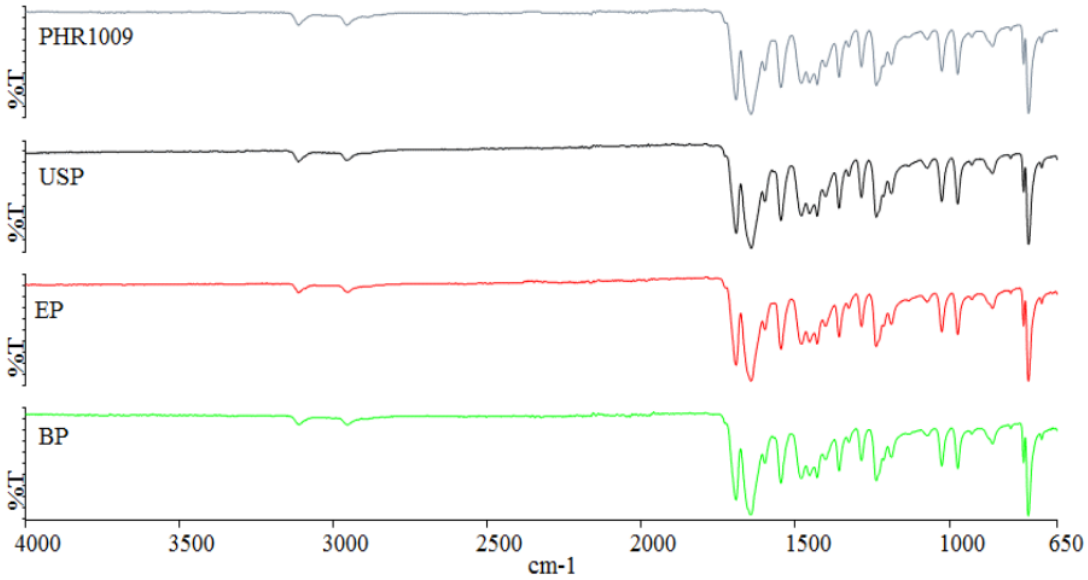
CERTIFIED PURITY BY MASS BALANCE

99.96 % U_{crm} = ± 0.7 %, k = 2.0
(as is basis)

Homogeneity assessment:	Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA. Analytical method: HPLC Sample size: 20 mg
Stability assessment:	Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation. Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

Identification Test:

INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)

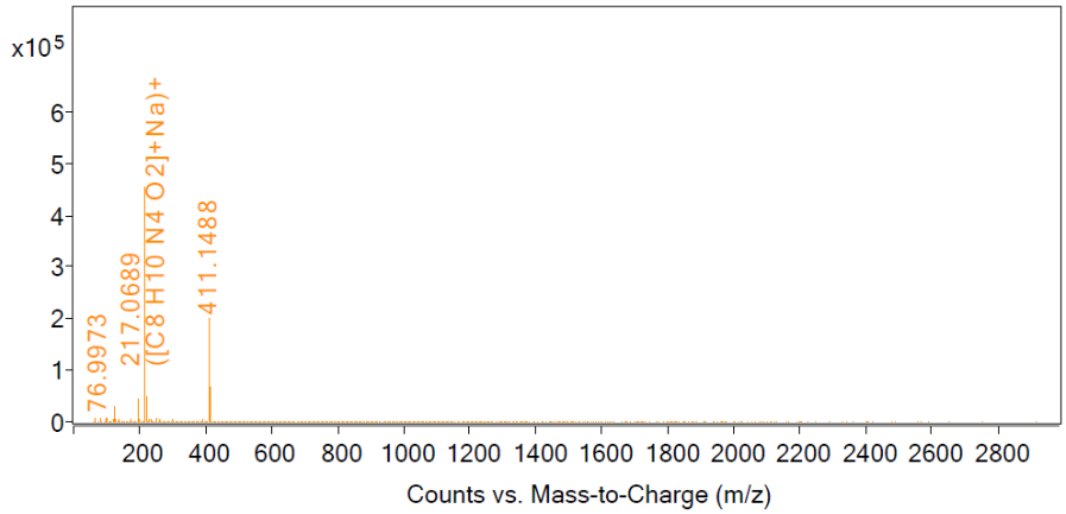


PHR1009 LRAC4115 vs USP R04330/EP Batch 4.0/BP Batch 3271

Indicative Values:

MASS SPECTRUM

Method: HR-QTOF; 4.0 kV ESI+; temperature: 325 °C



Theoretical value: 217.0701 m/z

The signal of the MS spectrum is consistent with the theoretical value and its interpretation is consistent with the structural formula.

MELTING POINT
Specification: 234-239 °C (EP)
Mettler Toledo FP900 Thermosystem with FP81 Measuring Cell
Mean of three measurements = **236.9 °C**

Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAC4115.1	04 February 2020	Original Release

Disclaimer:

The purchaser is required to determine the suitability of this product for any particular application. Sigma-Aldrich RTC makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by Sigma-Aldrich RTC. We do not guarantee that the product can be used for any particular application.

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The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the US and Canada.

F Linearity Tables

In Table 7-5, Table 7-6 and Table 7-7 the results from linearity tests are given, respectively as peak areas from FID-Polyarc, peak heights from FID-Polyarc and peak areas from MS.

Table 7-5 Theoretical conc. of AI and area results from FID/Polyarc.

Sample	Theoretical conc. AI [µg/mL]	Area (FID/Polyarc)
P	0,06	0,00
O	0,12	0,00
N	0,24	0,00
M	0,49	0,11
L	0,98	0,25
K	1,95	0,43
J	3,91	1,00
I	7,81	2,04
H	15,63	4,30
G	31,25	9,07
F	62,50	16,59
E	125,00	37,98
D	250,00	76,60
C	500,00	152,89
B	1000,00	309,45
A	2000,00	613,31

Table 7-6 Theoretical conc. of AI and height results from FID/Polyarc.

Sample	Theoretical conc. AI [µg/mL]	Height (FID/Polyarc)
P	0,06	0,00
O	0,12	0,00
N	0,24	0,00
M	0,49	0,05
L	0,98	0,11
K	1,95	0,22
J	3,91	0,49
I	7,81	1,06
H	15,63	2,28
G	31,25	4,89
F	62,50	9,16
E	125,00	21,28
D	250,00	43,68
C	500,00	87,29
B	1000,00	174,20
A	2000,00	327,11

Table 7-7 Theoretical conc. of AI and area results from MS.

Sample	Theoretical conc. AI [µg/mL]	Area (MS)
P	0,06	0,00
O	0,12	0,00
N	0,24	0,00
M	0,49	439,12
L	0,98	1983,17
K	1,95	4553,73
J	3,91	13204,61
I	7,81	38776,09
H	15,63	108486,00
G	31,25	302244,24
F	62,50	703477,76
E	125,00	2276278,90
D	250,00	5421080,82
C	500,00	9461459,93
B	1000,00	14412066,58
A	2000,00	20454356,75

G Response Factors for MS

Table 7-8 Results for Fit for Purpose tests of sample with 90% purity from GC/MS expressed as RF.

Area 90% X1						Area 90% X3						Area 90% X10				
	Area AI	Area Imp 1	Area Imp 2	Area Ext. St			Area AI	Area Imp 1	Area Imp 2	Area Ext. St			Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	11266848,61	941538,36	1751486,25	2537634,37		Inj 1	4403506,74	233987,66	399206,67	2537634,37		Inj 1	1270999,31	56061,66	113484,86	2537634,37
Inj 2	10501458,98	847775,98	1568179,27	2265742,22		Inj 2	4468513,47	237874,35	406916,81	2265742,22		Inj 2	1258833,49	55466,37	94542,11	2265742,22
Inj 3	10629764,84	860658,19	1600338,82	2276838,53		Inj 3	4421809,86	231492,47	397896,16	2276838,53		Inj 3	1276746,39	55757,18	82277,47	2276838,53
Average	10799357,47	883324,18	1640001,45	2360071,70		Average	4431276,69	234451,50	401339,88	2360071,70		Average	1268859,73	55761,74	96768,15	2360071,70
Variance	1,1202E+11	1,7221E+09	6,3868E+09	1,5785E+10		Variance	7,4912E+08	6,8956E+06	1,5837E+07	1,5785E+10		Variance	55767575,67	59072,09111	164794440,1	15784771347
SD	334690,47	41498,24	79917,48	125637,46		SD	27370,11	2625,96	3979,61	125637,46		SD	7467,77	243,05	12837,23	125637,46
%RSD	3,10	4,70	4,87	5,32		%RSD	0,62	1,12	0,99	5,32		%RSD	0,59	0,44	13,27	5,32
u	193233,63	23959,02	46140,38	72536,82		u	15802,14	1516,10	2297,63	72536,82		u	4311,52	140,32	7411,58	72536,82
U	386467,27	47918,04	92280,76	145073,64		U	31604,28	3032,19	4595,26	145073,64		U	8623,04	280,65	14823,15	145073,64

Response factor ext. st v. sample 90% x1			
	AI	Imp 1	Imp 2
Inj 1	0,23	2,70	1,45
Inj 2	0,22	2,67	1,44
Inj 3	0,21	2,65	1,42
Average	0,22	2,67	1,44
Variance	0,0000	0,0004	0,0001
SD	0,00	0,02	0,01
%RSD	2,23	0,76	0,80
u	0,00	0,01	0,01
U	0,01	0,02	0,01

Response factor ext. st v. sample 90% x3			
	AI	Imp 1	Imp 2
Inj 1	0,58	10,85	6,36
Inj 2	0,51	9,52	5,57
Inj 3	0,51	9,84	5,72
Average	0,53	10,07	5,88
Variance	0,0010	0,3176	0,1165
SD	0,03	0,56	0,34
%RSD	5,81	5,60	5,80
u	0,02	0,33	0,20
U	0,04	0,65	0,39

Response factor ext. st v. sample 90% x10			
	AI	Imp 1	Imp 2
Inj 1	2,00	45,27	22,36
Inj 2	1,80	40,85	23,97
Inj 3	1,78	40,83	27,67
Average	1,86	42,32	24,67
Variance	0,0094	4,3476	4,9480
SD	0,10	2,09	2,22
%RSD	5,21	4,93	9,02
u	0,06	1,20	1,28
U	0,11	2,41	2,57

Response factor AI v. imp 90% x1

	AI	Imp 1	Imp 2
Inj 1	1,00	11,97	6,43
Inj 2	1,00	12,39	6,70
Inj 3	1,00	12,35	6,64
Average	1,00	12,23	6,59
Variance	0,0000	0,0362	0,0129
SD	0,00	0,19	0,11
%RSD	0,00	1,56	1,73
u	0,00	0,11	0,07
U	0,00	0,22	0,13

Response factor AI v. imp 90% x3

	AI	Imp 1	Imp 2
Inj 1	1,00	18,82	11,03
Inj 2	1,00	18,79	10,98
Inj 3	1,00	19,10	11,11
Average	1,00	18,90	11,04
Variance	0,0000	0,0201	0,0029
SD	0,00	0,14	0,05
%RSD	0,00	0,75	0,49
u	0,00	0,08	0,03
U	0,00	0,16	0,06

Response factor AI v. imp 90% x10

	AI	Imp 1	Imp 2
Inj 1	1,00	22,67	11,20
Inj 2	1,00	22,70	13,32
Inj 3	1,00	22,90	15,52
Average	1,00	22,76	13,34
Variance	0,0000	0,0104	3,1077
SD	0,00	0,10	1,76
%RSD	0,00	0,45	13,21
u	0,00	0,06	1,02
U	0,00	0,12	2,04

Table 7-9 Results for Fit for Purpose tests of sample with 95% purity from GC/MS expressed as RF.

Area 95% X1

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	12139729,32	324896,26	533689,35	2537634,37
Inj 2	12119421,15	323237,52	527858,27	2265742,22
Inj 3	12166934,42	320810,79	528566,91	2276838,53
Average	12142028,30	322981,52	530038,18	2360071,70
Variance	3,7889E+08	2,8146E+06	6,7492E+06	1,5785E+10
SD	19465,21	1677,68	2597,93	125637,46
%RSD	0,16	0,52	0,49	5,32
u	11238,24	968,61	1499,92	72536,82
U	22476,49	1937,22	2999,83	145073,64

Area 95% X3

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	5168652,25	82173,90	114535,37	2537634,37
Inj 2	5095536,86	81244,30	110299,86	2265742,22
Inj 3	4785149,93	74644,14	99023,52	2276838,53
Average	5016446,34	79354,11	107952,92	2360071,70
Variance	2,7640E+10	1,1236E+07	4,2857E+07	1,5785E+10
SD	166252,80	3352,01	6546,53	125637,46
%RSD	3,31	4,22	6,06	5,32
u	95986,10	1935,28	3779,64	72536,82
U	191972,20	3870,56	7559,28	145073,64

Area 95% X10

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	1309849,38	17243,45	18665,27	2537634,37
Inj 2	1326039,38	16302,69	19454,81	2265742,22
Inj 3	1291781,49	16123,00	17179,06	2276838,53
Average	1309223,42	16556,38	18433,05	2360071,70
Variance	195796384,7	241413,4306	890139,5734	15784771347
SD	13992,73	491,34	943,47	125637,46
%RSD	1,07	2,97	5,12	5,32
u	8078,70	283,67	544,71	72536,82
U	16157,41	567,35	1089,43	145073,64

Response factor ext. st v. sample 95% x1

	AI	Imp 1	Imp 2
Inj 1	0,21	7,81	4,75
Inj 2	0,19	7,01	4,29
Inj 3	0,19	7,10	4,31
Average	0,19	7,31	4,45
Variance	0,0001	0,1287	0,0460
SD	0,01	0,36	0,21
%RSD	5,33	4,91	4,82
u	0,01	0,21	0,12
U	0,01	0,41	0,25

Response factor ext. st v. sample 95% x3

	AI	Imp 1	Imp 2
Inj 1	0,49	30,88	22,16
Inj 2	0,44	27,89	20,54
Inj 3	0,48	30,50	22,99
Average	0,47	29,76	21,90
Variance	0,0004	1,7710	1,0350
SD	0,02	1,33	1,02
%RSD	4,10	4,47	4,65
u	0,01	0,77	0,59
U	0,02	1,54	1,17

Response factor ext. st v. sample 95% x10

	1,94	147,17	135,95
Inj 1	1,71	138,98	116,46
Inj 2	1,76	141,22	132,54
Inj 3	1,80	142,45	128,32
Average	0,0095	11,9321	72,2269
Variance	0,10	3,45	8,50
SD	5,41	2,42	6,62
%RSD	0,06	1,99	4,91
u	0,11	3,99	9,81
U	1,94	147,17	135,95

Response factor AI v. imp 95% x1

	AI	Imp 1	Imp 2
Inj 1	1,00	37,36	22,75
Inj 2	1,00	37,49	22,96
Inj 3	1,00	37,93	23,02
Average	1,00	37,59	22,91
Variance	0,0000	0,0575	0,0136
SD	0,00	0,24	0,12
%RSD	0,00	0,64	0,51
u	0,00	0,14	0,07
U	0,00	0,28	0,13

Response factor AI v. imp 95% x3

	AI	Imp 1	Imp 2
Inj 1	1,00	62,90	45,13
Inj 2	1,00	62,72	46,20
Inj 3	1,00	64,11	48,32
Average	1,00	63,24	46,55
Variance	0,0000	0,3794	1,7646
SD	0,00	0,62	1,33
%RSD	0,00	0,97	2,85
u	0,00	0,36	0,77
U	0,00	0,71	1,53

Response factor AI v. imp 95% x10

	AI	Imp 1	Imp 2
Inj 1	1,00	75,96	70,18
Inj 2	1,00	81,34	68,16
Inj 3	1,00	80,12	75,20
Average	1,00	79,14	71,18
Variance	0,0000	5,2981	8,7501
SD	0,00	2,30	2,96
%RSD	0,00	2,91	4,16
u	0,00	1,33	1,71
U	0,00	2,66	3,42

Table 7-10 Results for Fit for Purpose tests of sample with 97% purity from GC/MS expressed as RF.

Area 97% X1

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	12274305,24	189024,40	265703,02	2537634,37
Inj 2	12318569,18	192575,15	265985,44	2265742,22
Inj 3	12286761,38	190268,99	261021,66	2276838,53
Average	12293211,94	190622,845	264236,707	2360071,704
Variance	3,4735E+08	2,1639E+06	5,1816E+06	1,5785E+10
SD	18637,44	1471,02	2276,30	125637,46
%RSD	0,15	0,77	0,86	5,32
u	10760,33	849,29	1314,22	72536,82
U	21520,66	1698,59	2628,45	145073,64

Area 97% X3

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	5207807,66	49873,47	54948,35	2537634,37
Inj 2	5201330,37	47654,96	52886,71	2265742,22
Inj 3	4864413,40	43353,47	48305,31	2276838,53
Average	5091183,809	46960,63233	52046,79	2360071,704
Variance	2,5719E+10	7,3261E+06	7,7077E+06	1,5785E+10
SD	160372,70	2706,68	2776,28	125637,46
%RSD	3,15	5,76	5,33	5,32
u	92591,22	1562,70	1602,89	72536,82
U	185182,44	3125,40	3205,77	145073,64

Area 97% X10

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	1334910,86	9435,80	9703,02	2537634,37
Inj 2	1330641,10	9785,44	10322,00	2265742,22
Inj 3	1304595,06	9802,44	11896,67	2276838,53
Average	1323382,339	9674,561667	10640,56233	2360071,704
Variance	179519448,7	28551,51731	852759,4815	15784771347
SD	13398,49	168,97	923,45	125637,46
%RSD	1,01	1,75	8,68	5,32
u	7735,62	97,56	533,15	72536,82
U	15471,24	195,11	1066,31	145073,64

Response factor ext. st v. sample 97% x1

	AI	Imp 1	Imp 2
Inj 1	0,21	13,42	9,55
Inj 2	0,18	11,77	8,52
Inj 3	0,19	11,97	8,72
Average	0,19	12,39	8,93
Variance	0,0001	0,5468	0,1992
SD	0,01	0,74	0,45
%RSD	5,44	5,97	5,00
u	0,01	0,43	0,26
U	0,01	0,85	0,52

Response factor ext. st v. sample 97% x3

	AI	Imp 1	Imp 2
Inj 1	0,49	50,88	46,18
Inj 2	0,44	47,54	42,84
Inj 3	0,47	52,52	47,13
Average	0,46	50,31	45,39
Variance	0,0005	4,2829	3,3885
SD	0,02	2,07	1,84
%RSD	4,60	4,11	4,06
u	0,01	1,19	1,06
U	0,02	2,39	2,13

Response factor ext. st v. sample 97% x10

	AI	Imp 1	Imp 2
Inj 1	1,90	268,94	261,53
Inj 2	1,70	231,54	219,51
Inj 3	1,75	232,27	191,38
Average	1,78	244,25	224,14
Variance	0,0073	304,7958	830,8124
SD	0,09	17,46	28,82
%RSD	4,78	7,15	12,86
u	0,05	10,08	16,64
U	0,10	20,16	33,28

Response factor AI v. imp 97% x1

	AI	Imp 1	Imp 2
Inj 1	1,00	64,94	46,20
Inj 2	1,00	63,97	46,31
Inj 3	1,00	64,58	47,07
Average	1,00	64,49	46,53
Variance	0,0000	0,1594	0,1508
SD	0,00	0,40	0,39
%RSD	0,00	0,62	0,83
u	0,00	0,23	0,22
U	0,00	0,46	0,45

Response factor AI v. imp 97% x3

	AI	Imp 1	Imp 2
Inj 1	1,00	104,42	94,78
Inj 2	1,00	109,15	98,35
Inj 3	1,00	112,20	100,70
Average	1,00	108,59	97,94
Variance	0,0000	10,2507	5,9335
SD	0,00	3,20	2,44
%RSD	0,00	2,95	2,49
u	0,00	1,85	1,41
U	0,00	3,70	2,81

Response factor AI v. imp 97% x10

	AI	Imp 1	Imp 2
Inj 1	1,00	141,47	137,58
Inj 2	1,00	135,98	128,91
Inj 3	1,00	133,09	109,66
Average	1,00	136,85	125,38
Variance	0,0000	12,0909	136,1162
SD	0,00	3,48	11,67
%RSD	0,00	2,54	9,30
u	0,00	2,01	6,74
U	0,00	4,02	13,47

Table 7-11 Results for Fit for Purpose tests of sample with 99% purity from GC/MS expressed as RF.

Area 99% X1

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	12511517,11	128767,43	51406,68	2537634,37
Inj 2	12417697,74	127524,00	50727,88	2265742,22
Inj 3	12631445,73	126999,07	51298,79	2276838,53
Average	12520220,19	127763,499	51144,44933	2360071,704
Variance	7,6526E+09	5,4986E+05	8,8705E+04	1,5785E+10
SD	87478,98	741,53	297,83	125637,46
%RSD	0,70	0,58	0,58	5,32
u	50506,01	428,12	171,95	72536,82
U	101012,03	856,24	343,91	145073,64

Area 99% X3

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	4986654,83	30016,78	9682,48	2537634,37
Inj 2	5376029,16	32867,83	11003,69	2265742,22
Inj 3	4901511,91	28894,40	9351,76	2276838,53
Average	5088065,3	30593,00333	10012,64633	2360071,704
Variance	4,2670E+10	2,7974E+06	5,0932E+05	1,5785E+10
SD	206566,73	1672,53	713,66	125637,46
%RSD	4,06	5,47	7,13	5,32
u	119261,35	965,64	412,03	72536,82
U	238522,71	1931,28	824,07	145073,64

Area 99% X10

	Area AI	Area Imp 1	Area Imp 2	Area Ext. St
Inj 1	1369792,42	6935,90	3434,97	2537634,37
Inj 2	1337670,22	6262,18	3162,68	2265742,22
Inj 3	1318264,77	6454,68	2151,49	2276838,53
Average	1341909,133	6550,919333	2916,379	2360071,704
Variance	451500628,1	80280,77771	304884,2929	15784771347
SD	21248,54	283,34	552,16	125637,46
%RSD	1,58	4,33	18,93	5,32
u	12267,85	163,59	318,79	72536,82
U	24535,71	327,17	637,58	145073,64

Response factor ext. st v. sample 99% x1

	AI	Imp 1	Imp 2
Inj 1	0,20	19,71	49,36
Inj 2	0,18	17,77	44,66
Inj 3	0,18	17,93	44,38
Average	0,19	18,47	46,14
Variance	0,0001	0,7727	5,2181
SD	0,01	0,88	2,28
%RSD	5,39	4,76	4,95
u	0,01	0,51	1,32
U	0,01	1,02	2,64

Response factor ext. st v. sample 99% x3

	AI	Imp 1	Imp 2
Inj 1	0,51	84,54	262,09
Inj 2	0,42	68,93	205,91
Inj 3	0,46	78,80	243,47
Average	0,46	77,42	237,15
Variance	0,0013	41,5328	545,9175
SD	0,04	6,44	23,36
%RSD	7,68	8,32	9,85
u	0,02	3,72	13,49
U	0,04	7,44	26,98

Response factor ext. st v. sample 99% x10

	AI	Imp 1	Imp 2
Inj 1	1,85	365,87	738,77
Inj 2	1,69	361,81	716,40
Inj 3	1,73	352,74	1058,26
Average	1,76	360,14	837,81
Variance	0,0047	30,1185	24383,0456
SD	0,07	5,49	156,15
%RSD	3,89	1,52	18,64
u	0,04	3,17	90,15
U	0,08	6,34	180,31

Response factor AI v. imp 99% x1

	AI	Imp 1	Imp 2
Inj 1	1,00	97,16	243,38
Inj 2	1,00	97,38	244,79
Inj 3	1,00	99,46	246,23
Average	1,00	98,00	244,80
Variance	0,0000	1,0746	1,3536
SD	0,00	1,04	1,16
%RSD	0,00	1,06	0,48
u	0,00	0,60	0,67
U	0,00	1,20	1,34

Response factor AI v. imp 99% x3

	AI	Imp 1	Imp 2
Inj 1	1,00	166,13	515,02
Inj 2	1,00	163,57	488,57
Inj 3	1,00	169,64	524,13
Average	1,00	166,44	509,24
Variance	0,0000	6,1907	227,4778
SD	0,00	2,49	15,08
%RSD	0,00	1,49	2,96
u	0,00	1,44	8,71
U	0,00	2,87	17,42

Response factor AI v. imp 99% x10

	AI	Imp 1	Imp 2
Inj 1	1,00	197,49	398,78
Inj 2	1,00	213,61	422,95
Inj 3	1,00	204,23	612,72
Average	1,00	205,11	478,15
Variance	0,0000	43,6836	9151,9526
SD	0,00	6,61	95,67
%RSD	0,00	3,22	20,01
u	0,00	3,82	55,23
U	0,00	7,63	110,47

H Analysis Report Examples

H.1 Purity and Repeatability

Analyserapport Chiron

Tidspunkt for analyse: 03/Mar/2021 04:18 **Lims ID:**
Analyse utført av: SYSTEM

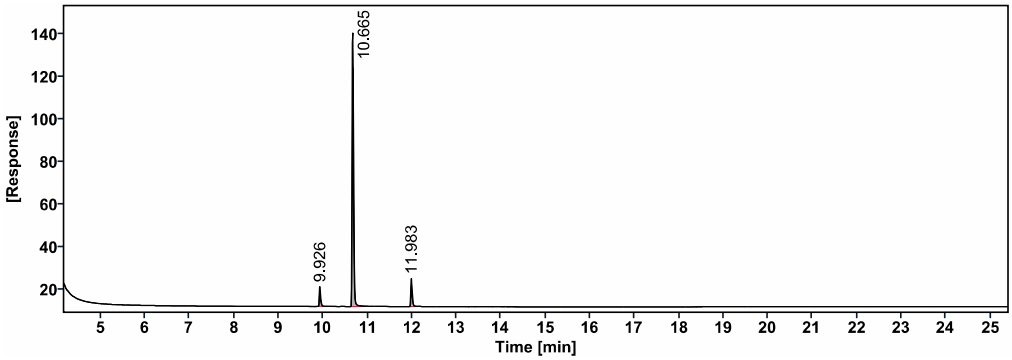
Filnavn: D:\CDSProjects\8890\Results\2021\Validation\Purity and repeatability.rslt\2021-03-03 04-12-50+01-00-15-r001.dx

Prøvenavn: A1

Analysemetode: Standard.amx **Sist endret:** 01/Mar/21
Beregningsmetode: *Standard_purity_undiluted.pmx **Sist endret:** 07/Apr/21

Annen Prøveinformasjon: SPJ8/034
Multiplier 1
Dilution 1

GC FID resultater

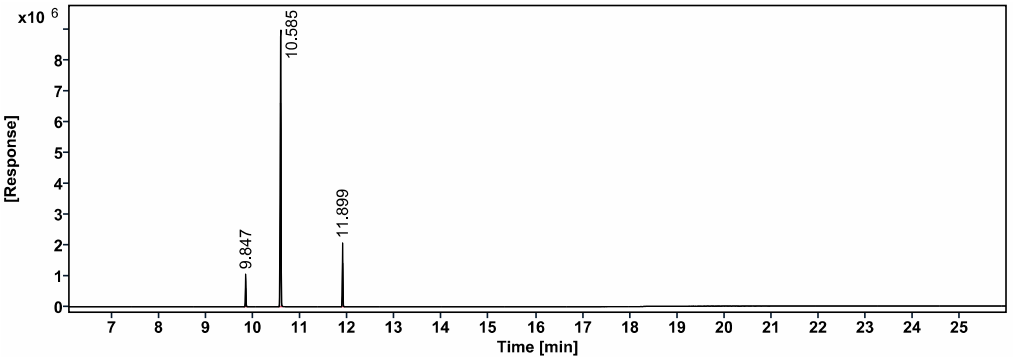


Komponent	*RT (min)*	*Areal*	*S/N*	*Areal%*	*Mengde*
Impurity	9.93	19.25	803.5	6.26	0.00
AI	10.66	260.98	11177.2	84.79	0.00
Impurity	11.98	27.56	918.4	8.95	0.00
			Sum:	100.00	0.00

matriks KJEMISK TEKNOLOGI | Utskriftstidspunkt: Friday, May 14, 2021 10:14 | Side 1 av 3

Analyserapport Chiron

GC-MS resultater



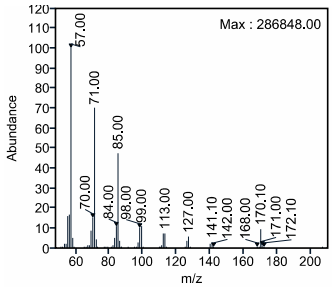
Komponent	RT (min)	Areal	S/N	Areal%	Mengde
Impurity	9.85	941538.36	4447.9	6.74	0.00
AI	10.59	11266848.61	46121.9	80.71	0.00
Impurity	11.90	1751486.25	11830.8	12.55	0.00
			Sum:	100.00	0.00

Analyserapport Chiron

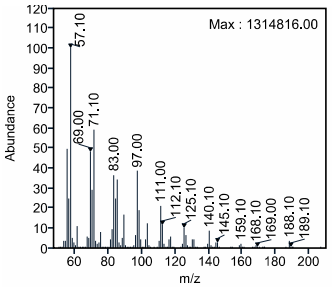
GC-MS Spektra

Signal: MS1Front TIC SCAN EI

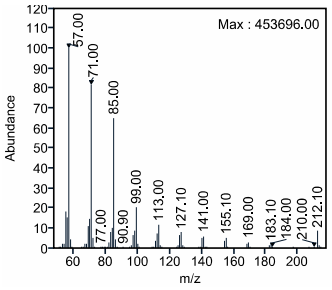
Peak RT: 9.847



Peak RT: 10.585



Peak RT: 11.899



H.2 Intermediate Precision

Analyserapport Chiron

Tidspunkt for analyse: 05/Mar/2021 05:45 **Lims ID:**
Analyse utført av: SYSTEM

Filnavn: D:\CDSProjects\8890\Results\2021\Validation\Intermediate precision day 1.rslt\2021-03-05 05-40-07+01-00-10-r002.dx

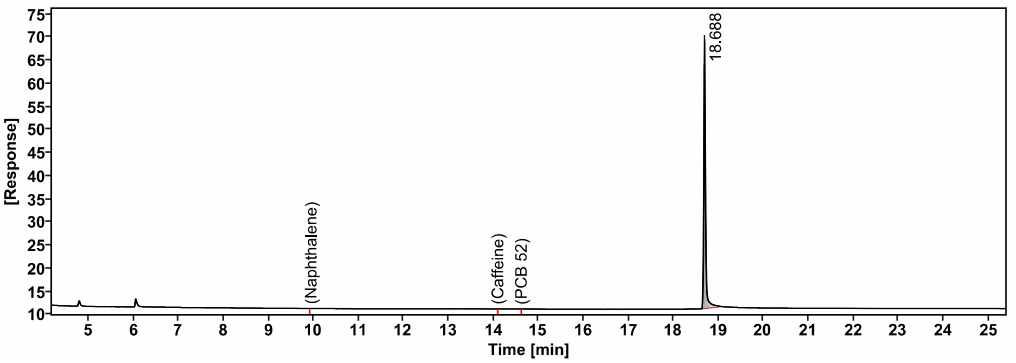
Prøvenavn: CRM/RM-101

Analysemetode: Standard.amx **Sist endret:** 01/Mar/21
Beregningsmetode: *Standard_intermediate_precision.pmx **Sist endret:** 24/Mar/21

Annen Prøveinformasjon: IPA 1000 ug/mL in ACN (SJP4/152)

Multiplier 1
Dilution 1

GC FID resultater



Komponent	RT (min)	Areal	S/N	Areal%	Mengde
Impurity			0.0	0.00	0.00
AI	18.69	159.57	4199.9	100.00	765.25
			Sum:	100.00	765.25

H.3 Linearity

Analyserapport Chiron

Tidspunkt for analyse: 09/Mar/2021 16:53 **Lims ID:**
Analyse utført av: SYSTEM

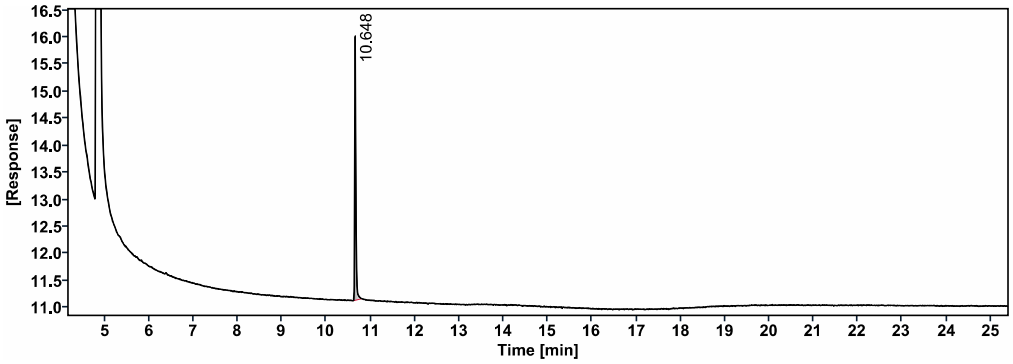
Filnavn: D:\CDSProjects\8890\Results\2021\Validation\Linearity.rslt\2021-03-09 16-48-01+01-00-12.dx

Prøvenavn: G

Analysemetode: Standard.amx **Sist endret:** 01/Mar/21
Beregningsmetode: *Standard_linearity.pmx **Sist endret:** 15/Mar/21

Annen Prøveinformasjon: SPJ8/046 **Multiplier** 1
Dilution 1

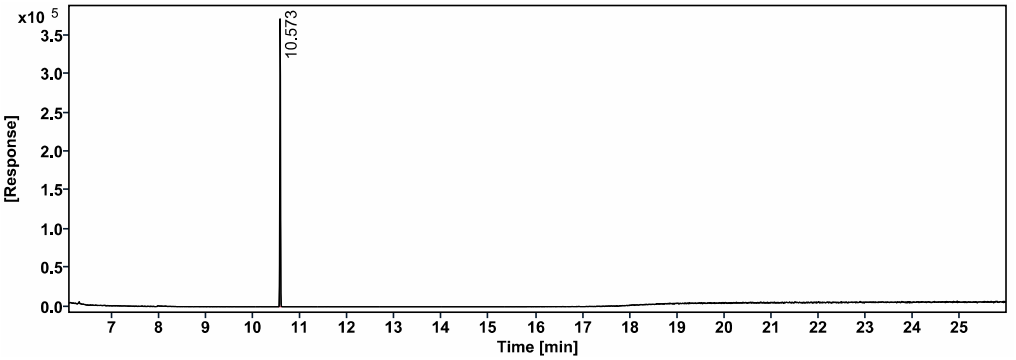
GC FID resultater



Komponent	RT (min)	Areal	S/N	Areal%	Høyde
AI	10.65	9.07	483.0	100.00	4.89
			Sum:	100.00	

Analyserapport Chiron

GC-MS resultater



Komponent	RT (min)	Areal	S/N	Areal%	Høyde
AI	10.57	302244.24	3657.0	100.00	380121.27
			Sum:	100.00	

H.4 Robustness

Analyserapport Chiron

Tidspunkt for analyse: 16/Mar/2021 16:31 **Lims ID:**
Analyse utført av: SYSTEM

Filnavn: D:\CDSProjects\8890\Results\2021\Validation\Robustness.rslt\2021-03-16 16-24-45+01-00-07-r001.dx

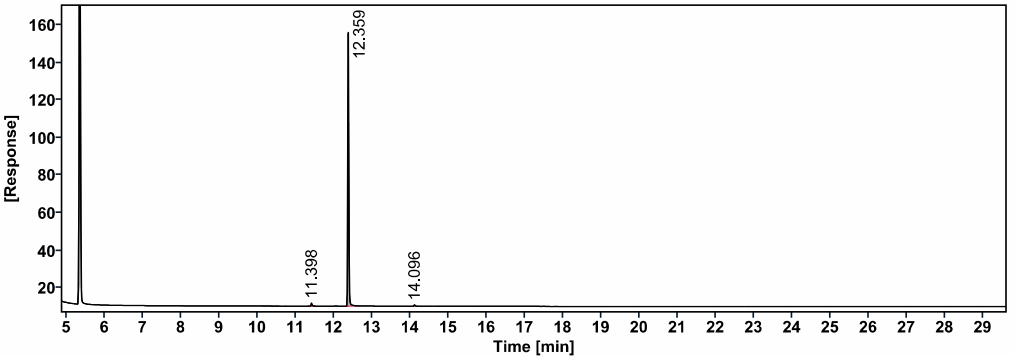
Prøvenavn: D1

Analysemetode: Robustness_gradient_1.amx **Sist endret:** 16/Mar/21
Beregningsmetode: *Standard_robustness.pmx **Sist endret:** 24/Mar/21

Annen Prøveinformasjon: SPJ8/034 (AI 99%)
Gradient temp. extra meth. 1

Multiplier 1
Dilution 1

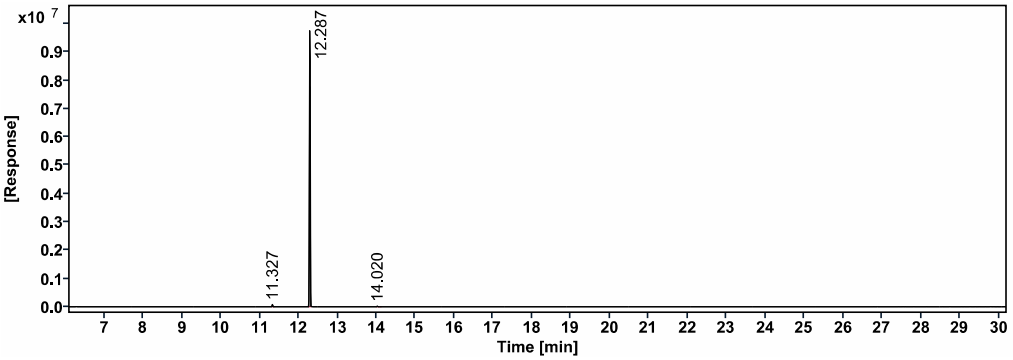
GC FID resultater



Komponent	RT (min)	Areal	S/N	Areal%	Mengde
Impurity	11.40	3.19	0.0	1.16	0.00
AI	12.36	271.48	0.0	98.29	0.00
Impurity	14.10	1.54	0.0	0.56	0.00
			Sum:	100.00	0.00

Analyserapport Chiron

GC-MS resultater



Komponent	RT (min)	Areal	S/N	Areal%	Mengde
Impurity	11.33	88240.14	0.0	0.59	0.00
AI	12.29	14784499.22	0.0	99.23	0.00
Impurity	14.02	26512.76	0.0	0.18	0.00
			Sum:	100.00	0.00

I Safety Data Sheets

Supelco®

www.sigmaaldrich.com

SAFETY DATA SHEET
according to Regulation (EC) No. 1907/2006

Version 6.5
Revision Date 26.03.2021
Print Date 21.04.2021
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Benzo[a]pyrene solution

Product Number : CRM40071
Brand : Supelco
REACH No. : Not applicable

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science AS
Drammensveien 123, 5th floor,
N-0277 OSLO

Telephone : +47 23 1760-70
Fax : +47 23 1760-10
E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(47)-22591300 (Giftinformasjonen)
+(47)-21930678 (CHEMTREC)
Brann og større ulykker 110
Ambulanse medisinsk nødtelefon - 113

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 2), H225
Eye irritation (Category 2), H319
Germ cell mutagenicity (Category 1B), H340
Carcinogenicity (Category 1B), H350
Specific target organ toxicity - single exposure (Category 3), Central nervous system, H336
Long-term (chronic) aquatic hazard (Category 3), H412

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

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Signal word	Danger

Hazard statement(s)

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H336	May cause drowsiness or dizziness.
H340	May cause genetic defects.
H350	May cause cancer.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P273	Avoid release to the environment.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

Supplemental Hazard information (EU)

EUH066	Repeated exposure may cause skin dryness or cracking.

Restricted to professional users.
Contains: benzo[a]pyrene. May produce an allergic reaction.

Reduced Labeling (<= 125 ml)

Pictogram

Signal word	Danger

Hazard statement(s)

H340	May cause genetic defects.
H350	May cause cancer.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

Supplemental Hazard information (EU)

EUH066	Repeated exposure may cause skin dryness or cracking.

2.3 Other hazards

This substance/mixture contains components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB).

SECTION 3: Composition/information on ingredients

3.2 Mixtures

Molecular weight : 252,31 g/mol

Component	Classification	Concentration

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acetone			
CAS-No. EC-No. Index-No. Registration number	67-64-1 200-662-2 606-001-00-8 01-2119471330-49-XXXX	Flam. Liq. 2; Eye Irrit. 2; STOT SE 3; H225, H319, H336 Concentration limits: >= 20 %: STOT SE 3, H336;	>= 90 - <= 100 %
benzo[a]pyrene Included in the Candidate List of Substances of Very High Concern (SVHC) according to Regulation (EC) No. 1907/2006 (REACH)			
CAS-No. EC-No. Index-No.	50-32-8 200-028-5 601-032-00-3 *	Skin Sens. 1; Muta. 1B; Carc. 1B; Repr. 1B; Aquatic Acute 1; Aquatic Chronic 1; H317, H340, H350, H360FD, H400, H410 Concentration limits: >= 0,01 %: Carc. 1B, H350; M-Factor - Aquatic Acute: 10	>= 0,1 - < 0,25 %

*A registration number is not available for this substance as the substance or its use are exempted from registration according to Article 2 REACH Regulation (EC) No 1907/2006, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

For the full text of the H-Statements mentioned in this Section, see Section 16.

SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air. Call in physician.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. Consult a physician.

In case of eye contact
After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed
After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

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SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides
Combustible.
Pay attention to flashback.
Vapors are heavier than air and may spread along floors.
Development of hazardous combustion gases or vapours possible in the event of fire.
Forms explosive mixtures with air at ambient temperatures.

5.3 Advice for firefighters

Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information

Remove container from danger zone and cool with water. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Keep away from heat and sources of ignition.
Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains. Risk of explosion.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up carefully with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling
Work under hood. Do not inhale substance/mixture. Avoid generation of vapours/aerosols.

Advice on protection against fire and explosion
Keep away from open flames, hot surfaces and sources of ignition.Take precautionary measures against static discharge.

Hygiene measures

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Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Keep container tightly closed in a dry and well-ventilated place. Keep away from heat and sources of ignition. Keep locked up or in an area accessible only to qualified or authorized persons.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
required

Body Protection
Flame retardant antistatic protective clothing.

Respiratory protection
required when vapours/aerosols are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.
Recommended Filter type: Filter type ABEK

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
Do not let product enter drains. Risk of explosion.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid
b)	Odor	No data available
c)	Odor Threshold	No data available
d)	pH	No data available

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e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	-17,0 °C - closed cup
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	No data available
l)	Vapor density	No data available
m)	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Autoignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
Vapors may form explosive mixture with air.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Warming.

10.5 Incompatible materials
Bases, Oxidizing agents, Reducing agents, Acetone reacts violently with phosphorous oxychloride.

10.6 Hazardous decomposition products
In the event of fire: see section 5

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SECTION 11: Toxicological information

11.1 Information on toxicological effects

Mixture

Acute toxicity
No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
No data available

Germ cell mutagenicity
No data available

Carcinogenicity
No data available

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available**Specific target organ toxicity - repeated exposure**
No data available

Aspiration hazard
No data available

11.2 Additional Information

Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Components

acetone

Acute toxicity
LD50 Oral - Rat - female - 5.800 mg/kg
Remarks:
(ECHA)
LC50 Inhalation - Rat - 4 h - 76 mg/l
Remarks:
Unconsciousness
Drowsiness

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Dizziness
(External MSDS)
LD50 Dermal - Rabbit - 20.000 mg/kg
Remarks:
(IUCLID)

Skin corrosion/irritation
Skin - Rabbit
Result: Mild skin irritation - 24 h
(Draize Test)
Remarks:
(RTECS)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Eye irritation - 24 h
(Draize Test)
Remarks:
(RTECS)

Respiratory or skin sensitization
Maximization Test - Guinea pig
Result: Not a skin sensitizer.
Remarks:
(ECHA)
Chronic exposure may cause dermatitis.

Germ cell mutagenicity

Mutagenicity (mammal cell test): chromosome aberration.
Chinese hamster ovary cells
Result: negative
Ames test
Salmonella typhimurium
Result: negative
In vitro mammalian cell gene mutation test
Mouse lymphoma test
Result: negative

Carcinogenicity

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
Inhalation - May cause drowsiness or dizziness. - Narcotic effects

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

benzo[a]pyrene

Acute toxicity
No data available

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Skin corrosion/irritation
Skin - Mouse
Result: Mild skin irritation
Remarks:
(RTECS)

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitization
May cause allergic skin reaction. Classified according to Regulation (EU) 1272/2008, Annex VI (Table 3.1/3.2)

Germ cell mutagenicity
May cause genetic defects.
Ames test
Salmonella typhimurium
Result: positive
Remarks:
(Lit.)
Mutagenicity (mammal cell test): chromosome aberration.
Chinese hamster ovary cells
Result: positive
Remarks:
(National Toxicology Program)
sister chromatid exchange assay
Chinese hamster ovary cells
Result: positive
Remarks:
(National Toxicology Program)

Mouse - male - Bone marrow
Result: positive
Remarks:
(National Toxicology Program)

Carcinogenicity
Presumed to have carcinogenic potential for humans

Reproductive toxicity
May damage the unborn child.
May damage fertility.

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

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SECTION 12: Ecological information

12.1 Toxicity

Mixture
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB).

12.6 Other adverse effects
No data available

Components

acetone

Toxicity to fish	flow-through test LC50 - Pimephales promelas (fathead minnow) - 6.210 mg/l - 96 h (OECD Test Guideline 203)
Toxicity to daphnia and other aquatic invertebrates	static test LC50 - Daphnia pulex (Water flea) - 8.800 mg/l - 48 h Remarks: (ECHA)
Toxicity to algae	static test NOEC - M.aeruginosa - 530 mg/l - 8 d (DIN 38412) Remarks: (maximum permissible toxic concentration) (IUCLID)
Toxicity to bacteria	static test EC50 - activated sludge - 61,15 mg/l - 30 min (OECD Test Guideline 209)

benzo[a]pyrene
No data available

Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia magna (Water flea) - 0,25 mg/l - 48 h Remarks: (above the solubility limit in the test medium) (ECOTOX Database)
Toxicity to algae	static test ErC50 - Scenedesmus acutus - 0,005 mg/l - 72 h Remarks: (ECOTOX Database)

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SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number
ADR/RID: 1090 IMDG: 1090 IATA: 1090

14.2 UN proper shipping name
ADR/RID: ACETONE, SOLUTION
IMDG: ACETONE, SOLUTION
IATA: Acetone, SOLUTION

14.3 Transport hazard class(es)
ADR/RID: 3 IMDG: 3 IATA: 3

14.4 Packaging group
ADR/RID: II IMDG: II IATA: II

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user
No data available

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Authorisations and/or restrictions on use

REACH - Restrictions on the manufacture, placing on the market and use of certain dangerous substances, preparations and articles (Annex XVII) :

National legislation
Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances. : FLAMMABLE LIQUIDS

Other regulations
Observe work restrictions regarding maternity protection in accordance to Dir 92/85/EEC or stricter national regulations where applicable.

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Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment
For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

EUH066	Repeated exposure may cause skin dryness or cracking.
H225	Highly flammable liquid and vapor.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H336	May cause drowsiness or dizziness.
H340	May cause genetic defects.
H350	May cause cancer.
H360FD	May damage fertility. May damage the unborn child.
H400	Very toxic to aquatic life.
H410	Very toxic to aquatic life with long lasting effects.
H412	Harmful to aquatic life with long lasting effects.

Further information
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Naphthalene solution

Product Number : CRM48641
Brand : Supelco
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 91-20-3

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science AS
Drammensveien 123, 5th floor,
N-0277 OSLO

Telephone : +47 23 1760-70
Fax : +47 23 1760-10
E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone number

Emergency Phone # : +(47)-22591300 (Giftinformasjonen)
+(47)-21930678 (CHEMTREC)
Brann og større ulykker 110
Ambulanse medisinsk nødtelefon - 113

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Flammable liquids (Category 2), H225
Acute toxicity, Oral (Category 3), H301
Acute toxicity, Inhalation (Category 3), H331
Acute toxicity, Dermal (Category 3), H311
Specific target organ toxicity - single exposure (Category 1), H370
Long-term (chronic) aquatic hazard (Category 3), H412

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

The life science business of Merck operates as MilliporeSigma in the US and Canada

Pictogram	
Signal word	Danger
Hazard statement(s)	
H225	Highly flammable liquid and vapour.
H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled.
H370	Causes damage to organs.
H412	Harmful to aquatic life with long lasting effects.
Precautionary statement(s)	
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing.
P302 + P352 + P312	IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/doctor if you feel unwell.
P304 + P340 + P311	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor.
P370 + P378	In case of fire: Use dry powder or dry sand to extinguish.
P403 + P235	Store in a well-ventilated place. Keep cool.
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.2 Mixtures

Component		Classification	Concentration
Methanol			
CAS-No. EC-No. Index-No. Registration number	67-56-1 200-659-6 603-001-00-X 01-2119433307-44-XXXX	Flam. Liq. 2; Acute Tox. 3; STOT SE 1; H225, H301, H331, H311, H370 Concentration limits: >= 10 %: STOT SE 1, H370; 3 - < 10 %: STOT SE 2, H371;	>= 90 - <= 100 %
Naphthalene			
CAS-No. EC-No. Index-No.	91-20-3 202-049-5 601-052-00-2	Flam. Sol. 2; Acute Tox. 4; Carc. 2; Aquatic Acute 1; Aquatic Chronic 1; H228, H302, H351, H400, H410	>= 0,025 - < 0,1 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

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SECTION 4: First aid measures

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Carbon oxides

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
Use water spray to cool unopened containers.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

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6.3 Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Store at room temperature.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Components with workplace control parameters

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Body Protection
Complete suit protecting against chemicals, Flame retardant antistatic protective clothing., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

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Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid
b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	No data available
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	9,7 °C - closed cup
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	No data available
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

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SECTION 10: Stability and reactivity

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Heat, flames and sparks.

10.5 Incompatible materials
No data available

10.6 Hazardous decomposition products
Other decomposition products - No data available
Hazardous decomposition products formed under fire conditions. - Carbon oxides
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: Not available

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SECTION 12: Ecological information

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects
Harmful to aquatic life with long lasting effects.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

SECTION 14: Transport information

14.1 UN number

ADR/RID: 1230	IMDG: 1230	IATA: 1230

14.2 UN proper shipping name

ADR/RID: METHANOL, SOLUTION
IMDG: METHANOL, SOLUTION
IATA: Methanol, SOLUTION

14.3 Transport hazard class(es)

ADR/RID: 3 (6.1)	IMDG: 3 (6.1)	IATA: 3 (6.1)

14.4 Packaging group

ADR/RID: II	IMDG: II	IATA: II

14.5 Environmental hazards

ADR/RID: no	IMDG Marine pollutant: no	IATA: no

14.6 Special precautions for user
No data available

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SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.2 Chemical safety assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H225	Highly flammable liquid and vapour.
H228	Flammable solid.
H301	Toxic if swallowed.
H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled.
H302	Harmful if swallowed.
H311	Toxic in contact with skin.
H331	Toxic if inhaled.
H351	Suspected of causing cancer.
H370	Causes damage to organs.
H371	May cause damage to organs.
H400	Very toxic to aquatic life.
H410	Very toxic to aquatic life with long lasting effects.
H412	Harmful to aquatic life with long lasting effects.

Further information

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 8.2
Revision Date 24.02.2021
Print Date 21.04.2021
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Caffeine

Product Number : PHR1009
Brand : Sigma-Aldrich
Index-No. : 613-086-00-5
REACH No. : 01-2119433305-48-XXXX
CAS-No. : 58-08-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science AS
Drammensveien 123, 5th floor,
N-0277 OSLO

Telephone : +47 23 1760-70
Fax : +47 23 1760-10
E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +(47)-22591300 (Giftinformasjonen)
+(47)-21930678 (CHEMTREC)
Brann og større ulykker 110
Ambulanse medisinsk nødtelefon - 113

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008
Acute toxicity, Oral (Category 4), H302

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008
Pictogram

Signal word Warning

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Hazard statement(s)	
H302	Harmful if swallowed.
Precautionary statement(s)	
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P301 + P312	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.
P501	Dispose of contents/ container to an approved waste disposal plant.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram	
Signal word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Synonyms	:	Caffeine free base
Formula	:	C8H10N4O2
Molecular weight	:	194,19 g/mol
CAS-No.	:	58-08-2
EC-No.	:	200-362-1
Index-No.	:	613-086-00-5

Component		Classification	Concentration
Caffeine			
CAS-No. EC-No. Index-No.	58-08-2 200-362-1 613-086-00-5	Acute Tox. 4; H302	<= 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

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SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice
Show this material safety data sheet to the doctor in attendance.

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed
After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Water Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Carbon oxides
Nitrogen oxides (NOx)
Combustible.
Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
In the event of fire, wear self-contained breathing apparatus.

5.4 Further information
Suppress (knock down) gases/vapors/mists with a water spray jet. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

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6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed. Dry.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Full contact
Material: Nitrile rubber
Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).
Splash contact
Material: Nitrile rubber

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Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

Body Protection
protective clothing

Respiratory protection
required when dusts are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.
Recommended Filter type: Filter type P2

The entrepeneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure
Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: Crystalline powder Color: colorless
b)	Odor	odorless
c)	Odor Threshold	Not applicable
d)	pH	No data available
e)	Melting point/freezing point	No data available
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	No data available
l)	Vapor density	No data available
m)	Relative density	1,23 at 18 °C
n)	Water solubility	No data available
o)	Partition coefficient: n-octanol/water	No data available
p)	Autoignition temperature	No data available

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q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

SECTION 10: Stability and reactivity

10.1 Reactivity
The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability
The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
Violent reactions possible with:
Strong oxidizing agents

10.4 Conditions to avoid
no information available

10.5 Incompatible materials
no information available

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - male and female - 367,7 mg/kg
(OECD Test Guideline 401)
Remarks:
(Regulation (EC) No 1272/2008, Annex VI)
LC50 Inhalation - Rat - male and female - 4 h - 4,94 mg/l
(OECD Test Guideline 403)
LD50 Dermal - Rat - male and female - > 2.000 mg/kg
(OECD Test Guideline 402)

Skin corrosion/irritation
Skin - Rabbit
Result: No skin irritation - 4 h
(OECD Test Guideline 404)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: No eye irritation

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(OECD Test Guideline 405)

Respiratory or skin sensitization
Local lymph node assay (LLNA) - Mouse
Result: negative
(OECD Test Guideline 429)

Germ cell mutagenicity
In vitro mammalian cell gene mutation test
mouse lymphoma cells
Result: negative
Chromosome aberration test in vitro
Human lymphocytes
Result: negative
Ames test
Escherichia coli/Salmonella typhimurium
Result: negative
Chromosome aberration test in vitro
Chinese hamster lung cells
Result: positive

Mouse - male
Result: negative
Remarks:
(ECHA)

Human
Result: negative
Remarks:
(ECHA)

Mouse - male
Result: negative
Remarks:
(ECHA)
OECD Test Guideline 474
Mouse - male and female - Red blood cells (erythrocytes)
Result: Positive results were obtained in some in vivo tests.

Carcinogenicity

IARC: No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

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11.2 Additional Information

Repeated dose toxicity - Mouse - male and female - Oral - 90 d - NOAEL (No observed adverse effect level) - 167,4 - 179,4 mg/kgRemarks:
(ECHA)

Not available

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

After absorption of toxic quantities:

Diarrhea
Vomiting
agitation
Headache

Systemic effects:

drop in blood pressure
tachycardia

Handle in accordance with good industrial hygiene and safety practice.

Liver - Irregularities - Based on Human Evidence

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Leuciscus idus (Golden orfe) - ca. 87 mg/l - 96 h (DIN 38412 part 15)
	static test NOEC - Leuciscus idus (Golden orfe) - 46 mg/l - 96 h (DIN 38412 part 15)
Toxicity to daphnia and other aquatic invertebrates	static test EC50 - Daphnia magna (Water flea) - 182 mg/l - 48 h (DIN 38412)
Toxicity to algae	static test ErC50 - Desmodesmus subspicatus (green algae) - > 100 mg/l - 72 h (OECD Test Guideline 201)
	static test NOEC - Desmodesmus subspicatus (green algae) - 6,25 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to bacteria	EC50 - activated sludge - > 1.000 mg/l - 3 h (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 22 d Result: 90 - 100 % - Readily biodegradable.

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(OECD Test Guideline 301A)

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Other adverse effects
Discharge into the environment must be avoided.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product
See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number
ADR/RID: - IMDG: - IATA: -

14.2 UN proper shipping name
ADR/RID: Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

14.3 Transport hazard class(es)
ADR/RID: - IMDG: - IATA: -

14.4 Packaging group
ADR/RID: - IMDG: - IATA: -

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA: no

14.6 Special precautions for user

Further information
Not classified as dangerous in the meaning of transport regulations.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

REACH - Restrictions on the manufacture, : Not applicable

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placing on the market and use of certain dangerous substances, preparations and articles (Annex XVII)

National legislation

Seveso III: Directive 2012/18/EU of the European Parliament and of the Council on the control of major-accident hazards involving dangerous substances. : Not applicable

: Not applicable

Other regulations

Observe work restrictions regarding maternity protection in accordance to Dir 92/85/EEC or stricter national regulations where applicable.

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

For this product a chemical safety assessment was not carried out

SECTION 16: Other information

Full text of H-Statements referred to under sections 2 and 3.

H302 Harmful if swallowed.

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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Safety Data Sheet

Prepared in accordance with Commission Regulation (EU) 2015/830

Stock Number: 33257
Revision Date: 18-09-2019
Supercedes: None
2 Letter ISO country code/language code: NO/NN

PCB 52 Stock Solution

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier: **PCB 52 Stock Solution**
Stock Number: 33257

Other means of identification:
Synonyms: None Known
REACH Registration No.: None Known
Molecular formula: C2H3N

1.2 Relevant identified uses of the substance or mixture and uses advised against:
Relevant identified uses: For Laboratory use only
Uses advised against: Uses other than recommended use.

1.3 Details of the Supplier of the Safety Data Sheet:

Manufacturer	**Supplier**
Restek Corporation	Restek GmbH
110 Benner Circle	Schaberweg 23
Bellefonte, Pa. 16823	Bad Homburg
USA	Germany 61348
00 1 814-353-1300	0049-(0)6172 2797-0
00 1 814-353-1309	info@restekgmbh.de
sds@restek.com	

1.4 Emergency telephone number:
00 1 800-424-9300 (CHEMTREC within the US)
+1 703-741-5970 (CHEMTREC International)
00 1 703-741-5970 (Outside USA)

Poison Centre contact information:
National Poisons Information Service (NPIS)
Email: director.birmingham.unit@npis.org
Website: http://www.npis.org/

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture:

Classification according to Regulation (EC) No 1272/2008 [CLP]:
Flammable Liquid Category 2
Serious Eye Damage/Eye Irritation Category 2

Acute Toxicity - Dermal Category 4
Acute Toxicity - Oral Category 4

2.2 Label elements:

Labelling according to Regulation (EC) No 1272/2008 [CLP]:

Hazard pictograms:

Signal Word:	Danger
Hazard Statements:	H225 - Highly flammable liquid and vapour H302+H312 - Harmful if swallowed or in contact with skin H319 - Causes serious eye irritation
Precautionary Statements:	P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 - Keep container tightly closed. P280 - Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P370+P378 - In case of fire: Use an appropriate extinguisher (see section 5) to extinguish.
Supplemental Hazard information (EU):	None Known
2.3 Other hazards:	This substance does not meet the PBT or vPvB criteria of REACH, Annex XIII

SECTION 3: Composition/information on ingredients

3.1 Substances:
Not applicable

3.2 Mixtures:

Chemical Name	%	CAS No.	EC No. REACH Registration No.	Classification in accordance with (EC) No 1272/2008	M Factor	SCL	Acute Toxicity Estimates
acetonitrile	99.995	75-05-8	200-835-2 None Known	Acute Tox. 4 (Dermal); H312 Acute Tox. 4 (Inh Dust/Mist); H332 Acute Tox. 4 (Oral); H302 Eye Irrit. 2; H319 Flam. Liq. 2; H225	No data available	No data available	Not determined

For full text of H-statements see Section 16.

SECTION 4: First aid measures

4.1 Description of first aid measures:

Inhalation: Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen.

Eye contact: Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin Contact: Wash with soap and water. Remove contaminated clothing, launder immediately, and discard contaminated leather goods. Get medical attention immediately.

Ingestion: Do not induce vomiting and seek medical attention immediately. Drink two glasses of water or milk to dilute. Provide medical care provider with this SDS.

Self protection of the first aider: No data available

4.2 Most important symptoms and effects, both acute and delayed: Harmful if swallowed or in contact with skin Causes serious eye irritation

4.3 Indication of any immediate medical attention and special treatment needed: None Known

SECTION 5: Firefighting measures

5.1 Extinguishing media:

Suitable extinguishing media:	Use alcohol resistant foam, carbon dioxide, or dry chemical extinguishing agents. Water may be ineffective but water spray can be used extinguish a fire if swept across the base of the flames. Water can absorb heat and keep exposed material from being damaged by fire.
Unsuitable extinguishing media:	None Known
5.2 Special hazards arising from the substance or mixture:	Vapors may be ignited by heat, sparks, flames or other sources of ignition at or above the low flash point giving rise to a fire (Class B). Vapors are heavier than air and may travel to a source of ignition and flash back.
Hazardous Combustion Products:	Carbon dioxide, Carbon monoxide
5.3 Advice for firefighters:	Do not enter fire area without proper protection including self- contained breathing apparatus and full protective equipment. Use methods for the surrounding fire.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures:

Non-emergency personnel:	Non-emergency personnel should be kept clear of the area
Emergency responders:	Exposure to the spilled material may be severely irritating or highly toxic. Follow personal protective equipment recommendations found in Section 8 of this SDS. Personal protective equipment needs must be evaluated based on information provided on this sheet and the special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred, and the expertise of employees in the area responding to the spill. Never exceed any occupational exposure limits.
6.2 Environmental precautions:	No data available

6.3 Methods and material for containment and cleaning up:

Small spills:	Refer to information provided for large spills
Large spills:	Prevent the spread of any spill to minimize harm to human health and the environment if safe to do so. Wear complete and proper personal protective equipment following the recommendation of Section 8 at a minimum. Dike with suitable absorbent material like granulated clay. Gather and store in a sealed container pending a waste disposal evaluation.
6.4 Reference to other sections:	Refer to section 13 for disposal information

SECTION 7: Handling and storage

7.1 Precautions for safe handling:	Highly toxic or corrosive material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area. Use spark-proof tools and

explosion-proof equipment

7.2 Conditions for safe storage, including any incompatibilities:

Conditions for safe storage: Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed. Keep away from sources of ignition

Materials to Avoid/Chemical Incompatibility: Strong oxidizing agents

7.3 Specific end use(s): For Laboratory use only

SECTION 8: Exposure controls/personal protection

8.1 Control parameters:

Occupational Exposure limit values:

Chemical Name	Norway - Occupational Exposure Limits - TWAs	Norway - Occupational Exposure Limits - STELs	Norway - Occupational Exposure Limits - Ceilings
acetonitrile	30 ppm TWA; 50 mg/m3 TWA	45 ppm STEL (value calculated); 75 mg/m3 STEL (value calculated)	No data available

DNEL: None Known

PNEC: None Known

8.2 Exposure controls:

Appropriate engineering controls: Local exhaust ventilation is recommended when generating excessive levels of vapours from handling or thermal processing.

Individual protection measures, such as personal protective equipment:

Eye and face protection: Wear chemically resistant safety glasses with side shields when handling this product. Do not wear contact lenses.

Skin Protection:

Hand protection: No information available

Other skin protection: Wear protective gloves. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work

Respiratory Protection: Respiratory protection may be required to avoid overexposure when handling this product. General or local exhaust ventilation is the preferred means of protection. Use a respirator if general room ventilation is not available or sufficient to eliminate symptoms.

Respirator Type(s):	None required where adequate ventilation is provided. If airborne concentrations are above the applicable exposure limits, use NIOSH/MSHA approved respiratory protection.
Thermal Hazards:	Not applicable
Environmental exposure controls:	No data available

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties:

Appearance:	No data available
Colour:	No data available
Odour:	Mild
Odour threshold:	No data available
pH:	Not applicable
Melting point / freezing point (°C):	
Melting point (°C):	No data available
Freezing point (°C):	No data available
Initial boiling point and boiling range (°C):	81
Flash point (°C):	6
Evaporation rate:	No data available
Flammability (solid, gas):	No data available
Upper/lower flammability or explosive limits:	
Upper flammable or explosive limit, % in air:	16
Lower flammable or explosive limit, % in air:	4.4
Vapour pressure:	No data available
Vapour density:	1.4
Relative density:	0.7857 g/cm3 at 20 °C
Solubility(ies):	Not determined
Partition coefficient: n-octanol/water:	No data available
Auto-ignition temperature (°C):	No data available
Decomposition temperature (°C):	No data available
Viscosity:	No data available

Explosive properties:	No data available
Oxidizing properties:	No data available

9.2 Other information:

Volatile Organic Chemicals:	100
Bulk Density:	6.559

SECTION 10: Stability and reactivity

10.1 Reactivity:	Not expected to be reactive
10.2 Chemical stability:	Stable under normal conditions.
10.3 Possibility of hazardous reactions:	None expected under standard conditions of storage
10.4 Conditions to avoid:	No data available
10.5 Incompatible materials:	Strong oxidizing agents
10.6 Hazardous decomposition products:	No data available

SECTION 11: Toxicological information

11.1 Information on toxicological effects:

Acute toxicity:

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
acetonitrile	No data available	DERMAL LD50 Rabbit 390 mg/kg	No data available

Classification has been based on toxicological information of the components in Section 3.

Skin corrosion/irritation:
Based on available data, the classification criteria are not met.

Serious eye damage/irritation:

pH	Not applicable

Classification is based on pH and the components listed in Section 3.

Respiratory or skin sensitisation:
Based on available data, the classification criteria are not met.

Germ cell mutagenicity:
Based on available data, the classification criteria are not met.

Carcinogenicity:
Based on available data, the classification criteria are not met.

Reproductive toxicity:
Based on available data, the classification criteria are not met.

STOT-single exposure:
Based on available data, the classification criteria are not met.

STOT-repeated exposure:
Based on available data, the classification criteria are not met.

Aspiration hazard:
Based on available data, the classification criteria are not met.

SECTION 12: Ecological information

12.1 Toxicity: Moderate ecological hazard. This product may be dangerous to plants and/or wildlife.

Ecological Toxicity Data:

Chemical Name	CAS No.	Aquatic EC50 Crustacea	Aquatic ERC50 Algae	Aquatic LC50 Fish
No data available				

12.2 Persistence and degradability: No data
12.3 Bioaccumulative potential: No data available
12.4 Mobility in soil: No data available
12.5 Results of PBT and vPvB assessment: No data available
12.6 Other adverse effects: None Known
12.7 Additional information: No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods:

Disposal methods: Spent or discarded material is a hazardous waste.
Dispose of by incineration following Federal, State, Local, or Provincial

regulations.

Waste codes / waste designations according to LoW: No data available

SECTION 14: Transport information

International carriage of dangerous goods by road (ADR), rail or inland waterways:

14.1. UN number: UN1648

14.2. UN proper shipping name: Acetonitrile

14.3. Transport hazard class(es): 3

14.4. Packing group: II

International carriage of dangerous goods by air (IATA):

14.1. UN number: UN1648

14.2. UN proper shipping name: Acetonitrile

14.3. Transport hazard class(es): 3

14.4. Packing group: II

14.5. Environmental hazards: No

14.6. Special precautions for user: No data available

14.7 Transport in bulk according to Annex II of MARPOL and the IBC Code: No data available

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture:

Chemical Name	EINECS	SVHC
acetonitrile	Yes	No

15.2 Chemical Safety Assessment No Chemical Safety Assessment has been carried out for this substance/mixture by the supplier.

SECTION 16: Other information

Revision Date: 18-09-2019

Indication of changes:	Any changes to the SDS compared to previous versions are marked by a vertical line in front of the concerned paragraph.
Abbreviations and acronyms:	CAS = Chemical Abstract Service DNEL= Derivative No Effect Level EC= European Community EINECS = European Inventory of Existing Chemical Substances MSHA = Mine Safety Health Administration NIOSH = National Institute of Occupational Safety & Health OEL = Occupational Exposure Limit PBT= Persistent, Bioaccumulative, Toxic PNEC= Predicted No Effect Concentration SCOEL= Scientific Committee on Occupational Exposure Limits TLV = Threshold Limit Value TWA= Time Weighted Average vPvB= Very Persistent, Very Bioaccumulative Wt.% = Weight Percent
Key literature references and sources for data:	No data available
Hazard phrase(s) referenced in section 3	H225 - Highly flammable liquid and vapour H302+H312+H332 - Harmful if swallowed, in contact with skin or if inhaled H319 - Causes serious eye irritation
Precautionary Statements:	
Prevention:	P210 - Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 - Keep container tightly closed. P240 - Ground/bond container and receiving equipment. P241 - Use explosion-proof electrical/ventilating/lighting equipment. P242 - Use only non-sparking tools. P243 - Take precautionary measures against static discharge. P264 - Wash thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P280 - Wear protective gloves/protective clothing/eye protection/face protection.
Response:	P301+P312 - IF SWALLOWED: Call a POISON CENTER/doctor/ if you feel unwell. P302+P352 - If on skin: Wash with plenty of water.

	P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
	P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P321 - Specific treatment (see Sections 4 to 8 on this SDS and any additional information on this label).
	P330 - Rinse mouth.
	P337+P313 - If eye irritation persists: Get medical advice/attention.
	P362+P364 - Take off contaminated clothing and wash it before reuse.
	P370+P378 - In case of fire: Use an appropriate extinguisher (see section 5) to extinguish.
Storage:	P233 - Keep container tightly closed.
	P403+P235 - Store in a well-ventilated place. Keep cool.
Disposal:	P501 - Dispose of contents/container to a suitable disposal site in accordance with local/national/international regulations.

Disclaimer:

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SAFETY DATA SHEET

1-Fluorododecane, Neat

The safety data sheet is in accordance with Commission Regulation (EU) 2015/830 of 28 May 2015 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

SECTION 1: Identification of the substance / mixture and of the company / undertaking

Date issued	06.05.2021

1.1. Product identifier

Product name	1-Fluorododecane, Neat
Article no.	C8239.12

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use categories nordic (UCN).	No information available.
Use of the substance / preparation	Reference Material Laboratory chemicals
Use of chemical, comments	This product is to be used for research and analytical purposes only. The product is in no way meant for, approved for or suitable for medical use, but purely analytical!

1.3. Details of the supplier of the safety data sheet

Producer

Company name	Chiron AS
Office address	Stiklestadveien 1
Postal address	Stiklestadveien 1
Postcode	N-7041
City	TRONDHEIM
Country	Norway
Telephone number	+47 73874490
Email	HMS@chiron.no
Website	www.chiron.no
Enterprise No.	924 361 360
Contact person	Jon E. Johansen

1.4. Emergency telephone number

Emergency telephone	Telephone number: 22 59 13 00 Description: Poison Control Center: Døgnåpen Telephone number: 110 Description: Fire : Telephone number: 113 Description: Medical Aid: Telephone number: 112 Description: Police: Description: Emergency numbers in Norway: Description: If not in Norway contact you local emergency numbers.

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP / GHS]	Acute Tox. 2; H300 Aquatic Chronic 4; H413

2.2. Label elements

Hazard pictograms (CLP)

Composition on the label	1-Fluorododecane ~ 100 %
Signal word	Danger
Hazard statements	H300 Fatal if swallowed. H413 May cause long lasting harmful effects to aquatic life.
Precautionary statements	P261 Avoid breathing dust / fume / gas / mist / vapours / spray. P262 Do not get in eyes, on skin, or on clothing. P264 Wash thoroughly after handling. P273 Avoid release to the environment. P280 Wear protective gloves / protective clothing / eye protection / face protection. P285 In case of inadequate ventilation wear respiratory protection. P273 Avoid release to the environment. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor / physician. P330 Rinse mouth. P405 Store locked up.
EC label	No

2.3. Other hazards

PBT / vPvB	No information available.
Other hazards	See also section 5, 11 and 12.

SECTION 3: Composition / information on ingredients

3.2. Mixtures

Substance	Identification	Classification	Contents	Notes
1-Fluorododecane	CAS No.: 334-68-9 EC No.: 206-381-1	Acute Tox. 2; H300 Aquatic Chronic 4; H413	~ 100 %	

Substance comments	The safety and toxicological aspects are not fully investigated for some reference materials, and specific data are therefore not available.

SECTION 4: First aid measures

4.1. Description of first aid measures

Inhalation	Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical advice if discomfort develops.
Skin contact	Flush skin with large amounts of water and remove contaminated clothing, watches etc. simultaneously. Get medical advice if discomfort develops.
Eye contact	Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid if irritation develops or presists.
Ingestion	Never give anything by mouth to an unconscious person. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Rinse nose, mouth and throat with water. Immediately call a POISON CENTER or doctor/physician.
Recommended personal protective equipment for first aid responders	Refer to protective measures listed in sections 7 and 8.

4.2. Most important symptoms and effects, both acute and delayed

General symptoms and effects	The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11.
Acute symptoms and effects	Dødelig ved svelging.

4.3. Indication of any immediate medical attention and special treatment needed

Other information	Treat symptomatically and supportive.

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media	Use extinguishing media most appropriate for the surrounding fire.

5.2. Special hazards arising from the substance or mixture

Fire and explosion hazards	No information available.

5.3. Advice for firefighters

Personal protective equipment	Use a self-containing breathing apparatus in pressure-demand and full protective

	gear. Refer to protective measures listed in sections 7 and 8.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Personal protection measures	Evacuate personnel to safe area. Wear a self-contained breathing apparatus when needed and appropriate personal protection. Refer to protective measures listed in sections 7 and 8.
For emergency responders	No information available.

6.2. Environmental precautions

Environmental precautionary measures	Do not allow to enter sewers or watercourses: This chemical is toxic to aquatic organisms.

6.3. Methods and material for containment and cleaning up

Clean up	Absorb in an inert material (sand, vermiculite, etc.) and collect into a suitable container. Thoroughly ventilate and clean the area after the material has been removed.

6.4. Reference to other sections

Other instructions	For disposal see section 13.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Handling	Do not breathe dust, vapor, mist or gas. Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Always keep away from sources of ignition.

Protective safety measures

Safety measures to prevent fire	Keep cool. Protect from sunlight. Store in a well-ventilated place. Use only non-sparking tools. Take precautionary measures against static discharge.
Preventitive measures to prevent aerosol and dust generation	Handle and open carefully.

7.2. Conditions for safe storage, including any incompatibilities

Storage	Keep containers tightly closed in a dry, cool and well-ventilated place. Avoid heat, light & moisture. The product's accompanying certificate contains specific storage information.

Conditions for safe storage

Requirements for storage rooms and vessels	Store locked up.

7.3. Specific end use(s)

Specific use(s)	This product should be used for research and analytical purposes.

SECTION 8: Exposure controls / personal protection

8.1. Control parameters

Control parameters comments	Contains no substances with administrative standards for pollution in the working atmosphere.

8.2. Exposure controls

Safety signs

Precautionary measures to prevent exposure

Technical measures to prevent exposure	Use adequate general or local exhaust ventilation to keep airborne concentrations low. Shower and eyewash facilities should be available at the workplace. Wash hands after using the product.

Eye / face protection

Suitable eye protection	Wear approved goggles or face shield.

Hand protection

Suitable gloves type	There are no data available for permeation time for this specific product, but nitrile gloves are recommended on a general basis.

Skin protection

Suitable protective clothing	Wear appropriate protective clothing when risk of direct contact or splashes.

Respiratory protection

Recommended type of equipment	Normally not necessary in ordinary use. Respiratory protection with filter P2 is recommended when exposed to particles of harmful substances.

Appropriate environmental exposure control

Product related measures to prevent exposure	Prevent the spillage from reaching watercourses or drains and pollute soil and vegetation.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Form	Liquid
Colour	Colourless.
Odour	No data available.
pH	Status: In delivery state Comments: No data available.

Boiling point / boiling range	Comments: No data available.
Flash point	Comments: No data available.
Explosion limit	Comments: No data available.
Vapour pressure	Comments: No data available.
Relative density	Comments: No data available
Partition coefficient: n-octanol/ water	Comments: No data available.
Viscosity	Comments: No data available.

9.2. Other information

9.2.2. Other safety characteristics

Comments	The certificate accompanying this product contains batch specific properties.

SECTION 10: Stability and reactivity

10.1. Reactivity

Reactivity	No data available.

10.2. Chemical stability

Stability	Stable under recommended storage conditions and recommendations for safe handling.

10.3. Possibility of hazardous reactions

Possibility of hazardous reactions	No information available.

10.4. Conditions to avoid

Conditions to avoid	No data available.

10.5. Incompatible materials

Materials to avoid	No data available.

10.6. Hazardous decomposition products

Hazardous decomposition products	No data available.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Other information regarding health hazards

Inhalation	May have an irritating effect on the mucous membranes.
Skin contact	May cause slight irritation to the skin.

Eye contact	May cause mild irritation.
Ingestion	Fatal if swallowed.
Mutagenicity	No information available.
Carcinogenicity, other information	No information available.
Reproductive toxicity	No information available.

11.2 Other information

Other information	To the best of our knowledge the toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1. Toxicity

Ecotoxicity	May cause long lasting harmful effects to aquatic life. Do not allow to enter waters, waste water or soil.

12.2. Persistence and degradability

Persistence and degradability description/evaluation	No information available.

12.3. Bioaccumulative potential

Bioaccumulation, comments	No data available.

12.4. Mobility in soil

Mobility	No information available.

12.5. Results of PBT and vPvB assessment

Results of PBT and vPvB assessment	No information available.

12.6. Endocrine disrupting properties

12.7. Other adverse effects

Additional ecological information	Do not allow this chemical to contaminate sources of drinking water, waste water or soil!

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Appropriate methods of disposal for the chemical	Comply with all local and national regulations. Contact local waste collection servicer. Should be prevented from entering drain. What EWC-code should be used depends on the prosesses the product has been a part of. The EWC-code is in the European waste list.
EWC waste code	EWC waste code: 160509 discarded chemicals other than those mentioned in 16

	05 06, 16 05 07 or 16 05 08

SECTION 14: Transport information

Dangerous goods	Yes

14.1. UN number

ADR/RID/ADN	2810
IMDG	2810
ICAO/IATA	2810

14.2. UN proper shipping name

Proper shipping name English ADR/RID/ADN	TOXIC LIQUID, ORGANIC, N.O.S.
ADR/RID/ADN	TOXIC LIQUID, ORGANIC, N.O.S.
IMDG	TOXIC LIQUID, ORGANIC, N.O.S.
ICAO/IATA	TOXIC LIQUID, ORGANIC, N.O.S.

14.3. Transport hazard class(es)

ADR/RID/ADN	6.1
Classificaton code ADR/RID/ADN	T1

14.4. Packing group

ADR/RID/ADN	II
IMDG	II
ICAO/IATA	II

14.5. Environmental hazards

14.6. Special precautions for user

14.7. Maritime transport in bulk according to IMO instruments

Product name	TOXIC LIQUID, ORGANIC, N.O.S.

Additional information

Hazard label ADR/RID/ADN	6.1
Hazard label IMDG	6.1
Hazard label ICAO/IATA	6.1

ADR/RID Other information

Tunnel restriction code	D/E
Transport category	2

Hazard No.	60

IMDG Other information

EmS	F-A, S-A

SECTION 15: Regulatory information

15.1. Safety, health and environmental regulations / legislation specific for the substance or mixture

References (laws/regulations)	Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). CLP regulation (EC) No 1272/2008, Annex II and III. ECHA – Information on Chemicals. European waste list (EWL) Producer's information about ingredients. Norwegian Labour Inspectorate: Occupational air requirement labelling. (December 2011).
Comments	Careful – this product is not yet fully tested.

15.2. Chemical safety assessment

SECTION 16: Other information

Supplier's notes	Information in this safety data sheet is based on information from the manufacturer and current regulations, and is only intended to be a description of the health, environmental and safety aspects of the product.
List of relevant H-phrases (Section 2 and 3)	H300 Fatal if swallowed. H413 May cause long lasting harmful effects to aquatic life.
Version	1
Prepared by	Marie-Sophie Zoch

SAFETY DATA SHEET

n-Dodecane, Neat

The safety data sheet is in accordance with Commission Regulation (EU) 2015/830 of 28 May 2015 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

SECTION 1: Identification of the substance / mixture and of the company / undertaking

Date issued	06.05.2021

1.1. Product identifier

Product name	n-Dodecane, Neat
Article no.	C1132.12

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use categories nordic (UCN).	No information available.
Use of the substance / preparation	Reference Material Laboratory chemicals
Use of chemical, comments	This product is to be used for research and analytical purposes only. The product is in no way meant for, approved for or suitable for medical use, but purely analytical!

1.3. Details of the supplier of the safety data sheet

Producer

Company name	Chiron AS
Office address	Stiklestadveien 1
Postal address	Stiklestadveien 1
Postcode	N-7041
City	TRONDHEIM
Country	Norway
Telephone number	+47 73874490
Email	HMS@chiron.no
Website	www.chiron.no
Enterprise No.	924 361 360
Contact person	Jon E. Johansen

1.4. Emergency telephone number

Emergency telephone	Telephone number: 22 59 13 00 Description: Poison Control Center: Døgnåpen Telephone number: 110 Description: Fire : Telephone number: 113 Description: Medical Aid: Telephone number: 112 Description: Police: Description: Emergency numbers in Norway: Description: If not in Norway contact you local emergency numbers.

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP / GHS]	Flam. Liq. 3; H226 Asp. Tox. 1; H304 Skin Irrit. 2; H315 Eye Irrit. 2; H319 STOT SE 3; H335

2.2. Label elements

Hazard pictograms (CLP)

Composition on the label	n-Dodecane ~ 100 %
Signal word	Danger
Hazard statements	H225 Highly flammable liquid and vapour. H304 May be fatal if swallowed and enters airways. H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation.
Precautionary statements	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P240 Ground and bond container and receiving equipment. P261 Avoid breathing dust / fume / gas / mist / vapours / spray. P280 Wear protective gloves / protective clothing / eye protection / face protection. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor / physician. P303+P361+P353 IF ON SKIN (or hair): Remove / Take off immediately all contaminated clothing. Rinse skin with water / shower.

	P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P312 Call a POISON CENTER or doctor / physician if you feel unwell. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice / attention. P405 Store locked up.
EC label	Yes

2.3. Other hazards

PBT / vPvB	No information available.
Other hazards	See also section 5, 11 and 12.

SECTION 3: Composition / information on ingredients

3.2. Mixtures

Substance	Identification	Classification	Contents	Notes
n-Dodecane	CAS No.: 112-40-3 EC No.: 203-967-9	Flam. Liq. 3; H226 Asp. Tox. 1; H304 Skin Irrit. 2; H315 Eye Irrit. 2; H319 STOT SE 3; H335	~ 100 %	

Substance comments	The safety and toxicological aspects are not fully investigated for some reference materials, and specific data are therefore not available.

SECTION 4: First aid measures

4.1. Description of first aid measures

Inhalation	Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Don't use mouth to mouth or mouth to nose, but use aids instead. Call a POISON CENTER or doctor/ physician if you feel unwell.
Skin contact	Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid if irritation develops or presists.
Eye contact	Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical aid if irritation develops or presists.
Ingestion	DO NOT INDUCE VOMITING! Immediately rinse mouth and provide fresh air. Aspiration risk. Get medical attention immediately!
Recommended personal protective equipment for first aid responders	In case of inadequate ventilation wear respiratory protection. Refer to protective measures listed in sections 7 and 8.

4.2. Most important symptoms and effects, both acute and delayed

General symptoms and effects	The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11.

Acute symptoms and effects	May be fatal if swallowed and enters airways. Irritating to skin. Causes serious eye irritation. Kan forårsake irritasjon av luftveiene.

4.3. Indication of any immediate medical attention and special treatment needed

Other information	Treat symptomatically and supportive.

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media	Use water spray only to cool containers. Do not direct water at material which has leaked out. Use dry chemical, carbon dioxide, or alcohol-resistant foam.
Improper extinguishing media	Do not use water.

5.2. Special hazards arising from the substance or mixture

Fire and explosion hazards	Highly flammable liquid and vapour. Will burn if involved in a fire. Flashback is possible over considerable distances. Vapours may ignite.

5.3. Advice for firefighters

Personal protective equipment	Use a self-containing breathing apparatus in pressure-demand and full protective gear.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures	Evacuate area. Eliminate all ignition sources if safe to do so. Avoid breathing vapors, mist or gas. Ensure adequate ventilation.
Personal protection measures	Refer to protective measures listed in sections 7 and 8.
For emergency responders	No information available.

6.2. Environmental precautions

Environmental precautionary measures	Prevent the spillage from reaching watercourses or the sewage system and contaminating the soil and vegetation. Inform respective authorities in case product reaches water or sewage system.

6.3. Methods and material for containment and cleaning up

Containment	Store locked up.
Clean up	Absorb in an inert material (sand, vermiculite, etc.) and collect into a suitable container. Use non-sparking tools. Prevent release to sewer: Risk of explosion.

6.4. Reference to other sections

Other instructions	Refer to protective measures listed in sections 7 and 8. For disposal see section 13.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Handling	Use only in well-ventilated areas. Avoid inhalation of vapour/spray. Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Empty containers may contain product residue; handle therefore with care.

Protective safety measures

Safety measures to prevent fire	Store in a well-ventilated place. Keep cool. Do not spray on an open flame or other ignition source. Take precautionary measures against static discharge. Use only non-sparking tools. Ground / bond container and receiving equipment.
Preventitive measures to prevent aerosol and dust generation	Handle and open carefully.

7.2. Conditions for safe storage, including any incompatibilities

Storage	Containers must be kept tightly closed. Always keep away from sources of ignition. Keep from contact with oxidizing products. Store as a flammable liquid. Store in freezer.

Conditions for safe storage

Additional information on storage conditions	Store locked up.

7.3. Specific end use(s)

Specific use(s)	This product should be used for research and analytical purposes.

SECTION 8: Exposure controls / personal protection

8.1. Control parameters

Control parameters comments	Contains no substances with administrative standards for pollution in the working atmosphere.

8.2. Exposure controls

Safety signs

Precautionary measures to prevent exposure

Instruction on measures to prevent exposure	Wash thoroughly after handling. Remove contaminated clothing and wash before reuse.
Technical measures to prevent exposure	All work with dangerous chemicals must be done using a fume cupboard or in a well-ventilated room. Shower and eyewash facilities should be available at the workplace.

Eye / face protection

Suitable eye protection	Wear approved goggles or face shield.

Hand protection

Suitable gloves type	There are no data available for permeation time for this specific product, but nitrile gloves are recommended on a general basis.

Skin protection

Suitable protective clothing	Wear appropriate protective clothing when risk of direct contact or splashes.

Respiratory protection

Recommended type of equipment	Respirator with filter A2 is recommended for organic gases and vapors with boiling point above 65 degrees Celsius.

Thermal hazards

Thermal hazards	Personal protective equipment must have thermal insulation capacity and mechanical strength appropriate to foreseeable conditions of use

Appropriate environmental exposure control

Product related measures to prevent exposure	Prevent the spillage from reaching watercourses or drains and pollute soil and vegetation.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Form	Liquid
Colour	No data available.
Odour	No data available.
pH	Status: In delivery state Comments: No data available.
Boiling point / boiling range	Comments: No data available.
Flash point	Comments: No data available.
Explosion limit	Comments: No data available.
Vapour pressure	Comments: No data available.
Relative density	Comments: No data available
Partition coefficient: n-octanol/ water	Comments: No data available.
Viscosity	Comments: No data available.

9.2. Other information

Physical hazards

Flammable aerosols	Classification: Highly flammable liquid and vapor.
Flammable liquids	Classification: Highly flammable liquid and vapor.

9.2.2. Other safety characteristics

Comments	The certificate accompanying this product contains batch specific properties.

SECTION 10: Stability and reactivity

10.1. Reactivity

Reactivity	Highly flammable liquid and vapour.

10.2. Chemical stability

Stability	Stable under recommended storage conditions and recommendations for safe handling.

10.3. Possibility of hazardous reactions

Possibility of hazardous reactions	Reacts with strong oxidizing agents.

10.4. Conditions to avoid

Conditions to avoid	Heat, flames and sparks.

10.5. Incompatible materials

Materials to avoid	Strong oxidants. Reducing agents. Avoid contact with strong acids and bases.

10.6. Hazardous decomposition products

Hazardous decomposition products	Carbon dioxide (CO2).

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Substance	n-Dodecane
Acute toxicity	**Effect tested:** LC50 **Route of exposure:** Oral **Value:** > 5000 mg/kg **Animal test species:** Rat **Test reference:** OECD Test-Guideline 401 **Effect tested:** LC50 **Route of exposure:** Inhalation. **Duration:** 4 hour(s) **Value:** 9.3 mg/l **Animal test species:** Rat **Test reference:** OECD Test-Guideline 403 **Effect tested:** LC50

	Route of exposure: Inhalation. **Duration:** 4 hour(s) **Value:** 5.6 mg/l **Animal test species:** Rat **Test reference:** OECD Test-Guideline 403

Other information regarding health hazards

Inhalation	May cause irritation to the respiratory tract.
Skin contact	Irritating to skin.
Eye contact	Causes serious eye irritation.
Ingestion	May be fatal if swallowed and enters airways.
Mutagenicity	In vivo tests are showing mutagenic effects.
Carcinogenicity, other information	No information available.
Reproductive toxicity	No information available.

11.2 Other information

Other information	To the best of our knowledge the toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1. Toxicity

Substance	n-Dodecane
Aquatic toxicity, fish	**Value:** > 1000 mg/l **Effect dose concentration:** LC50 **Exposure time:** 96 hour(s) **Species:** Oncorhynchus mykiss
Substance	n-Dodecane
Aquatic toxicity, algae	**Value:** 57100 mg/l **Effect dose concentration:** EC50 **Exposure time:** 72 hour(s) **Species:** Skeletonema Costatum
Ecotoxicity	The product is not classified as environmentally hazardous. This does not preclude the possibility that accidental major discharges or frequently repeated minor discharges can have a disruptive or harmful effect on the environment.

12.2. Persistence and degradability

Substance	n-Dodecane
Biodegradability	**Value:** 83 % **Method:** aerobic **Test reference:** OECD TG 301 F **Comments:** Easily biodegradable. **Test period:** 28 day(s)

12.3. Bioaccumulative potential

Substance	n-Dodecane
Bioconcentration factor (BCF)	**Value:** 52

12.4. Mobility in soil

Mobility	No information available.

12.5. Results of PBT and vPvB assessment

Results of PBT and vPvB assessment	No information available.

12.6. Endocrine disrupting properties

12.7. Other adverse effects

Additional ecological information	Do not allow this chemical to contaminate sources of drinking water, waste water or soil!

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Appropriate methods of disposal for the chemical	Comply with all local and national regulations. Waste must be disposed of at approved landfill/waste storage or treatment facility. What EWC-code should be used depends on the prosesses the product has been a part of. The EWC-code is in the European waste list.
EWC waste code	EWC waste code: 070104 other organicsolvents, washing liquids and mother liquors

SECTION 14: Transport information

Dangerous goods	Yes

14.1. UN number

ADR/RID/ADN	1993
IMDG	1993
ICAO/IATA	1993

14.2. UN proper shipping name

Proper shipping name English ADR/RID/ADN	FLAMMABLE LIQUID, N.O.S.
ADR/RID/ADN	FLAMMABLE LIQUID, N.O.S.
IMDG	FLAMMABLE LIQUID, N.O.S.
ICAO/IATA	FLAMMABLE LIQUID, N.O.S.

14.3. Transport hazard class(es)

ADR/RID/ADN	3
Classificaton code ADR/RID/ADN	F1

14.4. Packing group

ADR/RID/ADN	III
IMDG	III
ICAO/IATA	III

14.5. Environmental hazards

14.6. Special precautions for user

14.7. Maritime transport in bulk according to IMO instruments

Product name	FLAMMABLE LIQUID, N.O.S.

Additional information

Hazard label ADR/RID/ADN	3
Hazard label IMDG	3
Hazard label ICAO/IATA	3

ADR/RID Other information

Tunnel restriction code	D/E
Transport category	3
Hazard No.	30

IMDG Other information

EmS	F-E, S-E

SECTION 15: Regulatory information

15.1. Safety, health and environmental regulations / legislation specific for the substance or mixture

References (laws/regulations)	Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). CLP regulation (EC) No 1272/2008, Annex II and III. ECHA – Information on Chemicals. European waste list (EWL) Producer's information about ingredients. Norwegian Labour Inspectorate: Occupational air requirement labelling. (December 2011).
Comments	Careful – this product is not yet fully tested.

15.2. Chemical safety assessment

SECTION 16: Other information

Supplier's notes	Information in this safety data sheet is based on information from the manufacturer and current regulations, and is only intended to be a description of the health, environmental and safety aspects of the product.
List of relevant H-phrases (Section 2 and 3)	H226 Flammable liquid and vapour. H304 May be fatal if swallowed and enters airways. H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation.
Version	1
Prepared by	Marie-Sophie Zoch

SAFETY DATA SHEET

Hydrocarbons, Individual Compounds, Neat

The safety data sheet is in accordance with Commission Regulation (EU) 2015/830 of 28 May 2015 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

SECTION 1: Identification of the substance / mixture and of the company / undertaking

Date issued	01.07.2015
Revision date	06.05.2021

1.1. Product identifier

Product name	Hydrocarbons, Individual Compounds, Neat
Article no.	C1133.13, C1135.15, C1137.17, C1139.19

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use categories nordic (UCN).	No information available.
Use of the substance / preparation	Reference Material Laboratory chemicals
Use of chemical, comments	This product is to be used for research and analytical purposes only. The product is in no way meant for, approved for or suitable for medical use, but purely analytical!

1.3. Details of the supplier of the safety data sheet

Producer

Company name	Chiron AS
Office address	Stiklestadveien 1
Postal address	Stiklestadveien 1
Postcode	N-7041
City	TRONDHEIM
Country	Norway
Telephone number	+47 73874490
Email	HMS@chiron.no
Website	www.chiron.no
Enterprise No.	924 361 360

Contact person	Jon E. Johansen

1.4. Emergency telephone number

Emergency telephone	Telephone number: 22 59 13 00 Description: Poison Control Center: Døgnåpen Telephone number: 110 Description: Fire : Telephone number: 113 Description: Medical Aid: Telephone number: 112 Description: Police: Description: Emergency numbers in Norway: Description: If not in Norway contact you local emergency numbers.

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP / GHS]	Asp. Tox. 1; H304

2.2. Label elements

Hazard pictograms (CLP)

Composition on the label	n-Tridecane, δ13C: -33.47 ~ 100 %, n-Pentadecan ~ 100 %, n-Heptadecane, δ13C: – 25.72 ~ 100 %, n-Nonadecane, δ13C: -34.60 ~ 100 %
Signal word	Danger
Hazard statements	H304 May be fatal if swallowed and enters airways.
Precautionary statements	P261 Avoid breathing dust / fume / gas / mist / vapours / spray. P262 Do not get in eyes, on skin, or on clothing. P280 Wear protective gloves / protective clothing / eye protection / face protection. P285 In case of inadequate ventilation wear respiratory protection. P301+P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor / physician. P331 Do NOT induce vomiting. P405 Store locked up.
Supplemental label information	None.
EC label	Yes

2.3. Other hazards

PBT / vPvB	No information available.

Other hazards	See also section 5, 11 and 12.

SECTION 3: Composition / information on ingredients

3.2. Mixtures

Substance	Identification	Classification	Contents	Notes
n-Tridecane, δ13C: -33.47	CAS No.: 629-50-5 EC No.: 211-093-4	Asp. tox. 1; H304	~ 100 %	
n-Pentadecan	CAS No.: 629-62-9 EC No.: 211-098-1	Asp. Tox. 1; H304 EUH 066	~ 100 %	
n-Heptadecane, δ13C: –25.72	CAS No.: 629-78-7 EC No.: 211-108-4	Asp. tox. 1; H304	~ 100 %	
n-Nonadecane, δ13C: -34.60	CAS No.: 629-92-5 EC No.: 211-116-8	Asp. tox. 1; H304	~ 100 %	

Substance comments	This SDS applies to several products with the same health, safety and environmental properties. All components need not be present in all products. The safety and toxicological aspects are not fully investigated for some reference materials, and specific data are therefore not available.

SECTION 4: First aid measures

4.1. Description of first aid measures

Inhalation	Remove from exposure to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical advice if discomfort develops.
Skin contact	Flush skin with large amounts of water and remove contaminated clothing, watches etc. simultaneously. Get medical advice if discomfort develops.
Eye contact	Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid if irritation develops or presists.
Ingestion	Never give anything by mouth to an unconscious person. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Rinse nose, mouth and throat with water. Immediately call a POISON CENTER or doctor/physician.
Recommended personal protective equipment for first aid responders	Refer to protective measures listed in sections 7 and 8.

4.2. Most important symptoms and effects, both acute and delayed

General symptoms and effects	The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11.

4.3. Indication of any immediate medical attention and special treatment needed

Information on clinical testing	No information available.
Other information	Treat symptomatically and supportive.

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media	Use water spray only to cool containers. Do not direct water at material which has leaked out. Use dry chemical, carbon dioxide, or alcohol-resistant foam.
Improper extinguishing media	Do not use water.

5.2. Special hazards arising from the substance or mixture

Fire and explosion hazards	No information available.

5.3. Advice for firefighters

Personal protective equipment	Use a self-containing breathing apparatus in pressure-demand and full protective gear. Refer to protective measures listed in sections 7 and 8.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

Personal protection measures	Evacuate personnel to safe area. Wear a self-contained breathing apparatus when needed and appropriate personal protection. Refer to protective measures listed in sections 7 and 8.
For emergency responders	No information available.

6.2. Environmental precautions

Environmental precautionary measures	Avoid release of significant quantities of the product to water sources, sewers or the environment in general.

6.3. Methods and material for containment and cleaning up

Clean up	Absorb in an inert material (sand, vermiculite, etc.) and collect into a suitable container. Thoroughly ventilate and clean the area after the material has been removed.

6.4. Reference to other sections

Other instructions	For disposal see section 13.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Handling	Do not breathe dust, vapor, mist or gas. Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Always keep away from sources of ignition.

Protective safety measures

Safety measures to prevent fire	Keep cool. Protect from sunlight. Store in a well-ventilated place.
Preventitive measures to prevent aerosol and dust generation	Handle and open carefully.

7.2. Conditions for safe storage, including any incompatibilities

Storage	Keep containers tightly closed in a dry, cool and well-ventilated place. Avoid heat, light & moisture. The product's accompanying certificate contains specific storage information.

7.3. Specific end use(s)

Specific use(s)	This product should be used for research and analytical purposes.

SECTION 8: Exposure controls / personal protection

8.1. Control parameters

Control parameters comments	Contains no substances with administrative standards for pollution in the working atmosphere.

8.2. Exposure controls

Safety signs

Precautionary measures to prevent exposure

Instruction on measures to prevent exposure	Wash hands after using the product.
Technical measures to prevent exposure	Use adequate general or local exhaust ventilation to keep airborne concentrations low. Shower and eyewash facilities should be available at the workplace. Wash hands after using the product.

Eye / face protection

Suitable eye protection	Wear approved goggles or face shield.

Hand protection

Suitable gloves type	There are no data available for permeation time for this specific product, but nitrile gloves are recommended on a general basis.

Skin protection

Suitable protective clothing	Wear appropriate protective clothing when risk of direct contact or splashes.

Respiratory protection

Recommended type of equipment	Normally not necessary in ordinary use. Respiratory protection with filter P2 is recommended when exposed to particles of harmful substances.

Thermal hazards

Thermal hazards	Personal protective equipment must have thermal insulation capacity and mechanical strength appropriate to foreseeable conditions of use

Appropriate environmental exposure control

Product related measures to prevent exposure	Prevent the spillage from reaching watercourses or drains and pollute soil and vegetation.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Form	Liquid
Colour	No data available.
Odour	No data available.
pH	Status: In delivery state Comments: No data available.
Boiling point / boiling range	Comments: No data available.
Flash point	Comments: No data available.
Explosion limit	Comments: No data available.
Vapour pressure	Comments: No data available.
Relative density	Comments: No data available
Partition coefficient: n-octanol/ water	Comments: No data available.
Viscosity	Comments: No data available.

9.2. Other information

9.2.2. Other safety characteristics

Comments	The certificate accompanying this product contains batch specific properties.

SECTION 10: Stability and reactivity

10.1. Reactivity

Reactivity	No data available.

10.2. Chemical stability

Stability	Stable under recommended storage conditions and recommendations for safe handling.

10.3. Possibility of hazardous reactions

Possibility of hazardous reactions	No information available.

10.4. Conditions to avoid

Conditions to avoid	No data available.

10.5. Incompatible materials

Materials to avoid	No data available.

10.6. Hazardous decomposition products

Hazardous decomposition products	No data available.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Substance	n-Tridecane, δ13C: -33.47
Acute toxicity	**Type of toxicity:** Acute **Effect tested:** LC50 **Route of exposure:** Inhalation. **Duration:** 8 t **Value:** > 41 ppm **Animal test species:** Rat

Other information regarding health hazards

Inhalation	May have an irritating effect on the mucous membranes.
Skin contact	May cause slight irritation to the skin.
Eye contact	May cause mild irritation.
Ingestion	May be fatal if swallowed and enters airways.
Sensitisation	No data available.
Mutagenicity	No information available.
Carcinogenicity, other information	No information available.
Reproductive toxicity	No information available.

11.2 Other information

Other information	To the best of our knowledge the toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1. Toxicity

Ecotoxicity	The product is not classified as environmentally hazardous. This does not preclude the possibility that accidental major discharges or frequently repeated minor discharges can have a disruptive or harmful effect on the environment.

12.2. Persistence and degradability

Persistence and degradability description/evaluation	No information available.

12.3. Bioaccumulative potential

Bioaccumulation, comments	No data available.

12.4. Mobility in soil

Mobility	No information available.

12.5. Results of PBT and vPvB assessment

Results of PBT and vPvB assessment	No information available.

12.6. Endocrine disrupting properties

12.7. Other adverse effects

Additional ecological information	Do not allow this chemical to contaminate sources of drinking water, waste water or soil!

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Appropriate methods of disposal for the chemical	Comply with all local and national regulations. Contact local waste collection servicer. Should be prevented from entering drain. What EWC-code should be used depends on the prosesses the product has been a part of. The EWC-code is in the European waste list.
EWC waste code	EWC waste code: 160509 discarded chemicals other than those mentioned in 16 05 06, 16 05 07 or 16 05 08

SECTION 14: Transport information

Dangerous goods	No

14.1. UN number

14.2. UN proper shipping name

14.3. Transport hazard class(es)

14.4. Packing group

14.5. Environmental hazards

14.6. Special precautions for user

14.7. Maritime transport in bulk according to IMO instruments

ADR/RID Other information

Hazard No.	33

SECTION 15: Regulatory information

15.1. Safety, health and environmental regulations / legislation specific for the substance or mixture

References (laws/regulations)	Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). CLP regulation (EC) No 1272/2008, Annex II and III. ECHA – Information on Chemicals. European waste list (EWL) Producer's information about ingredients. Norwegian Labour Inspectorate: Occupational air requirement labelling. (December 2011).
Comments	Careful – this product is not yet fully tested.

15.2. Chemical safety assessment

SECTION 16: Other information

Supplier's notes	Information in this safety data sheet is based on information from the manufacturer and current regulations, and is only intended to be a description of the health, environmental and safety aspects of the product.
List of relevant H-phrases (Section 2 and 3)	EUH 066 Repeated exposure may cause skin dryness or cracking. H304 May be fatal if swallowed and enters airways.
Information added, deleted or revised	06.05.2021 Updated acc. CLP Regulation and REACH. Compounds added to the sagfty datasheet. Contact person in section 1.3 updated.
Revision responsible	06.05.2021 Marie-Sophie Zoch
Last update date	06.05.2021
Version	2
Prepared by	Inge Fenstad

J Popular Scientific Article

VALIDATION OF AN ANALYTICAL METHOD

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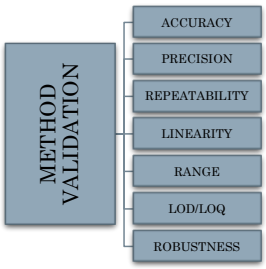
SOLFRID MARGRETE RYDSÅ

How can you be sure your analytical method can produce reliable results? By performing a validation of the method. The term validation means to verify and document that an analytical method is suitable for the purpose. This is usually done according to various guidelines, depending on the method, instrument and laboratory performing the analysis [1, 2]. Some of the parameters that are often examined in method validation are accuracy, precision, repeatability, linearity, range, limit of detection (LOD), limit of quantification (LOQ) and robustness.



Accuracy and Precision

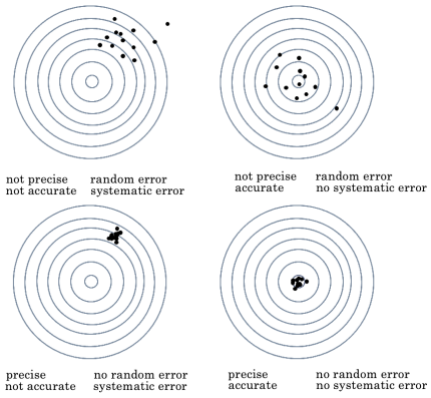
Values of accuracy says something about how close your data is to the "true value" [1]. Evaluation of accuracy is often done by analyzing a certified reference material (CRM). When the content of analyte is determined, the difference from the theoretical concentration is calculated. For example, accuracy can be given as a percentage value, where the determined concentration are divided by the theoretical concentration:

$$\%accuracy = \frac{actual\ conc.}{theoretical\ conc.} \cdot 100\%$$

Precision on the other hand gives an indication of whether or not your data is consistent [1]. The level of precision is usually shown through the standard deviation, often expressed as percentage relative standard deviation (%RSD):

$$\%RSD = \frac{\sigma}{\bar{x}} \cdot 100\%$$

The relation between the two concepts accuracy and precision are illustrated well in the following figure of "targets" [1].



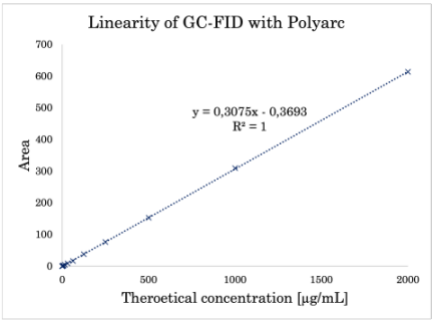
Relationship between precision and accuracy.

Repeatability

Quite closely related to precision is the term instrumental repeatability [3]. It covers analytical variation between assays that are operated over as short a period of time as possible. Repeatability is often measured from the standard deviation and should be measured from at least 6 samples to avoid a large uncertainty.

Linearity and Range

Preferably, the relationship between your theoretical analyte concentration and results should be linear [3]. When plotting results graphically, a correlation coefficient (R^2) can be retrieved from the linear trend line of the slope. The closer this value is to $R^2 = 1$, the more linear relationship has been achieved.

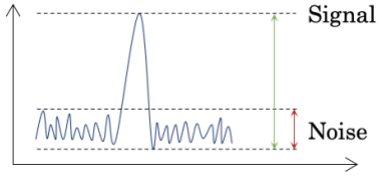


Graphic illustration of a linear relationship.

The term range defines within which concentration range your samples are expected to be found [2]. Evaluation of range can be done from for example LOQ up to the highest concentration used in your experiments.

LOD and LOQ

The limits of detection and quantification are often based on the signal-to-noise ratio (S/N) of peaks.

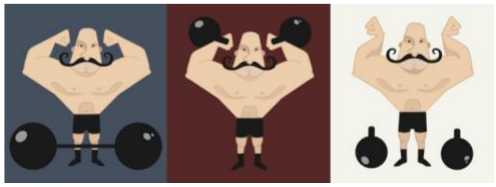


Definitions of peak signal and noise.

LOD covers peaks that can be distinguished from the baseline noise, but not quantified [1]. It will provide an indication that an analyte is present in the sample, but not at which concentration. LOQ on the other hand covers the lowest concentration of analyte possible to quantify [1].

Robustness

One of the most important parameters, but often least noticed, is the robustness of the instrument [3]. How well does your instrument perform when certain method parameters are changed? Your instrument should be able to produce the same results within acceptable variations in the method. A robust instrument will produce accurate analyzes even if the injector temperature changes a few degrees, or if your gradient for the column are varied [1].



Robustness is achieving equally good results with changes in the method. (fig. by Voelker [4])

When all of the parameters chosen for your validation are tested, evaluated and fulfilled, you have proved that your instrument can provide accurate analysis and that it is "fit for purpose". Lastly, a report is generated to confirm that your analytical method and procedures are suitable for the intended use [1].

References

[1] K. Dettmer-Wilde and W. Engewald, *Practical Gas Chromatography : A Comprehensive Reference*, 1st ed. 2014. ed. Berlin, Heidelberg: Springer Berlin Heidelberg : Imprint: Springer, 2014.

[2] E. Lundanes, L. Reubsaet, and T. Greibrokk, *Chromatography : basic principles, sample preparations and related methods.* Weinheim: Wiley-VCH, 2014.

[3] J. M. Joachim Ermer, *Method Vaildation in Pharmaceutical Analysis. A Guide to Best Practice.* Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA, 2005.

[4] P. Voelker, "Robustness Enhances Lab Optimization Analysis Processes – Part 3: Gas Chromatography," in *analyteguru.com* vol. 2021, ed: Thermo Fischer Scientific Inc., 2016.