Analytical validation applied to simultaneous determination of solvents dichloromethane (DCM), methyl isobutyl ketone (MIBK), tetrahydrofuran (THF) and toluene (TOL) in urine by headspace extraction and injection on chromatographic system with a flame ionization detector

E D M Muna¹ and R P Pereira¹
¹ Instituto Militar de Engenharia, IME – Praça Gen. Tibúrcio, 80 - Urca - Rio de Janeiro - RJ - Brazil
E-mail: al_dobloni@hotmail.com, dobloni@ime.eb.br

Abstract. The determination of the volatile organic solvents dichloromethane (DCM), methyl isobutyl ketone (MIBK), tetrahydrofuran (THF) and toluene (TOL) is applied on toxicological monitoring of employees in various industrial activities. The gas chromatography technique with flame ionization detector and headspace injection system has been applied. The analytical procedure developed allows the simultaneous determination of the above-mentioned solvents and the accuracy of the method was tested following the INMETRO guidelines through the DOQ-CGRE 008 Rev.04- July/2011.

1. Introduction
The importance of determination of the volatile organic compounds such as dichloromethane (DCM), methyl isobutyl ketone (MIBK), tetrahydrofuran (THF) and toluene (TOL) is associated with the risk they offer to the health of workers exposed to those substances during their journey labor. The biological exposure index (BEI) values, originates from studies conducted by the American Conference of Governmental Industrial Hygienists (ACGIH), where the analyte concentration levels found in urine were correlated with their symptomatology. The BEI values are related to exposures of eight hours per day, five days per week, with concentrations in urine of 300 µg/L for DCM, 1000 µg/L for MIBK, 2000 µg/L for THF and 30 µg/L for TOL [1].

DCM is associated to cancer predisposition, heart diseases, central nervous system and liver failure, skin and eyes irritation. Exposure occurs primarily by inhalation and skin absorption [2]. The MIBK is irritating to the eyes, nose, throat and skin, causing headaches and nausea, and also effects on liver and kidneys. Exposure to high concentrations can be life-threatening due to the narcotic effects of this substance [3]. The THF has similar toxicological effects to MIBK, however, with less intensity [4]. TOL is the most toxic of the mentioned species and is the one presenting greater use, being the major substituent of benzene in various industrial activities where the use of this compound was prohibited. It is a strong central nervous system depressor, leading to mental confusion, loss of coordination and unconsciousness, which may lead to death in cases of acute exposure [5].

In the present work, the technique utilized was gas chromatography with flame ionization detector, and injection system by headspace extraction. This approach was adopted due to the chemical
characteristics of the analytes and the complexity of the matrix. Despite the theoretical and methodological discussion, the method was developed experimentally. The main goal of the present work involves the analytical validation aiming to assign metrological reliability to the measurements made, which requires a set of experiments planned to compose statistical representation of the chemical assay [6, 7].

2. Materials and Methods

2.1. Materials
Reverse-osmosis type quality water (purified with a Milli-RO plus Milli-Q station from Millipore Milford, MA) and pure solvents for the composition of the patterns.

2.2. Sample and standards preparation
Sample preparation consists of collecting an aliquot of 8 mL, adding 1 mL of internal standard (fluorobenzene in aqueous solution) and sealing the headspace vial, subsequently submitting to extraction programming of the headspace equipment.

The small number of steps in sample manipulation can be considered a great advantage, which considerably reduces the contribution of uncertainty sources associated with the sample preparation step.

The standards were prepared from pure substances diluted appropriately in water containing 5% methanol. In the case of reproducing the sample behavior, a spiked urine sample was prepared, ensuring its homogeneity, which was used in the execution of matrix experiments.

2.3. Chromatographic conditions
A headspace injection system (Perkin Elmer Turbo Matrix 40 Trap) coupled to gas chromatograph with flame ionization detector (Perkin Elmer Clarus 500) were used to quantify the analytes in urine. The analyses were performed under the following chromatographic conditions: Column, DB-624 with 60 m length, 250 µm in diameter and 1.4 µm of inner film.

The temperature of the FID was 220°C, and the injector temperature was 200°C. The oven temperature was programmed to 45°C for 5.5 minutes, ascending 15°C/min to 140°C, standing for 4 minutes, subsequently rising 40°C/min to 200°C and following for the cleaning stage, ascending 100°C/min to 240°C waiting for 2 minutes. Helium was the carrier gas with a flow of 1.4 mL/min for 17 minutes followed by an increase of 100 mL/min to 1.8 mL/min.

The parameters of headspace injection were: incubation time of 60 minutes, temperature of 80 °C, needle temperature 200 °C and time of pressurization of 1 minute. The analytes exhibited retention times of 7,441 min (DCM); 9,699 min (THF); 12,528 min (MIBK); 12,823 min (TOL) and 10,813 min for the internal standard fluorobenzene.

3. Results and Discussion

3.1. Analytical validation

3.1.1. Analytical Range
The measurement range was selected based on values indicated by the BEI/ACGIH (American Conference of Governmental Industrial Hygienists).
The IBE values applies to exposures of eight hours per day, five days a week, with values in urine, 300 µg/L of DCM, 1000 µg/L of MIBK, 2000 µg/L of THF, and 30 µg/L for TOL.

Table 1 shows the analytical range selected in accordance with toxicological limits.

Table 1. Analytical curve in µg/L (ppb).

| Levels | Dichloromethane | Methyl isobutyl ketone | Tetrahydrofuran | Toluene |
|--------|----------------|-----------------------|-----------------|---------|
| 1      | 150            | 450                   | 900             | 15      |
| 2      | 300            | 900                   | 1800            | 30      |
| 3      | 450            | 1350                  | 2700            | 45      |
| 4      | 600            | 1800                  | 3600            | 60      |
| 5      | 750            | 2250                  | 4500            | 75      |

3.1.2. Selectivity

The selectivity evaluates the ability of an analytical method of providing specific responses for each analyte, respecting the variation of concentrations.

This evaluation was performed through the t test applied to the angular coefficients of matrix without curve (standards in water) and curve with matrix (standards in urine).

Table 2 lists $t_{\text{calc}}$ and $t_{\text{tab}}$ values considered for evaluation.

Table 2. Values of $t_{\text{calc}}$ and $t_{\text{tab}}$.

|       | Dichloromethane | Methyl isobutyl ketone | Tetrahydrofuran | Toluene |
|-------|----------------|-----------------------|-----------------|---------|
| $t_{\text{calc}}$ | -0.107 | 0.317 | -0.017 | 0.577 |
| $t_{\text{tab}}$ |        | 2.160 |        |        |

The $t_{\text{tab}}$ values refer to a total number of 15 replicates (3 per level) and confidence interval of 95%. All values were observed to be lower than the reference, pointing to the inexistence of matrix effects.

3.1.3. Linearity

The linearity is the capacity of a analytical method to provide specific responses for each concentration within a stipulated range. To make this assessment, it is used the calculus of the coefficient of determination (R Squared).

For the linearity evaluation, it was used the analytical curve without matrix addition, with 3 replicates per level, being evaluated the values for $R^2$ and dispersion of the residues through the Cochran's test. Table 3 shows the values obtained for $R^2$ and Cochran.
Table 3. Values of $R^2$ and Cochran's test.

| $R^2$ / $C$ | Dichloromethane | Methyl isobutyl ketone | Tetrahydrofuran | Toluene |
|------------|-----------------|------------------------|-----------------|---------|
| $R^2$      | 0.9954          | 0.9966                 | 0.9959          | 0.9930  |
| $C_{\text{calc}}$ | 0.5455        | 0.4753                 | 0.4999          | 0.3395  |
| $C_{\text{tab}}$ |               |                        |                 | 0.6838  |

These results indicate linearity within the studied range and the homocedasticity of the residues (free residues trend over the analytical range) which is observed for $C_{\text{calc}}$ lower than $C_{\text{tab}}$.

Figures 1 to 4, below, show the residues dispersion obtained from the calibration curves for each analyte.

**Figure 1**: Dichloromethane residues dispersion.

**Figure 2**: Tetrahydrofuran residues dispersion.

**Figure 3**: Methylisobutylketone residues dispersion.

**Figure 4**: Toluene residues dispersion.
3.1.4. Limits of detection (LOD) and quantification (LOQ)
The detection limit relates the minimum signal from which can be considered a positive signal related to the presence of the analyte.

The importance of determining the limits of detection and quantification, allows the recognition of the metrological intervals of the analytical method. Allowing to evaluate the capacity of the method to attend a particular application.

For the determination of LOD and LOQ, 7 replicates were obtained of the lowest level of the curve applying the standard deviation method. Table 4 shows the LOD and LOQ obtained statistically.

| Limits  | Dichloromethane | Methyl isobutyl ketone | Tetrahydrofuran | Toluene |
|---------|-----------------|------------------------|-----------------|--------|
| LOD     | 25.75           | 68.86                  | 155.12          | 1.63   |
| LOQ     | 85.83           | 229.54                 | 517.07          | 5.44   |

The theoretical values obtained from the quantification limit were found below the lowest point of the curve for the analytes studied, ensuring metrological reliability in measurements close to the first point of the analytical curve.

3.1.5. Recovery
The recovery represents the study of the analytical system performance compared to samples containing known concentrations of the target analyte. The limitation of this procedure is that the added analyte is not necessarily in the same way as in the sample. The presence of analytes added into a more easily detectable form may result in overly optimistic evaluation of retrieval.

The success of the evaluation of recovery is the simulation capability of the actual conditions of the samples. In this case, the characteristics of the analytes and low concentrations studied, ensured the prospect of recovery with the expected success.

For the recovery evaluation, 3 replicates of fortified samples in the low, medium and high ranges were obtained. Table 5 presents the average recovery values obtained for the series of substances studied.

| Rec | Dichloromethane | Methyl isobutyl ketone | Tetrahydrofuran | Toluene |
|-----|-----------------|------------------------|-----------------|--------|
| %   | 97.60           | 99.48                  | 99.54           | 99.21  |
3.1.6. Repeatability

Repeatability is the relative standard deviation (RSD) evaluate of the replicates used in the composition of the curve. It has relation to the evaluation of the residue dispersion. The RSD values have great importance in the evaluation of homocedasticity, showing tendencies along the calibration curve. The repeatability must be uniform across the analytical range, indicating that responsiveness is equivalent at all concentrations.

Repeatability study was conducted from the matrix without curve and in triplicate per level. The relative standard deviation (RSD) for each concentration level was calculated for all analytes under study, there was no transposition of the acceptability criterion limit, which in this case is 10%.

4. Conclusions

The values obtained in the statistical analysis ensure metrological confidence in the measurements performed and the applicability of this methodology for occupational toxicological monitoring of the mentioned substances.

The framework of the values in the BEI/ACGIH within the analytical curve allows the anticipated deflagration of toxic conditions which individuals may be subjected. The observation of quantized values, even lower than the limit, presents an important toxicological monitoring tool.

The substances studied have toxicological effects observed in the short-term when there is transposition of the reference values, however, there are still uncertainties about the effects associated with long-term exposure to lower doses of the referenced values.

The technique presents considerable complexity, however, the operating cost is relatively low compared to other analytical techniques. CRMs (certified reference materials) can be utilized aiming to expand the metrological traceability to attend national and international quality standards.

Acknowledgments

We thank Serviço Social da Indústria – Federação das Indústrias do Estado do Rio de Janeiro (FIRJAN) for the support during the development of the present work.

References

[1] American Conference of Governmental Industrial Hygienists (ACGIH) 2010 *Threshold limit values and biological exposure indices for 2010* (Cincinnati)

[2] Osha analytical methods manual 1995. *Methylene Chloride: Method 80* (Salt Lake)

[3] Osha analytical methods manual 2000. *2-Butanone (MEK) Hexone (MIBK): Method 1004* (Salt Lake)

[4] CDC – Centers for Disease Control and Prevention in <www.cdc.gov/niosh/ipcsneng/neng0578.html> accessed in April 2015

[5] Osha analytical methods manual 1998. *Toluene: Method 111* (Salt Lake)

[6] Kolb B and Ettre L S 2006 *Static headspace-gas chromatography* 2nd ed (New Jersey: Wiley-Interscience)

[7] DOQ-CGCRE-008 2011 *Orientações sobre validação de métodos analíticos* (Rio de Janeiro: Inmetro)

[8] Diego C N et al 2008 *J. Braz. Chem. Soc.* São Paulo, v. 19, n. 6, 1201 – 1206

[9] E D M Muna et al 2015 *J. Phys.: Conf. Ser.* 575 012031