Development of an Innovative Gas Chromatography–Mass Spectrometry Method for Assessment of Formaldehyde in the Workplace Atmosphere

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Received: 18 August 2016; accepted: 01 September 2016

World consumption of formaldehyde (FA) is forecast to grow at an average annual rate of about 4% from 2015 to 2020 with world production to exceed 52 million tons in 2017. From the first day of January 2016, the Commission Regulation No. 91/2015 established the FA classification through an indication from European Chemical Agency as category 2 mutagenic and category 1B carcinogen. A novel method for the determination of gaseous FA in air is presented herewith. The sampling was carried out using a miniaturized cartridge by means of a medium-flow pumping system (1.0 L min⁻¹, 5–60 min) and absorption of FA vapors on 2,4-dinitrophenylhydrazine. Cartridge desorption removing the excess derivatizing agent based upon solid-phase extraction was performed by an innovative xyz robotic system on-line with fast gas chromatography (GC)–mass spectrometry (MS). Through the generation of known concentration of FA, we evaluated the precision (relative standard deviation for n = 10, 8.8%), lower limit of quantification (0.072 μg/cartridge), and linearity (from 0.125–64 μg/cartridge with correlation coefficient of 0.99) of the method. The described procedure combines the efficiency of fast GC–MS systems with both the high throughput of autosampler and the quantitative accuracy of FA-dinitrophenylhydrazine for measuring American Conference of Governmental Industrial Hygienists TLV Ceiling.

Keywords: Formaldehyde, fast gas chromatography, air sampling, automation

Introduction

Formaldehyde (FA), a colorless gaseous substance with a sharp smell, is the first member of a homologous series of aliphatic aldehydes. About 70% of FA application is the production of resins. China is the single-largest market, accounting for 42% of world demand in 2015 [1]. As of 2012, the global FA production registered a 4.7% of increase with world production to exceed 52 million tons in 2017 [2]. FA has been determined by the European Chemical Agency Risk Assessment Committee to be “presumed human carcinogen, classification is largely based on animal evidence.” The American Conference of Governmental Industrial Hygienists has established a threshold limit value of 370 μg m⁻³ (0.3 ppm) calculated as the concentration that should not be exceeded during any part of the working exposure.

The existing methods to detect gaseous FA are based on active or passive sampling using 2,4-dinitrophenylhydrazine (DNPH), pentafluoropophenylhydrazine, and O-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine as reagents whether on filters or solid sorbent and later analyzed by liquid or gas chromatography (GC) [3–5]. In the last 10 years, the developing interest in hyphenated techniques in analytical chemistry with resultant solvent and sample savings, sample enrichment, faster sample preparation, and easier automation has developed for the proliferation of xyz autosamplers. Although most chromatography laboratories use autosamplers as the standard form of sample injection, the modern instrumentation permits automation beyond injection. The evolution of robotic samplers has been driven by the addition of specialized hardware modules and the development of versatile and user-friendly software [6–8].

This article reports the first contribution to the determination of airborne FA sampled with miniature DNPH cartridge desorbed by innovative xyz autosampler on-line to fast GC–mass spectrometry (MS). The introduction of dedicated, automated, and robotic systems allowed a friendly use of MS apparatus for high-throughput screening so as to reduce the costs of the monitoring campaigns.

Experimental

The air sampling was performed by Sep-Pak XpSure Aldehyde Sampler Plus Short cartridge DNPH (XpoSure) silica sorbent (Cat. No. WAT047205, Waters, Milford, USA) connected to GilAir Plus pumps (Sensidyne, St. Petersburg, USA) at 1 L min⁻¹.

Cation exchanger Plus Short cartridges packed with hydrophilic weak silica (Cat. No. WAT020550, Sep-Pak Accell CM, Waters), polymeric Oasis mixed-mode cation-exchange sorbent (Cat. No. 186003516, Waters), and polymeric Oasis weak cation-exchange (WCX) phase (Cat. No. 186003518, Waters) were compared to retained DNPH.

Full automation of the analysis procedure was achieved using a new Flex GC xyz autosampler (EST Analytical, Fairfield, Ohio, USA) equipped with 98-sample trays for 12 × 32 mm screw neck cap and preslit PTFE–silicone septum vial, a bar-code reader, a 45-position tray for Fast Fit Assemblies (FFA), and a Multi Tools Exchange (MTX); these last two are patented.

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Development of GC–MS for Assessment of FA

by Chromline (Prato, Italy). FFA is formed by magnetic adaptors that make the Plus Short cartridge robust and identifiable by its barcode and allows change in automatic mode between the tray and the vial for desorption. The MTX automatically exchange tools in this operating sequence: a 5.0-mL syringe PTFE plunger and needle tip b (Cat. No. 2600040, ILS Innovative Labor Systeme GmbH, Stützerbach, Germany) for elution of FA-dinitrophenylhydrazone from FFA-XpoSure cartridge (2 mL ethyl acetate containing 1 μg of FA-dinitrophenylhydrazone-3,5,6-dibutylcarbamate, Cat. No. GBOSMX18, Giotto Biotech, Sesto Fiorentino, Italy) and a 10-μL syringe for 1 μL GC injection. In the case of Sep-Pak Accell CM cation exchanger, a previous conditioning cycle by three sequential elutions of 2 mL each with water, ethanol, and diethyl ether was required as indicated by the manufacturer.

The analysis was performed by fast GC with a Shimadzu GC–MS QP 2010 (Shimadzu, Kyoto, Japan), using a MEGA-5 MS FAST column (10 m × 0.10 mm × 0.1 μm film thickness). The initial column temperature was set to 80 °C and then increased at 30 °C min⁻¹ to 190 °C, 5 °C min⁻¹ to 200 °C, and 30 °C min⁻¹ to 300 °C. The injector (200 °C) with Merlin Microseal Septa (Cat. No. 61-12, Merlin Instrument Company, Newark, USA) was set in splitless mode. Helium at a flow rate of 0.9 mL min⁻¹ was used as carrier.

Calibration curves (0.125–64 μg/carrigade) were constructed by plotting the peak–area ratio of the base peak of the FA-2,4-dinitrophenylhydrazone (Cat. No. 5667, Fluka, Sigma-Aldrich) at m/z 210 (retention time [RT], 5.094 min) to the base peak of IS at m/z 186 (RT, 2.927 min). A linear regression plot was generated, and the instrumental limit of detection (LOD) is then calculated according to the following formula:

\[ C_{FA \ air} = C_{sol} / V \]

where \( C_{sol} \) is the mass of the FA in aqueous solution injected (μg) and \( V \) is volume (L) of the air.

**Results and Discussion**

Short sampling periods for the quick assessment of brief acute exposure monitoring of FA in the workplace atmosphere together with routine automated analysis have aroused the interest of the authors of this article and have been investigated as a possible alternative to conventional methods. Thus, to achieve a successful method, three fundamental requisites were satisfied by the authors.

The first objective was to develop and optimize the cartridge desorption and the sequential removal of the excess derivatizing agent based upon solid-phase extraction. Two milliliters of ethyl acetate of high purity was used for elution from FFA-XpoSure cartridge in consideration of the 0.8 mL of cartridge volume. The influence of flow rate desorption was investigated between 0.1 and 2.00 mL min⁻¹, and the recovery was observed to increase with a decrease the flow rate. Decreasing the flow rate below 0.4 mL min⁻¹ exhibited no further significant improvement in the percentage recovery (96%), and therefore, this flow rate was selected for the elution of 2 mL vial of the FA-2,4-dinitrophenylhydrazone. Then, the solution was aspirated in the FFA-cation exchange cartridge and elute at flow rate of 2 mL min⁻¹ in the same vial. The FFA-Oasis MCX cartridge (Figure 1) was selected over the two weak cation-exchange sorbents, thanks to its acidity (pH <1) that allowed the elution of 82% of the FA-2,4-dinitrophenylhydrazone (pH 7) and the retention over the 99% of the DNPH (pH 12) without condition and equilibrate operation [10] (Figure 2).

Figure 1. FFA-Plus Short cartridge: 1. pre-assembled (a. stainless steel hub for magnetic plunger, b. barcode case, c. septum, d. Plus Short Cartridge, e. needle); 2. assembled; 3. 45-position tray for FFA; 4. syringe autosampler
The second objective was the minimization of GC analysis time using narrow bore capillary column together with the structurally informative MS fragmentation pattern, for this allowed assays in very short time windows. In this investigation, the GC analysis of FA derivative was carried out in less than 6.00 min with a speed gain of almost 6 times in comparison with traditional GC procedure to maintain sufficient resolving power for separation of the compounds of interest (Figure 3). The degradation product of DNPH, identified as 2,4-dinitroaniline [11, 12], was directly adjoining that of FA-2,4-dinitrophenylhydrazone when 5% phenylmethylpolysiloxane capillary column was used. We observed that the separation of these two peaks is difficult because of the disturbance by GC–nitrogen phosphorus detection proposed by other authors and when the cation-exchange resin was not used [13–16].

Finally, the last goal was the high-throughput automated analysis, thanks to the flexibility of xyz robotic system. All sampling management processes were available with connection with open source Laboratory Information Management System developed by Bika Lab System (Western Cape, South Africa) that allows a user-programmable suite, and so customized processing steps could be created easily by the analyst. A number of sample preparation steps immediately before sample injection have been automated, allowing “just in time” sample preparation. This technology allows to analyze 216 samples in 24 h.

Figure 2. Oasis MCX: base isolation

Figure 3. Chromatogram without (a) and with (b) Oasis MCX purification. GC–MS electron impact ionization spectrum: 1. IS, 2. 2,4-dinitroaniline, 3. FA-2,4-dinitrophenylhydrazone, 4. DNPH
In light of what has been indicated above, the authors present the final results. The linearity ($0.125 – 64 \mu g/cartridge, \ y = 0.0613x – 0.00023$) showed a correlation coefficient of 0.99, and LOQ resulted in $0.072 \mu g/cartridge$. The precision of the assay (as a coefficient of variation, CV%) was estimated as within session repeatability by analysis of $1 \mu g/cartridge$ FA samples (Table 1) with total uncertainty of measurements of 22%.

A study was carried out in Tuscany, using 104 workers occupationally exposed to FA vapors in anatomy laboratories. Average and range of personal exposure levels were 0.11 ppm (0.03 – 1.45 ppm) for medical doctors and 0.05 ppm (0.02 – 0.49 ppm) for technicals.

Conclusions

For the first time, the authors present an analytical method for sampling and analysis of FA in work environments that is robust, sensitive, and simple using full automation. The sensitivity attained permits the evaluation of FA concentration with reduced sampling periods as well, producing an instantaneous measurement of FA levels. The quality of the fast GC–MS approach allows for excellent resolution, even with short analysis time, to resolve the analytes of interest from compounds that would interfere with the assay. The use of this innovative xyz autosampler configuration provides better traceability of the sampling and high throughput of the analysis.

Acknowledgments. The authors wish to take this opportunity to express their sincere appreciation to Regione Toscana especially Bernardo Perazzi, for his collaboration that has given us the chance to conduct this study and collect the required data.

References

1. Sriram P.; Alvarado M.; Bagdjian V.; Am X. Formaldehyde: Chemical Economics Handbook. 2015. IHS. https://www.ihs.com/products/formaldehyde-chemical-economics-handbook.html. Accession date: 28 February 2017.
2. Formaldehyde: 2016 World Market Outlook and Forecast up to 2020. Merchant Research and Consulting Ltd. http://mcgroup.co.uk/researches/formaldehyde. Accession date: 28 February 2017.
3. Wang, Q.; O'Reilly, J.; Pawliszyn, J. J. Chrom. A. 2005, 1071, 147–154.
4. Wu, L. J.; Que Hee, S. S. Am. Ind. Hyg. Assoc. J. 1995, 56, 362–367.
5. Hafkenscheid, T. L.; van Oosten, J. A. Anal. Bioanal. Chem. 2002, 372, 658–663.
6. Hindshaw, J. V. LCGC North Am. 2015, 33, 318–325.
7. Pace, M.; Dugheri, S.; Gigliano-Candela, R.; Strisciullo, G.; Franchi, E.; Degli Esposti, F.; Perchiazzi, N.; Boccalon, P.; Arcangeli, G.; Cupelli, V. Acta Chim. 2009, 21, 379–397.
8. Pace, M.; Dugheri, S.; Boccalon, P.; Arcangeli, G.; Dolara, P.; Cupelli, V. Ind. Health 2010, 48, 217–221.
9. Pieraccini, G.; Bartolucci, G.; Pacenti, M.; Boccalon, P.; Arcangeli, G.; Dolara, P.; Cupelli, V. J. Environ. Monit. 2010, 3, 654–660.
10. Subramanian, P.; Breuer, G. M.; Reynolds, S. J. Int. J. Environ. Anal. Chem. 2000, 76, 215–239.
11. Dalene, M.; Persson, P.; Skarping, G. J. Chromatogr. 1992, 626, 284–288.
12. Jeong, J. Y.; Paik, N. W. J. Occup. Environ. Hyg. 2005, 2, 244–250.
13. Suliman, F. E.; Soma, Y. J. Environ. Monit. 2000, 2, 470–475.