

Development and Validation of Analytical Methodology for the Determination of Volatile Organic Compounds for Production and Certification of Reference Material

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ABSTRACT

An analytical method has been developed and validated for the determination of benzene, toluene, ethylbenzene and xylenes (BTEX) using gas chromatography - ion trap mass spectrometry (GC - IT - MS - ID) with isotope dilution. The limits of detection (LOD) and limits of quantification (LOQ) were always lower than 5 and 15 ug/g, respectively. The linear correlation coefficients were always greater than 0.99. The certified reference material was used for determination of the accuracy and precision of the method. The obtained results were satisfactory and agreed with the evaluated performance parameters.

1. INTRODUCTION

The volatile organic compounds (VOCs) comprise one of the main classes of contaminants in air, soil and groundwater; among these compounds spots the aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (*ortho*-, *meta*- and *para*-). These compounds, also called BTEX, have a high level of toxicity and can be harmful to public health. The risks to health associated with the exposure to volatile organic compounds may vary from irritation in the airways, fatigue until the development of serious diseases. In particular, benzene is a volatile environmental pollutant commonly found in crude petroleum products such as gasoline [1, 2]. Megatons of benzene are produced every year for use as an industrial solvent or a starting material in the manufacture of pesticides, plastics and numerous chemicals [3-5]. Benzene is of particular concern, since it's the most waters-soluble BTEX compound and a human carcinogen [6].

For this reason, the analysis of these contaminants with a metrological focus is considered essential because the level of contamination can be known with a high reliability on the results. The chemical metrology becomes an important part on this context, whose main goal is to provide quality and reliability for the measurements where the traceability and estimate the measurement uncertainty are essential.

According to the *Comité Consultatif pour la Quantité de Matière* (CCQM), the definition for primary measurement methods is: "methods that have the highest metrological qualities for what a complete uncertainty statement can be done, in terms of SI units and whose results are, therefore, accepted with no reference to a standard or measuring quantity". Primary measurement methods defined for amount of substance, for example, are gravimetry, coulometry, titration, calorimetry (DSC) and isotope dilution mass spectrometry (IDMS) [7].

The objective of this study was to implement and validate an analytical method for the determination of VOCs through a primary method (isotope dilution), using as analytical technique the gas chromatography - ion trap mass spectrometry (GC-IT-MS-ID) with isotope dilution and the estimate of measurement uncertainty associated with the analysis.

2. EXPERIMENTAL

2.1. Reagents and Materials

The commercial standards and solvents used in the preparation of the solutions were: benzene (Merck, 99.9 %), toluene (Tedia, 99.8 %), ethylbenzene (Sigma-Aldrich 99.9 %), *o*-xylene, *m*-xylene and *p*-xylene (Sigma-Aldrich, 99.6 %, 99.7 % and 99.9 %, respectively), benzene d-6, *o*-xylene d-10, *m*-xylene d-10 and *p*-xylene d-10 (Cambridge Isotope Laboratory, 99.5 %, 98 %, 98 % and 98 %, respectively).

respectively), methanol (Tedia HPLC/Spectrum). Certified Reference Material was purchased from the Korean Research Institute of Standards and Science (KRISS).

The purity of the commercial standards was determined by Differential Scanning Calorimetry – DSC and is being assured by GC-FID (gas chromatography with flame ionization detector) using two columns of different polarities. The standard solutions for the native and deuterated BTEX had been prepared in methanol. Calibration curves were constructed and prepared by diluting the stock solutions with methanol. The dilutions had been accomplished by gravimetric techniques through the preparation of solution-standard of compounds of interest in eight levels of equidistant concentrations. The method of internal standardization was applied (isotope dilution) by adding known amounts of the deuterated analytes of the respective analytes under investigation.

2.2. Instrumentation

Analyses were carried out using CP-3800 gas chromatograph coupled with a Saturn 2200 ion-trap spectrometer (Varian). The GC was equipped with a ZB-WAX (30m x 0.32mm x 0,5µm) capillary column. Helium was used as a carrier gas at 1 ml/min constant flow rate. All injections were performed on split mode (1:50). Oven temperature was programmed as follow: 40 °C for 1 min; 2 °C min⁻¹ to 48 °C; 30 °C min⁻¹ to 70 °C; 4°C min⁻¹ to 80 °C; 40 °C min⁻¹ to 150 °C, with 10 min final hold.

The injection volume was 1µL and injection was in split mode. Operational ion-trap conditions are listed in Table 1.

Table 1: GC-IT-MS ion trap operacional conditions for the analytical method for BTEX – Ionization parameters.

Injector temperature	220 °C
Ion Trap temperature	220 °C
Transfer line temperature	230 °C
Manifold temperature	50 °C
Axial modulation voltage	4.0 V
Multiplier offset	0 V
Emission current	10 µA
Electron multiplier voltage	1500 V

3. RESULTS

Quantification was performed using the technique of internal standardization, where it was used their patterns deuterated of analytes of interest as internal

standard. The selected ions from the selected-ion monitoring (SIM) for the quantification of analytes of interest and internal standards are presented in Table 2.

Table 2: Ion selection for quantification of analytes.

Analytes	Quantification ions (m/z)	Confirmed ions (m/z)
Benzene	78	77, 52, 51
Toluene	91	92, 65
Ethylbenzene	91	106, 77, 65
<i>o</i> -xylene	91	106, 77, 65
<i>m</i> -xylene	91	106, 77, 65
<i>p</i> -xylene	91	106, 77, 65

The method developed for the determination of analytes benzene, toluene, ethylbenzene and the xylene isomers was validated in accordance with the guidelines of the document INMETRO DOQ-CGCRE-008 [8].

The performance parameters evaluated are: selectivity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), limit of repeatability, limit of reproducibility and uncertainty.

3.1. Selectivity

As condition for the method selectivity, the absence of peaks in the region of the retention time for the investigated compounds was observed [9]. Fig.1 shows a typical chromatogram of the sample. The peaks of the analytes and it's retention times are indicated in Fig. 1.

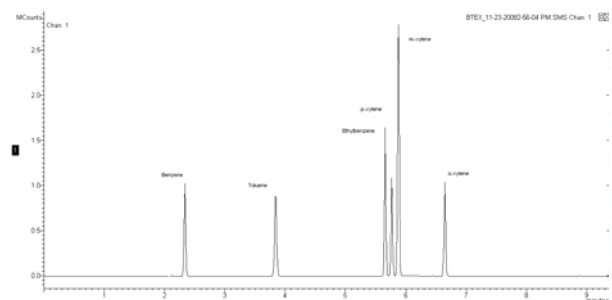


Fig.1: Chromatogram of the sample.

3.2. Linearity

Linearity was determined using the regression analysis. The linearity of the curves was in the

working range of 30 µg/g to 170 µg/g. The correlation coefficients were greater than 0.999 for the BTEX analyzed. The correlation coefficients (R^2), linear coefficient (b) and angular coefficient (a) of the calibration curves are listed in Table 3.

Table 3: The correlation coefficients (R^2), linear Coefficient and Angular Coefficient for each analyte.

Analyte	R^2	a	b
Benzene	0.99931	-0.07400	1.02225
Toluene	0.99933	-0.00728	1.26473
Ethylbenzene	0.99870	0.00769	1.09348
o-xylene	0.99907	0.03131	1.16948
m-xylene	0.99899	0.04383	1.08919
p-xylene	0.99847	0.01166	0.98280

3.3. Repeatability and Reproducibility

Repeatability and Reproducibility were evaluated from the calculation of the relative standard deviation (RSD) of the Certified Reference Material (CRM). This value was obtained by analysis of variance (ANOVA) from each result of two analysts in two different days (18 true replicates). The variance error was associated to repeatability and the sum of error and analyst variances were associated to reproducibility. The standard deviation of repeatability (s_r) and reproducibility (s_R) were calculated by expected mean squares (EMS) for the terms in the Two-Way ANOVA model. The limits of repeatability (r) and reproducibility (R) were calculated from Eq. (1).

$$r = R = t_{(95\%,df)} * \sqrt{2} * s_x, \quad (1)$$

were s_x is the standard deviation of x ($x = r$ or R).

3.5. Accuracy

According to the INMETRO DOQ-CGCRE-008, in the accuracy evaluation using certified reference materials (CRM's), the values obtained by the laboratory - mean and standard deviation of a series of tests on replicate - should be compared with the certified values at the reference material. For this comparison may be used: relative error, hypothesis test, z-score and normalized error. The accuracy of the method was determined using the Certified Reference Material of VOC through the calculation of the relative error (RE), expressed in percentage through Eq. 2. The results of accuracy for each analyte are shown in Table 4.

$$RE = \frac{X_{lab} - X_t}{X_t} * 100, \quad (2)$$

where X_{lab} is the experimental value or the arithmetic average of the experimental values and X_t is the value accepted as true.

3.6. Limit of Quantification (LOQ) and Limit of Detection (LOD)

These parameters were determined by analysis of the signal to noise ratio (S/N). The signal to noise ratio accepted as estimative of LOD and LOQ had been 3:1 and 10:1, respectively [10].

In the Table 4 the validation parameters were summarized.

Table 4. Validation parameters.

Analyte	r (%)	R (%)	Accuracy (%)	LOQ (ppb)	LOD (ppb)
Benzene	0.86	1.86	-1.4	4.4	1.5
Toluene	5.07	6.73	1.22	12.7	4.2
Ethylbenzene	4.44	4.80	3.05	7.7	2.6
o-xylene	3.57	4.87	1.71	7.5	2.5
m-xylene	5.10	5.26	-	7.5	2.5
p-xylene	3.99	4.41	0.33	7.4	2.5

3.7. Uncertainty

The uncertainty determination for BTEX analysis was evaluated based on the Guide to the Expression of Uncertainty in Measurement and on the EURACHEM/CITAC [11, 12].

The values of measurement uncertainty were expressed in terms of expanded uncertainty (U), which was determined by multiplying the coverage factor (k) by the combined standard uncertainty (u_c) of the input quantity (Eq. 3). The combined standard uncertainty is given by Eq. 4.

$$U = u_c * k \quad (3)$$

$$u_c = \sqrt{(u_{Aratio})^2 + (u_{cc})^2 + (u_{ms})^2 + (u_{mIS})^2 + (u_p)^2 + (u_{acc})^2} \quad (4)$$

where u_{Aratio} is the area ratio uncertainty; u_{cc} is the calibration curve uncertainty; u_{ms} is the sample mass uncertainty; u_{mIS} is the internal standard mass uncertainty; u_p is the analyte purity uncertainty; and u_{acc} is the accuracy uncertainty.

The input quantities are those that contribute for the uncertainty of the analytical method, this being

better evidenced in the cause and effect diagram (Fig. 2) and at the Fig. 3 that presents the contribution of the input quantity.

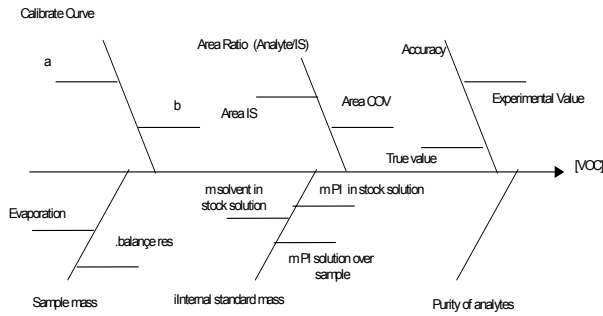


Fig.2: Cause and effect diagram illustrating the input quantity for the method.

The results obtained from purity of the commercial standards are shown in Table 5.

Table 5: Results of purity of DSC

Compound	Purity (%)
Benzene	99.970
Toluene	99.980
Ethylbenzene	99.980
<i>o</i> -xylene	99.527
<i>m</i> -xylene	99.827
<i>p</i> -xylene	99.920

4. DISCUSSION

Due to co-eluting the isomers *meta*- and *para*-, the separation of them becomes difficult. The method developed allowed the separation with good resolution and the quantification of them.

According to Fig. 3 we can see that the greater contributions come from the uncertainty of accuracy and repeatability.

The value of the uncertainty of accuracy can be considered high when compared with other sources of uncertainty. This is due to the fact of it being the sum of the uncertainties of the analysis.

Among the uncertainties of the input quantity, the ones provided from the gravimetric primary method present lower values among all the uncertainties. This result is in accordance with what is expected

from this technique, since its only uncertainty source is the balance resolution.

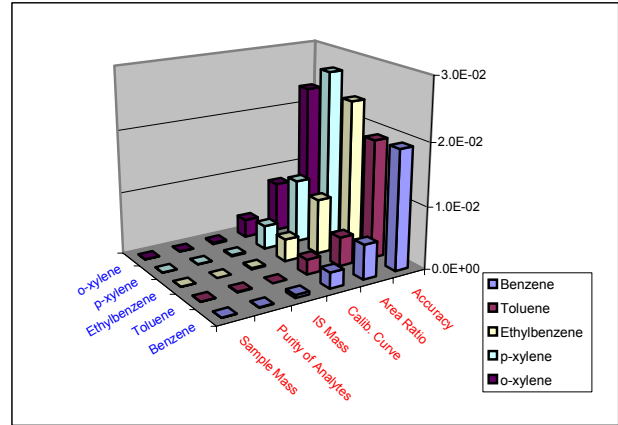


Fig.3: Contributions of the uncertainty sources.

The uncertainty value of the area ratio, which can be considered low in relation to the other uncertainty sources, indicates a good repeatability of the chromatograph used, since this uncertainty is associated to the standard deviations of the analyses.

The internal standard purity, differently from the standard purity, it is not a source of uncertainty in this analysis, since, independently from its value, the internal standard is added to the points of the calibration curve and to the sample at the same concentration, in which both are divided by the same factor, thus not causing uncertainty in the analysis.

5. CONCLUSIONS

The analytical method developed provided rapid analysis of volatile organic compounds benzene, ethylbenzene, *ortho*-, *meta*- and *para*-xylene. This analytical method has proved proficient, since it enabled analysis of the CRM accurately.

The estimate of the measurement uncertainty provided, for each VOC analyzed, knowledge of the range in which the result is included. The assessment of the purity of commercial standards used and the use of IDMS gave the results reliability and traceability to the International System of Units.

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