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Note

Optimization of high-performance liquid chromatographic analysis with UV detection: light-scattering detection to establish the coelution of UV- and non-UV-absorbing constituents

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The high-performance liquid chromatographic (HPLC) analysis of complex mixtures with UV detection can sometimes be difficult to achieve, *e.g.*, with a mixture of trace amounts of a UV-absorbing solute in the presence of large amounts of non-UV-absorbing compounds. However, the choice of UV detection is valuable owing to the specificity and sensitivity of this method in the routine analysis of UV-absorbing solutes at trace levels.

However, quantitative UV measurements can be altered by coelution of non-UV-absorbing and UV-absorbing products, ghost peaks linked to the injection solvent which differs from the mobile phase composition, peaks resulting from the residual solvent in the sample studied, etc. A wavelength change sometimes gives information about other compounds that do not absorb at the wavelength of the solute determination. In spite of this, UV detection does not always permit the optimization of the analytical parameters (repeatability, sensitivity, etc.) in order to obtain a validation method. Hence non-detection of the major products does not enable one to ascertain that the elution of the UV-absorbing product traces and that of the major compounds are not concomitant. Connection of a universal detector to the specific UV detector provides a solution to the problem.

The determination of trace amounts of cetylpyridinium chloride (CPC) in an alcoholic solution illustrates this problem, as large amounts of surfactant are included in cosmetic or pharmaceutical samples, either as an emollients or as lubricants. Several chromatographic methods have been reported for the determination of CPC using various chromatographic systems[1–4], including a rapid and specific HPLC procedure directly applied to CPC in mouthwash[1]. The variability of the results we obtained with this procedure made it necessary to optimize the proposed determination.

For this study, refractive index detection is limited owing to the very long equilibration time required for the detection limit and because it is impossible to use gradient elution for the analysis of complex mixtures. Light-scattering detection

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(LSD), which is more sensitive than refractive index detection, is easy to use and compatible with gradient elution[5,6]. Scattered light produced by microparticles permits a universal detection method for non-volatile solutes. The development of LSD has been described in several papers and many applications have been published in both HPLC[7,8] and supercritical fluid chromatography (SFC)[9–11]. This paper describes for the first time the compatibility of LSD with selected salted eluents. It also gives an explanation for the variability of the determination of CPC in mouthwash using the HPLC conditions described by Meyer and Takahashi[1] and presents a specific and rapid new procedure.

EXPERIMENTAL

Chemicals

The mobile phase solvents used were reversed-phase HPLC-grade methanol (Prolabo, Paris, France), HPLC-grade acetonitrile (Fisons, Loughborough, U.K.) and distilled water (Cooperation Pharmaceutique Française, Melun, France). Other reagents were of analytical-reagent grade.

Apparatus

Chromatography was carried out using a Knauer (Berlin, F.R.G.) Model 64 pump, a Rheodyne (Cotati, CA, U.S.A.) Model 7125 valve, and a Shimadzu (Touzart et Matignon, Vitry-sur-Seine, France) CR 3A integrator.

Three different detectors were used: a Model SF 769 UV spectrophotometer (Kratos, Ramsey, NJ, U.S.A.) set at 258 nm, a differential refractometer (LDC, Riviera Beach, FL, U.S.A.) and a Model Sedex 45 light scattering detector, (Sedere Vitry-Sur-Seine, France). LSD uses the following principle: the effluent is nebulized by an inert gas (nitrogen) and the solvent is vaporized in a warm tube. The non-volatile solutes give a mist of small particles which scatter the light. Scattered light is measured at 120° to the collimated light source[6,8,12].

Columns

The columns used were 10-μm μBondapak CN (150 × 3.9 mm I.D.) purchased from Waters Assoc. (Milford, MA, U.S.A.) and 7-μm Zorbax CN (150 × 4.6 mm I.D.) purchased from DuPont (Wilmington, DE, U.S.A.).

Mobile phases

Solution S was a 3.6 g l^{-1} aqueous solution of tetramethylammonium hydroxide pentahydrate (Aldrich, Strasbourg, France) adjusted to pH 4.2 with acetic acid. Solution T was a 10^{-3} mol l^{-1} aqueous solution of triethylamine (Aldrich) adjusted to pH 4.2 with trifluoroacetic acid. The eluents were as follows: A, mixture of 700 ml of methanol and 300 ml of solution S; B, mixture of 700 ml of acetonitrile and 300 ml of solution S; C, mixture of 700 ml of methanol and 300 ml of solution T; and D, mixture of 700 ml of acetonitrile and 300 ml of solution T.

Samples

Solution M_1 was prepared by dissolving cetylpyridinium chloride (CPC) at 500 ppm (0.05%) in 95% ethanol. Solution M_2 was a 50 ppm (0.005%) solution of CPC

obtained by diluting solution M_1 10-fold with 95% ethanol. Solution M_3 was a 40 000 ppm solution of hydrogenated polyoxyethylenated castor oil (HPCO) in 95% ethanol.

A standard mixture M_4 was prepared by mixing 10 ml of M_1 and 20 ml of M_3 with 70 ml of distilled water in a 100-ml flask.

The analyte was a commercial mouthwash used as received.

RESULTS AND DISCUSSION

The CPC analysis performed with a μBondapak CN column, with a methanol–water mobile phase containing tetramethylammonium (eluent A) as defined by Meyer and Takahashi[1] led to the chromatograms in Fig. 1 and to the quantitative results in Table I.

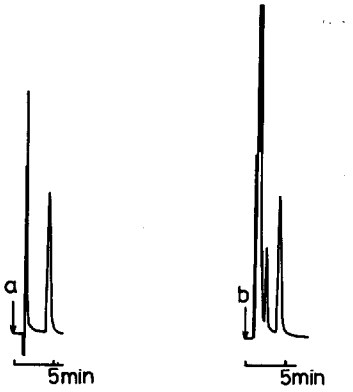

Fig. 1. Typical chromatograms of CPC on μBondapak CN (150 × 4.6 mm I.D.) with a UV detector. Eluent A, methanol–aqueous buffer; flow-rate, 1.5 ml min^{-1}; pressure, 134 bar; injection loop, 20 μl; detection, UV at 258 nm. (a) Standard solution M_2 of CPC; (b) commercial mouthwash.

The method is rapid and simple and the CPC peak is easily identified as the lasted peak elutes. However, the repeatability determined from eight replicate injections was unsatisfactory both for the standard mixture and for the mouthwash [relative standard deviation (R.S.D.) 8.6% and 6.1%, respectively].

No difference in the CPC UV peak is observed in the chromatogram of standard M_2 (Fig. 1a) and mouthwash (Fig. 1b). Standard M_4 and mouthwash solution differ from standard M_2 owing to the large amount of hydrogenated polyoxyethylenated castor oil (HPCO). This seems to be the cause of the above high R.S.D.s for standard M_4 and the mouthwash compared with that of standard M_2 (2.9%). The cause of the variability should be established by using another detection mode. Therefore, we first attempted to carry out a chloroform extraction of CPC prior to its determination in the standard M_4 or mouthwash solution; the extraction selectivity and extraction yield were poor and did not improve the repeatability. Next, we connected the light-scattering detector to the specific UV detector, and then all the non-volatile compounds including UV-absorbing solutes and non-UV-absorbing solutes were detected. This form of detection requires readily evaporated eluent in order to obtain a low enough background noise. Therefore, tetramethylam-

TABLE I

REPEATABILITY FOR STANDARD SOLUTION M_2, STANDARD MIXTURE M_4 AND THE COMMERCIAL MOUTHWASH

Column, μBondapak CN (150 × 3.9 mm I.D.); eluent A, methanol–aqueous buffer; detection, UV at 258 nm.

Peak-area units

Standard solution M_2 (CPC 50 ppm)	*Standard mixture M_4 (CPC 50 ppm, castor oil 8000 ppm)*	*Commercial mouthwash*
709 091	642 874	714 576
717 410	779 244	679 613
730 641	725 461	627 895
753 395	801 945	616 727
774 929	749 800	601 250
750 376	769 679	610 277
766 105	757 587	610 791
748 382	613 207	604 006
Mean: 743 791	729 974	633 142
R.S.D.: 2.9%	8.6%	6.1%

monium acetate salt in eluent A must be replaced with a more volatile salt, triethylammonium trifluoroacetate, which constitutes eluent C.

This is the first time we have demonstrated the application of LSD in eluents containing salts. Using an organic–aqueous mobile phase containing a 10^{-3} mol l^{-1} concentration of salt, the evaporation temperature in the diffusion tube remains below 50°C; this possibility is only afforded by the Sedex 45 detector. Other applications using various salts will be presented later[13]. Such a modification of the mobile phase does not produce a difference in the CPC retention. Indeed, UV detection gives for the standard solution M_4 or the mouthwash the same pattern as Fig. 1. Fig. 2 displays the same analysis as Fig. 1 but with LSD.

LSD proved conclusively that the elution of HPCO was quicker than that of CPC. However, the high concentration of HPCO in relation to the concentration of CPC in mouthwash and elution spreading produced by the diverse components present in the HPCO mixture led to partial co-elution of CPC with castor oil. This co-elution cannot be revealed using the UV detection mode as HPCO is a non-UV-absorbing mixture. It was co-elution in Meyer and Takahashi's UV method[1] that caused the variability in our results.

The UV signal consists of two contributions: a direct UV CPC signal and a UV signal given by the change in the refractive index of the effluent at the elution time of CPC in standard solution M_2 and the refractive index of the effluent containing castor oil at the elution time of CPC in standard solution M_4 (or mouthwash). The latter contribution was not constant and resulted in variability in the determination of CPC. Changes in the chromatographic elution conditions, discriminating without greatly modifying the procedure, should allow CPC and HPCO elutions to be carried out.

First, replacement of methanol with acetonitrile permitted a more rapid elution

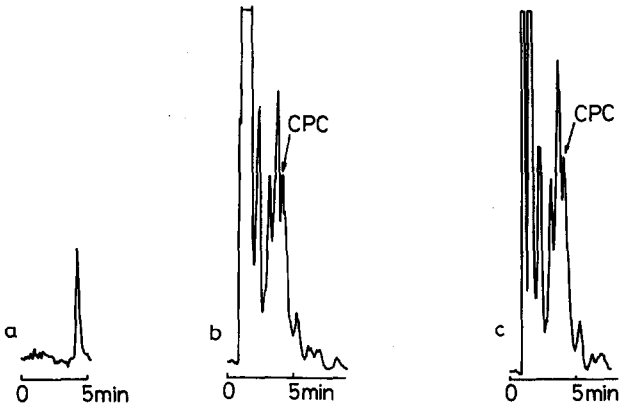

Fig. 2. Chromatograms of CPC on μBondapak CN (150 × 4.6 mm I.D.) with a light-scattering detector. Eluent C, methanol–aqueous buffer; flow-rate, 1.5 ml min^{-1}; pressure, 134 bar; injection loop, 20 μl. LSD conditions: evaporation temperature, 40°C; nebulizer gas, 2.5 bar. (a) Standard solution M_2 of CPC (50 ppm); (b) commercial mouthwash; (c) standard mixture M_4 of CPC (50 ppm) and HPCO (8000 ppm).

of the compounds apart from CPC. Second, replacement of μBondapak CN with Zorbax CN increased the separation of HPCO and CPC. Under these conditions an acceptable chromatogram was obtained with rapid elution. The choice of Zorbax CN rather than μBondapak CN resulted from comparisons made by Goldberg[14] which showed that the stationary phases are different in terms of classification of polar and non-polar phases. Our previous similar work[15,16] demonstrated the great difference between Zorbax and μBondapak the hydrophobic contribution to retention being greater with Zorbax.

Typical chromatograms of the commercial mouthwash solution obtained using the three different detection modes are presented in Fig. 3. When considering the universal detection modes, clearly LSD (Fig. 3b) afforded greater more sensitivity

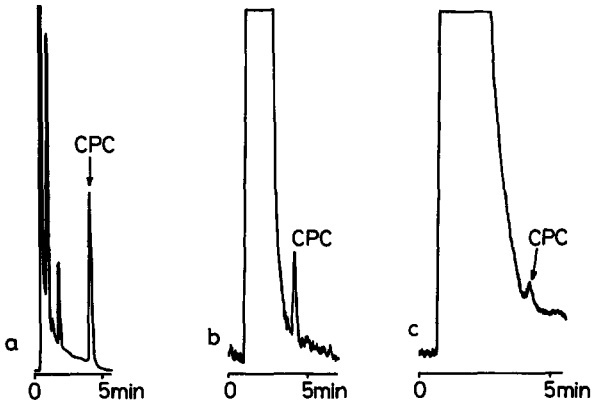

Fig. 3. Typical chromatograms of commercial mouthwash on Zorbax CN (150 × 4.6 mm I.D.) with three different detection modes. Flow-rate, 1.5 ml min^{-1}; pressure, 41 bar; injection loop, 20 μl. (a) UV detection at 258 nm. Eluent B = acetonitrile–aqueous buffer. (b) Light-scattering detection. Nebulizer gas, 2.5 bar; evaporation temperature, 40°C. Eluent D = acetonitrile–aqueous buffer. (c) Differential refractometric detection. Eluent B = acetonitrile–aqueous buffer.

than refractive index detection (Fig. 3c), despite the unfavourable elution conditions (addition of salt to the mobile phase). Moreover, the variation of the refractive index of the effluents was very large and the CPC peak was partially masked by lack of resolution; ethanol in the mouthwash preparation was the major cause of the large refractive index change or disturbance.

With the new chromatographic conditions and UV detection, a calibration graph for CPC determination was established with seven reconstituted solutions of increasing CPC concentration from 5 to 75 ppm containing a constant concentration of HPCO (8000 ppm). The graph was linear with an acceptable regression value (R = 0.9997) and passed very close to the origin. Now, using the new chromatographic conditions, the repeatability established using six injections of a commercial mouthwash was better; the R.S.D. obtained (2.8%) compared favourably with that for the standard CPC solution under the same conditions.

It appears that the effects of HPCO on the determination of CPC using the new chromatographic conditions were negligible; the chromatographic procedure is satisfactory and capable of achieving excellent separations.

CONCLUSION

Variability in the UV determination of low CPC concentrations in a mouthwash by HPLC was caused by the co-elution of UV-absorbing and non-UV-absorbing compounds. Universal detection connected with UV detection afforded information enabling the variability problem to be solved. The LSD mode was superior to the refractive index mode for two reasons: a higher detection limit and the injection solvent, being of a different nature from that of the eluent, does not produce a disturbance in the chromatogram. Using LSD new elution conditions were established and the routine UV determination of CPC in mouthwash became an accurate and rapid analysis.

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