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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FREE FORMALDEHYDE IN COSMETICS

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SUMMARY

An improved, sensitive method for the determination of formaldehyde in cosmetics and other commercial products is reported. The procedure is based on dilution of the sample with tetrahydrofuran–water (9:1), followed by precolumn derivatization with 2,4-dinitrophenylhydrazine and direct reversed-phase high-performance liquid chromatography. The formaldehyde derivative is stabilized in the reaction medium by addition of phosphate buffer and neutralization and detected in less than 10 min by the standard additions method. The method also appears to be suitable for the direct evaluation of the formaldehyde donors used in cosmetics as preservatives.

INTRODUCTION

An EEC Council Directive[1] allows the use of formaldehyde as a preservative in cosmetic products at a maximum concentration of 0.2%; if the concentration exceeds 0.05%, the addition of formaldehyde must be declared on the label. In order to ascertain whether cosmetic products conform to this regulation and to prevent undesirable effects, rapid and reliable analytical methods are required. The official EEC method is based on condensation of free formaldehyde with ammonium acetate and acetylacetone to form fluorescent 3,5-diacetyl-1,4-dihydrolutidine, which is selectively detectable[2]. Although this method is sensitive, it is not suitable when formaldehyde donors are present in the cosmetic formula because additional formaldehyde is released during analysis.

One approach, based on headspace diffusion and direct reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH) followed by high-performance liquid chromatography (HPLC), allows the detection of free formaldehyde[3] in the presence of formaldehyde donor preservatives[4]. With this method, however, the 2,4-DNPH derivative must be extracted and each analysis takes several hours. Other methods involving UV or fluorescence spectroscopy, HPLC and gas chromatography after

derivatization with different reagents have been reported[5-21]. However, they all require sample pretreatments that are not suitable for routine control and stability studies.

The aim of this study was to overcome these problems by developing a rapid and reliable method based on direct reversed-phase HPLC of untreated samples after precolumn derivatization with 2,4-DNPH using the standard additions method. The method has the following advantages: (i) treatment and extraction steps are avoided through sample dilution with tetrahydrofuran (THF)–water (9:1)[22]; (ii) the formaldehyde 2,4-DNPH derivative which is formed is compatible with the medium and the mobile phase and is stable at neutral pH; (iii) standard additions before derivatization allow the evaluation of the matrix effect and the equilibrium rate of decomposition of formaldehyde donors during sample preparation, derivatization and HPLC steps; (iv) free formaldehyde can be determined in less than 15 min in any complex matrix[23].

EXPERIMENTAL

Materials and reagents

Bronopol (2-bromo-2-nitropropane-1,3-diol) was obtained from Formenti (Milan, Italy), formaldehyde (40% RPE) from Carlo Erba (Milan, Italy), Germall 115 {N,N'-methylenebis[N'-(1-hydroxymethyl)-2,5-dioxo-4-imidazolidinylurea]} from Medolla (Milan, Italy) and 2,4-DNPH from Carlo Erba. Reagents and solvents were of analytical-reagent grade from Merck (Darmstadt, F.R.G.).

A 0.1% solution of 2,4-DNPH was prepared by dissolving 0.25 g of 2,4-DNPH in 100 ml of 32% hydrochloric acid, heating until dissolved and then diluting to 250 ml with water in a volumetric flask.

Apparatus

A Perkin-Elmer Series 410 liquid chromatograph equipped with a Rheodyne 7125 valve, UV LC-95 detector and 3700 data station was used. The column was LiChrosorb RP-8 (10 μm, 250 mm × 4 mm I.D.) from Merck with acetonitrile–water (1:1) as eluent at a flow-rate of 1 ml/min and UV detection at 345 nm.

Standard solutions

Formaldehyde solution (40%, measured iodimetrically) was diluted to 0.004–0.0001% with THF–water (9:1). The solutions were freshly prepared and stored in a refrigerator.

Samples

About 1 g of each cosmetic sample, accurately weighed, was diluted to 10 ml in a screw-capped tube with THF–water (9:1) or THF and stirred in a vortex mixer until completely homogeneous.

Derivatization procedure

A 1-ml volume of standard or sample solution was added to 0.4 ml of 0.1% 2,4-DNPH solution, stirred for 60 s in a vortex mixer and allowed to stand for 2 min at room temperature. The solution was then stabilized by adding 0.4 ml of 0.1 M phosphate buffer (pH 6.8) and 0.7 ml of 1 M sodium-hydroxide solution NaOH. Aliquots of 6 μl were injected into the HPLC system.

RESULTS AND DISCUSSION

Derivatization procedure

The use of an acidic solution of 2,4-DNPH as the derivatizing agent for carbonyl groups is well known and widely used; the reaction occurs rapidly at room temperature, yielding a UV-absorbing derivative detectable by HPLC after extraction with an organic medium.

To avoid this step, the reaction yield obtained using the same aqueous–organic medium for sample preparation and for the mobile phase was investigated. The results in Fig. 1 show that the THF–water and acetonitrile–water mixtures allow the formaldehyde derivative obtained by derivatization with 2,4-DNPH to be evaluated without memory effects. Consequently, the combined use of a THF–water mixture and reversed-phase HPLC can be successfully employed for the direct determination of formaldehyde without need for sample pretreatment and extraction steps.
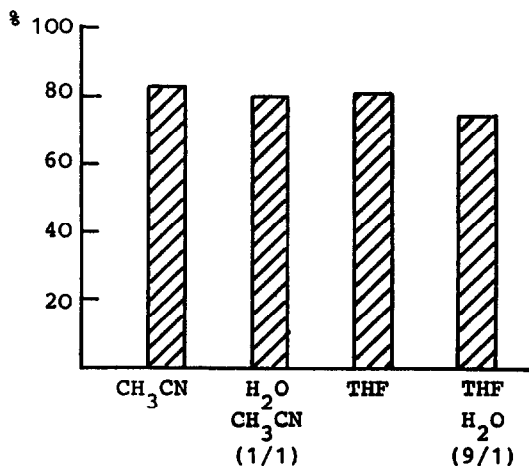

Fig. 1. Reaction yield of 4.0 μg/ml formaldehyde standard solution derivatized in the aqueous–organic medium used for sample preparation and as the mobile phase and injected directly into the HPLC system.

The stability of the 2,4-DNPH derivative is a function of reaction time, as shown in Fig. 2. The product formation reaches a maximum within 3 min (a), after which its concentration decreases as a function of time because of its instability in the acidic medium (b). Stability of the reaction over a period of 60 min was obtained by addition of phosphate buffer and by neutralization with 1 M sodium hydroxide solution.

Fig. 3 shows the progress of the reaction during the first 3 min of reaction with the standard solution of formaldehyde in THF–water (4 μg/ml). After only 30 s the yield is substantial and the maximum is attained within 3 min. This period appears to ensure reproducible measurements.

HPLC

Fig. 4 compares the chromatograms of (a) a THF–water (9:1) blank, (b) a standard solution of formaldehyde in THF–water (9:1), (c) a commercial cosmetic emulsion diluted with THF–water (9:1) and (d) the same cosmetic sample to which
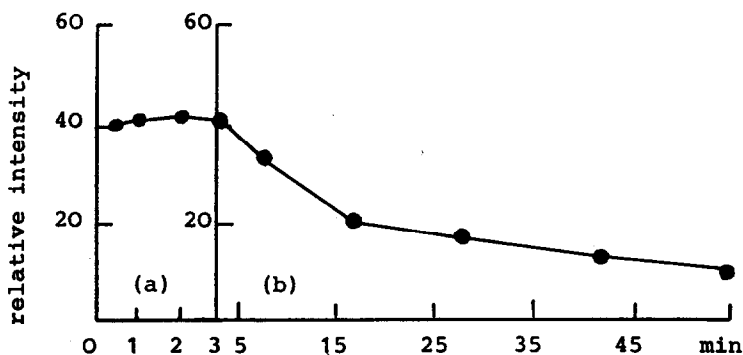
Fig. 2. Effect of addition of phosphate buffer and neutralization on stability of 2,4-DNPH derivative as a function of reaction time using a solution of formaldehyde in THF–water (9:1).

a known amount of formaldehyde standard had been added followed by derivatization and stabilization as described above. The patterns show the resolution of the method, with capacity factors of $k' = 2.35$ for the formaldehyde derivative and 1.67 for the unreacted 2,4-DNPH, respectively. Under the chromatographic conditions used there is no interference from other carbonyl compounds that also react with 2,4-DNPH.

Peaks were characterized by the absorbance ratio method using the stop flow technique or by measuring the peak-area ratio at two different wavelengths (345 and 254 nm).

For quantitative determinations, the standard additions method before derivatization was employed for the simultaneous evaluation of both the matrix effect and the derivatization rate. Calibration graphs and correlations for concentrations in the range 2–40 μg/ml with an average coefficient of variation of less than 1.5% can easily be obtained. Fig. 5 shows the calibration graphs for formaldehyde in the standard solution and added to a cosmetic emulsion sample; the identical slopes confirm the usefulness of the method and the absence of a matrix effect. The detection limit was 0.2 μg/ml (twice the signal-to-noise ratio).
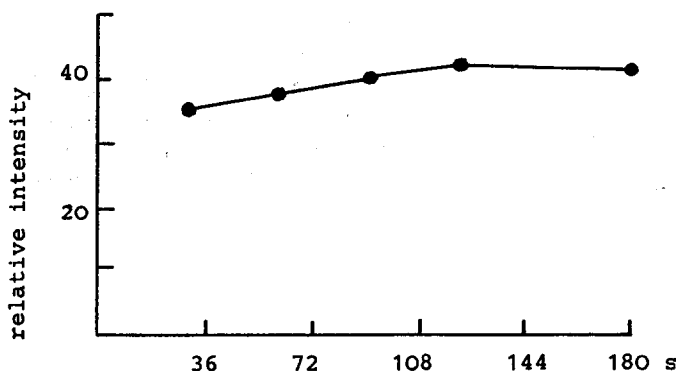

Fig. 3. Effect of increasing time of reaction on reaction rate and yield using a standard solution of formaldehyde in THF–water (9:1).
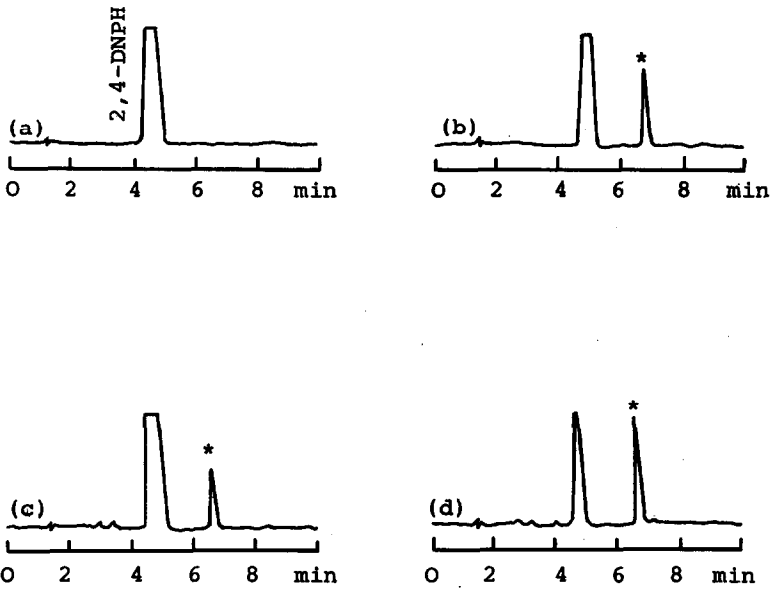
Fig. 4. Chromatographic patterns of (a) THF–water (9:1) blank, (b) formaldehyde standard, (c) cosmetic emulsion and (d) cosmetic emulsion after standard additions. The 2,4-DNPH peak represents the excess of derivatization agent and can be considered as a marker for reproducible measurements. (★), formaldehyde derivative.

Application

Recovery trials, carried out on typical commercial products diluted 1:10 or 1:50 with THF–water (9:1) depending on their formulative complexity and formaldehyde content, showed the reproducibility and flexibility of the method, which appears to be suitable for rapid and sensitive formaldehyde investigations (Table I).
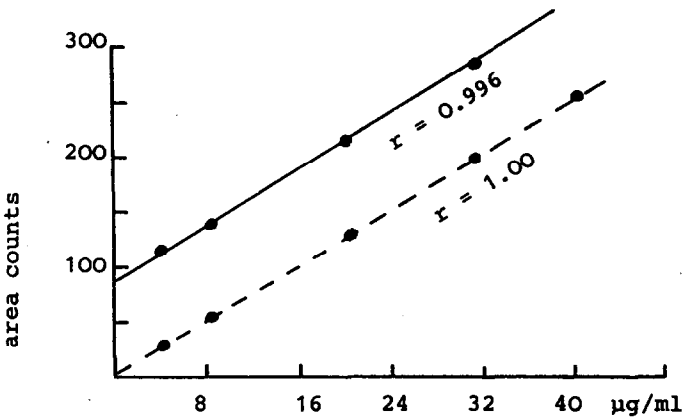

Fig. 5. Calibration graphs and correlations for formaldehyde standard solution (broken line) and added to cosmetic emulsion sample (solid line) after 2,4-DNPH precolumn derivatization.

TABLE I

RECOVERY TRIALS ON FORMALDEHYDE ADDED TO COSMETIC PRODUCTS BEFORE THE DERIVATIZATION STEP

Cosmetic product	Formaldehyde added (%)	Recovery (%)[a]
Emulsion	0.04	94 ± 3.8
Detergent	0.04	97 ± 2.7
Shampoo	0.04	99 ± 2.2
Disinfectant	0.04	97 ± 2.3
Toothpaste	0.04	99 ± 1.9

[a] ± Relative standard deviation ($n = 10$).

TABLE II

STABILITY OF FORMALDEHYDE DONOR PRESERVATIVES IN REACTION MEDIUM AND RELEASED FORMALDEHYDE LEVELS IN STANDARD SOLUTIONS

Formaldehyde donor preservative	Compatibility with method	Maximum dose authorized[1] (%)	Formaldehyde released (%)
Germall 115	+	0.6	0.043
Germall II	+	0.3	0.047
Bronopol	+	0.1	0.00054
Bronidox	+	0.1	0.000006
MDMHydantoin	+	0.2	0.025
Quaternium 15	−	0.2	N.D.
Benzylformal	+	0.2	0.06
Monochloroacetamide	+	0.3	0.00035
Dimethoxane	+	0.2	N.D.

The method can be used to study preservatives known to be formaldehyde donors[24,25]. Table II reports the stability of these compounds in the reaction medium and released free formaldehyde levels in standard solutions. Quaternium 15 appears to be the only compound that cannot be directly determined because of its instability with respect to the pH required for the 2,4-DNPH derivatization reaction.

Fig. 6 shows the formaldehyde released from a Bronopol cosmetic preservative[26] in an emulsion sample after standing for 5 weeks.
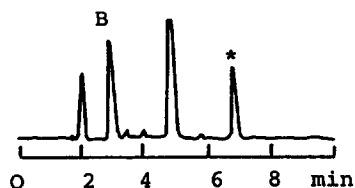

Fig. 6. Chromatographic pattern (at 216 nm) of formaldehyde (★) released from Bronopol (B) preservative in an emulsion sample after standing for 5 weeks.

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