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Note

High-performance liquid chromatographic determination of free formaldehyde in cosmetics preserved with Dowicil 200

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The 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.

The official EEC method for formaldehyde determination is based on condensation of free formaldehyde with ammonium acetate and acetylacetone, to form the fluorescent 3,5-diacetyl-1,4-dihydrolutidine compound which is selectively detectable[2]. Although the method is sensitive, it is not suitable when formaldehyde donors are present in the cosmetic formula, because additional formaldehyde is released during analysis.

We recently reported a rapid and reliable method for the determination of free formaldehyde in cosmetics[3]; the procedure is based on sample dilution with a tetrahydrofuran (THF)–water solvent mixture[4], followed by precolumn derivatization with 2,4-dinitrophenylhydrazine (2,4-DNPH)[5] and direct high-performance liquid chromatographic (HPLC) analysis[6].

Our studies showed the applicability of this method when preservative donors are present in the cosmetic formula: only Dowicil 200 [*cis*-1-(chloroallyl)-3,5,7-triaza-1-azoniaadamantane chloride] is not compatible with the procedure, because of its instability in the acidic media[7].

This preservative, increasingly employed for antimicrobial protection of personal care formulations[8], is a quaternary derivative of urotropine which releases formaldehyde by a hydrolytic process[9].

To overcome this problem, the method was modified by incorporating a precolumn step before 2,4-DNPH derivatization. In this way, Dowicil 200 is completely retained on a cationic stationary phase, while free formaldehyde is eluted and derivatized without memory effects[10,11]. The procedure allows the rapid and reliable determination of free formaldehyde released by the preservative in untreated cosmetic products, simply diluted with a THF–water (9:1) solvent mixture.

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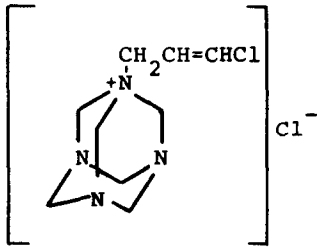

Dowicil 200

EXPERIMENTAL

Materials

Formaldehyde (40% RPE) and 2,4-DNPH were obtained from Carlo Erba (Milan, Italy), Dowicil 200 from Dow Chemical (Midland, MI, U.S.A.) and analytical-reagent grade reagents and solvents from Merck (Darmstadt, F.R.G.). Bond Elut SCX columns (500 mg of sorbent, 2.8 ml) (silica functionalized with benzenesulphonylpropyl cation-exchange groups) were supplied by Analytichem International (Harbor City, CA, U.S.A.).

Apparatus

A Perkin-Elmer Series 410 liquid chromatograph equipped with a Rheodyne 7125 valve, UV LC-95 detector and LCI-100 data station was used. The LiChrosorb RP-8 column (250 mm × 4 mm I.D., 10 μm) (Merck) was eluted with acetonitrile–water (50:50) at a flow-rate of 1 ml/min and with UV detection (345 nm).

2,4-DNPH solution (0.1%)

2,4-DNPH (0.25 g) was dissolved in 40 ml of 32% HCl with heating in a 250-ml volumetric flask and then diluted to volume with water.

Standards

Formaldehyde solution (40%), iodimetrically controlled, was diluted to the range 0.004–0.001% with THF–water (9:1). The solution was freshly prepared and stored in a refrigerator. Dowicil 200 aqueous standard solution (0.2%) was stored at room temperature in darkness and diluted 1:100 before analysis.

Samples

About 1 g of each commercial cosmetic sample, carefully weighed, was diluted 1:100 with THF–water (9:1) in a volumetric flask and stirred in a vortex mixer until completely homogeneous.

SCX column step

The column[12] was connected with a syringe by means of a PTFE tube and rinsed with 2–3 volumes of THF–water (9:1). A 1-ml volume of standard or sample solution was added to the top of the column and the eluate was collected by light suction; the

column was then washed with 1 ml of solvent mixture (THF–water, 9:1) and the eluate was added to the preceding one.

Derivatization procedure

A 1-ml volume of standard or sample solution was added to 0.4 ml of 0.1% 2,4-DNPH, 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. Aliquots of 6 μl were injected into the HPLC system.

RESULTS AND DISCUSSION

Dowicil 200 is a highly unstable molecule in acidic media (pH < 4) in which formaldehyde is quickly released[7]. For this reason, we studied a new approach to the quantitative evaluation of free formaldehyde in its solutions; determination is performed in the absence of the preservative, which is captured from the solution before analysis.

Fig. 1 shows the simple apparatus employed for this purpose, with a polypropylene column filled with silica functionalized with benzenesulphonylpropyl cation-exchange groups (SCX).

Formaldehyde analysis

We first verified the compatibility of the system with the analysis of standard formaldehyde solutions. Fig. 2 shows the analysis of a standard solution of formaldehyde with and without the SCX column step in comparison with blanks. The system does not absorb or release formaldehyde, as also confirmed by the correlations

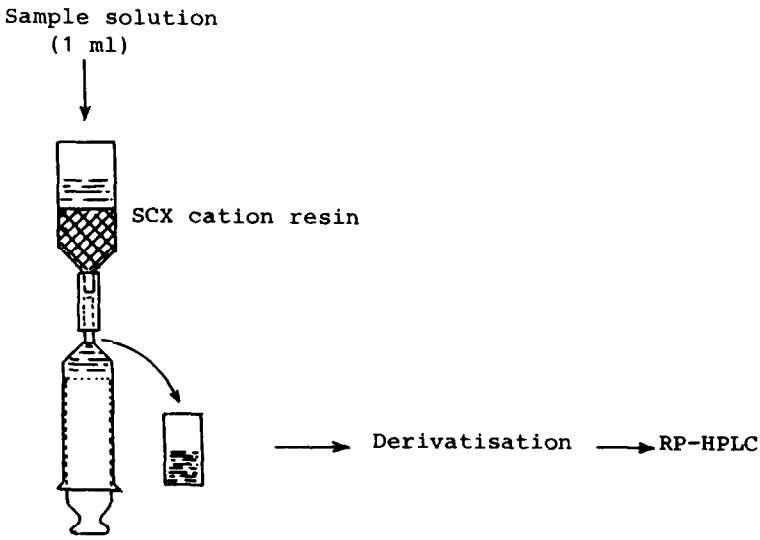

Fig. 1. Apparatus for determination of released formaldehyde.

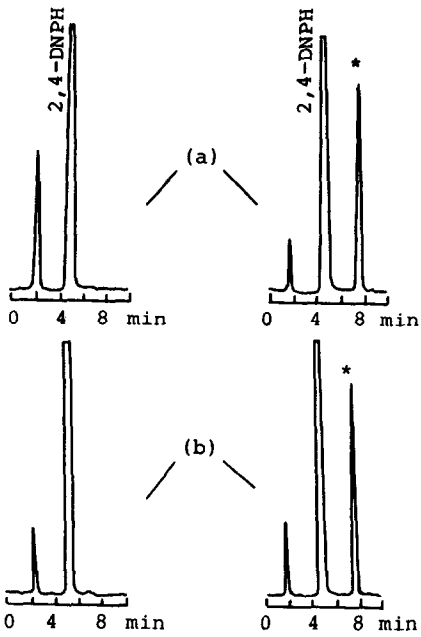

Fig. 2. HPLC of a derivatized formaldehyde standard solution (10 μg/ml) obtained (a) with and (b) without the SCX column step in comparison with blanks. The asterisks (in all figures) indicate the formaldehyde derivative peaks.

obtained with the same formaldehyde solutions (concentration range 4–60 μg/ml) before ($y = 18.61x + 34.15, r = 0.999$) and after the column step ($y = 18.74x + 33.02$, $r = 0.999$).

We also verified the applicability of the column system to the determination of formaldehyde in cosmetic samples. Fig. 3 shows the chromatographic patterns of a cosmetic emulsion, simply diluted in a THF–water (9:1) solvent mixture, containing formaldehyde, derivatized (a) before and (b) after the SCX column step, in comparison

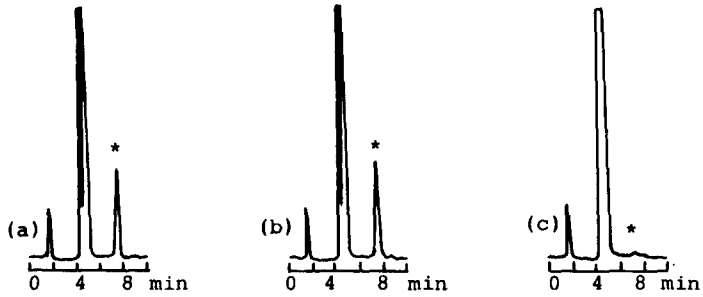

Fig. 3. Chromatographic patterns of a cosmetic emulsion containing formaldehyde derivatized (a) before and (b) after the SCX column step, in comparison with (c) the same sample without added formaldehyde.

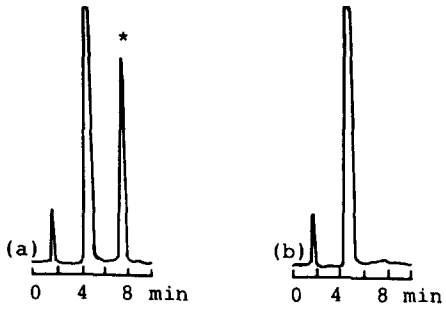

Fig. 4. HPLC of a freshly prepared Dowicil 200 standard solution derivatized (a) directly and (b) after elution from an SCX column.

with the same sample without added formaldehyde. No matrix effect and no interference of the SCX column in the quantitative evaluation of formaldehyde resulted.

Dowicil 200 analysis

A freshly prepared Dowicil 200 solution does not contain formaldehyde, the release of which is due to a time-dependent hydrolytic process. Fig. 4 shows the HPLC analysis of a freshly prepared standard solution of Dowicil 200 derivatized (a) directly and (b) after passage through the SCX column. In the first instance we found a large amount of formaldehyde because of the instability of the molecule in the acidic reaction medium, whereas in the latter instance no formaldehyde was detectable, as expected. Clearly, the preservative is not hydrolysed during the column step, although a small amount of H^+ is released into the medium. Under these experimental conditions, the pH of the solution remains stable at about 4.5–5. In order to ascertain that the preservative was completely blocked on the cation-exchange resin, we repeatedly derivatized each eluate; the good reproducibility (relative standard deviation = 1.2%) of the data obtained confirmed our hypothesis.

It is important to stress that the column must be used for only one sample; however, it is possible to regenerate the resin by the following washing sequence:1 volume of 0.5 *M* methanolic hydrochloric acid, 1 volume of water, 1 volume of methanol and 1–2 volumes of water to neutrality. No significant variation in quantitative measurements was found using regenerated columns.

Fig. 5 shows the chromatographic patterns of a cosmetic emulsion preserved with Dowicil 20 analysed (a) at zero time and (b) after 24 h storage. No matrix effect was detected.

Fig. 6 shows the kinetics of release of formaldehyde from a 0.2% solution of Dowicil 200, stored at room temperature in darkness for 200 days and performed with the described procedure: the plateau formaldehyde concentration is about 300 μg/ml.

Starting from these results we studied the applicability of the procedure to the evaluation of free formaldehyde in different cosmetic products preserved with Dowicil 200. First we considered the influence of formulation components such as salts and other cationic moieties, on the blocking step on the cation-exchange column, and in particular on the amount of H^+ exchanged with the resin. The results showed that with

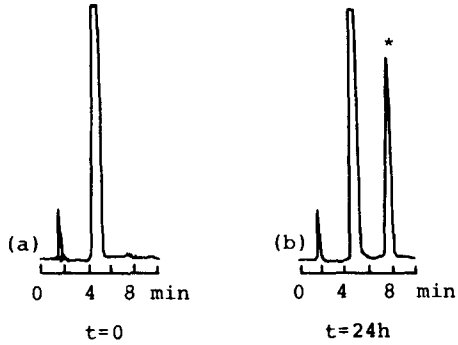

Fig. 5. Chromatographic patterns of a cosmetic emulsion preserved with 0.2% Dowicil 200 analysed (a) at zero time and (b) after 24-h storage.

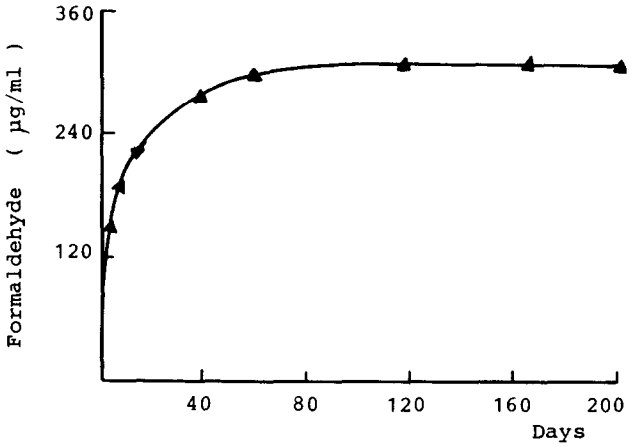

Fig. 6. Kinetics of formaldehyde release in a 0.2% Dowicil 200 aqueous solution stored at room temperature in darkness.

TABLE I

FORMALDEHYDE RELEASED IN COSMETICS PRESERVED WITH DOWICIL 200 STORED FOR 1 MONTH AT ROOM TEMPERATURE IN DARKNESS

Sample	*Dowicil 200 added (%)*	*Formaldehyde (µg/ml)*	*Relative standard deviation (%) (n=5)*
Bath foam	0.2	398.5	4.3
Cleansing milk	0.2	481.6	0.9
Lotion	0.2	525.2	2.4
Anti-dandruff shampoo	0.2	310.0	2.7
Sunscreen emulsion	0.2	405.5	3.0
Standard solution	0.2	250.0	2.5

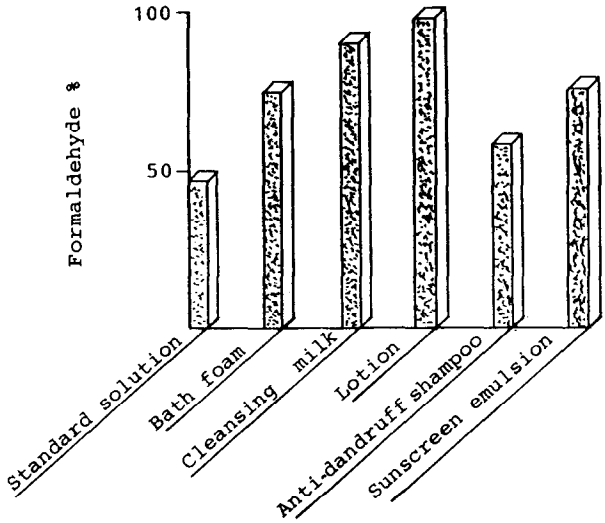

Fig. 7. Formaldehyde levels in Dowicil 200 standard solution (0.2%) and in different cosmetic samples preserved with Dowicil 0.2%, stored for 1 month at room temperature in darkness.

the dilution conditions employed (1:100), the pH of the eluate remained at *ca.* 4.5–5 for all kinds of formulations considered (emulsions, lotions, shampoos, bath foams, gels tonics, balsams, tanning and suncare products, etc.) and no interference was found in formaldehyde evaluation.

The applicability of the method was studied on five different commercial formulations, free of formaldehyde but with Dowicil 200 added (0.2%). Table I summarizes the results obtained. The samples were analysed after storage for 1 month at room temperature in darkness.

Fig. 7 shows the amount of formaldehyde found in each cosmetic sample compared with the value for a Dowicil 200 aqueous standard solution at the same concentration and stored under the same conditions for the same period of time. It is clear that the formulation significantly influences the hydrolysis of the molecule and the consequent release of formaldehyde.

ACKNOWLEDGEMENT

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