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Quantitative determination of formaldehyde in cosmetics using combined headspace–solid-phase microextraction–gas chromatography

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Synopsis

The objective of this research was the application of headspace (HS)–solid-phase microextraction (SPME) for the quantitation of formaldehyde present in raw materials and cosmetic formulations. The formaldehyde was derivatized *in situ* first with pentafluorophenylhydrazine (PFPH), to form a derivative hydrazone. The formed hydrozone was adsorbed on a SPME fiber during headspace extraction under controlled conditions (time, temperature, volume, etc.). After the adsorption step, the SPME fiber was directly transferred into the gas chromatography (GC) injection port in which the analytes were thermally desorbed. Deuterated acetone was used as an internal standard (IS) in order to quantitate the formaldehyde content. For the experiment, a gas chromatograph equipped with a flame ionization detector (GC/FID) was employed. A gas chromatograph/mass spectrometer (GC/MS) was used for the qualitative confirmation of results in this work.

INTRODUCTION

For decades, formaldehyde was one of the most widely used preservatives in personal care products due to its versatility, cost, and efficiency. In recent years, the potential for carcinogenic and respiratory sensitization from formaldehyde has become widely understood. This has led to a movement in the industry that has imposed regulations, restrictions, and formaldehyde's usage being banned.

In surfactant and cosmetic industries several analytical procedures have been developed for qualitative as well as quantitative analysis. One of the most well known procedures for qualitative analysis is the *phloroglucinol test,* in which formaldehyde reacts in an alkaline medium with phloroglucinol to produce a reddish-brown color complex. For the quantitation of formaldehyde, several methods have been employed, one of which is based on *Nash reagent.* In this determination, formaldehyde is condensed with ammonia and acetylacetone to form the lutidine derivative 3,5-diacetyl-1,4-dihydrolutidine (1,2). Nash reagent is sensitive not only to formaldehyde, but also to other aldehydes. If chromophore compounds are present in the product, they could interfere with the spectrophotometric determination. Formaldehyde can also be determined colorimetri-

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cally with chromotropic acid (3,4). *The chromotropic acid test* is based on the reaction of formaldehyde with a solution of chromotropic acid (1,8-dihydroxynaphthalene-3, 6-disulfonic acid) to produce a purple species in solution. The mechanism of this reaction has not been fully elucidated (5). One difficulty with this technique is that some perfume ingredients used in cosmetics liberate aldehydes in an acid medium and give a false-positive test.

Other techniques have been reported for the determination of carbonyl compound by derivatization with 2,4-dinitrophenylhydrazine (DNPH) utilizing gas chromatography (6). In another technique, high-performance liquid chromatography (HPLC) has been used, after the derivation of formaldehyde by DNPH. This technique has been reported by Wu *et al.* (7).

The Conway microdiffusion technique has been employed for the determination of free formaldehyde (8). This method is based on the principle of gas diffusion from a relatively large volume of solution under analysis to a very small volume of aqueous trapping solution until the free formaldehyde concentration of the test solution is same as in the absorbent solution.

The contribution of the present work is attributed to the application of the solid-phase microextraction (SPME) procedure for the determination of formaldehyde in cosmetic products. SPME is a powerful alternative to traditional techniques for the extraction of volatile or semivolatile organic compounds. The method, invented in the early nineteen nineties by Prof. Janusz Pawliszyn (9) from the University of Waterloo in Ontario, utilizes a small segment of fused silica fiber coated with an appropriate material and mounted on a syringe-like device for extraction of analytes from various matrices and introduced to a chromatographic system. No solvents are used in the process. Analyte extraction and pre-concentration are combined in a single step. The technique itself has been thoroughly described (10–12) for qualitative analysis as well as for quantitative determination (13–15).

EXPERIMENTAL

REAGENTS AND MATERIALS

The reagents and materials used were water (HPLC grade, J. T. Baker Inc, Phillipsburg, NJ); 37% formaldehyde solution (Sigma, St. Louis, MO); 97% pentafluorophenylhydrazine (Aldrich, St. Louis, MO); sodium chloride (Extra Pure, EM Industries, Damstadt, Germany); deuterated acetone (Aldrich, Milwaukee, WI); formaldehyde-free sodium lauryl sulfate (Sulfochem SLS-BZ, Chemron, Paso Robles, CA); and formaldehyde-free sodium laureth sulfate (Sulfochem ES-2DX-BZ, Chemron).

INSTRUMENTS AND EQUIPMENT

The instruments and equipment used were SPME fiber, polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm, catalog no. 57326-U (Supelco, Bellefonte, PA); a headspace vial, 10 ml (Supelco); a block heater (Alltech Associates Inc, Deerfield, IL); a gas chromatograph (HP 6890) equipped with FID (Agilent Technologies, Wilmington, DE); a mass spectrometer (HP-5973, Agilent); SPME septa (Pre-drilled septa,

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Supleco); an inlet liner for SPME (0.75 mm ID, Supelco); and a capillary column (HP-1 methyl siloxane, 30 m × 0.25 mm × 0.25 μm film thickness, Agilent).

PREPARATION OF SOLUTIONS

Preparation of 25% sodium chloride solution. The proper amount of sodium chloride was dissolved in HPLC-grade water.

Preparation of 1.5 mM pentafluorophenylhydrazine (PFPH). The proper amount of PFPH was dissolved in HPLC-grade water. This solution was used as a derivatization agent in the present work.

Preparation of 0.5000 mM, 0.2500 mM, 0.1250 mM, 0.0625 mM, 0.0313 mM, and 0.0010 mM of formaldehyde stock standard solutions

These solutions were prepared using 37% formaldehyde solution (assayed as per EPA method 8315A) and diluted with formaldehyde-free sodium lauryl sulfate (Sulfochem SLS-BZ).

Preparation of 0.5 mM deuterated acetone stock internal standard solution. The proper amount of acetone was dissolved with 25% aqueous solution of sodium chloride.

Preparation of surfactants and cosmetic products (formaldehyde-free) spiked with formaldehyde

To determine the recovery and precision of the current method, samples of raw materials and cosmetic products that were spiked with formaldehyde (0.05%) were analyzed ten times. The coefficient of variation (CV%) and recovery for each spiked sample was calculated. Another spiked sample (15 μg/ml) of surfactant was also analyzed eight times to determine the limit of detection (LOD), with calculated signal-to-noise ratio = 3 (S/N = 3).

PROCEDURE

Hydrozone. Pentafluorophenylhydrazine reacts with aldehydes and the ketones group by nucleophilic addition to the carbonyl group followed by elimination of water and the formation of pentafluorophenylhydrozone. The addition of acid, in general, is recommended to promote protonation of the carbonyl because hydrazines are weak nucleophiles. However, this phenomenon was not observed during the study (see Figure 1).

Calibration standard for hydrozone derivative. We prepared at least five concentration levels of spiked formaldehyde in formaldehyde-free sodium lauryl sulfate. We added into a

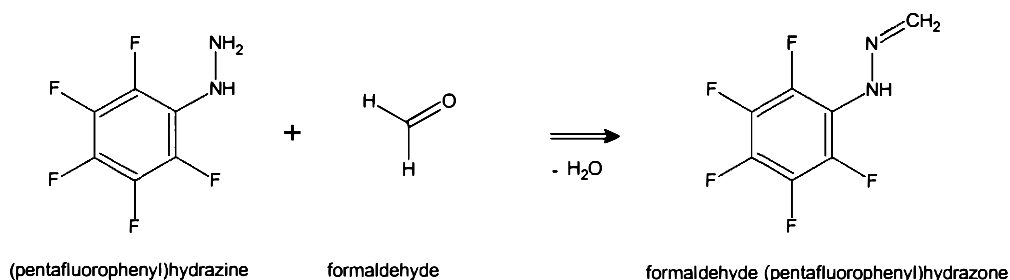

Figure 1. Reaction between pentafluorophenylhydrazine and formaldehyde to form the respective hydrazone.

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10-ml PTFE screw-capped vial 1 ml of 1.5 mM of PFPH solution, 0.5 ml of 0.5 mM deuterated acetone, and 0.5 ml of 0.5 mM formaldehyde concentration. We placed the vial into a temperature-controlled heating block at 35°C after sonication for five minutes. We inserted SPME fiber in the headspace of the vial for an adsorption period of 15 minutes. After the adsorption step, the fiber was directly inserted into the GC injector, in which the analytes were thermally desorbed at 250°C. To obtain a complete desorption, the SPME fiber was allowed to stay in the injection port for five minutes. For successive analysis of samples in this work, the SPME fiber was always first exposed onto the GC injector port as a blank run before the next experiment, to make sure the fiber was clean as well as to avoid the carryover effects. We repeated the experiment using 0.25 mM, 0.125 mM, 0.0625 mM, 0.0313 mM, and 0.0010 mM of formaldehyde concentrations in the same manner as before, to establish the calibration curve. The experiments were performed in triplicate. A plot was generated by using formaldehyde concentration vs peak area ratio: formaldehyde derivative/IS derivative (Table I, Figure 2).

Sample preparation for the determination of formaldehyde content. Various categories of cosmetic products and raw materials were used in this study: nail polish, shower gel, make-up foundation, and surfactant. All raw materials and cosmetics samples employed in this work were prepared as follows: We weighed 0.50 g of sample into a 50-ml volumetric flask, added 20 ml of water, and sonicated for 15 minutes. We diluted to volume with water and mixed well (filtering if necessary). We pipetted 0.5 ml of the above solution into a 10-ml headspace PTFE-capped vial, then added 1 ml of 1.5 mM of PFPH solution and 0.5 ml of 0.5 mM deuterated acetone. We sonicated for five minutes and placed the vial into a temperature-controlled heating block at 35°C. We inserted SPME fiber into the headspace of the vial for an adsorption period of 15 minutes. After the adsorption step, the fiber was directly inserted into the GC injector, in which the analytes were thermally desorbed at 250°C. To obtain a complete desorption, the SPME fiber was allowed to stay in the injection port for five minutes.

Chromatographic conditions

- Column: non-polar HP-1
- Injector temperature: 250°C, splitless
- Carrier gas: helium
- Oven temperature: 100°C (5 min), 10°C/min, to 300°C.
- Detector temperature (FID): 275°C
- Flow: 1.2 ml/min

Table I
Concentration vs Peak Area Ratio

Concentration (µg/ml)	Peak area ratio
17.7000	9.538
8.8500	4.590
4.4250	2.128
2.2125	1.231
1.1063	0.692
0.0350	0.026
0.0000	0.000

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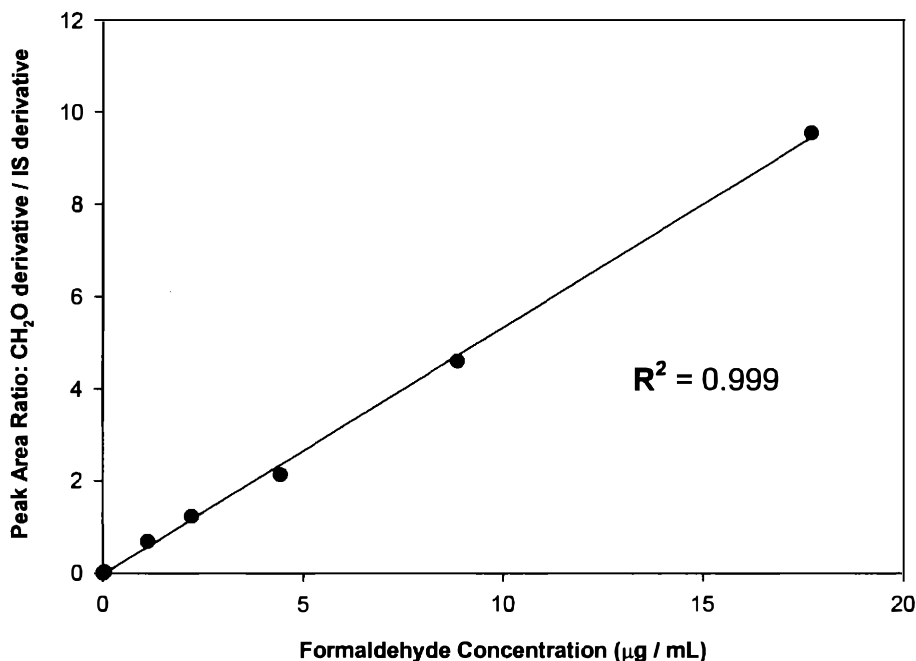

Figure 2. Calibration curve for quantitative determination of formaldehyde via HS-SPME-GC method.

RESULTS AND DISCUSSION

SPME is a powerful alternative to traditional techniques for the extraction of volatile or semivolatile organic compounds (9,16). It is a fast, simple, precise, and sensitive technique that requires no solvent (17). The adsorption time profile of the formaldehyde derivative on the SPME fiber was investigated, and it was observed that the equilibrium time was around 60 minutes. The selected exposure time in the current study does not represent the equilibrium time. Conventional sample preparation methods intend to remove the analytes from the sample, but SPME does not work in that manner. With SPME, the amount of analyte removed by the fiber is proportional to the concentration of compound in the sample. This permits the use of SPME on a quantitative basis before reaching equilibrium, allowing much shorter exposure times. For the purpose of current experiments, 15 minutes of extraction time yielded sufficient extraction (*ca* 75%) of the analyte. For quantitation purposes there are several parameters that need to be controlled in order to ensure optimum performance of the SPME: ionic strength, temperature, and time of adsorption and desorption.

Adding 25% sodium chloride solution can enhance the ionic strength of a solution, reducing the solubility of the analyte. Fluctuations of temperature can change the equilibrium distribution of analytes in the sample and the headspace. The amount of analyte adsorbed on the fiber is proportional to the time of extraction. By using a 0.75-mm ID inlet liner in the GC injector port, the linear velocity increased through the liner, which sharpened the peaks.

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Table II
Precision and Recovery in Cosmetic Products and Raw Materials (n = 10)

Sample tested	Recovery (%)	CV% (r)[b]
Nail polish recovery (0.05%)[a]	89.00%	9.5
Shower gel recovery (0.05%)	98.01%	4.1
Makeup foundation recovery (0.05%)	90.42%	6.3
Surfactant recovery (0.05%)	91.18%	5.6

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

The addition of an internal standard in the experiment was implemented to compensate for any undesired variation in the extraction condition, including the change of the fiber properties due to irreversible adsorption of some of the matrix components (18). This method was evaluated with respect to the linearity, run precision, limit of detection, and percent recovery. This study found a linear relationship between the amounts of formaldehyde-PFPH derivative adsorbed by coated SPME fiber and its concentration in the solution. The calibration curve (Figure 2) obtained by plotting peak area ratio versus concentration showed a high correlation coefficient, $R^2 > 0.999$, for the formaldehyde-PFPH derivative and accuracy, expressed in terms of the standard error of estimate, of 0.1353. Table II shows the precision and recovery of various samples, including nail polish, shower gel, makeup foundation, and surfactant. Table III shows the data for the limit of detection of the current method (*vide infra*).

During this study some interference occurred. This interference was due to the internal standard peak coeluted with compounds present in the matrix of some cosmetic products. To eliminate this drawback, the researchers have developed an alternative SPME method, combined with the isotope dilution mass spectrum technique, in which a stable labeled isotope analogue was employed as an internal standard. This paper was published in the *Journal of Chromatography A* (March 2004) (19). Both methods have their advantages. The present method is simple, low-cost, and can be used for routine analysis. The alternative method is more accurate and sensitive; however, it is relatively more expensive (labeled isotope analogues are not cheap) and GC/MS capability is also required. It has been reported by Hoshika *et al.* (20) and Stashenko *et al.* (21), that high sensitivity and selectivity can be accomplished by using an electron capture detector, resulting from the five halogen atoms on the PFPH moiety. A typical chromatogram obtained for this work is shown in Figure 3.

Table III
Limit of Detection (LOD) of Formaldehyde in Spiked Surfactant

Analyte	LOD in current research[a] (μg/ml)
Formaldehyde	0.04

[a] Spiked concentration = 15 μg/ml; n = 8.

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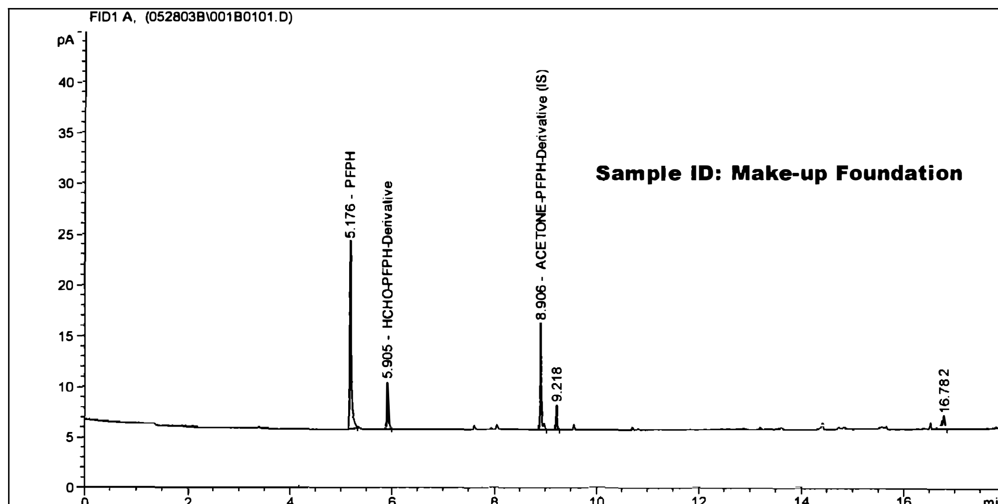

Figure 3. Typical chromatogram obtained after HS-SPME of the cosmetic sample.

CONCLUSION

Our work has demonstrated that SPME is fast, precise, and highly sensitive, and is an alternative procedure for the determination of formaldehyde in cosmetic products such as surfactant systems, foundations, and nail-polish products.

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REFERENCES

(1) Identification and determination of free formaldehyde, *Off. J. Eur. Commun.*, **L 185/18** (June 6, 1982).
(2) C. H. Wilson, Fluorometric determination of formaldehyde in cosmetic products, *J. Soc. Cosmet. Chem.*, **25**, 67–71 (1974).
(3) E. Sawicki, T. R. Hauser, and S. McPherson, Spectrophotometric determination of formaldehyde and formaldehyde-releasing compounds with chromotropic acid, 6-amino-1-naphthol-3-sulfonic acid (J-acid), and 6-anilino-1-naphthol-3-sulfonic acid (phenyl J-acid), *Anal. Chem.*, **34**, 1460 (1962).
(4) P. W. West and B. Sen. Spectrophotometric determination of traces of formaldehyde, *J. Anal. Chem.*, **153**, 12–18 (1956).
(5) J. Zhang, D. Thickett, and L. Green, Two tests for the detection of volatile organic acids and formaldehyde, *J. Am. Inst. Conservat.*, **33**, 47–53 (1994).
(6) Y. Hoshika, Y. Takata, Gas chromatographic separation of carbonyl compounds as their 2,4-dinitrophenylhydrazines using glass capillary columns, *J. Chromatogr.*, **120**, 379–389 (1976).
(7) P. W. Wu, C. C. Chag, and S. S. Chou, Determination of formaldehyde in cosmetics by HPLC method and acetylacetone method, *J. Food Drug Anal.*, **11**, 8–15 (2003).
(8) M. I. Feldman, Determination of free formaldehyde in the presence of its compounds with amino acids and proteins, *Biochemistry*, **23**, 867–872 (1958).
(9) R. P. Belardi and J. Pawliszyn, The application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns, *Water Poll. Res. J. Canada*, **24**, 179–189 (1989).

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From: SCC Media Library & Resource Center (library.scconline.org)

(10) C. L. Arthur and J. Pawliszyn, Solid-phase microextraction with thermal desorption using fused silica optical fibers, *Anal. Chem.,* 62, 2145–2148 (1990).

(11) B. Schafer, P. Hennig, and W. Engewald, Methodological aspects of headspace SPME: Application of the retention index system, *J. High Resol. Chromatogr.,* 20, 217–221 (1997).

(12) K. Wada and T. Shibamoto, Isolation and identification of volatile compounds from a wine using solid phase extraction, gas chromatography, and gas chromatography/mass spectrometry, *J. Agric. Food Chem.,* 45, 4363–4366 (1997).

(13) D. D. C. Garcia, M. Reichenbacher, K. Danzer, C. Hurlbeck, C Bartzsch, and K. Feller, Analysis of bouquet components using headspace–solid-phase microextraction–capillary gas chromatography, *J. High Resol. Chromatogr.,* 21, 373–377 (1998).

(14) E. Ralf and K. Levsen, Determination of pesticides in aqueous samples by solid-phase microextraction in-line coupled to gas chromatography–mass spectrometry, *J. Am. Soc. Mass Spectrum,* 6, 1119–1130 (1995).

(15) D. Garcia, S. Magnaghi, and M. Reichenbacher, Systematic optimization of the analysis of wine bouquet components by solid-phase microextraction, *J. High Resol. Chromatogr.,* 19, 257–262 (1996).

(16) Z. Zhang, M. J. Yang, and J. Pawliszyn, Solid-phase microextraction, *Anal. Chem.,* 66, 844A–853A (1994).

(17) C. L. Arthur, D. W. Potter, K. D. Buchholz, S. Motlagh, and J. Pawliszyn, Solid-phase microextraction for the direct analysis of water: Theory and practice, *J. Liq. Chromatogr. Gas Chromatogr.,* 10, 656–661 (1992).

(18) M. J. Yang, M. L. Orton and J. Pawliszyn, Quantitative determination of caffeine in beverages using a combined SPME-GC/MS method, *J. Chem. Ed.,* 74, 1130–1132 (1997).

(19) R. T. Rivero and V. Topiwala, Quantitative determination of formaldehyde in cosmetics using a combined solid-phase microextraction-isotope dilution mass spectroscopy method, *J. Chromatogr. A* 1029, 217–222 (2004).

(20) Y. Hoshika and G. Muto, Sensitive gas chromatographic determination of lower aliphatic carbonyl compounds as their pentafluorophenylhydrazones, *J. Chromatogr.,* 152, 224–227 (1978).

(21) E. E. Stashenko, M. A. Puertas, W. Salgar, W. Delgado and J. R. Martinez, Solid-phase microextraction with on-fiber derevitation applied to the analysis of volatile carbonyl compounds, *J. Chromatogr.* A, 886, 175–182 (2000).

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