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BRACKETINS VERSUS MULTIPOINT CALIBRATION IN DETERMINATION OF ISOCYANATES IN AGGLOMERATED CORK STOPPERS

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ABSTRACT

A calibration method was developed for the quantification of isocyanates in cork stoppers agglomerates. Bracketing and multipoint calibration curve was applied to a in-house optimized UPLC/DAD analytical method. The calibration curves were obtained by regression analysis using experimental points that covers the range of concentrations expected in real samples. A contaminated real sample was injected and MDI concentration was 6.2 % and 6.4% when calibration methods to calculate the content were multipoint or bracketing, respectively. According with our results both methods are complementary and necessary to guarantee the reliably of results obtained by chromatographic procedure.

Keywords: MDI, 1,2PP, UPLC-DAD, Calibration Curve, Bracketing.

INTRODUCTION

The chemical class of isocyanates is of extreme commercial importance due to their use in the manufacture of polyurethanes. Nowadays polyurethanes are used in several applications such as adhesives, coatings, foams, thermoplastics resins, printing inks, foundry moulds and rubbers [1].

With all these application and materials, human and environmental exposure is high. Isocyanates are usually present in workplace air and this contamination is well documented. The effects on humans are, in higher doses toxic effects on mucous membranes and in lower doses, as 1ppm, stimulate functional changes and inflammatory processes in lung tissues. Occupational asthma is the most severe manifestation of isocyanates workplace exposure. Therefore some countries limited worker exposure to 5ppb. However the limitation in materials and articles in contact with foodstuffs is questionable [1][2].

Polyurethane based polymers are used as adhesives in agglomerated cork stoppers. These cork stoppers are better for wine than screw caps or foam mimetizing cork because they improve wine quality.

Agglomerated cork stoppers are currently used for still wines, semi-sparkle and gaseous wines, beer and cider. Methylene diphenyl diisocyanate (MDI) is presently the isocyanate used in the production of polyurethane based adhesive in use due to its lowest toxicity comparing with toluene diisocyanate (TDI) previously employed [3]. However, free monomeric MDI can migrate from agglomerated cork stoppers to beverages therefore it needs to be under control. The presence of these compounds are usually investigated by HPLC with Fluorescence or UV-Vis detector depending on the derivatising agent. Ultra Performance Liquid Chromatography with Diode Array Detector (UPLC-DAD) method is replacing HPLC [1]. UPLC has the advantage of decreasing time and solvent consumption, although optimization is necessary because column and solvent conditions are not the same in both systems.

To determine the accurate content of isocyanate the choice of calibration methodology to obtain the calibration function is crucial to calculate the accurate amounts of an analyte as a function of an instrumental signal. In order to establish the relationship between isocyanate concentration and instrumental response a calibration experiment was designed. In this process, the relevant experimental parameters are specified: a) calibration range; b) composition; c) including uncertainty of the reference standards for calibration; d) parameters of the analytical method; e) measurement conditions; f) number and sequence of calibration measurements [4][5]. The choice of the calibration method was decided on priori information about the experimental design, which includes “measurement uncertainties” provided by measurement standards and the measuring system. In this work two different methods bracketing and multipoint calibration curve are used to optimize the calibration function to determine the content of isocyanate by UPLC/DAD.

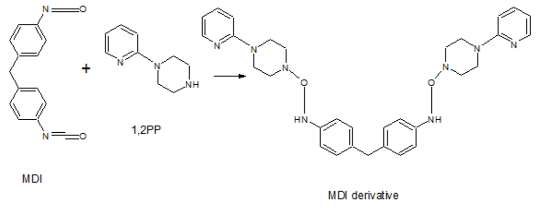

Fig.1: Derivatization reaction

MATERIALS AND METHODS

Materials

All solvents used were HPLC or superior grade. Diphenylmethan-4,4’-diisocyanate (4,4’-MDI), from EHRENSTORFER; Dicloromethane (DCM), from Riedel-de Haën; Acetonitrile (AcN), from Biosolve; Dimethyl sulfoxide (DMSO), from Sigma-Aldrich; 1-(2-pyridyl)-piperazine (1,2PP), from Aldrich; Ammonium acetate (NH4Ac), from Merk; Acetic acid, from Panreac. The ultra pure water used for all purposes was obtained with a Milli-Q Element system from Millipore (Interface, Portugal).

All stock standard solutions were prepared at a concentration of 500 µg/mL in DCM and derivatized with 50 µL of 1,2PP. Intermediate standard solutions were prepared daily, by dilution, at concentration of 80 µg/mL in AcN:DMSO (95:5). Working standards were prepared daily, by dilution with AcN, to a concentration range of 8 to 0.4 µg/mL.

Every standard were filtered to a vial by a GHP 0.2µm syringe filter.

All samples were prepared by the same person and with the same laboratory conditions.

Analytical equipment

Acquity Ultra Performance LC system coupled with a Diode Array Detector (DAD) (Waters, Milford, MA, USA) was used for the analysis of target compounds.

Table 1: UPLC-DAD parameters for the separation and detection of MDI

<table>
<tr><th>Parameter</th><th>Analytical Conditions</th></tr>
<tr><td>Derivatising agent</td><td>1-(2-pyridyl)-piperazine (1,2PP)</td></tr>
<tr><td>Column</td><td>Acquity UPLC BEH 18
150 x 2.1 mm 1.7µm</td></tr>
<tr><td>Column Temperature</td><td>40°C</td></tr>
<tr><td>Tray Temperature</td><td>15°C</td></tr>
<tr><td>Solvent A</td><td>H_2O 0.1% NH_4Ac
pH 6.0 acetic acid</td></tr>
<tr><td>Solvent B</td><td>Acetonitrile</td></tr>
<tr><td>Flow</td><td>0.4 mL/min</td></tr>
<tr><td>Solvent gradient</td><td><table>
<tr><th>Time (min)</th><th>%A</th><th>%B</th></tr>
<tr><td>0</td><td>65</td><td>35</td></tr>
<tr><td>0,01</td><td>65</td><td>35</td></tr>
<tr><td>4,69</td><td>30</td><td>70</td></tr>
<tr><td>5,16</td><td>5</td><td>95</td></tr>
<tr><td>7,03</td><td>5</td><td>95</td></tr>
<tr><td>7,05</td><td>65</td><td>35</td></tr>
<tr><td>10</td><td>65</td><td>35</td></tr>
</table></td></tr>
<tr><td>Run Time</td><td>10 min</td></tr>
<tr><td>UV-detection</td><td>254 nm</td></tr>
</table>

RESULTS AND DISCUSSION

The design of calibration process was carried out according with new version of VIM calibration definition and implied two steps [6]. The first step consisted of measuring signal intensities using reference standards with known concentrations and associated uncertainties so as to construct a calibration graph. In a second step, the concentration was deduced from the calibration graph using the measurement of the signal intensity from an “unknown sample”. A so-called inverse calibration was therefore performed.

The Limit of Detection (LoD) and Limit of Quantification (LoQ) was obtained according to Eurachem Guideline. LoD was achieved using a serial of spiked dilutions until chromatographic signal disappeared. LoD and LoQ were calculated according to (1) and (2) respectively, when 30 independent sample blanks fortified at lowest acceptable concentration were measured once each.

(1)

(2)

A reliable value for limit of detection and limit of quantification were 11 ppb and 20 ppb respectively. These values are adequate for isocyanate concentration in real samples.

To estimate the isocyanate content quadratic regression was used as first approach. The curve fitting equation was obtained by generating a five point calibration curve with appropriate concentrations and preliminary results are presented in Figure 2 a).

The second method involved bracketing, the estimated concentration of the isocyanate between adjacent concentrations of two standards. It assumes that a linear algebraic interpolation of the analyzer response can be made between the concentrations of these two standards. This was calculated by choosing the difference in the concentrations of the two bracketing standards to be small enough that the uncertainty in a linear response is small compared with the uncertainty of overall process, results are presented in Figure 2b).

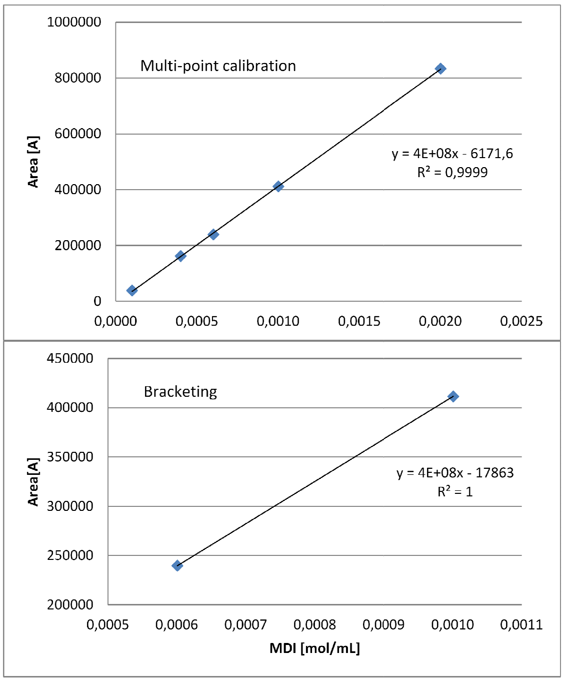

Fig. 2: a) Multi-point calibration and b) bracketing data with linear regression curves

Due to acute toxicity and sensitizing properties rigorous analytical control process, including method validation are needed to guarantee the absence of free monomeric isocyanates compounds in the samples and suitable for routine determination of MDI in food contact materials as a vapor aerosols or adsorbed on particulate matter [7].

A better fitting was obtained with bracketing when the same slope was found in both methodologies. The choice of concentration to build the multipoint calibration curve was according with ISO standards and covers the expected concentrations of real samples [8]. The bracketing standards were chosen after the estimation of concentration of isocyanate in sample calculated by multi-point calibration approach. The difference between concentrations of bracketing standards was selected to give the minimal residuals.

In Figure 3 a chromatogram of a real sample containing MDI is presented. The retention time was checked against pure commercial standards (>99%) demonstrating that peak can be attribute to MDI in the sample. MDI content was calculate by extrapolation of area to concentration through the calibration function depending the result obtained on the method used. 6.2661% and 6.4351% was obtained for MDI values when multipoint or bracketing was applied, respectively. In a collaborative study the same magnitude of MDI concentration was achieved by different laboratories.

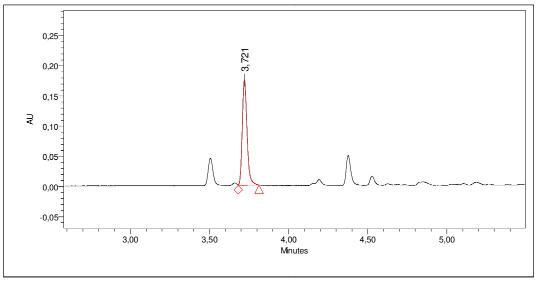

Fig. 3: Chromatogram of unknown sample. The retention time of MDI was 3,721 min.

CONCLUSIONS

An analytical method to measure MDI in cork stoppers is crucial for safety reasons and consumer and market acceptance. Metrology plays therefore a crucial role in overall measurement process. Calibration methodology is therefore critical to obtain an accurate value. This work is a contribution to improve analytical measurement procedure of MDI in food contact materials. According with our results both methods multipoint calibration point curve and bracketing calibration should be applied to obtain a measurement value with low associated uncertainty.

REFERENCES

[1] M. Schulz and T. Salthammer, “Sensitive determination of airborne diisocyanates by HPLC: 4,4’-Diphenylmethane-diisocyanate (MDI)”, Fresenius Journal Analytical Chemistry, 362, 289–293, 1998.

[2] OSHA Analytical Laboratory (1989) Methylene Bisphenyl Isocyanate (MDI), OSHA Method # 47, Salt Lake Technical Center, Salt Lake City.

[3] P. Tremblay, J. Lesage, C. Ostiguy and H. V. Tra, “Investigation of the competitive rate of derivatization of several secondary amines with phenylisocyanate (PHI), hexamethylene-1,6-diisocyanate (HDI), 4,4’-methylenebis(phenyl isocyanate) (MDI) and toluene diisocyanate (TDI) in liquid medium”, Analyst, 128, 142–149, 2003.

[4] K. Danzer, L.A. Currie, IUPAC. Guideline for calibration in analytical chemistry, Pure Appl. Chem. 70 (1998) 993−1014.

[5] LGC/VAM/2003/032, Preparation of Calibration Curves — A Guide to Best Practice, Laboratory of the Government Chemist (LGC), Cambridge, 2003.

[6] International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM) JCGM 200:2008.

[7] T. Salthammer, C. Wismach and H. Miertzsch, “Absorption and fluorescence of 1-(2-pyridyl)-piperazine andfour diisocyanate derivatives in solution”, Journal of Photochemistry and Photobiology A: Chemistry, 107, 159-164, 1997.

[8] M. Thompson, S.L.R. Ellison, Accred. Qual. Assur. 10, 82, 2005.