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CERTIFICATION REPORT

The certification of the purity of three PAHs, benzo[a]pyrene (ERM®-AC051), indeno[1,2,3-cd]pyrene (ERM®-AC053) and 6-methylchrysene (ERM®-AC082)

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Abstract

This report describes the production of ERM-AC051, benzo[a]pyrene, ERM-AC053, indeno[1,2,3-cd]pyrene and ERM-AC082, 6-methylchrysene, which are pure materials certified for their purity. This material was produced following ISO Guide 34:2009 and is certified in accordance with ISO Guide 35:2017.
The materials were custom synthesized, purified and filled into glass vials.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017.
The material was characterised by applying different methods (HPLC, GC, DSC, qNMR, ICP-MS) in different laboratories of demonstrated competence and adhering to ISO/IEC 17025.
Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The main purpose of the material is for preparation of calibrant solutions. As any reference material, it can also be used for control charts or validation studies.
The CRMs are available in amber glass vials containing at least 25 mg powder which were sealed. The minimum amount of sample to be used is 1 mg.

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The certification the purity of three PAHs, benzo[a]pyrene (ERM®-AC051), indeno[1,2,3-cd]pyrene (ERM®-AC053) and 6-methylchrysene (ERM®-AC082)

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM-AC051, benzo[*a*]pyrene, ERM-AC053, indeno[1,2,3-*cd*]pyrene and ERM-AC082, 6-methylchrysene, which are pure materials certified for their purity. This material was produced following ISO Guide 34:2009 [1] and is certified in accordance with ISO Guide 35:2017 [2].

The materials were custom synthesized, purified and filled into glass vials.

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017 [2].

The material was characterised by applying different methods (HPLC, GC, DSC, qNMR, ICP-MS) in different laboratories of demonstrated competence and adhering to ISO/IEC 17025.

Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [3] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The main purpose of the material is for preparation of calibrant solutions. As any reference material, it can also be used for control charts or validation studies.

The CRMs are available in amber glass vials containing at least 25 mg powder which were sealed. The minimum amount of sample to be used is 1 mg.

The following values were assigned:

	Mass fraction	
	Certified value [1)] [g/g]	Uncertainty [2)] [g/g]
ERM-AC051, benzo[*a*]pyrene	0.979	0.007
ERM-AC053, indeno[1,2,3-*cd*]pyrene	0.996	+ 0.004 - 0.005 [3)]
ERM-AC082, 6-methylchrysene	0.983	0.005

1) Calculated as combination of the sum of impurities as detected by different methods subtracted from unity and quantitative nuclear magnetic resonance. The certified values and their uncertainties are traceable to the SI.
2) Expanded uncertainty with a coverage factor k = 2 according to the Guide for the Expression of Uncertainty in Measurement, corresponding to a level of confidence of about 95 %.
3) The asymmetric uncertainty interval is based on a standard uncertainty of 0.0022 g/g.

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Glossary

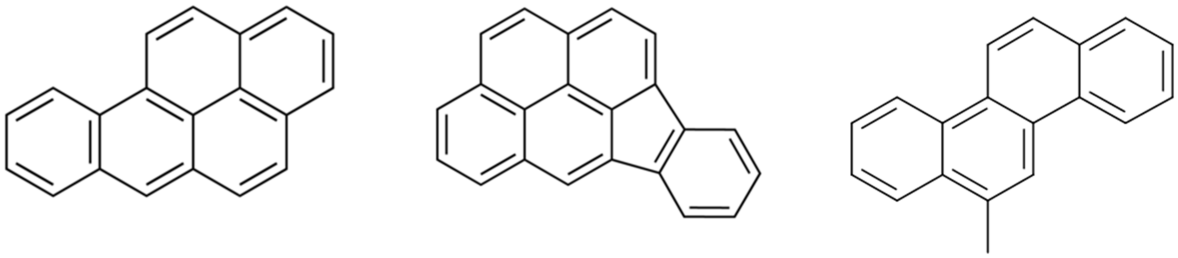
Δ_m	Absolute difference between the mean measured value and the certified value of a CRM
ANOVA	Analysis of variance
BCR	Community Bureau of Reference, the former reference materials programme of the European Commission
$CDCl_3$	Deuterochloroform
CRM	Certified reference material
DMSO	Dimethylsulfoxide
DSC	Differential scanning calorimetry
EPA	Environmental Protecion Agency of the USA
FID	Flame ionization detection
GC	Gas chromatography
GC-FID	Gas chromatography with flame ionization detection
GCxGC	Comprehensive two-dimensional gas chromatography
GC-MS	Gas chromatography with mass spectrometric detection
GMP	Good Manufacturing Practice
HPLC-UV	High performance liquid chromatography with ultra-violet detection
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LOD	Limit of detection
MS	Mass spectrometry
MS_{among}	Mean square among bottles from an ANOVA
MS_{within}	Mean square within a bottle from an ANOVA
n	Average number of replicates per bottle
PAH	Polycyclic hydrocarbon
qNMR	Quantitative nuclear magnetic resonance spectroscopy
RSD_{stab}	Relative standard deviation of all results of the stability study
RSD_{method}	Method repeatability, expressed as relative standard deviation
s_{bb}	Between-unit variability, expressed as relative standard deviation; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation; an additional index "rel" is added as appropriate
SI	International System of Units (Système International d'Unités)
u	Standard uncertainty
u_Δ	Combined uncertainty of Δ_m
U_Δ	Expanded uncertainty of Δ_m

u_{bb}	Uncertainty related to a possible between-bottle heterogeneity; an additional "rel" is added to denominate relative uncertainties
u^*_{bb}	Uncertainty of heterogeneity that could be hidden by method repeatability, expressed as relative uncertainty
u_{char}	Uncertainty of the characterisation; an additional "rel" is added to denominate relative uncertainties
u_{CRM}	Combined uncertainty of a certified value
U_{CRM}	Expanded uncertainty of a certified value; an additional "rel" is added to denominate relative uncertainties
u_{lts}	Uncertainty of stability; an additional "rel" is added to denominate relative uncertainties
u_m	Measurement uncertainty
x	Pre-defined shelf life
$\overline{x}$	Average of all time points in an isochronous stability study
x_i	Time point i in an isochronous stability study
$\overline{y}$	Average of all results of a homogeneity study
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Accurate measurements of polycyclic hydrocarbons (PAHs) are required by legislation in a number of matrices, amongst other food (Commission Regulation (EC) No. 208/2005 [4] amending Regulation (EC) No. 466/2001 [5], Directive 2005/10/EC and Commission Recommendation 2005/108/EC) and environmental samples (Air Quality Framework Directive 96/62/EC [6] and Daughter Directive 2004/107/EC [7], the Water Framework Directive 2000/60/EC [8] etc.). Any of these measurements require the availability of reliable calibrants. For many years, the European Commission's reference materials programme has been supplying these PAH calibrants as solid pure substances, amongst many other BCR-051R (benzo[*a*]pyrene), BCR-053 (indeno[1,2,3-*cd*]pyrene) and BCR-082R (6-methylchrysene). After the stock of these three PAHs was exhausted the Reference Materials Unit of the Directorate F of the European Commission's Joint Research Centre decided to produce new batches, labelled ERM-AC051, ERM-AC053 and ERM-AC082, and subsequently certified.



a) Benzo[*a*]pyrene b) Indeno[1,2,3-*cd*]pyrene c) 6-Methylchrysene

Figure 1: Structural formulas of the three PAHs.
a) Benzo[a]pyrene, CAS no. 50-32-8, molecular mass: 252.32 g/mol
b) Indeno[1,2,3-cd]pyrene, CAS no. 193-39-5, molecular mass: 276.34 g/mol
c) 6-Methylchrysene, CAS no. 1705-85-7, molecular mass: 242.33 g/mol

1.2 Design of the CRM project

The basic goal of the project was to obtain materials of a purity above 95 %. In this case impurities expressed in molar fraction, mass fraction and response fraction (chromatographic area percent) can be assumed to be roughly equivalent, as even response factors differing by an order of magnitude will not have a large impact on the final assessment of purity. If this is ensured, results from different methods can be combined even if they contain unidentified impurities.

During characterisation different methods were applied to be able to detect all impurities potentially present, including inorganic impurities, detected by inductively-coupled plasma mass spectrometry or optical emission spectrometry (ICP-MS or ICP-OES), organic impurities, detected by high-performance liquid chromatography (HPLC), gas chromatography (GC) and quantitative nuclear magnetic resonance (qNMR) as well as differential scanning calorimetry (DSC).

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer-Foundation (BIU), Grosshansdorf, DE

Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol, BE

2.3 Homogeneity and stability study

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE

2.4 Characterisation

Biochemisches Institut für Umweltcarcinogene, Prof. Dr. Gernot Grimmer-Foundation (BIU), Grosshansdorf, DE

Bundesanstalt für Materialforschung und –prüfung (BAM), Berlin, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAP-PL-2614.14)

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE

European Directorate for the Quality of Medicines (EDQM), Strasbourg, FR

LGC Ltd, Teddington, UK
(measurements under the scope of ISO/IEC 17025 accreditation UKAS 0423)

Spectral Service, Cologne, DE
(certified for GMP; City Government of Cologne, CGN/24.30.12-P/01/2018/043)

Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol, BE

3 Material processing and process control

The three PAHs, namely benzo[*a*]pyrene (ERM-AC051), indeno[1,2,3-*cd*]pyrene (ERM-AC053) and 6-methylchrysene (ERM-AC082) were synthesized on demand for this project. After an initial check of the purity at the JRC, the PAHs were manually weighed into the pre-cleaned brown glass vials in a glove box flushed with argon at VITO. At least 25 mg were filled into each vial, typically they contain between 26 and 30 mg of PAH. After filling and capping the vials were wiped twice using a paper tissue wetted with methanol to remove any residues on the outside. Every vial was labelled individually with a label containing the CRM identification, hazard labels and its number in the filling sequence. A flow chart of the processing is shown in Figure 2.

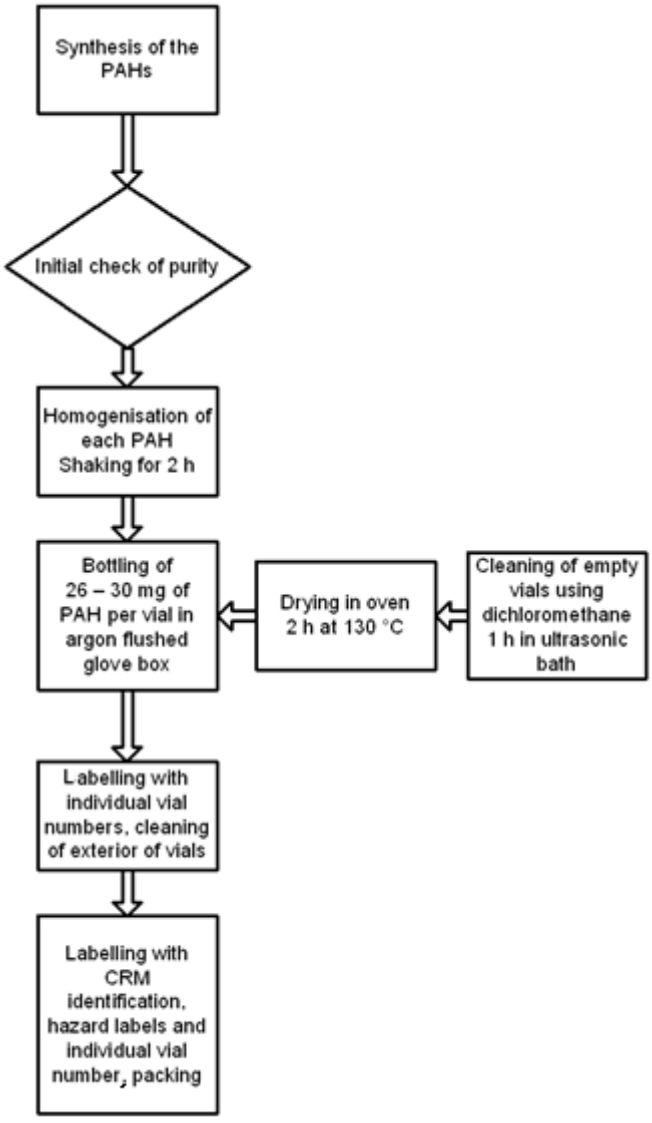


Figure 2: Processing of the three PAH CRMs

4 Homogeneity

A key requirement for any reference material aliquoted into vials is equivalence between those vials. In this respect, it is relevant whether the variation between vials is significant compared to the uncertainty of the certified value, but it is not relevant if this variation between vials is significant compared to the analytical variation. Consequently, ISO Guide 34 [1] requires RM producers to quantify the between vial variation. This aspect is covered in between-vial homogeneity studies.

The within-vial inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole vial.

4.1 Between-vial homogeneity

The between-vial homogeneity was evaluated to ensure that the certified values of the CRMs are valid for all vials of the material, within the stated uncertainties.

The number of vials selected corresponds to approximately the cube root of the total number of vials produced. For ERM-AC051, 15 vials were selected whereas 10 vials were used for ERM-AC053 and ERM-AC082. The vials were selected using a random stratified sampling scheme covering the whole batch for the between-vial homogeneity test. For this, the batch was divided into groups with equal number of vials per group and one vial was selected randomly from each group. Independent samples were taken from each selected vial, and analysed by differential scanning calorimetry (DSC). Triplicate analyses were performed on each vial of ERM-AC051 and duplicate analysis for each vial of ERM-AC053 and ERM-AC082. The results of the homogeneity study are shown in Annex A.

Regression analyses were performed to evaluate potential trends in the filling sequence. No trends in the filling sequence or the analytical sequence were observed at a 95 % confidence level. The datasets were assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the vial means.

Quantification of between-vial inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the (relative) between-vial variation ($s_{bb,rel}$) from the (relative) within-vial variation ($s_{wb,rel}$). The latter is equivalent to the method repeatability if the individual samples were representative for the whole vial.

Evaluation by ANOVA requires mean values per vial, which follow at least a unimodal distribution and results for each vial that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per vial was visually tested using histograms and normal probability plots. The results of all statistical evaluations are given in Table 1.

Table 1: Results of the statistical evaluation of the homogeneity studies. Outliers were tested on a 99 % confidence level, trends at a 95 % confidence level.

Material	Trends in the filling sequence	Outliers	Distribution of vial means
ERM-AC051	no	no	normal
ERM-AC053	no	no	normal
ERM-AC082	no	no	normal

It should be noted that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups

(MS_{among}) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-vial variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [9]. u^*_{bb} is comparable to the LOD of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–vial standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \text{Equation 1}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{among} - MS_{within}}{n}}}{\bar{y}} \qquad \text{Equation 2}$$

$$u^*_{bb} = \frac{\sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}}}{\bar{y}} \qquad \text{Equation 3}$$

MS_{within} mean of squares within-vial from an ANOVA

MS_{among} mean of squares between-vial from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n mean number of replicates per vial

$\nu_{MSwithin}$ degrees of freedom of MS_{within}

The results of the evaluation of the between-vial variation are summarised in Table 2. The resulting values from the above equations was/were converted into relative uncertainties.

Table 2: Results of the homogeneity studies

CRM	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{bb,rel}$ [%]
ERM-AC051	n.c.[1)]	0.002	0.002
ERM-AC053	0.061	0.048	0.061
ERM-AC082	n.c.[1)]	0.028	0.028

[1)] n.c.: cannot be calculated as $MS_{among} < MS_{within}$

When comparing the purity as determined by DSC with the purity obtained by other methods and the certified purity (see section 6) it is obvious that DSC overestimates the purity in case of ERM-AC051 and ERM-AC082. DSC cannot detect impurities that form solid solutions with the main component. Any heterogeneity originating from such impurities would therefore not be detected using this method and the uncertainty contribution of homogeneity would be underestimated. The uncertainty contribution of homogeneity is small compared to other sources of uncertainty for all materials. Even if it was larger, it would not significantly affect the uncertainty of the certified value (see section 7). Therefore, no additional efforts were made to further investigate the homogeneity. This is further confirmed by the good within-laboratory reproducibility observed in the characterisation of the materials.

The homogeneity study showed no outlying vial means or trends in the filling sequence. Therefore the between-vial standard deviation can be used as estimate of u_{bb}. As u^*_{bb} sets

the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-vial homogeneity and minimum sample intake

The within-vial homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole vial and thus should be used in an analysis. Using sample sizes equal or above the minimum sample intake guarantees the certified value within its stated uncertainty.

Despite the differences observed between the different methods applied for the purity determinations (see section 6) all measurements very good precision. As the laboratories applying the mass balance approach carried out determinations on 6 different subsamples of two different vials, this can be considered good evidence that the sample intakes used by the laboratories were sufficient. Typically, sample intakes of 0.25 - 5 mg have been used. Further the homogeneity data by differential scanning calorimetry (DSC) has been obtained on 1.16 – 1.80 mg subsamples. Summarising these findings, a minimum sample intake of 1 mg is established for the CRMs. Users will need to consider the uncertainty of their weighings when using such small sample intakes.

5 Stability

Time, temperature, light (including ultraviolet radiation) and water content were regarded as the most relevant influences on the stability of the materials. The influence of ultraviolet or visible light was minimised by storing the material in containers which reduces light exposure. In addition, materials are stored in the dark and dispatched in boxes, thus removing any possibility of degradation by light. The water content was adjusted to an optimum during processing

Stability testing is necessary to establish the conditions for storage (long-term stability) as well as the conditions for dispatch of the materials to the customers (short-term stability). During transport, especially in summer time, temperatures up to +60 °C can be reached and stability under these conditions must be demonstrated, if the samples are to be transported without any additional cooling.

The stability studies were carried out using an isochronous design [10]. In this approach, samples were stored for a particular length of time at different temperature conditions. Afterwards, the samples were moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1 Short-term stability study

For the short-term stability study, samples were stored at +18 °C and +60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -20 °C. Two vials of ERM-AC051 and ERM-AC053 and 4 vials of ERM-AC082 per storage time were selected using a random stratified sampling scheme. From each vial, two samples were measured by DSC. The measurements were performed under repeatability conditions. The results of the short-term stability study are shown in Annex B.

The data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test on a confidence level of 99 %. One outlying individual result for t=0 was found for ERM-AC051 (Table 3). As no technical reason for the outliers could be found all data were retained for statistical analysis.

In addition, the data were evaluated against storage time, and regression lines of mol fraction versus time were calculated, to test for potential increases/decrease of the purity due to shipping conditions. The slopes of the regression lines were tested for statistical significance. None of the trends was statistically significant at a 95 % confidence level for any of the temperatures.

The results of the measurements are shown in Annex B. The results of the statistical evaluation of the short-term stability are summarised in Table 3.

Table 3: Results of the short-term stability tests. The outliers of ERM-AC051 were retained.

CRM	Number of individual outlying results on a 99 % confidence level		Significance of the trend on a 95 % confidence level	
	18 ºC	60 ºC	18 ºC	60 ºC
ERM-AC051	1	1	no	no
ERM-AC053	0	0	no	no
ERM-AC082	0	0	no	no

Two statistical outliers were detected for ERM-AC051 and they were retained for the estimation of u_{STS}. None of the trends was statistically significant on a 95 % confidence level for any of the temperatures.

The material is stable at +18°C and +60°C for up to 4 weeks. The samples can be safely dispatched under conditions where the temperatures do not exceed +60 °C for up to 4 weeks, i.e. at ambient temperature.

5.2 Long-term stability study

For the long-term stability study, samples were stored at +4 °C for 0, 4, 8 and 12 months. The reference temperature was set to -20 °C. Three vials per storage time were selected using a random stratified sampling scheme. From each vial, two samples were measured by DSC. The measurements were performed under repeatability conditions. The results of the short-term stability study are shown in Annex C.

The long-term stability data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. Some outlying individual results were found (Table 4). As no technical reason for the outliers could be found all data were retained for statistical analysis.

In addition, the data were plotted against storage time and linear regression lines of mole fraction versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage). No significant trend was detected at a 95 % confidence level.

The results of the long-term stability measurements are shown in Annex C. The results of the statistical evaluation of the long-term stability study are summarised in Table 4.

Table 4: Results of the long-term stability tests. All outliers were retained.

CRM	Number of individual outlying results on a 99 % confidence level	Significance of the trend on a 95 % confidence level
ERM-AC051	1	no
ERM-AC053	0	no
ERM-AC082	2	no

None of the trends was statistically significant on a 99 % confidence level for any of the temperatures. The material can therefore be stored at +4 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during dispatch and storage were estimated, as described in [11] for each CRM. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{tt}$$ Equation 4

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum \left(t_i - \bar{t}\right)^2}} \cdot t_{sl}$$ Equation 5

s_{rel} relative standard deviation of all results of the stability study

t_i time elapsed at time point *i*

$\bar{t}$ mean of all t_i

t_{tt} chosen transport time (1 week at 60 ºC)

t_{sl} chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the +60 °C studies. The uncertainty describes the possible change during a dispatch at +60 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the +4 °C studies.

The results of these evaluations are summarised in Table 5.

Table 5: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of+ 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of +4 °C and 24 months

	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
ERM-AC051	0.018	0.024
ERM-AC053	0.065	0.124
ERM-AC082	0.038	0.070

As discussed for the homogeneity study, it is obvious that DSC overestimates the purity of the materials. This could lead to an overestimation of the stability of the compounds. If degradation would occur, it would most likely be an oxidative process, that would probably lead to degradation products which are more polar than the parent compounds. Such degradation products would probably not form solid solutions with the main component and would therefore be detectable by DSC. Due to this reason no further investigations on stability were done.

After the certification study, the materials will be included in the JRC's regular stability monitoring programme, to control its further stability.

5.4 Additional confirmation of stability

For the previous PAH CRMs, BCR-051R, BCR-053 and BCR-082R, no instability was detected over a period of 17 to 27 years. This confirms the finding of the long-term stability study.

Since the characterisation exercise took longer than planned, it was decided to check the material stability beyond the established 24 months shelf life. This was done by comparative analysis of number vials of the CRM stored at the normal storage temperature (+4 °C) and number references samples (placed at -20 °C directly after the material processing). Degradation at -20 °C is assumed to proceed at a different speed than at +4 °C, so this comparison is a useful confirmation of stability.

L5 performed two measurements each for all materials on samples stored at +4 and –20 °C, respectively. L6 performed 4 measurements on samples of ERM-AC082 from samples stored at +4 and -20 °C. All measurements were performed by qNMR and the results are shown in Table 6.

Table 6: Purities of samples stored at +4 and at -20 °C as determined by qNMR. Given are average values and their expanded uncertainties for the respective temperatures.

	L5		L6	
	Purity -20 °C [g/g]	Purity +4 °C [g/g]	Purity -20 °C [g/g]	Purity +4 °C [g/g]
ERM-AC051	0.987 ± 0.010	0.988 ± 0.010	not analysed	
ERM-AC053	0.995 ± 0.010	0.995 ± 0.010	not analysed	
ERM-AC082	0.983 ± 0.010	0.983 ± 0.010	0.9821 ± 0.0030	0.9843 ± 0.0036

The obtained results of the normal stock samples (stored at +4 °C) were compared with the values of the reference samples (stored at -20 °C) taking into account their uncertainties as stated by the laboratories. All purities for samples stored at +4 °C agreed with those of samples stored at -20 °C, demonstrating that no detectable change had occurred for 12 years and that the uncertainties stated in Table 5 are still valid.

6 Characterisation

For the characterisation of the purity of the three materials, several laboratories provided measurements using a variety of methods.

Two independent assessments of the purities were made:

- the mass balance approach, where individual impurities are quantified and the purity is determined as 1 – sum of impurities
- quantitative nuclear magnetic resonance (qNMR), which directly determines the purity of the target substance.

6.1 Selection of participants

Seven laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 or Good Manufacturing Practice (GMP) was obligatory. Where measurements are covered by the scope of accreditation/certification, the accreditation number is stated in the list of participants (Section 2).

The laboratory code (e.g. L1) is a random number and does not correspond to the order of laboratories in Section 2.

6.2 Study setup

For measurements by gas chromatography (GC), high-performance liquid chromatography (HPLC) and DSC, each laboratory received two vials of CRM and was requested to provide six independent results per method, three per vial. For qNMR, each laboratory received two samples stored at +4 °C and performed 1-3 measurements per vial. Laboratories were instructed to identify and quantify purities with a peak area percentage above 0.3 % whenever possible.

The vials for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The measurements had to be spread over at least two days to ensure intermediate precision conditions.

6.3 Methods used

The methods used for the mass-balance approach aimed at the quantification of organic impurities, inorganic impurities as well as residual solvents. In addition, quantitative NMR was used as a confirmation of the results of the mass balance. All methods used during the characterisation study are summarised in Annex D

<u>Organic impurities</u>

- GC utilizing flame ionisation detection (FID) and mass spectrometry (MS) detection. Columns of different polarity were used as well as two-dimensional GC (GCxGC-FID).
- HPLC with detection of absorption in the ultraviolet range (UV) employing different wavelengths for detection, different solvent mixtures and different gradients.

<u>Inorganic impurities</u>

- Inductively coupled plasma mass spectrometry (ICP-MS) and optical emission spectrometry (ICP-OES) after digestion was used for screening for 65 impurities

Residual solvents

- GC should also show residual solvents.
- DSC is also sensitive to residual solvents: Evaporation of solvent should lead to a disturbance of the heat flow curve, due to its evaporation enthalpy.

Confirmation of identity and determination of purity by direct methods

- Comparison of the retention times of the main compounds with retention times of commercial standards for the chromatographic methods was used as one method of confirming identity.
- DSC directly measures the purity of substances as long as impurities do not form solid solutions with the main compound.
- Elemental analysis (C, H, N, S) for a confirmation of the composition and detection of otherwise undetected impurities
- qNMR calibrated with substances with traceable values provided a second independent estimate of the purity of the substances.

All individual results of the participants, grouped per method are displayed in tabular and graphical form in Annex E.

6.4 Confirmation of identity

The identity of the substances is established by the processing, matching chromatographic signals, matching NMR signals and matching elemental composition.

6.4.1 Processing

The materials were derived from custom synthesis, which should result in the target substances as the main compound.

6.4.2 Chromatography

L3 used a commercial mixture of the 16 EPA PAHs to calibrate the HPLC and the GC x GC - FID method. The retention times of the main compounds in ERM-AC051, ERM-AC053 and ERM-AC053 matched those of the target compounds in the commercial mixture.

6.4.3 Elemental composition

Elemental analysis of hydrogen, nitrogen and carbon was used as proof of the identity of the compounds. Additionally, retention times observed in HPLC and GC matched with those of the respective compounds in calibrant mixtures. The results are summarised in Table 7.

Table 7: Results from elemental analysis. Errors are single standard deviations of 6 measurements. LOD: Limit of detection

		Mass fraction C [g/g]	Mass fraction H [g/g]	Mass fraction N [g/g]
ERM-AC051	Theoretical	0.952	0.0479	0
	Found	0.945 ± 0.012	0.0486 ± 0.0007	< LOD (0.0007)
ERM-AC053	Theoretical	0.9562	0.0438	0
	Found	0.955 ± 0.005	0.0454 ± 0.0011	< LOD (0.0007)
ERM-AC082	Theoretical	0.9418	0.0582	0
	Found	0.937 ± 0.009	0.0589 ± 0.00078	< LOD (0.0007)

Only trace amounts of other elements were found (see section 6.5.1). The elemental composition therefore matches the theoretical composition.

6.4.4 NMR

The NMR spectra of the main compounds matched the literature data for the target compounds in all laboratories.

6.4.5 Conclusion

NMR-data, retention times of liquid and two-dimensional gas-chromatography and overall elemental composition are in agreement with the assumed identity from the synthesis. The identity of the main compounds is therefore established beyond any doubt.

6.5 Quantification of the purity

6.5.1 Mass balance approach

Accurate determination of the mass fraction of impurities for the chromatographic methods would require identification of all impurities, calibration (or at least knowledge of their relative molecular mass and sensitivity) and subsequent quantification. However, as it was not possible to identify the impurities, results were reported in area-percent for the chromatographic methods and mass fractions for the inorganic impurities.

In the absence of reliable sensitivity factors for the impurities, area-fractions and mol-fractions are converted into mass fractions using a conversion factor of one. This is justified because of the high purity of the main compound: uncertainty of the response factors for the individual impurities results in a large uncertainty for each of them. However, as the amounts of these impurities are low, this amounts to a high uncertainty on a low mass fraction and does not lead to a high uncertainty of the purity of the main compound.

In order to obtain an overall estimate of the purity using the mass-balance approach, the impurities detected by GC-based methods, by HPLC-UV methods and the inorganic impurities were summed up and subtracted from unity.

Organic impurities

Some impurities detected by GC-MS could be tentatively identified as isomers and other structurally similar forms of the main compound or hydrogenated by-products of the synthesis.

The assumption was made that GC-based methods would detect a different set of impurities than HPLC-UV, therefore the impurities detected by GC-based methods were added to the impurities detected by HPLC-UV to obtain the total of organic impurities. This assumption was based on the different separation principles of the methods. Obviously, this can only be an approximation, because it is likely that some impurities are detected by both methods. The finally stated purity of the PAHs was probably underestimated, due to the calculation approach chosen, but could be considered as best estimate that can be achieved with the available data.

To estimate the mass fraction of impurities detected by a particular method (e.g. GC-based methods or HPLC-based methods), the following approach was taken:

The different datasets, including the chromatograms provided by the different laboratories were considered, choosing the dataset that detected the largest number of impurities (not necessarily being identical with the largest mass fraction of impurities). The impurities detected in this dataset were then taken as the best estimate of the total impurities detectable by this method. In order to obtain an estimate for the uncertainty associated with the purity estimate, a symmetric uncertainty interval was chosen such that it would cover any value reported in other datasets using the same method. This uncertainty interval was then considered to follow a rectangular distribution and consequently converted into a standard uncertainty. This standard uncertainty was then used as an estimate of the uncertainty of the impurity determination with this particular method. The uncertainty of the total impurities was calculated as the square root of the sum of squares of the uncertainties of the different methods contributing to the total impurities.

As a best estimate of impurities detectable by GC-based methods, GC-FID data was used. FID is generally considered to be the most universal detector with least differences in response to different compounds. Additionally, an analysis of the data and the chromatograms obtained revealed that overall GC-FID seemed to be able to detect the highest number of impurities (regarding the number of different impurities detected and the amount of impurities detected). GC-FID using a DB5 or DB17 column (i.e. columns of different polarity) did yield similar results. Therefore GC-FID data was used as a best estimate of all impurities that can be detected by GC-based methods.

Each laboratory checked for absence of peaks by injecting pure solvent.

Inorganic impurities

Results of the elemental analysis of nitrogen and sulphur are summed up with the inorganic impurities as determined by ICP-MS and ICP-OES to obtain the total of inorganic impurities.

The mass fractions of all detected elements were summed up. The standard uncertainty for this sum was estimated as 10 %.

The sum of the limits of detection of the non-detected elements was taken as upper limit of the mass fraction of the not-detected elements. For the calculation, the mass fraction of all not-detected elements was set to zero. This is justified as the sum of the LODs of not detected elements was only 1/5 to 1/20 of the sum of the detected impurities. The standard uncertainty of this estimate was assumed to follow a rectangular distribution between zero and the sum of all LODs and was therefore obtained by dividing the sum of all LODs by $\sqrt{3}$.

The final value for inorganic impurities was the sum of all detected impurities. The standard uncertainty of inorganic impurities was the combined uncertainty of the detected and not-detected impurities as described above.

Residual solvents

Residual solvents were assessed only in a qualitative manner. Each compound was subjected to a DSC analysis starting at room temperature. Evaporation of solvent should

lead to a disturbance of the heat flow curve, due to its evaporation enthalpy. The observed heat flow curves of the three compounds were smooth up to their melting point, therefore solvent residues should not be present.

This resulted in the following sets of data summarised in Table 8.

Table 8: Summary of impurities as determined by different methods and combined purity following the mass-balance approach

	ERM-AC051		ERM-AC053		ERM-AC082	
	Impurities [g/g]	u [g/g]	Impurities [g/g]	u [g/g]	Impurities [g/g]	u [g/g]
Impurities detected by GC-based methods	0.0081	0.0006	0.0006	0.0003	0.0065	0.0007
Impurities detected by HPLC-based methods	0.0172	0.0056	0.0027	0.0016	0.0095	0.0040
Inorganic impurities	0.0013	0.0001	0.0004	0.0001	0.0008	0.0001
Total impurities	0.0266	0.0060	0.0037	0.0016	0.0168	0.0041
Total purity	0.9734	0.0060	0.9963	0.0016	0.9832	0.0041

6.5.2 Direct determination of purity

DSC

DSC measurements were used as additional confirmation, but not taken into account for the calculation of the certified value. DSC will not detect impurities that form solid solutions with the main component. As it was to be expected that most of the impurities would be structurally very similar by-products of the synthesis of the PAHs, it could be assumed that some impurities would not be detected by DSC. It was also to be expected that those impurities that DSC was able to detect, would also have been detected by the other methods applied. Comparing the DSC results with those of the other methods, it was quite apparent that DSC overestimated the purity of the PAHs.

qNMR

qNMR measurements were performed by four laboratories using different internal standards and solvents. The values of the qNMR measurements themselves are traceable, generally via NIST SRM 350b (benzoic acid) to the International System of Unit (SI).

The values of the four laboratories agree within their respective uncertainties with the exception of the data from L1 for 6-methylchrysene, for which L1 found significantly lower values than obtained by mass balance and by the other qNMR measurements. The reason for this was elucidated by L6 which found that the purity values for 6-methylchrysene in deuterochloroform ($CDCl_3$) are concentration dependent, with lower concentrations having a lower purity (a 100-fold dilution resulted in a 60 fold increase of impurities). This was explained by reaction of the 6-methylchrysene with impurities of $CDCl_3$. This hypothesis was confirmed by mass spectrometric analysis of the solutions which found mono- and dichlorinated analogues of chrysene. Use of different batches of $CDCl_3$, yielded the same result. This effect would explain the lower values found by L1. On the other hand, also L7 used $CDCl_3$ and found significantly higher results than L1. The explanation could be the concentrations that were used: L7 used 13 – 28 mg/mL whereas L1 used 7 – 10 mg/mL. The

at least 30 % higher concentration used by L7 might explain the absence of an effect, although both laboratories did not notice any effect of the mass fraction used.

The data obtained using $CDCl_3$ on ERM-AC082 cast doubt on results obtained at lower concentrations in this solvent. The results of L1 which were obtained at a lower concentration were therefore excluded from the purity assessment, whereas the results from L7, which found no effect of concentration even at the threefold concentration than used by L1 were retained.

L5 and L6 used different solvents (D_6-dimethylsulfoxide (DMSO) D_8-toluene), which are not affected by this effect. The results of all labs are summarised in Table 9.

Table 9: Summary purities as determined by qNMR. Data from L1 for ERM-AC082 were not used for value assignment. Coverage factors used were 2 (L1, L5 and 7) and 2.26 (L6), respectively.

	Purity ERM-AC051		Purity ERM-AC053		Purity ERM-AC082	
	Purity [g/g]	U [g/g]	Purity [g/g]	U [g/g]	Purity [g/g]	U [g/g]
L1	0.9818	0.0100	0.9957	0.0080	*0.9665*	*0.0110*
L5	0.9873	0.0099	0.9951	0.0099	0.9831	0.0098
L6	Not analysed		Not analysed		0.9843	0.0036
L7	0.9868	0.0084	0.9943	0.0074	0.9825	0.0039

The unweighted mean of the four laboratory means was used as the combined value for qNMR. For the uncertainty budgets submitted by the laboratories, it seems that the integration, together with the weighing of the samples are the main contributions of the measurement uncertainties. These factors are independent of one another and therefore reduce by the square root of the number of results averaged. The combined uncertainty was therefore estimated as

$$u_{qNMR} = u\sqrt{\frac{u_{L1}^2+u_{L5}^2+u_{L6}^2+u_{L7}^2}{3^2}} \qquad \text{Equation 6}$$

u_{L1}, u_{L5}, u_{L6}, u_{L7} standard uncertainties of the results of L1-L7

Note that the denominator 3 in Equation 6 is the sensitivity coefficient of 1/3 , which comes from the division by 3 in averaging three results for each laboratory. The combined purity as determined by qNMR is shown in Table 10.

Table 10: Combined purity as determined by qNMR

Purity ERM-AC051		Purity ERM-AC053		Purity ERM-AC082	
Purity [g/g]	U [g/g]	Purity [g/g]	U [g/g]	Purity [g/g]	U [g/g]
0.9853	0.0054	0.9950	0.0053	0.9830	0.0037

6.5.3 Combination of qNMR and mass-balance approach

The results of the purity determined by the mass balance approach and by qNMR are shown in Figure 3.

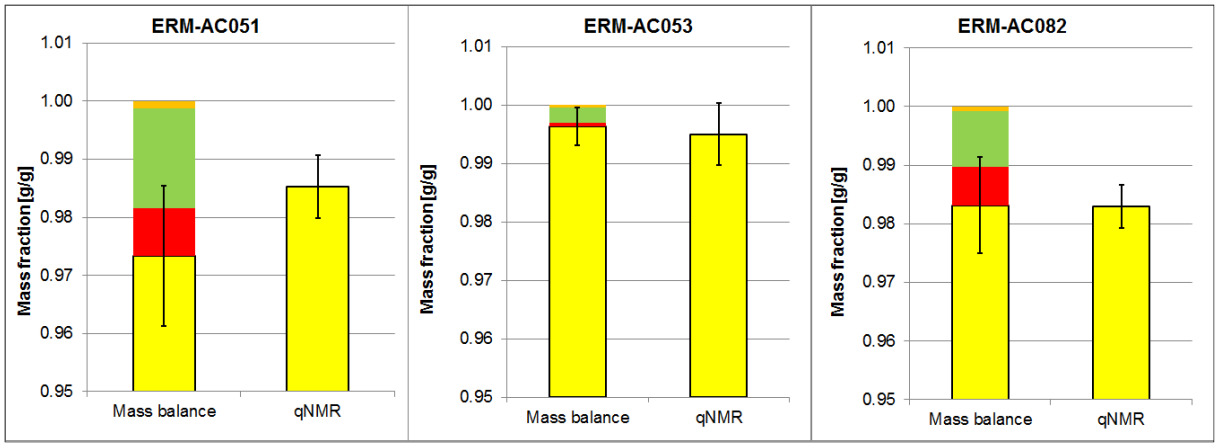


Figure 3: Comparison of the results of the purity determination by mass balance and qNMR. Yellow: Mass fraction of the main compound. Red: impurities detected by HPLC. Green: impurities detected by GC. Orange: inorganic impurities. The error bar on the main compound is the expanded uncertainty (k=2).

The results from the mass balance approach and from qNMR agree for all three materials within the respective uncertainties even if for ERM-AC051, the difference is slightly above 0.01 g/g. Whether this is due to double counting of some impurities in the mass-balance approach, due to some undetected interference in qNMR or just random fluctuation as reflected in the uncertainties is not known.

As the purity obtained by mass balance and by qNMR agree the purity of the three PAHs is equal to the average of the purities as determined by qNMR and the mass balance approach (Equation 7).

$$w_{purity} = \frac{w_{qNMR} + w_{mass\ balance}}{2}$$ Equation 7

w_{purity}	combined purity of the starting material
w_{qNMR}	purity as determined by qNMR
$w_{mass\ balance}$	purity as determined by the mass balance approach

The uncertainty was calculated according to Equation 8. Note that the denominator 2 in Equation 8 is the sensitivity coefficient of 1/2, which comes from the division by 2 in Equation 7.

The results are reported in Table 11:

$$u_{char} = \sqrt{\frac{u_{qNMR}^2}{2^2} + \frac{u_{Mmass\ balanceB}^2}{2^2}}$$ Equation 8

u_{char}	uncertainty of characterisation
u_{qNMR}	uncertainty derived from the purity determination by qNMR
$u_{mass\ balance}$	uncertainty derived from the impurity determination

Table 11: Combined purities of the materials

	Purity [g/g]	Combined standard uncertainty u_{char} [g/g]	Relative combined standard uncertainty $u_{char,rel}$ [%]
ERM-AC051	0.9794	0.0033	0.34
ERM-AC053	0.9956	0.0016	0.16
ERM-AC082	0.9831	0.0022	0.23

7 Value Assignment

Certified values were assigned for the purity.

Certified values are values that fulfil the highest standards of accuracy. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [3] were established.

7.1 Certified values and their uncertainties

The assigned uncertainty consists of uncertainties relating to characterisation, u_{char} (Section 6), potential between-vial inhomogeneity, u_{bb} (Section 4.1), and potential degradation during transport, u_{sts}, and long-term storage, u_{lts} (Section 5). The uncertainty related to inhomogeneity/degradation during transport was found to be negligible. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\,rel}$) with a coverage factor *k* given as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2 + u_{char,rel}^2} \qquad \text{Equation 9}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{sts} and u_{lts} were estimated as described in section 5.3

A coverage factor *k* of 2 was applied, to obtain the expanded uncertainties. The certified values and their uncertainties are summarised in Table 12.

For ERM-AC053 this resulted in an uncertainty interval exceeding a purity of 1 g/g. Therefore the upper limit of the uncertainty interval was truncated at 1 g/g, resulting in an asymmetric uncertainty interval.

Table 12: Certified values and their uncertainties for ERM-AC051, ERM-AC053 and ERM-AC082. The asymmetric uncertainty interval of ERM-AC053 is based on a standard uncertainty of 0.0022 g/g.

	Mass fraction [g/g]	$u_{char,rel}$ [%]	$u_{bb,rel}$ [%]	$u_{lts,rel}$ [%]	$u_{sts,rel}$ [%]	U_{CRM} [g/g]
ERM-AC051, benzo[*a*]pyrene	0.979	0.34	0.002	0.024	0.018	0.007
ERM-AC053, indeno[1,2,3-*cd*]pyrene	0.996	0.16	0.061	0.12	0.065	+ 0.004 - 0.005
ERM-AC082, 6-methylchrysene	0.983	0.23	0.028	0.070	0.038	0.005

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

The known synthetic route should result in the substances as the main compound. This assumption was confirmed by agreement of the NMR spectra with literature data, agreement of the elemental composition with the theoretical composition as well as the agreement of the retention time of the main compound with the retention times of standard solutions.

Therefore, the identity of the substances is established beyond any doubt.

Quantity value

The purity values have been obtained by a combination of subtraction of the sum of impurities from unity and direct quantification by qNMR. The sum of impurities as such is not a value traceable to the International System of Units (SI), as it was not possible to identify and calibrate each measurement for the individual impurities. Still, the purity of the compounds can be considered SI traceable, as the fraction of impurities was small and associated with a conservative uncertainty estimate.

The values by qNMR are traceable to the SI by the traceability of the standards used for quantification.

The purity values are therefore SI traceable, as they are combinations of two SI traceable values.

8.2 Commutability

Many measurement procedures include one or more steps which select specific (or specific groups of) analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions that define this concept. For instance, the CLSI Guideline C53-A [12] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

PAH calibrants are normally prepared by dissolving the solid substances in a solvent or they are directly purchased as solutions from commercial suppliers. There is no reason to assume that a solution prepared from ERM-AC051, ERM-AC053 or ERM-AC082 would behave differently from solutions prepared from solid PAHs of other suppliers or that it would behave differently from a commercially available calibrant solution. Therefore, ERM-AC051, ERM-AC053 and ERM-AC082 are considered commutable to commercially available calibrants or preparations in the individual laboratory.

9 Instructions for use

9.1 Safety information

The three substances are classified as hazardous. Specific hazards and safety measures are listed in the safety data sheets (SDS) available at https://crm.jrc.ec.europa.eu.

9.2 Storage conditions

The materials should be stored at +4 °C (between +1 and +9 °C) in the dark.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially for opened vials.

9.3 Minimum sample intake

The minimum sample intake for all materials is 1 mg.

It is recommended to use an appropriate balance in order to minimise weighing uncertainties when weighing such small quantities.

9.4 Use of the certified value

The main purpose of this material is for instrument calibration. This material can also be used to assess own calibrants.

For assessing own calibrants, the measured values of the CRMs are compared with the certified values following a procedure described in an ERM application note [13]. The procedure is described here in brief

- Calculate the absolute difference between the mean measured value and the certified value (Δ_m)
- Combine the measurement uncertainty (u_m) with the uncertainty of the certified value (u_{CRM}): $u_\Delta = \sqrt{u_m^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two (k = 2), corresponding to a confidence interval of approximately 95 %
- If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

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11 References

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Annexes

Annex A: Results of the homogeneity measurements

Annex B: Results of the short-term stability studies

Annex C: Results of the long-term stability studies

Annex D: Summary of methods used in the characterisation study

Annex E: Results of the characterisation measurements

Annex A: Results of the homogeneity measurements

Table A.1: Homogeneity data for ERM-AC051

	Purity [mol-%]		
Vial no.	**Replicate 1**	**Replicate 2**	**Replicate 3**
40	99.88	99.87	99.87
79	99.88	99.87	99.87
115	99.88	99.87	99.87
226	99.87	99.88	99.87
263	99.88	99.88	99.88
328	99.87	99.87	99.87
405	99.87	99.88	99.87
494	99.87	99.87	99.87
598	99.87	99.88	99.88
617	99.89	99.87	99.87
672	99.88	99.87	99.88
760	99.88	99.88	99.87
784	99.87	99.87	99.87
863	99.88	99.86	99.86
913	99.89	99.87	99.87

Table A.2: Homogeneity data for ERM-AC053

	Purity [mol-%]	
Vial no.	**Replicate 1**	**Replicate 2**
34	99.48	*99.6*
118	99.72	*99.62*
220	99.52	*99.78*
269	99.62	*99.48*
382	99.68	*99.61*
465	99.42	*99.66*
572	99.72	*99.58*
710	99.51	*99.58*
861	99.72	99.72
960	99.8	99.85

Table A.3: Homogeneity data of ERM-AC082

	Purity [mol-%]	
Vial no.	**Replicate 1**	**Replicate 2**
1	99.72	99.68
136	99.7	99.72
249	99.7	99.64
319	99.74	99.58
413	99.66	99.73
503	99.71	99.63
616	99.71	99.66
840	99.76	99.7
937	99.74	99.75
741	99.69	99.55

Annex B: Results of the short-term stability studies

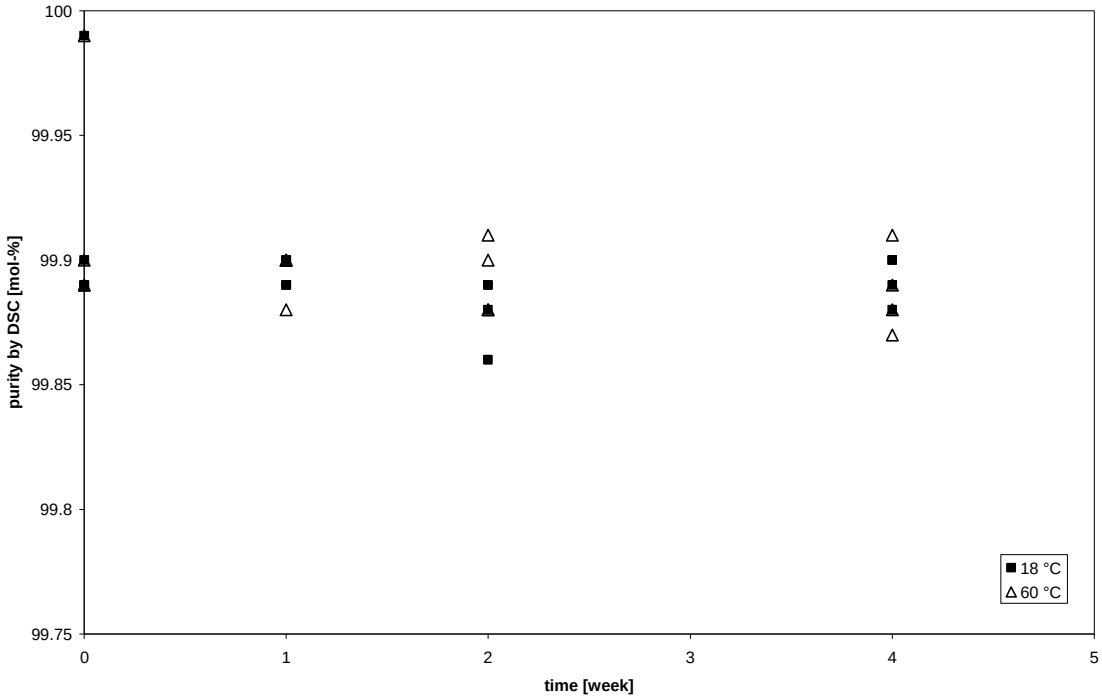

Figure B.1: Short-term stability graph for ERM-AC051

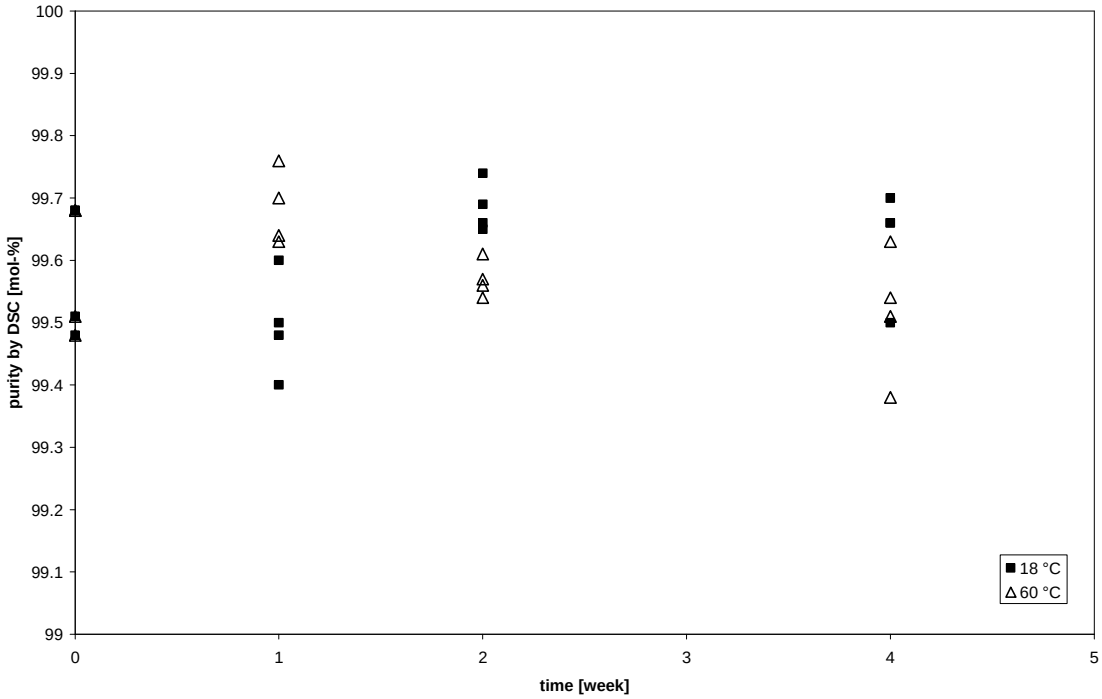

Figure B.2: Short-term stability graph for ERM-AC053

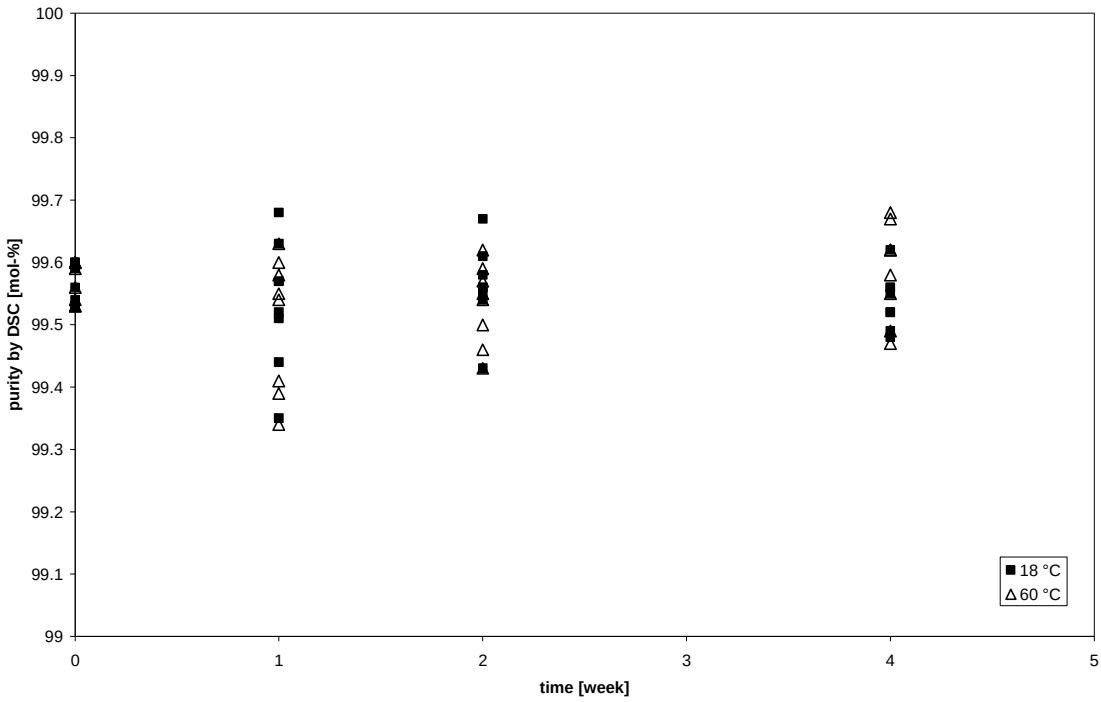

Figure B.3: Short-term stability graph for ERM-AC082

Annex C: Results of the long-term stability studies

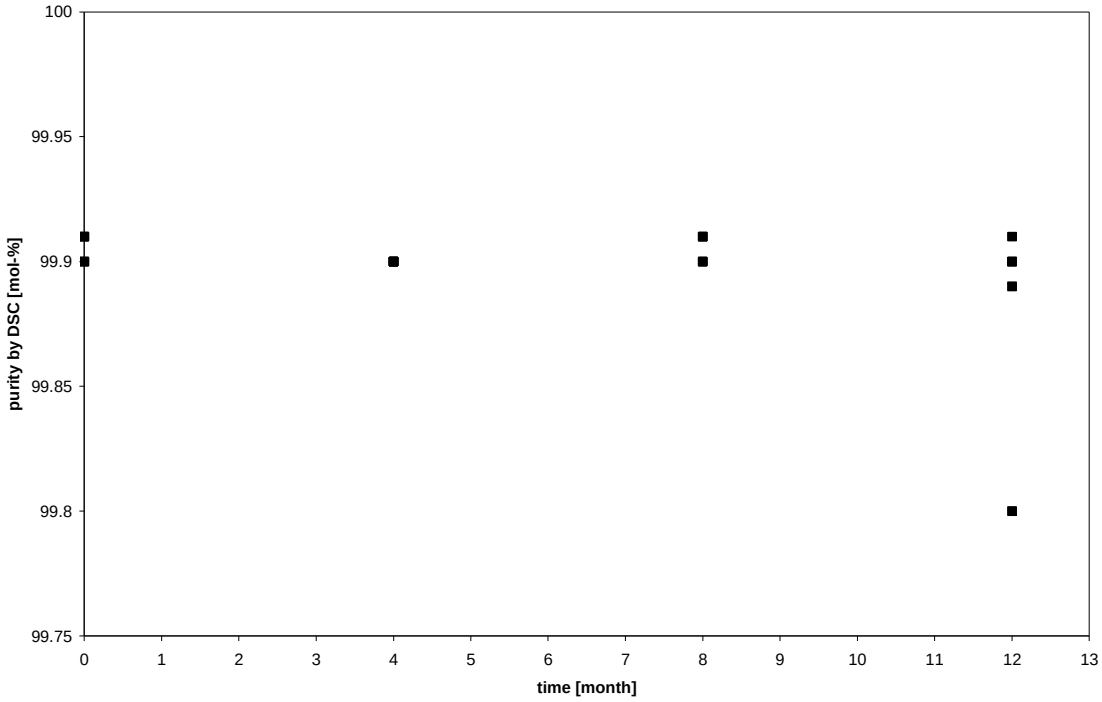

Figure C.1: Long-term stability graph for ERM-AC051, test temperature 4 ºC

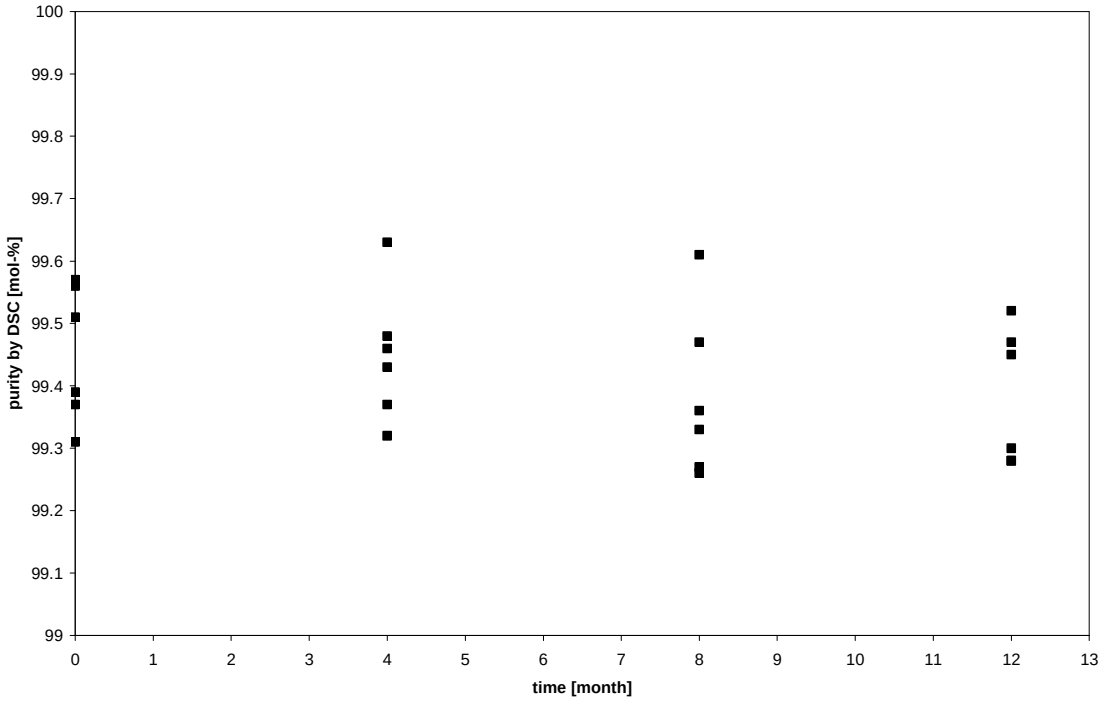

Figure C.2: Long-term stability graph for ERM-AC053, test temperature 4 ºC

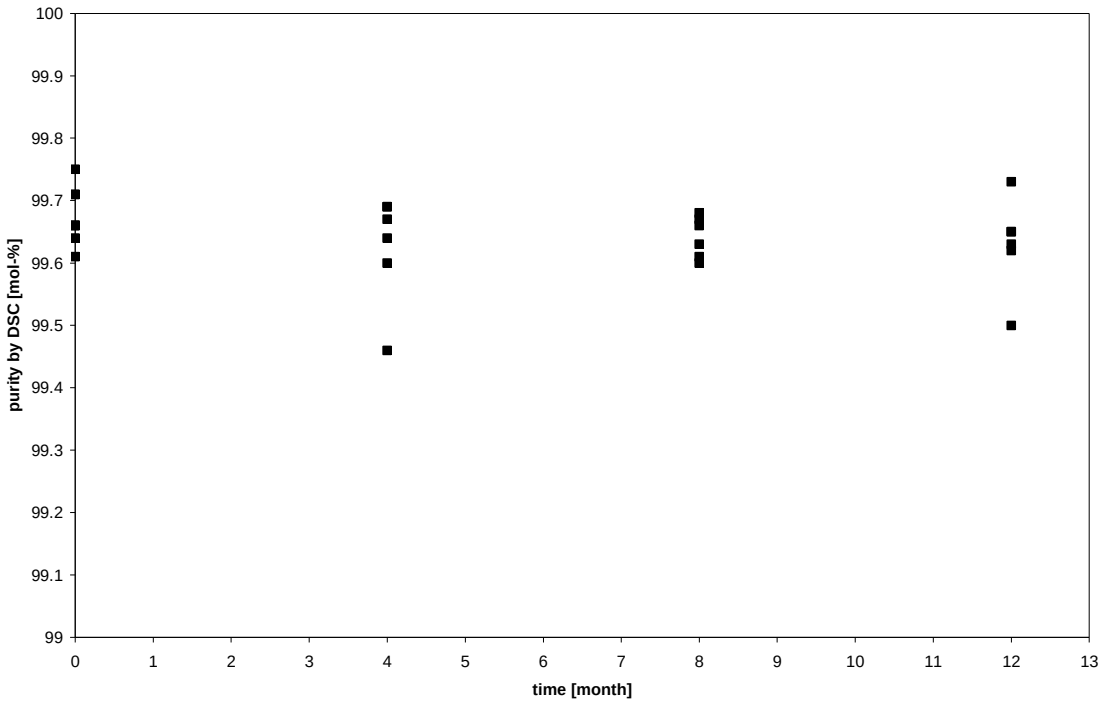

Figure C.3: Long-term stability graph for ERM-AC082, test temperature 4 ºC

Annex D: Summary of methods used in the characterisation study

Laboratory code - Method	Description
L1 - GC-FID DB5	Gas chromatography with flame ionisation detection, using a DB5 column Column dimensions (length x diameter x film thickness): 60 m x 0.32 mm x 0.25 µm Carrier gas: H_2, 2 mL/min Temperature programme: 1 min @ 100 ºC increase to 280 ºC with 50 ºC/min 55 min @ 280 ºC Detector temperature: 300 ºC
L1 - GC-FID DB17	Gas chromatography with flame ionisation detection, using a DB17 column Column dimensions (length x diameter x film thickness): 30 m x 0.32 mm x 0.25 µm Carrier gas: H_2, 2 mL/min Temperature programme: 1 min @ 100 ºC increase to 280 ºC with 50 ºC/min 55 min @ 280 ºC Detector temperature: 300 ºC
L3 – GCxGC FID	Comprehensive two-dimensional gas chromatography using flame ionization detection First dimensional column: RTX-1 (30 m x 0.32 mm x 0.25 µm) Second dimensional column: BPX50 (1.5 m x 0.10 mm x 0.1 µm) Carrier gas: H_2, 0.4 mL/min Temperature programme: 2 min @ 40 ºC increase to 100 ºC with 10 ºC/min 1 min @ 100 ºC increase to 330 ºC with 3 ºC/min 10 min @ 330 ºC Sample intake: 2 – 5 mg

Laboratory code - Method	Description
L1 - GC-MS DB5MS The results coded L1-GC-MS-DB5MS and L1 - GC-MS DB17 have been obtained on different instruments, but using the same sample solution. They are considered independent, as different instruments and columns were used.	Gas chromatography with mass spectrometric detection, using a DB5MS column Column dimensions (length x diameter x film thickness): 30 m x 0.25 mm x 0.25 µm Carrier gas: He, 1 mL/min Temperature programme: 3 min @ 80 ºC increase to 120 ºC with 30 ºC/min increase to 310 ºC with 5 ºC/min 10 min @ 310 ºC Injector temperature: 80 ºC on column Detector temperature: 220 ºC
L1 - GC-MS DB17 The results coded L1-GC-MS-DB5MS and L1 - GC-MS DB17 have been obtained on different instruments, but using the same sample solution. They are considered independent, as different instruments and columns were used.	Gas chromatography with mass spectrometric detection, using a DB17 column Column dimensions (length x diameter x film thickness): 60 m x 0.25 mm x 0.25 µm Carrier gas: He, 2 mL/min Temperature programme: 3 min @ 80 ºC increase to 120 ºC with 30 ºC/min increase to 310 ºC with 5 ºC/min 10 min @ 310 ºC Injector temperature: 80 ºC on column Detector temperature: 220 ºC

Laboratory code - Method	Description
L4 - GC-MS DB17MS	Gas chromatography with mass spectrometric detection, using a DB17MS column Column dimensions (length x diameter x film thickness): 30 m x 0.25 mm x 0.25 µm Carrier gas: He, 1.5 mL/min Temperature programme: 1.5 min @ 80 ºC increase to 260 ºC with 10 ºC/min increase to 320 ºC with 3 ºC/min 10 min @ 320 ºC Injector temperature (PTV injection): 80 ºC (0.01 min), increase to 330 ºC with 8 ºC/s, hold 5 min Ion source temperature: 250 ºC Sample intake: 2 – 5 mg
L1 - HPLC-UV x nm	High performance liquid chromatography with UV detection at x=254, 270, 274, 285, 294 nm, using an Ultrasep ES PAH column. 6-methylchrysene Column dimensions (length x diameter): 125 mm x 2 mm Flow: 0.25 mL/min Column temperature: 25 ºC Solvent: Acetonitirile/chloroform 10:1 (v:v) Gradient: time (min) / acetonitrile (%) / water (%) 0 - 22 / 80 / 20 22 - 26 / from 80 to 90 / from 20 to 10 26 - 60 / 90 / 10

Laboratory code - Method	Description
L2 - HPLC-UV 254 nm	High performance liquid chromatography with UV detection at 254 nm, using a Nacalai Tesque Cosmosil 5 PYE column Column dimensions (length x diameter): 150 mm x 4.6 mm Column temperature: 30 ºC Gradient: time (min) / water (%) / methanol (%) 0 - 2 / 20 / 80 2 – 10 / from 20 to 0 / from 80 to 100 10-25 / 0 / 100
L3 - HPLC-UV 250 nm	High performance liquid chromatography with UV detection at 250 nm, using a Vydac 201TP5415 column Column temperature: 35 ºC Solvent: Acetonitirile/water Gradient: time (min) / acetonitrile (%) / water (%) 0 - 20 / from 50 to 100 / from 50 to 0 20 - 29 / 100 / 0 Sample intake: 5 mg
L4 - HPLC-UV 254 nm	High performance liquid chromatography with UV detection at 254 nm, using a Supelcosil LC-PAH column Column dimensions (length x diameter): 150 mm x 4.6 mm Particle size: 5 µm Flow: 1.5 mL/min Gradient: time (min) / water:acetonitrile (90:10 v:v) (%) / acetonitrile (%) 0 - 5 / 60 / 40 5 – 30 / from 60 to 0 / from 40 to 100 30 - 45 / 0 / 100 Sample intake: 2 – 5 mg

Laboratory code - Method	Description
L1 - DSC	Differential scanning calorimetry 0.7 to 1.7 mg samples sealed into 50 µL aluminium pans
L4 - DSC	Differential scanning calorimetry Sample intake 1.16 – 1.8 mg
L1 - ICP-MS	Measurement of inorganic impurities (65 trace elements) by ICP-MS (with collision cell in He-mode) after high pressure microwave digestion using nitric acid Temperature programme: time (min) temperature (ºC) 16 20 - 160 15 160 - 250 45 250 Starting pressure: 50 bar, increasing to 100 bar at 250 ºC
L1 - ICP-OES	Measurement of S and P by ICP-OES after high pressure microwave digestion using nitric acid Temperature programme: time (min) temperature (ºC) 16 20 - 160 15 160 - 250 45 250 Starting pressure: 50 bar, increasing to 100 bar at 250 ºC
L1 – COMB-COND	Determination of H, C, N using a Elementar vario MACRO (Elementar, DE) based on catalytic combustion of the sample with oxygen at 960 °C, separation of the reaction gases with absorption columns and quantification via thermal conductivity detection.
L1 – COMB-UVF	Determination of S with a TN/TC 3000b (Thermo Electron, Waltham, US): Samples were dissolved in toluene, were combusted and the S content is quantified by UV fluorescence.

Laboratory code - Method	Description
L1-qNMR	Dissolution of 10 mg substance and 10 mg internal standard were dissolved in 1.0 and 1.5 mL CDCl3. Internal standard: 1,3,5 trioxane (Merck 8.21190.0100) assayed in-house by qNMR against NIST SRM 350a (bezoic acid) Instrument and settings: Bruker Avance 600: spectral window: 9615 Hz; relaxation delay 60 s; excitation pulse: 7 µs Evaluated line: ^{1}H resonance frequency at 600.20 MHz
L5-qNMR	Dissolution of 10-15 mg substance and 8-10 mg internal standard were dissolved in 1.4 mL $(CD_3)_2SO$ from Euriso-top, Saarbruecken (DE). Internal standard: CRM for qNMR TraceCert®: ethyl 4-(dimethylamino)benzoate (Fluka 42582) (benzo[a]pyrene)/ 1,2,4,5 tetramethylbenzene (Fluka 74658) (indeno(1,2,3-*cd*]pyrene; 6-methylchrysene) Instrument and settings: Bruker Avance III 600: spectral window: 14423 Hz (benzo[a]pyrene indeno(1,2,3-*cd*]pyrene)/12335 Hz (6-methylchrysene); relaxation delay 1 s (benzo[a]pyrene indeno(1,2,3-*cd*]pyrene)/ 30 s (6-methylchrysene); excitation pulse: 12 µs
L6-qNMR	Dissolution of 8 mg substance and 3 mg internal standard were dissolved in 1 mL C_7D_8 (deuterotoluene) from Sigma-Aldrich. Internal standard: CRM for qNMR TraceCert®: dimethyl terephthalate (Fluka 070328) Instrument and settings: Bruker Avance 600: spectral width: 20 ppm; relaxation delay 30 s
L7-qNMR	Dissolution of 20 mg substance and 10 mg internal standard in 0.7, 1.0 and 1.4 mL CD2Cl2 (benzo(a)pyrene, indeno(1,2,3-*cd*]pyrene) or CDCl3 (6-methylchrysene) Internal standard: CRM for qNMR TraceCert®: 1,2,4,5-tetramethylbenzene (Sigma 74658) Instrument and settings: Bruker Ascend 400: spectral window 812.8 Hz; relaxation delay 1 s

Annex E: Results of the characterisation measurements

Table E.1: Summary of data for ERM-AC051. Values in bold were used as estimate for purity for the respective methods.

Laboratory code - Method	Mean purity [g/g]	Standard deviation [g/g]
L1 - HPLC-UV 254 nm[1)]	0.98941	0.00008
L1 - HPLC-UV 270 nm[1)]	0.98837	0.00005
L1 - HPLC-UV 285 nm[1)]	0.99260	0.00014
L2 - HPLC-UV 254 nm	0.98892	0.00029
L3 - HPLC-UV 250 nm	0.99033	0.00015
L4 - HPLC-UV 254 nm	**0.98285**	**0.00130**
L1 - GC-MS DB5MS	0.99550	0.00026
L1 - GC-MS DB17	0.99190	0.00030
L3 - GCxGC	0.99318	0.00041
L4 - GC-MS DB17MS	0.99562	0.00015
L1 - GC-FID DB5	**0.99187**	**0.00006**
L1 - GC-FID DB17	0.99293	0.00046
L1 – inorganic[2)]	**0.99866**	**0.00014**
L1 - DSC	0.99907	0.00021
L4 - DSC	0.99874	0.00004
L1-qNMR	**0.98188**	**0.00679**
L5-qNMR	**0.98728**	**0.00206**
L7-qNMR	**0.98679**	**0.00385**

1) The three results with laboratory code 4 have been obtained in the same analytical runs, using a diode array detector. Results from three selected wavelengths of these runs have been reported. They are not considered as independent results.

2) purity considering only inorganic impurities

Table E.2: HPLC data for ERM-AC051. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for HPLC methods.

Laboratory	L1 - HPLC-UV 254 nm	L1 - HPLC-UV 270 nm	L1 - HPLC-UV 285 nm	L2 - HPLC-UV 254 nm	L3 - HPLC-UV 250 nm	**L4 - HPLC-UV 254 nm**
Main peak %	98.941 ± 0.008	98.837 ± 0.005	99.260 ± 0.014	98.892 ± 0.029	99.033 ± 0.015	**98.285 ± 0.13**
Impurity 1 %	0.028 ± 0.004	0.037 ± 0.005	0.040 ± 0.000	0.092 ± 0.008	0.087 ± 0.006	**0.202 ± 0.042**
Impurity 2 %	0.085 ± 0.010	0.053 ± 0.008	0.023 ± 0.005	1.013 ± 0.030	0.740 ± 0.017	**0.092 ± 0.022**
Impurity 3 %	0.020 ± 0.000	0.020 ± 0.000	0.042 ± 0.004		0.138 ± 0.021	**0.325 ± 0.087**
Impurity 4 %	0.110 ± 0.000	0.307 ± 0.005	0.515 ± 0.005			**0.035 ± 0.005**
Impurity 5 %	0.705 ± 0.010	0.610 ± 0.011	0.075 ± 0.005			**0.130 ± 0.000**
Impurity 6 %	0.082 ± 0.004	0.103 ± 0.005	0.010 ± 0.000			**0.780 ± 0.017**

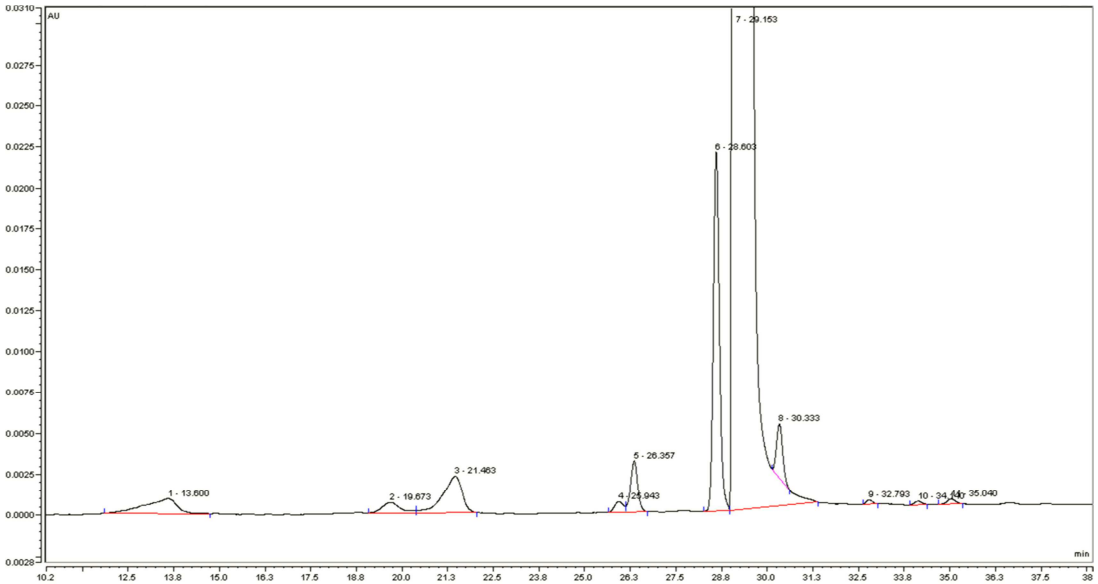

Figure E.1: Example chromatogram of 'L4 HPLC-UV 254 nm'. These data were used as best estimate of impurities detectable by HPLC-UV-methods for ERM-AC051.

Table E.3: GC data for ERM-AC051. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for GC methods.

Laboratory	**L1 - GC-FID DB5**	L1 - GC-FID DB17	L1 - GC-MS DB5MS	L1 - GC-MS DB17	L3 - GCxGC FID	L4 - GC-MS DB17MS
Main peak %	**99.187 ± 0.006**	99.293 ± 0.047	99.550 ± 0.026	99.190 ± 0.030	99.318 ± 0.041	99.562 ± 0.015
Impurity 1 %	**0.125 ± 0.003**	0.117 ± 0.007	0.053 ± 0.005	0.123 ± 0.005	0.093 ± 0 .008	0.307 ± 0.008
Impurity 2 %	**0.133 ± 0.001**	0.146 ± 0.009	0.070 ± 0.006	0.148 ± 0.008	0.085 ± 0 .004	0.128 ± 0.008
Impurity 3 %	**0.527 ± 0.004**	0.425 ± 0.047	0.327 ± 0.018	0.543 ± 0.027	0.327 ± 0 .023	
Impurity 4 %	**0.018 ± 0.001**	0.013 ± 0.002			0.179 ± 0.018	
Impurity 5 %	**0.011 ± 0.001**	0.006 ± 0.002				

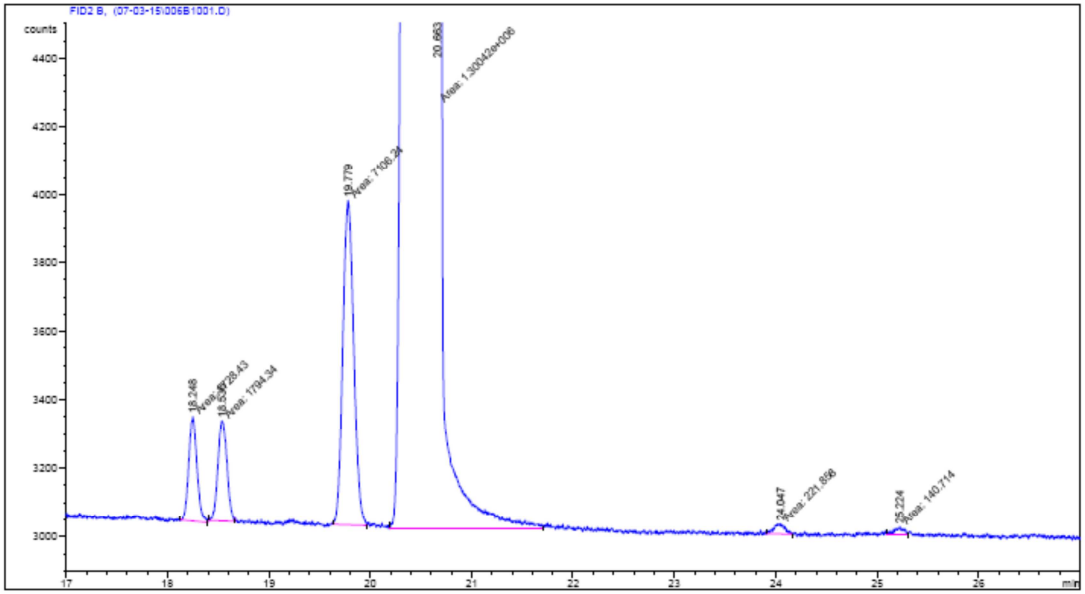

Figure E.2: Example chromatogram of 'L1 GC-FID DB5'. These data were used as best estimate of impurities detectable by GC-methods for ERM-AC051.

Table E.4: Average results by DSC and qNMR for ERM-AC051. Error for DSC is the standard deviation of six determinations. Errors for qNMR are expanded uncertainties (k=2)

Laboratory	L1 - DSC	L4 - DSC	**L1 - qNMR**	**L5 - qNMR**	**L7 - qNMR**
Purity %	99.907 ± 0.021	99.87378	**99.19 ± 0.98**	**98.73 ± 0.49**	**98.68 ± 0.84**

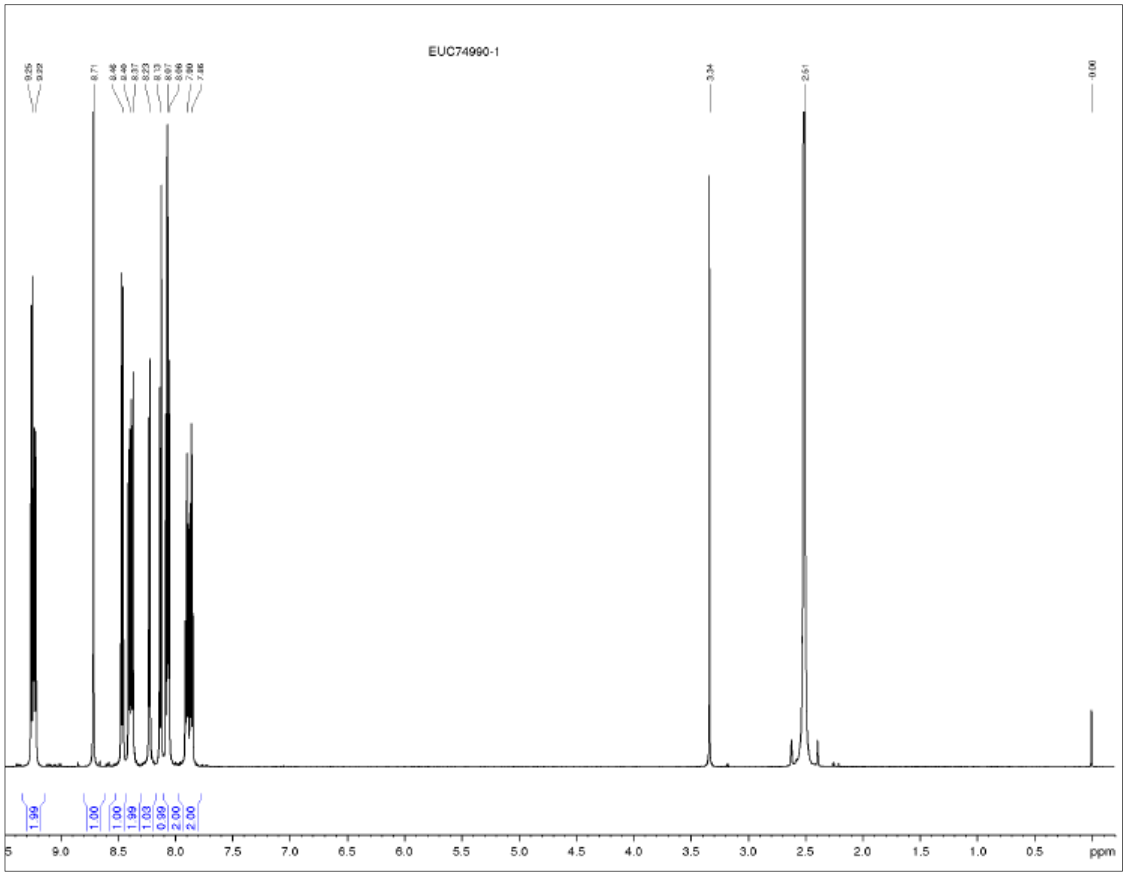

Figure E.3: Example ^{1}H NMR spectrum (L5).

Table E.5: Data for the inorganic impurities of ERM-AC051

Inorganic impurities	Reported impurities [g/g]	Impurity value used in calculations [g/g]	Standard uncertainty [g/g]
S (S analyser)	0.00104	Not used	Not used
Detected 15 elements including S (ICP-OES, ICP-MS)	**0.00134**	**0.00134**	**0.00013**
Sum of not detected 53 elements [g/g] (ICP-OES, ICP-MS)	**0.00007**	**0.00000**	**0.00004**

Table E.6: Summary of data for ERM-AC053. Values in bold were used as estimate for purity for the respective methods

Method	Mean purity [g/g]	Standard deviation [g/g]
L1 - HPLC-UV 254 nm[2)]	0.99750	0.00021
L1 - HPLC-UV 274 nm[2)]	**0.99727**	**0.00021**
L1 - HPLC-UV 294 nm[2)]	0.99812	0.00018
L2 -HPLC-UV 254 nm	1.00000	n.d. [1)]
L3 - HPLC-UV 250 nm	0.99780	0.00032
L4 - HPLC-UV 254 nm	0.99683	0.00040
L1 - GC-MS DB5MS[3)]	0.99985	0.00008
L1 - GC-MS DB17[3)]	1.00000	n.d. [1)]
L3 - GCxGC	0.99838	0.00040
L4 - GC-MS DB17MS	1.00000	n.d. [1)]
L1 - GC-FID DB5	0.99943	0.00040
L1 - GC-FID DB17	**0.99943**	**0.00019**
L1 - inorganic[4)]	**0.99960**	**0.00006**
L1 - DSC	0.99470	0.00083
L4 - DSC	0.99630	0.00094
L1 - qNMR	**0.99565**	**0.00295**
L5 - qNMR	**0.99505**	**0.00102**
L7 - qNMR	**0.99429**	**0.00319**

1) n.d. not defined either because all results were 1.0000 or because only 2 measurements were performed (l5-qNMR)

2) The three results with laboratory code 1 have been obtained in the same analytical runs, using a diode array detector. Results from three selected wavelengths of these runs have been reported. They are not considered as independent results.

4) Purity considering only inorganic impurities

Table E.7: HPLC data for ERM-AC053. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for HPLC methods.

Laboratory	L1 - HPLC-UV 254 nm	**L1 - HPLC-UV 274 nm**	L1 - HPLC-UV 294 nm	L2 - HPLC-UV 254 nm	L3 - HPLC-UV 250 nm	L4 - HPLC-UV 254 nm
Main peak %	99.75 ± 0.028	**99.727 ± 0.021**	99.812 ± 0.018	100.000 ± 0.000	99.780 ± 0.032	99.683 ± 0.040
Impurity 1 %	0.010 ± 0.000	**0.022 ± 0.004**	0.010 ± 0.000		0.013 ± 0.003	0.024 ± 0.017
Impurity 2 %	0.010 ± 0.000	**0.008 ± 0.004**	0.022 ± 0.004		0.054 ± 0.012	0.085 ± 0.026
Impurity 3 %	0.030 ± 0.006	**0.010 ± 0.000**	0.087 ± 0.008		0.043 ± 0.013	0.010 ± 0.000
Impurity 4 %	0.107 ± 0.012	**0.040 ± 0.006**	0.040 ± 0.011		0.016 ± 0.001	0.132 ± 0.050
Impurity 5 %	0.053 ± 0.010	**0.010 ± 0.000**	0.020 ± 0.000		0.101 ± 0.026	0.022 ± 0.004
Impurity 6 %	0.023 ± 0.005	**0.020 ± 0.000**				0.048 ± 0.017
Impurity 7 %		**0.082 ± 0.012**				0.015 ± 0.005
Impurity 8 %		**0.050 ± 0.006**				
Impurity 9 %		**0.020 ± 0.010**				

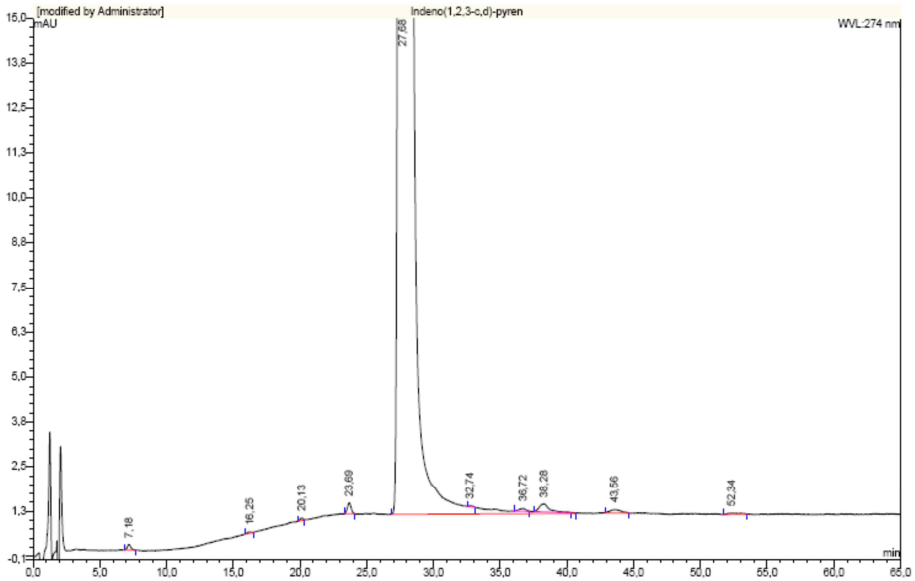

Figure E4 Example chromatogram of 'L1 HPLC-UV 274 nm'. These data were used as best estimate of impurities detectable by HPLC-UV-methods for ERM-AC053.

Table E.8: GC data for ERM-AC053. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for GC methods.

Laboratory	L1 - GC-FID DB5	**L1 - GC-FID DB17**	L1 - GC-MS DB5MS	L1 - GC-MS DB1[)]	L3 - GCxGC FID	L4 - GC-MS DB17MS
Main peak %	99.943 ± 0.040	**99.943 ± 0.019**	99.985 ± 0.008	100.000 ± 0.000	99.838 ± 0.04	100.000± 0.000
Impurity 1 %	0.007 ± 0.004	**0.007 ± 0.003**	0.006 ± 0.003		0.068 ± 0.026	
Impurity 2 %	0.003 ± 0.002	**0.010 ± 0.005**	0.012 ± 0.004		0.044 ± 0.017	
Impurity 3 %	0.016 ± 0.011	**0.012 ± 0.004**			0.049 ± 0.014	
Impurity 4 %	0.006 ± 0.004	**0.005 ± 0.003**				
Impurity 5 %	0.020 ± 0.016	**0.017 ± 0.007**				
Impurity 6 %	0.006 ± 0.005	**0.007 ± 0.003**				

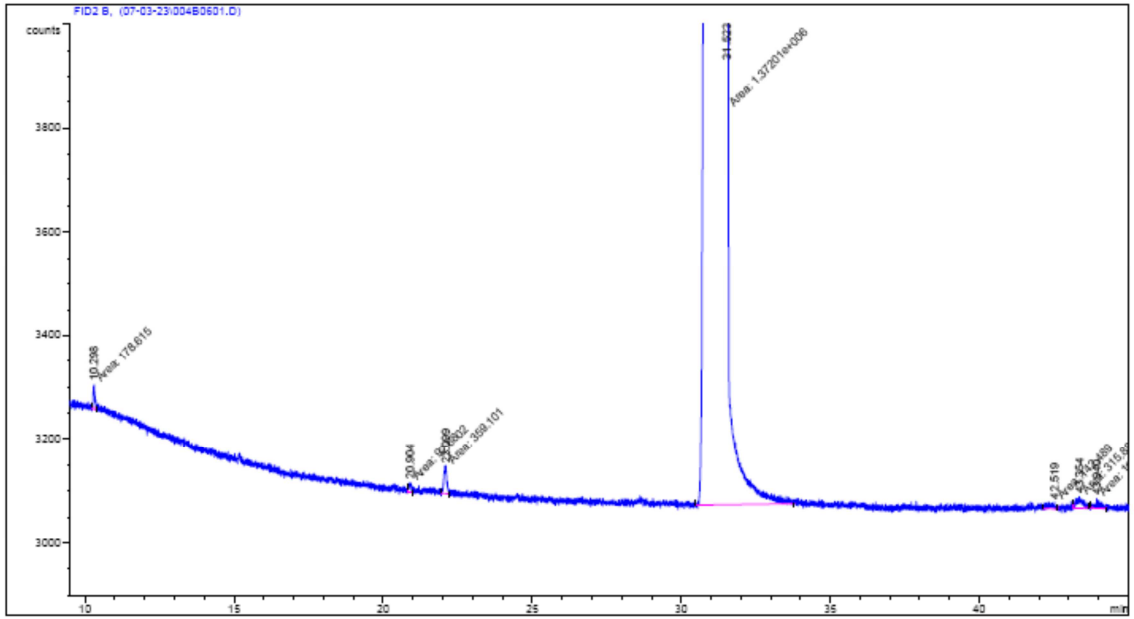

Figure E.5: Example chromatogram of 'L1 GC-FID DB5'. These data were used as best estimate of impurities detectable by GC-methods for ERM-AC053.

Table E.9: Average results by DSC and qNMR for ERM-AC053. Error for DSC is the standard deviation of six determinations. Errors for qNMR are expanded uncertainties (k=2)

Laboratory	L1 - DSC	L4 - DSC	**L1 - qNMR**	**L5 – qNMR**	**L7 – qNMR**
Purity %	99.470 ± 0.083	99.630 ± 0.094	**99.57 ± 1.00**	**99.51 ± 1.00**	**99.43 ± 0.74**

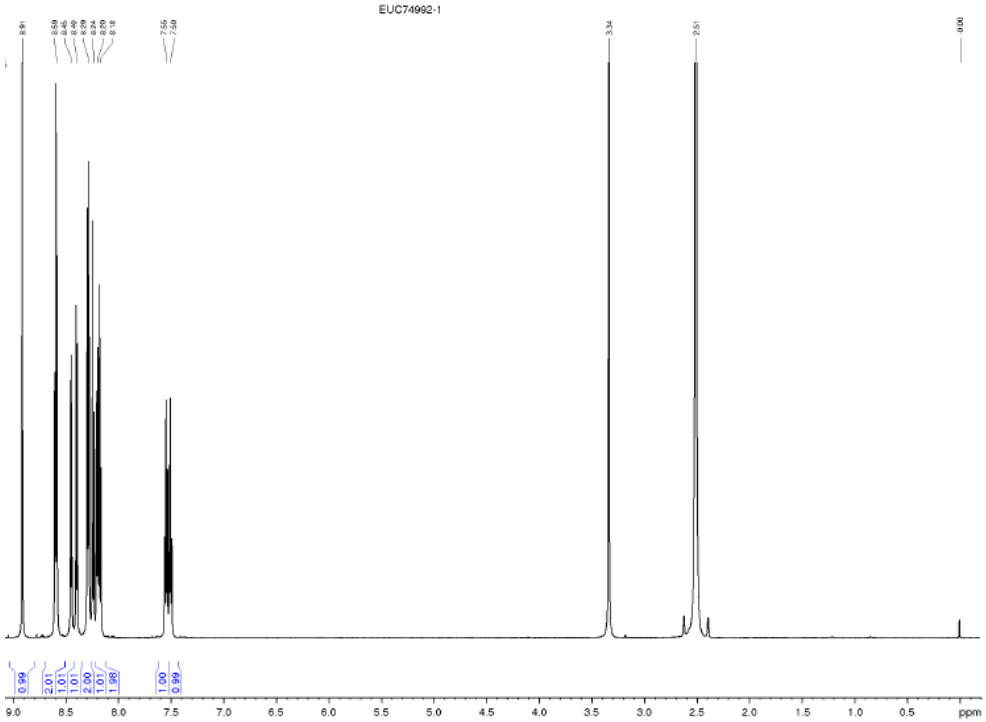

Figure E.6: Example ^{1}H NMR spectrum (L5).

Table E.10: Data for the inorganic impurities of ERM-AC053

Inorganic impurities	Reported impurites [g/g]	Impurity value used in calculations [g/g]	Standard uncertainty [g/g]
S (S analyser) [g/g]	0.00003	Not used	Not used
Detected 12 elements including S (ICP-OES, ICP-MS)	**0.00040**	**0.00040**	**0.00004**
Sum of not detected 55 elements [g/g] (ICP-OES, ICP-MS)	**0.00007**	**0.00000**	**0.00004**

Table E.11: Summary of data for ERM-AC082. Values in bold were used as estimate for purity for the respective methods

Method	Mean purity [g/g]	Standard deviation [g/g]
L1 - HPLC-UV 254 nm[1)]	**0.99045**	**0.00001**
L1 - HPLC-UV 270 nm[1)]	0.99380	0.00020
L2 - HPLC-UV 254 nm	0.99742	0.00021
L3 - HPLC-UV 250 nm	0.98658	0.00081
L4 - HPLC-UV 254 nm	0.99025	0.00018
L1 - GC-MS DB5MS[2)]	0.99690	0.00028
L1 - GC-MS DB17[2)]	0.99732	0.00056
L4 - GC-MS DB17MS	0.99640	0.00015
L3 - GCxGC	0.99628	0.00083
L1 - GC-FID DB5	**0.99350**	**0.00013**
L1 - GC-FID DB17	0.99477	0.00039
L1 – inorganic[2)]	**0.99920**	**0.00008**
L1 - DSC	0.99648	0.00051
L4 - DSC	0.99690	0.00040
L1 – qNMR [3)]	0.96645	0.00812
L5 - qNMR	**0.98318**	**0.00433**
L6 - qNMR	**0.98325**	**0.00228**
L7 - qNMR	**0.98249**	**0.00100**

1) The two results with laboratory code 1 have been obtained in the same analytical runs, using a diode array detector. Results from two selected wavelengths of these runs have been reported. They are not considered as independent results.
2) purity considering only inorganic impurities
3) Not used because of reaction of methylchrysene with impurities in $CDCl_3$

Table E.12: HPLC data for ERM-AC082. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for HPLC methods.

Laboratory	**L1 - HPLC-UV 254 nm**	L1 - HPLC-UV 270 nm	L2 - HPLC-UV 254 nm	L3 - HPLC-UV 250 nm	L4 - HPLC-UV 254 nm
Main peak %	**99.045 ± 0.012**	99.38 ± 0.02	99.742 ± 0.021	98.658 ± 0.081	99.025 ± 0.018
Impurity 1 %	**0.010 ± 0.000**	0.018 ± 0.004	0.117 ± 0.02	0.054 ± 0.005	0.035 ± 0. 005
Impurity 2 %	**0.010 ± 0.000**	0.010 ± 0.000	0.038 ± 0.008	0.105 ± 0.009	0.118 ± 0 .004
Impurity 3 %	**0.040 ± 0.000**	0.020 ± 0.000	0.052 ± 0.012	0.170 ± 0.014	0.240 ± 0 .006
Impurity 4 %	**0.025 ± 0.037**	0.082 ± 0.004	0.052 ± 0.013	0.380 ± 0.009	0.16 ± 0. 006
Impurity 5 %	**0.127 ± 0.065**	0.063 ± 0.005		0.198 ± 0.02	0.060 ± 0.000
Impurity 6 %	**0.292 ± 0.155**	0.140 ± 0.009		0.049 ± 0.007	0.010 ± 0.000
Impurity 7 %	**0.120 ± 0.035**	0.050 ± 0.000		0.233 ± 0.020	0.172 ± 0.013
Impurity 8 %	**0.042 ± 0.016**	0.205 ± 0.016		0.157 ± 0.023	0.178 ± 0.008
Impurity 9 %	**0.057 ± 0.114**	0.010 ± 0.000			

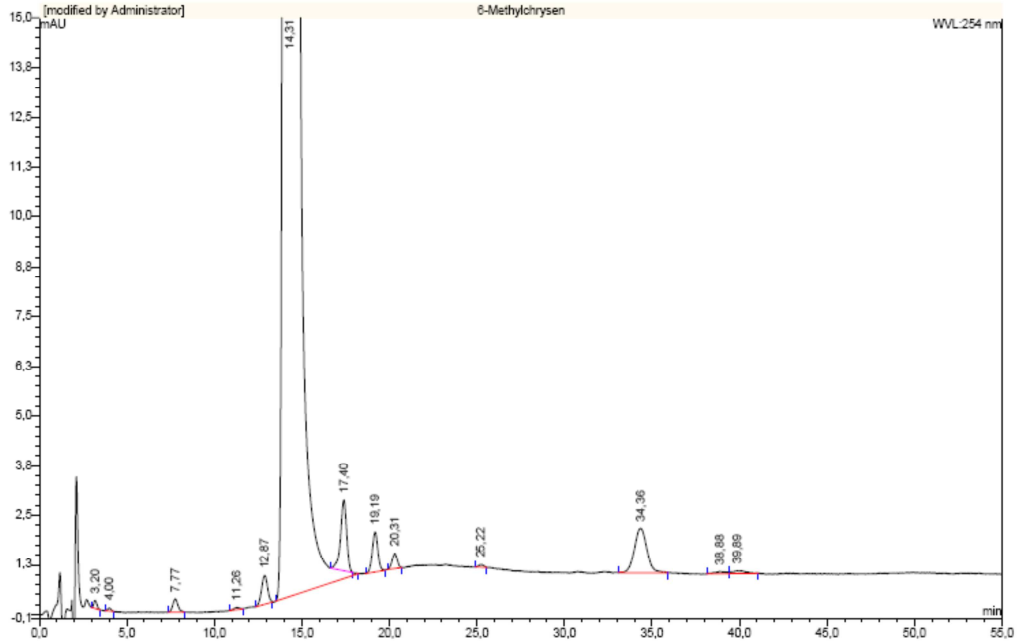

Figure E.7: Example chromatogram of 'L1 HPLC-UV 254 nm'. These data were used as best estimate of impurities detectable by HPLC-UV-methods for ERM-AC082.

Table E.13: GC data for ERM-AC082. Given are averages and standard deviations of the area percentages of six determinations. The column in bold was used as estimate for purity for GC methods.

Laboratory	**L1 - GC-FID DB5**	L1 - GC-FID DB17	L1 - GC-MS DB5MS	L1 - GC-MS DB17	L3 - GCxGC FID	L4 - GC-MS DB17MS
Main peak %	**99.35 ± 0.013**	99.477 ± 0.039	99.69 ± 0.028	99.732 ± 0.056	99.628 ± 0.083	99.64 ± 0.015
Impurity 1 %	**0.083 ± 0.005**	0.078 ± 0.004	0.032 ± 0.004	0.062 ± 0.010	0.061 ± 0 .014	0.198 ± 0.008
Impurity 2 %	**0.215 ± 0.005**	0.215 ± 0.005	0.085 ± 0.010	0.158 ± 0.021	0.165 ± 0 .035	0.115 ± 0.005
Impurity 3 %	**0.110 ± 0.006**	0.045 ± 0.023	0.070 ± 0.011	0.048 ± 0.029	0.059 ± 0 .017	0.048 ± 0.004
Impurity 4 %	**0.060 ± 0.000**	0.043 ± 0.008	0.035 ± 0.005		0.086 ± 0.027	
Impurity 5 %	**0.030 ± 0.009**	0.012 ± 0.004	0.088 ± 0.012			
Impurity 6 %	**0.153 ± 0.012**	0.125 ± 0.018				

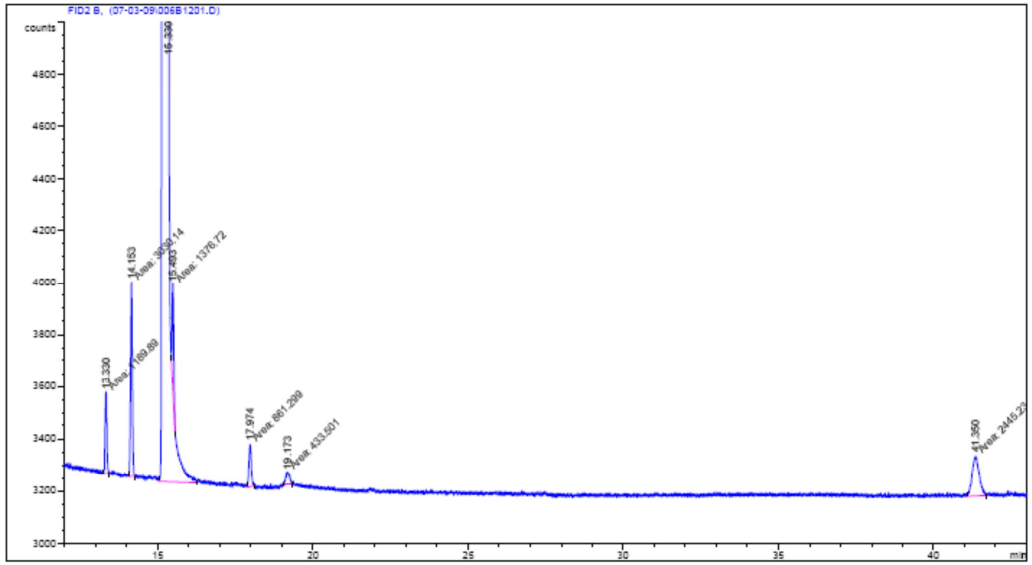

Figure E.8: Example chromatogram of 'L1 GC-FID DB5'. These data were used as best estimate of impurities detectable by GC-methods for ERM-AC082.

Table E.14: Average results by DSC and qNMR for ERM-AC082. Error for DSC is the standard deviation of six determinations. Errors for qNMR are expanded uncertainties (k=2)

Laboratory	L1 - DSC	L4 - DSC	L1 - qNMR	**L5 – qNMR**	**L6 – qNMR**	**L7 – qNMR**
Purity %	99.648 ± 0.051	99.690 ± 0.040	96.65 ± 0.97	**98.32 ± 0.98**	**98.325 ± 0.36**	**98.25 ± 0.39**

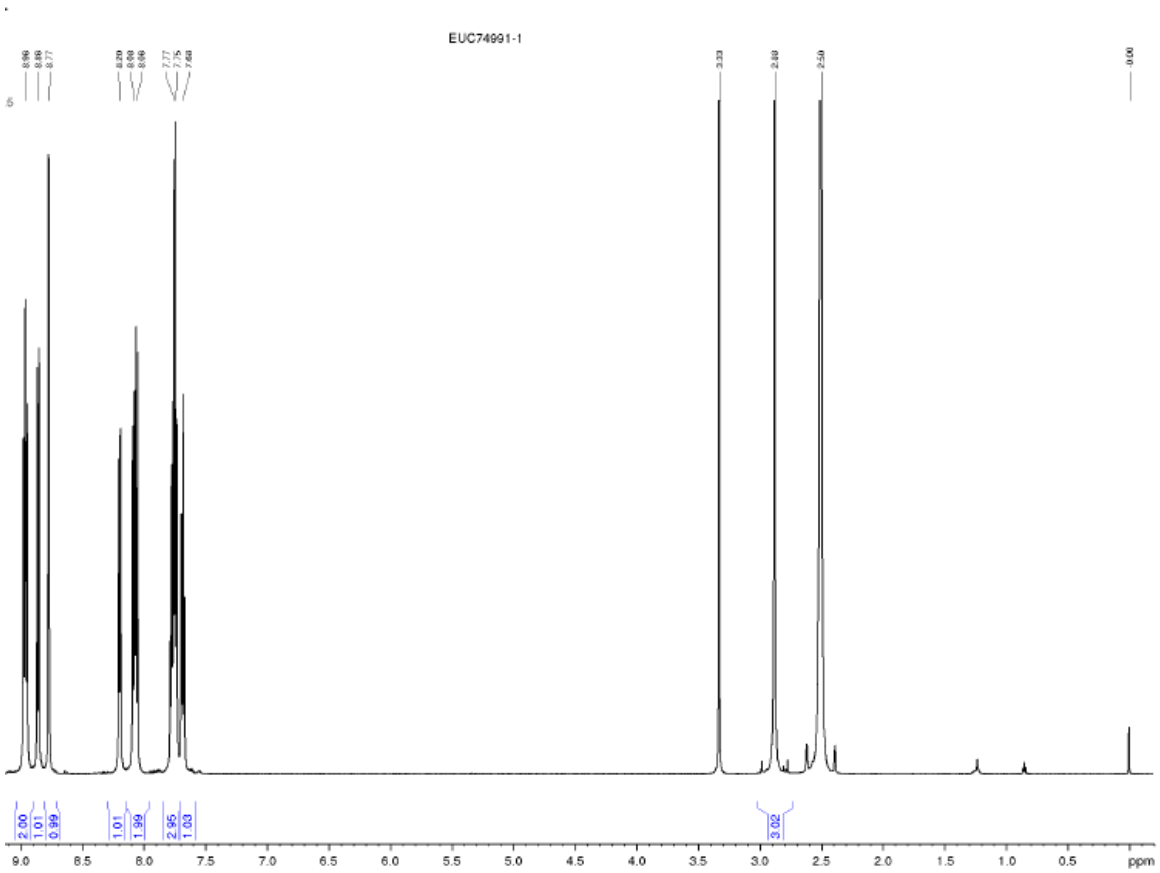

Figure E.9: Example ^{1}H NMR spectrum (L5).

Table E.15: Data for the inorganic impurities of ERM-AC082

Inorganic impurities	Reported impurites [g/g]	Impurity value used in calculations [g/g]	Standard uncertainty [g/g]
S (S analyser)	0.00039	Not used	Not used
Detected 18 elements including S (ICP-OES, ICP-MS)	**0.00080**	**0.00080**	**0.00008**
Sum of not detected 50 elements [g/g] (ICP-OES, ICP-MS)	**0.00006**	**0.00000**	**0.00004**

European Commission

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