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Reversed-phase high-performance liquid chromatography applied to the direct analysis of untreated heterophasic systems

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SUMMARY

A simple and rapid approach for the direct analysis of multi-component heterophasic matrices is reported. The procedure is based on sample dilution with tetrahydrofuran–water (9:1) followed by direct reversed-phase high-performance liquid chromatography. The method is suitable for quality control and stability studies of drugs, food and cosmetic products.

INTRODUCTION

Heterophasic systems are non-Newtonian fluids with a thixotropic profile[1,2]. We recently observed that tetrahydrofuran (THF)–water allows the homogeneous dilution of non-ideal fluids suitable for direct investigation of complex matrices such as drugs and cosmetic emulsions[3]. As this polar medium is compatible with the mobile phases usually employed for reversed-phase high-performance liquid chromatography (RP-HPLC), we have studied the analysis of multi-component untreated formulations.

The approach is based on sample dilution with THF–water (9:1) followed by direct RP-HPLC using the standard addition method[4,5]. The procedure is compatible with the precolumn derivatization step before analysis[6,7]. The method allows qualitative and quantitative determinations and the simultaneous evaluation of matrix effects[8–14].

EXPERIMENTAL

Materials and reagents

Prevan [3-acetyl-6-methyl-2*H*-pyran-2,4(3*H*)dione sodium salt], dehydroacetic acid sodium salt, was obtained from Formenti (Milan, Italy), benzalkonium chloride from Sigma (St. Louis, MO, U.S.A.) and formaldehyde and 2,4-dinitrophenylhydrazine (2,4-DNPH) from Carlo Erba (Milan, Italy). Reagents and solvents were of analytical-reagent grade.

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A 0.1% solution of 2,4-DNPH was prepared by dissolving 0.25 g in 40 ml of 32% hydrochloric acid, heating until dissolved and then diluting to 250 ml with water.

Apparatus

A Haake RV-12 absolute rotational viscometer equipped with a PG-142 speed programmer and data station was used. The variable test parameters were set using NV (sensor system) as the measuring head and 0–256 min^{-1} as the speed value (D = shear rate: 0–1384 s^{-1}).

A Perkin-Elmer Series 410 liquid chromatograph equipped with a Rheodyne 7125 valve, LC-235 diode-array UV detector and LC-100 data station was used. LiChrosorb RP-8 (10 μm) and LiChrosorb RP-Select B (5 μm) columns were obtained from Merck (Darmstadt, F.R.G.).

Standards

Prevan was diluted to 1.5 mg/ml with THF–water (9:1) in a screw-capped tube. Benzalkonium chloride was diluted to 0.25–0.002 mg/ml with THF–water (9:1) in a screw-capped tube. Formaldehyde solution (40%, iodimetrically controlled) was diluted to 0.004–0.0001% with THF–water (9:1) in a screw-capped tube.

Samples

About 1 g of each cosmetic emulsion sample, carefully weighed, was diluted to 10 ml with THF–water (9:1) in a screw-capped tube 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. Aliquots of 6 μl were injected into the HPLC system.

HPLC

Method 1. A LiChrosorb RP-8 (10 μm) column was used with acetonitrile–0.01 M phosphate buffer (pH 4.7) (1:1) as eluent at a flow-rate of 1 ml/min and UV detection at 300 nm.

Method 2. A LiChrosorb RP-Select B (5 μm) column was used with acetonitrile–0.02 M phosphate buffer (pH 3.5) (0.8:0.2) as eluent at a flow-rate of 1.2 ml/min and UV detection at 210 nm.

Method 3. A LiChrosorb RP-8 (10 μm) column was used with acetonitrile–water (1:1) as eluent at a flow-rate of 1 ml/min and UV detection at 345 nm.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of dilution with THF–water (9:1) on the disappearance of thixotropy in a cosmetic emulsion; a 1:10 dilution allows reticle homogeneity and Newtonian behaviour of the sample, which can be directly injected into the RP-

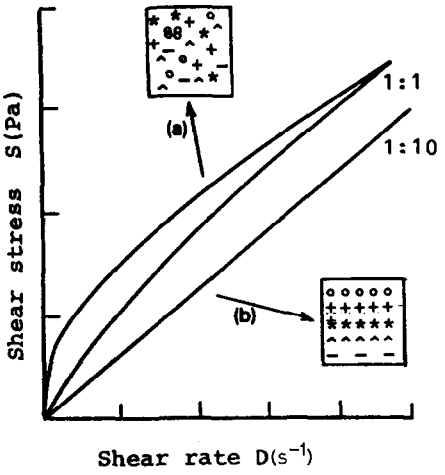

Fig. 1. Rheological behaviour (flow curves) of a typical heterophasic system: effect of dilution with THF–water (9:1) on the disappearance of thixotropy (a) and reticle homogeneity (b) of a commercial cosmetic emulsion sample.

HPLC system and investigated without a memory effect[3]. Fig 2 compared the chromatographic patterns of the cosmetic preservative Prevan obtained from the same emulsion sample, (a) directly injected after THF–water dilution and (b) injected after the usual sample pretreament; chromatographic resolution and the absence of a matrix effect may be seen. The matrix effect can be routinely evaluated by the standard addition method by comparing the slopes of the standard and sample plus standard curves.

The combined action of THF–water and RP-HPLC avoiding sample pretreatment allows more sensitive detection for the study of the modifications that occur in the finished product due to chemical or microbiological effects.

Fig. 3 shows an example of the direct analysis of benzalkonium chloride obtained at 210 nm from a cosmetic product diluted 1:10 with THF–water; the chroma-

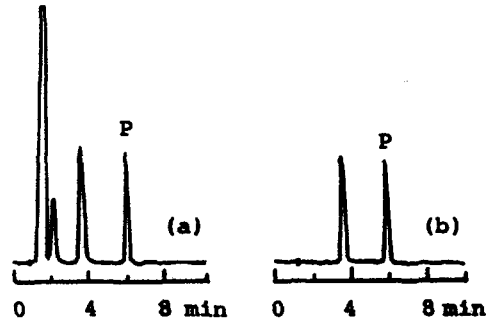

Fig. 2. Chromatographic patterns of cosmetic preservative Prevan (P) obtained from (a) the direct injection of a cosmetic emulsion diluted 1:10 with THF–water (9:1) and (b) the injection of same cosmetic emulsion conventionally pretreated (method 1).

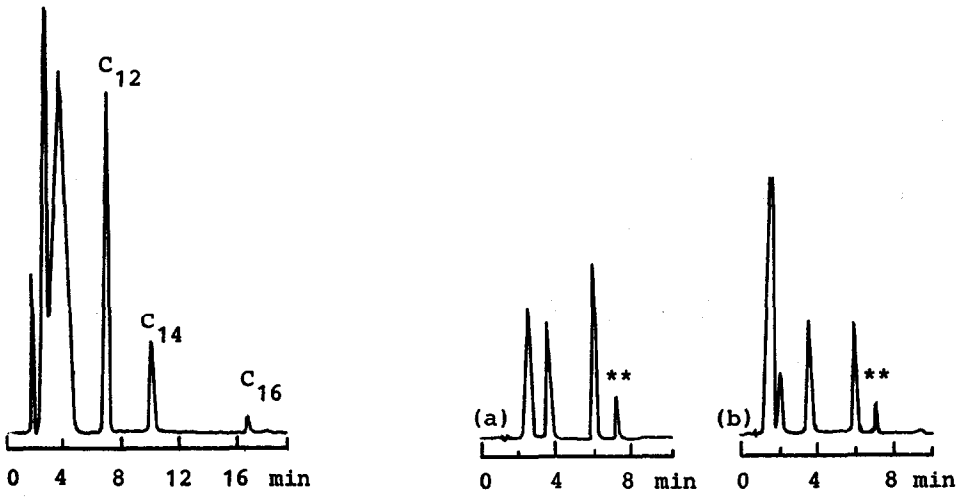

Fig. 3. HPLC resolution of C_{12}, C_{14} and C_{16} alkyl chain components of benzalkonium chloride obtained from an commercial product diluted 1:10 with THF–water (9:1) (method 2).

Fig. 4. Chromatographic evidence of an interaction product (peaks with asterisks) formed by the effect of formaldehyde on a typical cosmetic preservative system containing Prevan: (a) aqueous standard solution: (b) cosmetic emulsion. Method 1, UV detection at 300 nm.

tographic pattern reveals not only the C_{12} and C_{14} alkyl chain components but also, at a retention time of 16.8 min, a peak corresponding to the C_{16} alkyl component, which is normally undetected[14].

Fig. 4 shows an example of the appearance at the native level of an interaction product formed in a cosmetic emulsion between the dehydroacetic acid sodium salt (Prevan) and formaldehyde released from preservatives[11].

The procedure may also be employed in conjunction with precolumn derivatization before analysis; Fig. 5 shows the free formaldehyde detected at 345 nm after 2,4-DNPH derivatization of a cosmetic emulsion diluted 1:100 with THF–water (9:1) and injected directly into the HPLC system[7].

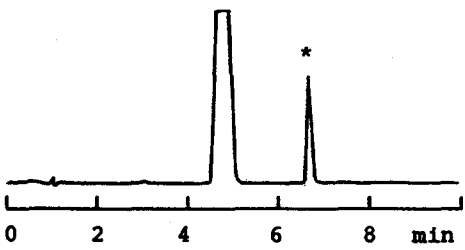

Fig. 5. Chromatographic pattern of formaldehyde (peak with asterisk) obtained after 2,4-DNPH precolumn derivatization of a cosmetic emulsion sample diluted 1:100 with THF–water (9:1) (method 3).

CONCLUSIONS

The combination of THF–water (9:1) dilution of the sample and RP-HPLC allows the direct evaluation of multi-component heterophasic systems such as drug, food and cosmetic products. The long-term effects of direct injections of untreated complex matrices on the chromatographic resolution, evaluated by repeated injections and calibrations, show statistically significant correlations and reproducibility of the data with a mean column lifetime of 100 analyses. The procedure is rapid and sensitive and appears to be a potentially powerful method suitable for quality control and stability studies.

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