Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1029 (2004) 217–222
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Quantitative determination of formaldehyde in cosmetics using a combined solid-phase microextraction–isotope dilution mass spectrometry method

René Thomas Rivero*, Vinod Topiwala

Analytical Lab, Coty Research and Development Center, 410 American Road, Morris Plains, NJ 07950-2451, USA

Received 26 September 2003; received in revised form 18 December 2003; accepted 22 December 2003

Abstract

Solid-phase microextraction (SPME) in conjunction with isotope dilution mass spectrometry (ID-MS) was employed for the analysis of formaldehyde in cosmetic products. The formaldehyde is derivatized in situ with pentafluorophenyl hydrazine. The formed hydrazone is adsorbed over a poly(dimethylsiloxane)–divinylbenzene-coated fiber and analyzed using gas chromatography–mass spectrometry. The adsorption–time profiles and salting effect were studied. The quantitation was performed by using a stable isotope labeled analogue as an internal standard. The precision, recovery and detection limits were determined with spiked samples. The relative standard deviations from different spiked cosmetic samples were all less than 10% and the recoveries were between 89.00 and 101.23%. The limit of detection was of 0.39 μg/l. Compared with other techniques, the study shown here provides a simple, fast and reliable method for the analysis of formaldehyde in cosmetic products.

Keywords: Cosmetics; Derivatization, GC; Isotope dilution; Formaldehyde; Carbonyl compounds; Pentafluorophenyl hydrazine

1. Introduction

Formaldehyde is a commonly found ingredient in cosmetic products. It is used to preserve cosmetic raw materials, or it is liberated by a formaldehyde-donor in consumer products. Based on its hydrophilic property, it is fairly common to find it in watery concoctions like shampoos, conditioners, shower gels, etc. In recent years, there has been a tendency in the industry to restrict and regulate the use of formaldehyde in consumer products.

For the assessment of formaldehyde in cosmetics, derivatizations prior to detection by a chromatography or a spectroscopic technique are commonly employed. An example would be the formation of a lutidine derivative: 3,5-diacetyl-1,4-dihydrolutidine followed by high-performance liquid chromatography (HPLC)–ultraviolet (UV) detection [1]. The lutidine derivative method allows the quantitation of free formaldehyde in the presence of its donators in cosmetic samples. Another commonly use method for determining formaldehyde is based on the derivatization with 2,4-dinitrophenylhydrazine (2,4-DNPH) [2]. The 2,4-DNPH method permits the quantitation of formaldehyde and other aldehydes in industrial surfactants. The chromotropic acid test is also employed, in which a solution of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) react with formaldehyde to produce a purple species [3] and its absorbance is measured spectrophotometrically at 570 nm. The mechanism of this reaction has not been fully elucidated [4]. The application of the above-mentioned techniques have also been reported in the environmental field [5,6].

All the methods stated earlier involve complex procedures for sample preparations (i.e. solvent extraction, filtration, etc.) and therefore are very laborious. A new technique called solid-phase microextraction (SPME) was invented in the early 1990s by Pawliszyn [7] at the University of Waterloo, Ontario, Canada. SPME involves the extraction of the analyte from a liquid, the headspace above a liquid or solid, or a gaseous phase. The analyte partitions between the sample matrix and the fiber coating until an equilibrium is reached. Finally, the analyte is desorbed from the fiber into a capillary GC column. SPME has been demonstrated

* Corresponding author. Tel.: +1-973-490-8720; fax: +1-973-644-9175.
E-mail address: rene_rivero@cotyinc.com (R.T. Rivero).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.12.054

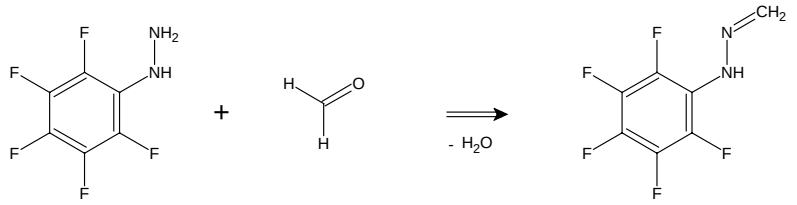

Fig. 1. Reaction between pentafluorophenyl hydrazine and formaldehyde to form the respective hydrazone.

to be an excellent option compared to the traditional technique. SPME is a simple, effective adsorption–desorption and environment-friendly technique that eliminates the need for solvents or the aggravation of concentrating volatile or semi-volatile compounds [8]. Controlling and monitoring the analyte parameters of temperature, time and technique are critical to accomplish a quantitatively reproducible SPME result.

The contribution of the present work combines the application of the SPME with isotope dilution mass spectrometry (ID-MS) for the determination of formaldehyde in cosmetic products. The technique of ID-MS is playing an increasingly important role in trace analysis [9]. ID-MS has greater accuracy than other calibration methods (i.e. external standard, standard addition, etc.) and also has the capacity to compensate for matrix effects. Isotope dilution methodology is becoming the method of preference for quantitative mass spectrometry because of the potential to render the lowest variance factors due to sample manipulation or instrumental error. In this new approach, formaldehyde is first derivatized with pentafluorophenyl hydrazine (PFPH) in situ. Once the hydrazone has been formed (see Fig. 1), equilibrium begins to develop between the aqueous phase and gaseous phase (headspace). This allows the analyte to be adsorbed on the SPME-coated fiber located in the headspace of the vial.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical-reagent grade unless otherwise specified. We used water (J.T. Baker, Phillipsburg, NJ, USA), unlabeled formaldehyde solution, 37% (Sigma, St. Louis, MO, USA), labeled formaldehyde (^{13}C, 99%) at 20% solution (Cambridge Isotope Labs., MA, USA), pentafluorophenyl hydrazine, 97% (Aldrich, St. Louis, MO, USA), sodium chloride (Extra Pure, Merck, Darmstadt, Germany), sodium lauryl sulfate, formaldehyde-free (Sulfochem SLS-BZ, Chemron, Paso Robles, CA, USA) and phosphoric acid, 85% (Fisher Scientific, Pittsburgh, PA, USA).

2.2. Instruments and equipments

We used the following: SPME fiber, poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB), 65 μm, catalog no. 57326-U (Supelco, Bellefonte, PA, USA), headspace vial, 10 ml (Supleco), block heater (Alltech Associates, Deerfield, IL, USA), HP 6890 gas chromatograph coupled to a HP-5973 mass spectrometer (Agilent Technologies, Wilmington, DE, USA), SPME septa (pre-drilled septa, Supleco), inlet liner for SPME (0.75 mm i.d., Supleco) and capillary column HP-1 methyl siloxane, 30 m × 0.25 mm, 0.25 μm film thickness (Agilent Technologies).

2.3. GC–MS analysis

The GC–MS analyses were performed on a Hewlett-Packard 6890 GC system coupled to a Hewlett-Packard HP 5973 quadrupole mass spectrometer. Helium was the carrier gas at a flow of 1.2 ml/min. The separation of compounds was performed on a HP-1 column. The column temperature was held at 100 °C for 5 min and increased to 300 °C at 10 °C/min. The ion source and transfer line were 230 and 280 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy. The ions characteristic of the analyte and isotopically labeled analogue were detected by selected ion monitoring (SIM) and total ion monitoring. The injector temperature was 250 °C and the desorption time of the SPME fiber was 5 min.

2.4. Determination of adsorption–time profile and calibration curve

PDMS–DVB SPME fiber (65 μm) was selected because of its high load capacity and adsorbs the pentafluoro hydrazone derivative with greater reproducibility. One milliliter of 1.5 mM PFPH prepared with 0.03 M of phosphoric acid solution [10], 0.5 ml of formaldehyde standard solution (4 μg/ml) and 0.5 ml of the stable isotope labeled formaldehyde ($^{13}CH_2O$) solution (4 μg/ml) were placed in a 10 ml PTFE-capped vial containing 0.6 g of sodium chloride. The vial was sonicated for 10 min prior to being placed in a heating block maintained at 35 °C. The SPME-coated fiber (65 μm) was inserted in the headspace of the vial. To obtain an adsorption–time profile to determine the appropriate time for further headspace extraction of the analyte, the SPME fiber was exposed to the headspace of the prepared solution for 1, 5, 10, 15, 30, 40, 60, and 70 min, respectively [11]. A fresh solution was used for each time interval. The GC area counts from the thermal desorptions versus the time that the fiber was exposed to the solution was plotted to obtain

an exposure time profile. To confirm that the desorption was complete when the SPME fiber was inserted into the GC injection port, different desorption times were studied to investigate their desorption efficiencies. For the duration of the analyses in this work, the SPME-coated fiber was always first exposed onto the GC injector port as a blank run before the next experiment to ensure that the fiber was clean, as well as to avoid carryover effects.

In addition to the 4 μg/ml sample tested, different standard concentrations of formaldehyde ranged from 0.100 to 10.000 μg/ml and doped with a fixed amount (0.5 ml) of isotope labeled analogue (4 μg/ml) were analyzed in the same manner as before to establish the calibration curve. After the headspace extraction of the formaldehyde derivative standard solutions for 15 min, the SPME-coated fiber was inserted for 5 min into the injector of the GC–MS system for analysis. The selective ion monitoring in mass spectrometer utilized *m*/*z* 210–211 while total ion monitoring utilized *m*/*z* 50–300. The experiments were performed in triplicate. The ion abundance ratio of the formaldehyde pentafluorophenyl hydrazone and its corresponding isotope labeled analogue derivative were plotted versus the formaldehyde concentration in the standard solutions (i.e. *m*/*z* 210/211 versus concentration).

2.5. Sample preparation for the determination of formaldehyde content

Various categories of cosmetic products and raw materials were employed in this study: nail polish, shower gel, mascara, body cream, and surfactant. All raw materials and cosmetics samples employed in this work were prepared as follows: weigh 3 g of sample into a 10 ml volumetric flask, add 5 ml of aldehyde-free water and sonicate for 15 min. Dilute to volume with water and mix well (filter if necessary). Pipette 0.5 ml of the above solution into a 10 ml PTFE-capped vial containing 0.6 g of sodium chloride, then add 1 ml of 1.5 mM PFPH solution, and 0.5 ml of the stable isotope labeled formaldehyde ($^{13}CH_2O$) solution (4 μg/ml). Sonicate for 10 min and place the vial into a heating block maintained at 35 °C. The SPME-coated fiber (65 μm) was inserted in the headspace of the vial and allowed to adsorb for a period of 15 min. The SPME-coated fiber was then inserted into the GC injection port and allowed to desorb for an elapsed period of 5 min. The ion abundance ratio of formaldehyde pentafluorophenyl hydrazone (*m*/*z* 210) and its corresponding isotope labeled analogue hydrazone derivative (*m*/*z* 211) was used together with the previous calibration data to quantitatively determine the formaldehyde content in the samples.

2.6. Preparation of surfactant and cosmetic products (formaldehyde-free) spiked with formaldehyde

To determine the precision and recovery of the current technique, samples of raw materials and cosmetic products that were spiked with formaldehyde (20 μg/ml) were analyzed 10 times based on the procedure mentioned earlier. The repeatability relative standard deviation (R.S.D.) and recovery for each spiked sample were then calculated. Another spiked sample (20 μg/l) of surfactant was also analyzed eight times to determine the limit of detection (LOD), calculated as three times the background noise (signal-to-noise ratio, $S/N = 3$) [12,13].

3. Results and discussion

To deposit PFPH derivative onto the SPME-coated fiber, a solution containing the analyte, PFPH reagent, internal standard and salt was placed in a 10 ml PTFE screw-capped vial and the solution was sonicated for 10 min. As shown in Fig. 2, the mass of formaldehyde–PFPH derivative adsorbed through the headspace on the SPME fiber increased as the adsorption time increased. The equilibrium time was around 60 min. The selected exposure time in the current study does not represent the equilibrium time. The SPME technique has the ability to be used quantitatively before equilibrium is reached, although constant convection and temperature in the system need to be ensured to obtain reproducible data. This condition requires good temperature control and a vibration-free environment (for static conditions) [14,15]. For the purpose of the current experiment, a 15 min extraction time and 35 °C were employed, since it was not necessary to reach equilibrium, as previously stated, and a 15 min extraction time yielded sufficient extraction (ca. 80%) of the analyte. The efficiency of the thermal desorption of the SPME fiber was also determined. At temperature of 250 °C, the desorption efficiency was found to be 99.98% when the desorption time was 5 min. Fig. 3 showed a typical total ion chromatogram and mass spectrum of a nail polish sample spiked at 20 μg/ml. Further experiments

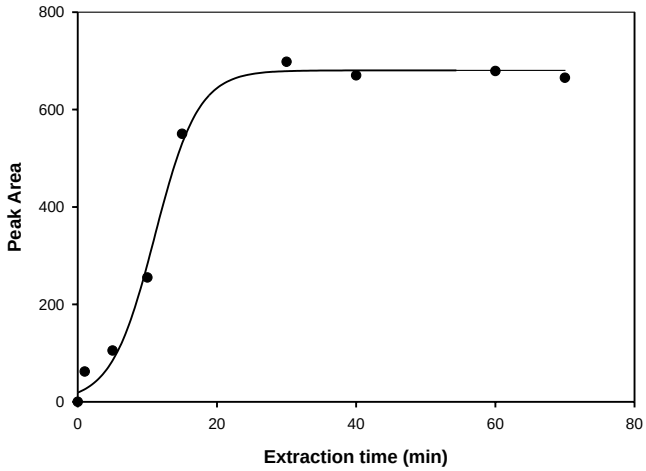

Fig. 2. Adsorption–time profile for formaldehyde using headspace SPME-coated fiber. Sample volume 0.5 ml, spiking level 4 μg/ml and temperature 35 °C.

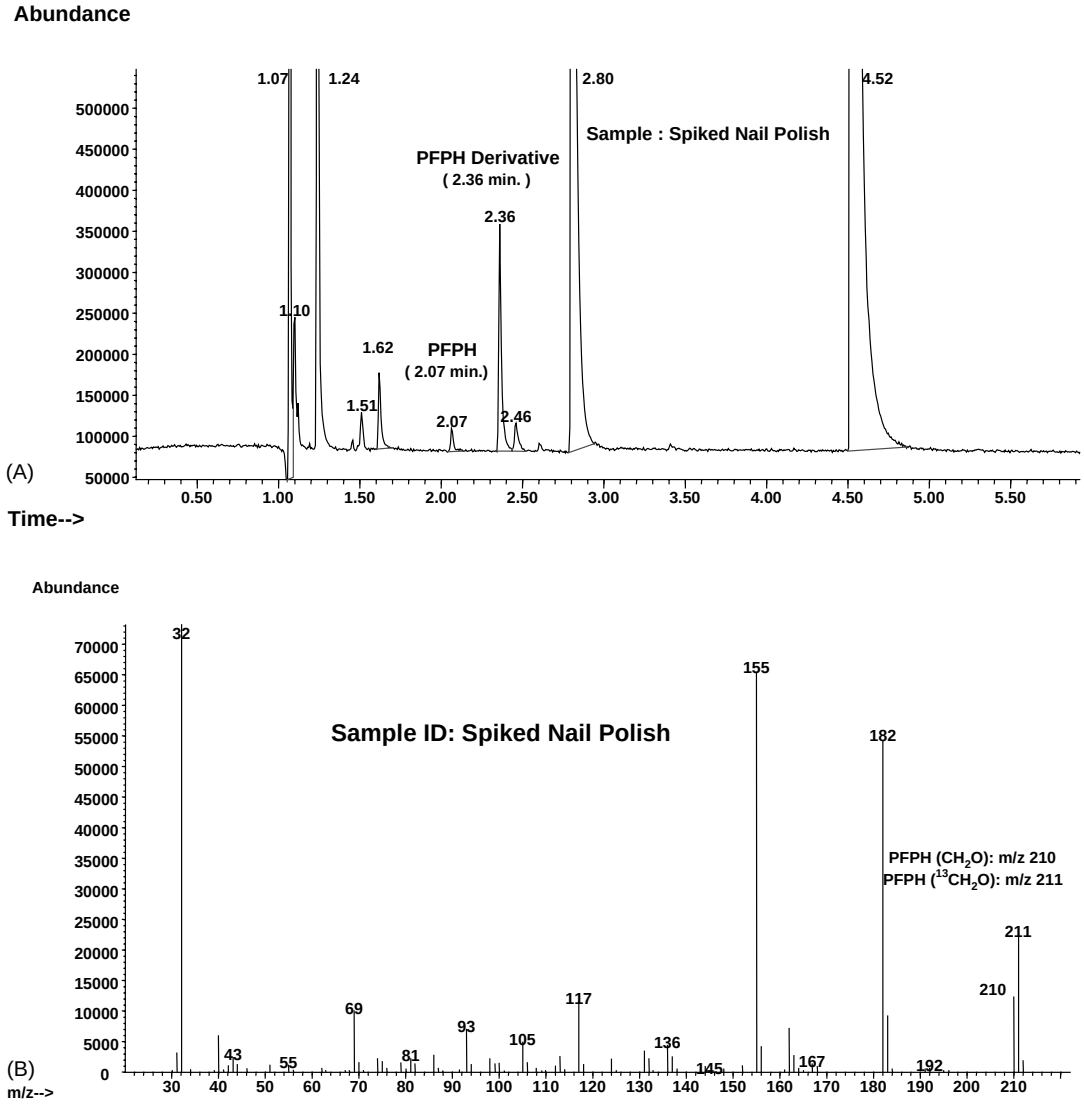

Fig. 3. (A) Typical total ion chromatogram of nail polish obtained after SPME–ID-MS method. (B) Typical mass spectrum of formaldehyde derivative obtained after SPME–ID-MS method.

showed that the peak of unreacted PFPH was still observed after the derivatization reaction occurred with a spiked sample at a level of 200 μg/ml (shower gel sample). Samples that exhibit a concentration level of more than 200 μg/ml should be re-run, reducing the size of the sample and/or increasing the dilution volume. This ensures that the concentration is within the calibration curve range. Therefore, increasing the amount of reagent was not necessary in this research.

During this work, the experimental parameters were studied and optimized to achieve better adsorption and desorption processes including all factors affecting the equilibrium between the analyte in the sample and on the SPME fiber. The formaldehyde derivative was exposed to different types of coating fibers, such as PDMS–DVB, and PDMS. It was found that the amount adsorbed under the same conditions on the PDMS–DVB is approximately twice the amount observed with the PDMS-coated fiber. This result corroborates the supplier's recommendation. The salting effect on the adsorption of the formaldehyde to the SPME fiber was studied to select the best salt for the experiment. Salts with different ionic strengths were employed: sodium chloride, potassium chloride, potassium bromide and ammonium chloride. All salts were used in saturated concentrations to reduce the solubility of the analyte. By using the integrated peak area provided by GC–MS to monitoring the salting effect, it was observed that sodium chloride has the greatest effect on the peak area of the formaldehyde. According to the results, an addition of 30% of sodium chloride was employed to optimize the effectiveness of the adsorption step. Besides the salting effect, the influences of different extraction temperatures were also investigated and Fig. 4 showed the results. The data showed the dependence of extraction temperature as expected. However, as mentioned previously, a 15 min extraction time at 35 °C yielded sufficient efficiency and

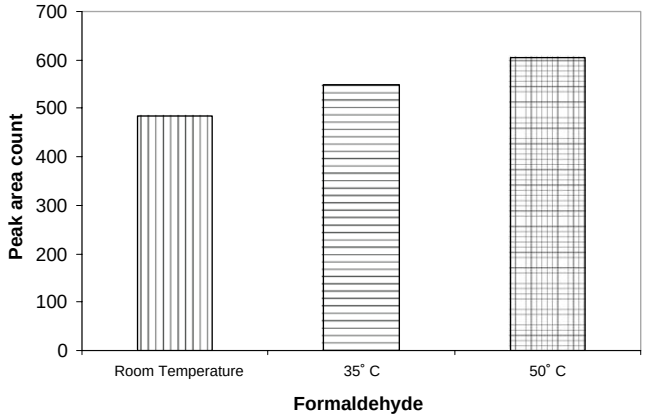

Fig. 4. Effects of extraction temperature of spiked cosmetic sample. Extraction time 15 min, spiking level 20 μg/ml and temperature 35 °C.

provided acceptable sensitivity (Table 2). A narrower liner (0.75 mm i.d.) was employed to increase the flow around the fiber, resulting in efficient removal of desorbed analyte and the sharpening of the peaks [15].

In this work, we achieved a high accuracy for the quantification of formaldehyde by employing a graphical method using a calibration curve [16]. The non-zero intercept observed in the calibration curve ($y = 0.2231x + 0.1033$) is attributed to the so-called spectral overlap as a result of the incomplete labeling of the internal standard (labeled formaldehyde, 99%). The calibration curve showed a correlation coefficient $r^2 = 0.9973$ for the formaldehyde–PFPH derivative and accuracy expressed in terms of the standard error of estimate of 0.0506. The x axis of the calibration curve was the concentration of formaldehyde in μg/ml; the y-axis was the ion abundance ratio of the PFPH formaldehyde derivative to its PFPH stable labeled isotope analogue derivative (m/z 210/211). Table 1 showed the data of precision and recovery for various samples, including nail polish, shower gel, body cream, and surfactant. Table 2 showed the data for the of limit of detection, and it was found that the current method had better sensitivities than the lutidine HPLC method [1], 2,4-DNPH HPLC method [2] and chromotropic acid method [3]. Besides the data from all the spiked cosmetic products used in this work, samples of mascara, make-up, and shower gel of different brands were analyzed using the current procedure without the spiking of formaldehyde. No formaldehyde was detected in the make-up sample, and the mascara

Table 1
Precision and recovery in cosmetic products and raw material

Sample tested	Recovery (%)	R.S.D.% (r)[a]
Nail polish recovery (20 μg/ml)[b]	89.00	7.8
Shower gel recovery (20 μg/ml)	97.21	3.8
Body cream recovery (20 μg/ml)	92.42	4.2
Surfactant recovery (20 μg/ml)	101.23	5.1

[a] R.S.D.% (r) = 100(S_r/x), where S_r is the repeatability standard deviation and x the observed mean of the data.
[b] Spiked concentration.

Table 2
The comparison of the LOD between different methods

Analyte	LOD in current research[a] (μg/l)	LOD in lutidine method[b] (μg/l)	LOD in 2,4-DNPH method[c] (μg/l)	LOD in chromotropic acid method[d] (μg/l)
Formaldehyde	3.9	40	100	50

[a] Spiked concentration = 20 μg/l, $n = 8$.
[b] Ref. [1].
[c] Ref. [2].
[d] Ref. [3].

exhibits an expected low concentration of formaldehyde (9 μg/ml). In the shower gel sample, it was determined that the ion abundance ratio fell above the calibration range and a close examination of the total ion chromatogram revealed the absence of the unreacted PFPH peak.

This result suggested that the level of formaldehyde and/or other carbonyl groups exceed the reagent capacity to react completely. The shower gel was re-tested. This time the sample size and dilution volume were properly adjusted in order to obtain an ion abundance ratio within the calibration curve range. The presence of the unreacted PFPH peak in the total ion chromatogram was observed. The level of formaldehyde found in the re-tested sample was 988 μg/ml which is about 20% higher than the original analysis. This corroborates the assumption that an excessive amount of analyte(s) was/were present in the original sample and gave the doubtful result.

During this study, it was observed that cosmetic products with complex matrices (i.e. nail polish) interfere with the determination of formaldehyde. This can be attributed to the interaction of formaldehyde with some matrix components, such as pigments, polymers, etc. However, the percent recovery for all sample studied were above 85% (Table 1).

4. Conclusions

In conclusion, the research shown here demonstrated that the analysis of formaldehyde in cosmetic products provides acceptable precision and sensitivity with a simple one step procedure. For samples containing a higher level of formaldehyde, the sample size and dilution volume can be adjusted to assure the sufficiency of PFPH reagent to produce the corresponding formaldehyde derivative. This method is suitable for use in the routine analysis of cosmetic products because it is not time-consuming and no solvents are required.

Acknowledgements

The authors gratefully acknowledge the technical assistance and support provided by Coty Research and Development Center and especially to Ralph Macchio, Paul Chapin, Frank Lucia and William Feuer.

References

[1] H. Engelhardt, R. Klinkner, Chromatographia 20 (1985) 559.
[2] J.R. Dahlgran, M.N. Jameson, J. Assoc. Off. Anal. Chem. 71 (1988) 560.
[3] P.W. West, B. Sen, J. Anal. Chem. 153 (1956) 12.
[4] J. Zhang, D. Thickett, L. Green, J. Am. Inst. Conserv. 33 (1994) 47.
[5] ASTM Standard, Determination of formaldehyde and other carbonyl compounds in air (active sampler methodology), Method ASTM D 5197-97, American Society for Testing and Materials, Gaithersburg, MO, 1997.
[6] ASTM Standard, Determining formaldehyde concentration in air and emission rates from wood products using a large chamber, Method ASTM E 1333-96, American Society for Testing and Materials, Gaithersburg, MO, 1996.
[7] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[8] C.L. Arthur, D.W. Potter, K.D. Buchholz, S. Motlagh, J. Pawliszyn, LC·GC 10 (1992) 656.
[9] M. Sargent, C. Harrington, R. Harte (Eds.), Guidelines For Achieving High Accuracy in Isotope Dilution Mass Spectrometry (IDMS), RSC Analytical Methods Committee, Cambridge, UK, 2002, p. 2.
[10] H. Heck, E.L. White, M. Casanova-Schmitz, Biomed. Mass Spectrom. 9 (1982) 347.
[11] S. Tsai, C. Chang, J. Chromatogr. A 1015 (2003) 143.
[12] P.C. Meier, R.E. Zund, Statistical Methods in Analytical Chemistry, 2nd ed., Wiley–Interscience, New York, 2000.
[13] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, 3rd ed., Ellis Horwood, Chichester, 1993, p. 115.
[14] J. Pawliszyn, Applications of Solid-Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999.
[15] J. Pawliszyn, Solid-Phase Microextraction—Theory and Practice, Wiley–VCH, New York, 1997.
[16] D.A. Schoeller, J. Clin. Pharmacol. 26 (1986) 396.