

# Development of a primary reference material for the analysis of BTEX and chlorobenzenes in environmental samples

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## ABSTRACT

Benzene, toluene, ethylbenzene, and xylene (BTEX) and chlorobenzenes are measured in all environments as part of world official pollution control programs. The health impact of these analytes increased the relevance of the validity and comparability of the measurement results. Therefore, equipment performance, implementation of test methods, and accuracy of results need to be verified through internal and external quality control programs. Herein, a new certified reference material (CRM) for benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene, and m, p-xylene has been produced and characterized by an approach that is metrologically valid and complies with ISO 17034:2016 and ISO 33405:2024 standards. The reference material was prepared gravimetrically and certified values were calculated based on masses of pure components. Homogeneity results proved that the between-ampule heterogeneity was negligible in comparison with the method precision. The stability of the material was evaluated for both long-term storage and dispatch and no measurable loss of analytes was observed within one year period. The developed certified reference material with small uncertainties values will support regulatory bodies, environmental protection agencies, and testing laboratories in their efforts to improve the quality of motoring results and compliance with regulations.

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## 1. INTRODUCTION

Measurement results are used as legal evidence for compliance with concentration levels of regulated pollutants established by regulatory organizations. Thus, the need for reliable and accurate measurements of controlled toxic substances has increased rapidly. Benzene and its derivatives including benzene, toluene, ethylbenzene, and xylene (BTEX) and chlorobenzenes are among the most commonly worldwide regulated pollutants. These compounds are utilized as solvents or reactants in many different industrial sectors to prepare widely used products including paint, rubber, detergent, resin, and pesticides [1], [2]. The large use of these substances and the lack of good waste management control lead to ecosystem contamination and human exposure [3], [4]. Human exposure to BTEX can cause neurological conditions, cancer, and harm to the kidney, heart, liver, lungs, and respiratory system, among other side effects [5], [6]. Therefore, the effective monitoring of BTEX and chlorobenzenes in environmental systems is assumed to be of prime importance in controlling the adverse effects of these pollutants on human and animal health. But there are many challenges affecting the accuracy and precision of BTEX and chlorobenzenes measurement results including their low concentration in the environmental samples and availability of ISO17034 certified

reference materials (CRMs) needed for calibration and performance check of measuring instruments, validation of analytical methods, internal quality control and interlaboratory comparisons [7]–[12]. Certified reference materials have become a necessity, especially for the quantitative measurement of these analytes in real samples. Although CRMs are needed for these materials, there are no reference materials characterized by primary methods for both BTEX and chlorobenzenes described in our manuscript. In the current paper, the process of preparation and characterization of BTEX and chlorobenzenes reference material is described. The purity of starting materials was established through the assessment of mass fractions of potential impurities in the material including water content as well as organic impurities [13]–[15]. The reference material was formulated gravimetrically and certified values were calculated based on the mass of each component. Additionally, value assignment, homogeneity, and stability evaluation of the prepared reference material were also highlighted.

## 2. RESEARCH METHOD

### 2.1. Reagent and chemicals

The starting materials for the CRMs were purchased from Merck (Germany) including high pure benzene, toluene, chlorobenzene, ethylbenzene, o, m, p-xylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene were purchased from Merck (Germany). High-performance liquid chromatography (HPLC) grade solvents, and methanol were purchased from Sigma-Aldrich (Germany). Karl Fisher reagents were purchased from Scharlau (Spain). No additional purification was required for organic solvents used in all processes.

### 2.2. Identity and organic impurities determination

Agilent 6890N gas chromatography consists of a flame ionization detector (FID), a 5975 inert XL mass selective detector, and an automated injector (7683B). The suspected organic impurities were separated using a DB-VRX column, (60 m, ID 0.25 mm, and 0.25  $\mu\text{m}$  film thickness) from J&W Scientific (USA). Helium was used as a carrier gas at constant flow (1.0 mL/min). The oven temperature was programmed as follows: 50  $^{\circ}\text{C}$  for 5 min increased by 5  $^{\circ}\text{C}/\text{min}$  to 240  $^{\circ}\text{C}$  with a final hold for 5 min. The injector, FID detector, and mass spectrometer (MS) interface temperatures were held constant at 250, 300, and 290  $^{\circ}\text{C}$ , respectively. The mass spectrometer was used at default operating conditions for the ion source, quadrupole. Three samples from each material were injected directly into gas chromatography (GC)-MS without any further treatments. Agilent Chemstation software was used for processing and analysis of chromatographic data.

### 2.3. Water content determination

The water content of each starting material was determined using a Metrohm 852 Titrando coulometric KF system; the samples were transferred to a titration cell for measurement without any additional preparations. Five samples from each material were analyzed and the mean value was used as an indicator of water content.

### 2.4. Blending and preparation of material

The total amount of all substances in a sample is equal to the mass fraction of the target component plus the mass fractions of all impurities. Hence, the sum of the measured mass fractions of water and organic impurities is used to estimate the purity of the compounds by subtracting it from 100%. The corrected weight of each compound was calculated and the mixture for each of the ten compounds was filled gravimetrically in the order of decreasing density as follows: ethylbenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, chlorobenzene, benzene, toluene, o, m, and p-xylene. The mixture was homogenized for 6 h before bottling in the final form. The material was packed in 2 mL brown glass ampoules under precisely regulated conditions to avoid any loss of material due to the volatility of the solution. The prepared batch consisted of approximately 100 ampoules, which were grouped into ten groups and assigned unique codes based on the order of filling. Each group contained ten ampoules. All ampoules were stored at a controlled temperature of about 4  $^{\circ}\text{C}$  for further studies.

### 2.5. Homogeneity testing

In order to determine the heterogeneity of the prepared reference material, the homogeneity experiment was designed in compliance with ISO 33405:2024. This standard recommends selecting ten units for evaluating both within and between ampoules homogeneity of small batches. Thus, 10 ampoules were selected in a random order taking into account the filling sequence including the first and last filled ampoule, selected ampoules were divided into three sub-samples and then analyzed by gas chromatograph three times

with the same conditions. Samples were randomly tested to evaluate the effect of the filling order of the ampules. Analysis of variance (ANOVA) was used to evaluate the data in order to estimate the uncertainty resulting from the heterogeneity effect and assess sample homogeneity.

## 2.6. Stability testing

The resulting mixture was subjected to isochronous stability studies in order to assess the materials' stability during transportation and storage. A stratified random sampling approach was applied to select seventeen ampules from the prepared patch, the ampules were divided into eight groups. Two groups were kept at 4 and 40 °C, while one ampule was kept at the reference temperature of -20 °C. One ampoule in each group was relocated to the reference temperature at the two-storage temperature after 3, 6, 9, and 12 months for the long-term stability study. Six samples were stored at 40 °C for the short-term stability study, the ampoules were transferred to reference temperature after 1, 2, 3, 4, 5, and 6 weeks. The ampules used to estimate the initial value for t=0 were initially kept at the reference temperature (-20 °C). After the study was completed, all ampules were tested under repeatability conditions in one batch. The data were statistically analyzed to estimate shelf life and uncertainty due to the instability of materials.

## 3. RESULTS AND DISCUSSION

### 3.1. Characterization of materials

The identity and purity of the starting materials were confirmed by their mass and area normalization methods using GC-MS and GC-FID, respectively. The chromatographic separation of suspected organic impurities was achieved with a nonpolar DB-VRX column. The peaks of target compounds were well resolved without interferences by impurities with baseline resolution higher than 1.5 except for m and p-xylenes. The quantification of detected organic impurities was estimated based on an appropriate approximation of their peak areas the total chromatogram peaks areas. The purities of benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene, m, and p-xylene used for the preparation of reference material were based on chromatographic purities in combination with water content by coulometric Karl Fischer titration method. The weight of each compound was calculated and the mixture for each of the ten compounds was filled gravimetrically in the order of decreasing density to avoid loss of analytes by evaporation. The gravimetrically prepared concentrations of benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene were confirmed by GC-MS as illustrated in Figure 1, whereas the concentration of m and p-xylene was not confirmed due to difficulties in chromatographic separation and presented as a reference value.

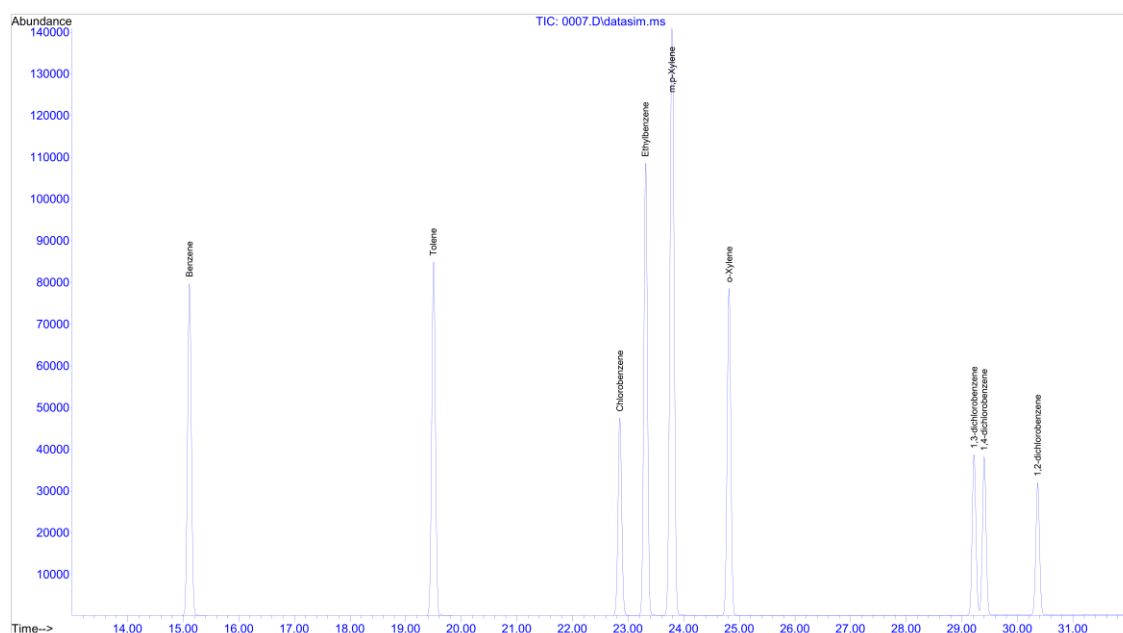


Figure 1. Gas chromatographic chromatogram of BTEX and chlorobenzenes by DB-VRX column and mass selective detection.

### 3.2. Determination of water content

Using a coulometric Karl Fischer titrator with a diaphragmed cell generator and 10  $\mu$ A polarization current, the water content of the raw materials was measured. The endpoint was automatically detected at low stop drift (10  $\mu$ g/min) for accurate determination of water mass fraction. One gram of each material was precisely weighed and added to the titration chamber. Each material was measured three times, and the mean values were calculated and used to correct the target value of each material. In all materials, the highest impurity content is represented by the water content.

### 3.3. Homogeneity study

Ten ampoules were tested to determine the homogeneity of the prepared batch; three subsamples from each ampoule were subjected to GC-FID analysis under repeatability conditions. Grubb's test was used to examine the homogeneity study data for outliers and ANOVA statistical tool was used to evaluate significant variations in blended mass fractions. The calculated  $F$  value was compared to the tabulated value for each analyte at a confidence level of approximately 95%. The  $P$ -value was deemed to be statistically significant when  $P$ -value  $< 0.05$  [16]–[18]. Based on the  $P$ -value and  $F$  test, no statistically significant variability was detected between the ampoules, as indicated by the results of the homogeneity test shown in Table 1. For all analytes, the  $F$ -distribution is smaller than the  $F$ -critical value and the  $P$ -value is greater than 0.05. The value of uncertainty resulting from material heterogeneity ( $u_{bb}$ ) for each analyte was measured using the mean squares  $MS_{\text{between}}$  and  $MS_{\text{within}}$  from ANOVA tables. The uncertainty due to materials heterogeneity was combined with uncertainties from stability and characterization to estimate expanded uncertainty associated with each certified value [16]–[18]. A remarkable degree of concordance among the prepared ampoules was demonstrated by the small values of uncertainty due to materials heterogeneity ( $u_{bb}$ ). The batch that was being certified was deemed sufficiently homogenous since there was a good agreement amongst ampoules for all analytes.

Table 1. Summary of homogeneity, stability, certified values, and expanded uncertainties of BTEX and chlorobenzenes reference material

Analyte	Homogeneity Results						Stability Results		Characterization Results	
	$MS_{\text{between}}$	$MS_{\text{within}}$	$F$	$P$ -value	$F$ critical	$u_{bb}$	$u_{st}$	$u_{ch}$	Concentration ( $\mu$ g/g)	$U_{\text{exp}}$ ( $\mu$ g/g)
Benzene	1.58E+10	1.10E+10	1.43	0.24	2.39	0.0015	0.0007	9.99	999.21	9.99
Toluene	6.67E+10	5.26E+10	1.27	0.31	2.39	0.0021	0.0013	9.99	998.66	9.99
Chlorobenzene	1.11E+10	9.73E+09	1.14	0.38	2.39	0.001	0.0008	10.00	999.82	10.00
Ethylbenzene	2.60E+10	1.61E+10	1.61	0.18	2.39	0.0016	0.0006	10.02	1001.67	10.02
p-Xylene	1.49E+11	9.74E+10	1.53	0.2	2.39	0.002	0.0009	9.99	998.76	9.99
m-Xylene	1.49E+11	9.74E+10	1.53	0.2	2.39	0.002	0.0009	10.00	999.93	10.00
o-xylene	1.86E+10	1.30E+10	1.43	0.24	2.39	0.0014	0.0007	10.02	1,002.06	10.02
1,2-dichlorobenzene	5.40E+09	5.38E+09	1.01	0.47	2.39	0.0008	0.0008	10.03	1,003.01	10.03
1,3-dichlorobenzene	3.91E+09	5.74E+09	0.68	0.72	2.39	0.0007	0.0007	10.00	999.79	10.00
1,4-dichlorobenzene	3.15E+09	2.96E+09	1.06	0.43	2.39	0.0005	0.0005	9.98	998.41	9.98

### 3.4. Stability study

Stability studies are carried out to determine the conditions for both dispatch and storage, as well as to investigate both short- and long-term stability. The ampoules were stored at 4 and 40  $^{\circ}$ C for six weeks and twelve months, respectively, whereas reference ampoules were kept at reference temperature ( $-20$   $^{\circ}$ C). After a year, all samples were tested simultaneously in duplicates with the same conditions in a single batch. This design reduced the possibility of variations in the GC-FID response. The Grubbs test was used at 95 and 99% confidence levels to screen the data for single and double outliers. The resulting peak areas were plotted against time, and t-test statistics were used to determine whether the regression line showed any significant trends. All materials did not significantly degrade at 4  $^{\circ}$ C, according to the results, but at 40  $^{\circ}$ C, some materials did significantly change. As a result, the materials certificate defied the storage temperature of 4  $^{\circ}$ C. The evaluation of the short-term stability study revealed that no significant deterioration was found for transportation at 4 and 40  $^{\circ}$ C. Although the material can be transported at both conditions, it was advised to dispatch and transport material at  $\leq 4$   $^{\circ}$ C for material protection. The calculation of shelf life and uncertainty resulting from material instability was done using regression results from a long-term stability study [16]–[19]. Estimated uncertainty was included in the combined expanded uncertainty of the assigned value of each analyte. The assigned value's combined uncertainty included the estimated uncertainty. When stored at  $\leq 4$   $^{\circ}$ C,

the prepared CRMs are thought to be stable for a period of 12 months. To preserve confidence in the certified values, stability testing will be carried out throughout the duration that the material is available.

### 3.5. Value assignment and uncertainty of reference values

The purity of the starting materials was estimated by employing the mass balance approach. The mass fractions of all found impurities were converted to percentage units (%) and summed in order to generate a single quantitative estimate for all impurities [8], [13], [20]. The impurities estimates were used to estimate the purity of the starting materials. The reference material was prepared gravimetrically and certified values were calculated based on the corrected purity and mass of each component. Every step in the production process contributes to the certified values uncertainty, which has an impact on laboratory uncertainty budgets. Accordingly, the uncertainty of the certified values was estimated using the uncertainty components resulting from the characterization of impurities ( $u_{ch}$ ), sample inhomogeneity ( $u_{bb}$ ), and long-term instability ( $u_{lts}$ ), in compliance with ISO 33405:2024 and GUM [16], [21], [22]. The measurement of water content ( $u_w$ ) and organic impurities ( $u_{oi}$ ) was used to determine the uncertainty resulting from impurity characterization. The expanded uncertainty ( $U_{EXP}$ ) is expressed as two times the root of the sum of the squares of  $u_{ch}$ ,  $u_{bb}$ , and  $u_{lts}$  at a confidence level of approximately 95 % [16], [23]–[25].

## 4. CONCLUSION

In compliance with ISO 17034:2016 and ISO 33405:2024 standards, a new certified reference material has been certified for BTEX and chlorobenzene mass fractions. Eight analytes including benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene was certified without major problems in inhomogeneity, stability, and characterization. For m and p-xylene, the gravimetrically prepared concentration is not confirmed by the GC technique due to the separation problem. The reference material was characterized using a mass balance approach with different analytical techniques. The mass fractions of all analytes were assigned with a small relative expanded uncertainty of  $\pm 1\%$ . Due to the lack of availability of traceable CRMs in the market for analysis of BTEX and chlorobenzenes, the material will be useful for establishing traceability to SI units, quality control, and supporting laboratories to demonstrate their method proficiency.

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