Validação analítica aplicada à determinação simultânea dos solventes diclorometano (DCM), metil isobutil cetona (MIBC), tetrahidrofurano (THF) e tolueno (TOL) em urina através de extração de headspace e injeção em sistema cromatográfico com detector de ionização em chama.

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.

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Resumo: A determinação dos solventes orgânicos voláteis diclorometano (DCM), metil isobutil cetona (MIBC), tetrahidrofurano (THF) e tolueno (TOL) é aplicada no monitoramento toxicológico de trabalhadores de diversas atividades industriais. Para tal, foi aplicada a técnica de cromatografia a gás com detector de ionização em chama e sistema de injeção por headspace. O procedimento analítico desenvolvido viabiliza a determinação simultânea dos solventes citados tendo a acurácia do método testada seguindo as orientações do INMETRO através do DOQ-CGRE 008 Rev.04-julho/2011.

Palavras-chave: Metrologia química, validação, toxicologia ocupacional, Urina.

Abstract: The determination the volatile organic solvent 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.

Keywords: Chemical metrology, validation, Occupational toxicology; Urine.
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 originate 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 MIBC, 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. METHODOLOGY

Sample preparation consists of collecting an aliquot, adding internal standard 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 chromatographic method was developed with a static headspace extraction system (Perkin Elmer, Turbo Matrix 40 Trap) and a gas chromatograph with flame ionization detector (Perkin Elmer, Clarus 500). Capillary column DB-624 with 60 m length, 250 µm in diameter and 1.4 µm of inner film was used in the experiments.

The analytes exhibited retention times of 7,441 min (DCM); 9,699 min (THF); 12,528 min (MIBC); 12,823 min (TOL) and 10,813 min for the internal standard fluorobenzene. The standards were prepared from pure substances diluted appropriately in water containing 5% methanol. In the case of reproducing the sample behavior, a diluted urine sample was prepared, ensuring its homogeneity, which was used in the execution of matrix experiments.

3. ANALYTICAL VALIDATION

3.1. Analytical range

The measurement range was selected based on values indicated by the BEI/ACGIH (American Conference of Governmental Industrial Hygienists) study.
Hygienists). Table 1 shows the analytical range studied.

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

<table>
<thead>
<tr>
<th>Levels</th>
<th>DCM</th>
<th>MIBK</th>
<th>THF</th>
<th>TOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>450</td>
<td>900</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>900</td>
<td>1800</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>450</td>
<td>1350</td>
<td>2700</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>1800</td>
<td>3600</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>750</td>
<td>2250</td>
<td>4500</td>
<td>75</td>
</tr>
</tbody>
</table>

**3.2. 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 \( t_{\text{tab}} \).**

<table>
<thead>
<tr>
<th>t</th>
<th>DCM</th>
<th>MIBK</th>
<th>THF</th>
<th>TOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{\text{calc}} )</td>
<td>-0.107</td>
<td>0.317</td>
<td>-0.017</td>
<td>0.577</td>
</tr>
<tr>
<td>( t_{\text{tab}} )</td>
<td>2.160</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

**3.3. Linearity**

For the linearity evaluation, the analytical curve without matrix addition was used, 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 and compared.

**Table 3. Values of \( R^2 \) and Cochran’s test.**

<table>
<thead>
<tr>
<th>( R^2 )</th>
<th>DCM</th>
<th>MIBK</th>
<th>THF</th>
<th>TOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{calc}} )</td>
<td>0.5455</td>
<td>0.4753</td>
<td>0.4999</td>
<td>0.3395</td>
</tr>
<tr>
<td>( C_{\text{tab}} )</td>
<td>0.6838</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

**3.4. Limit of detection (LOD) and quantification (LOQ)**

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.

**Table 4. LOD and LOQ in µg/L (ppb).**

<table>
<thead>
<tr>
<th>Limits</th>
<th>DCM</th>
<th>MIBK</th>
<th>THF</th>
<th>TOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD.</td>
<td>25.75</td>
<td>68.86</td>
<td>155.12</td>
<td>1.63</td>
</tr>
<tr>
<td>LOQ.</td>
<td>85.83</td>
<td>229.54</td>
<td>517.07</td>
<td>5.44</td>
</tr>
</tbody>
</table>

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 calibration curve.

**3.5. Recovery**

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.
Table 5. Recovery (%)

<table>
<thead>
<tr>
<th></th>
<th>Rec</th>
<th>DCM</th>
<th>MIBK</th>
<th>THC</th>
<th>TOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>97.60</td>
<td>99.48</td>
<td>99.54</td>
<td>99.21</td>
<td></td>
</tr>
</tbody>
</table>

3.6. Repeatability

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 and, for the analytes and concentration levels under study, there was no transposition of the acceptability criterion limit, which in this case is 10%.

4. CONCLUSION

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 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 aiming to attend national and international quality standards.

REFERENCES


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