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Note

Determination of 10-camphorsulphonates in pharmaceutical formulations by high-performance liquid chromatography

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Salts of 10-camphorsulphonic acid (CSA) are used in pharmaceutical preparations as an aqueous soluble form of camphor. The most frequently found are camphorsulphonates of sodium, codeine, piperazine, ephedrine and ethylmorphine, which are used in tablets, suppositories, oral drops, syrups and injections. A specific study of their determination in pharmaceutical forms has not yet been reported.

10-Camphorsulphonic acid has been used as a counter ion in reversed-phase chromatography[1–5]; (+)- and (−)-antipodes of 10-camphorsulphonic acid have been used for the separation of enantiomeric amines or amino alcohols by ion-pair formation[6–10], and themselves have been separated by the same procedure with chiral amines[11,12]. These separations of optical isomers require the use of low-polarity solvents, which are often incompatible with commercial dosage forms (syrups, injections, oral drops) containing water, and which therefore cannot be used.

Moreover, the raw material for the preparation of camphorsulphonates for medical use is synthetic camphor, a mixture of optical isomers. Our problem was to find a method for use in routine work that would achieve a separation suitable for quantification without the separation of enantiomers. The low absortivity of camphorsulphonic acid and of camphorsulphonates requires the use of a UV-absorbing counter ion. In these systems, the counter ion has a double function: to permit the separation in a reversed-phase system (now the most popular mode of liquid chromatography) and to improve the detection at the measured wavelength.

We chose phenethylammonium as a UV-absorbing counter ion with a high molar absortivity because it is known to yield good sensitivity, linearity and precision in the quantification of alkylsulphonate ions[13,14]. This chromatographic system has also been well described in several other papers[15–20].

The purpose of this paper is to present a method for the determination of 10-camphorsulphonates in commercial dosage forms. The developed method is based on ion-pair reversed-phase high-performance liquid chromatography (RP-HPLC) using phenethylammonium as a highly UV-absorbing counter ion.

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EXPERIMENTAL

Apparatus

The high-performance liquid chromatograph used was a Waters Assoc. 6000 A equipped with a U6K universal injector, a Waters Assoc Lambda-Max M480 detector and a Hewlett-Packard 3390 A recorder–integrator. The column used was a μBondapak C_{18} (30 cm × 3.9 mm I.D.; 10 μm) (Waters Assoc.).

Chemicals and reagents

All solvents and chemicals were of analytical-reagent or reagent grade and were used without further purification. (1*R*)-(−)-10-Camphorsulphonic acid, (1*S*)-(+)-10-camphorsulphonic acid and 2-phenethylamine were purchased from Aldrich (Strasbourg, France). Racemic 10-camphorsulphonic acid, perchloric acid, phenol (internal standard) and methanol were obtained from Prolabo (Paris, France).

Chromatographic conditions

The mobile phase was 2.5 m*M* 2-phenethylamine in water containing 50% (v/v) methanol. The pH of the aqueous solution was adjusted to 3.00 (±0.05) with dilute perchloric acid before adding methanol. The mobile phase was filtered through a 0.45-μm Millipore filter before use. The temperature was 20–25°C and the flow-rate was 1.0 ml min^{-1}. Before commencing the day's analysis, mobile phase was pumped through the column for 1 h at 1 ml min^{-1} to establish stable baseline conditions. The UV detector was set at 260 nm, the chart speed was 1 cm min^{-1} and the volume injected was 10 μl.

Preparation of standards

A stock solution of (1*S*)-(+)-10-camphorsulphonic acid was prepared by dissolving an accurately weighed amount of 464.6 mg of CSA into enough mobile phase to obtain a final volume of 100.0 ml (20 m*M* solution).

To prepare the stock solution of the internal standard, an accurately weighed amount of 22.5 mg of phenol was dissolved and diluted with mobile phase to 20.0 ml (12 m*M* solution).

Serial dilutions of the stock solution of (+)-10-camphorsulphonic acid and phenol (as constant internal standard) were prepared and diluted with the mobile phase.

Calibration solutions were prepared in the range 1–15 m*M* of (+)-10-camphorsulphonic acid and each contained 0.6 m*M* of phenol.

Preparation of samples

Depending on the theoretical amount of camphorsulphonate present in the sample, the volume of mobile phase has to be adjusted to obtain a concentration similar to that of one of the calibration solutions; the volume of stock solution of internal standard (phenol) has to be adjusted correspondingly.

Tablets. The tablets were broken and extracted under magnetic stirring with mobile phase for 10 min, the suspension was centrifuged and the supernatant was collected in a volumetric flask, then the bottom layer was stirred once more with the mobile phase for another 10 min. The supernatants were combined, the internal

TABLE I

INTRA-ASSAY VARIATION FOR THREE CONCENTRATIONS OF CSA

CSA concentration (mM)	*Standard deviation of peak-area ratios (n = 5)*	*Relative standard deviation (%) (n = 5)*
1	0.00261	1.09
9	0.0167	0.88
15	0.0275	0.77

standard solution was added to the volumetric flask and the mixture was diluted with mobile phase. The solution was filtered through paper before use.

Suppositories. The suppositories were dissolved in mobile phase by heating at 60°C for *ca.* 15 min in a water-bath, then cooled to separate the mass of excipient. The liquid phase was collected and the mass was extracted once more for 15 min. The combined solutions were filtered through paper; all the glassware was washed with mobile phase and the internal standard solution was added. The resulting solution was diluted to volume with mobile phase.

Liquids. All liquid forms (syrups, oral drops, injections) were merely diluted with the mobile phase and the appropriate volume of internal standard solution was added.

Linearity, precision and sensitivity

The peak-area ratios of 10-camphorsulphonic acid to the internal standard (phenol) were calculated in the 1–15 m*M* range. The calibration graph was plotted as peak-area ratio *versus* CSA concentration. The results of linear regression analysis were slope 0.799, intercept −0.013 and correlation coefficient $r = 0.9998$ ($n = 10$).

The precision of the proposed method was evaluated by assaying repeated injections using three concentrations (1, 9 and 15 m*M*) of CSA. The within-day variation was investigated by five replicate analyses for each concentration; the results are shown in Table I. The inter-assay variation was determined by replicate analyses of the three concentrations over a 5-day period (Table II). The assays showed good precision at low and high concentrations. The average reproducibility was greater than 99% in the intra-assay and close to 98% in the inter-assay mea-

TABLE II

INTER-ASSAY VARIATION OVER A 5-DAY PERIOD

Replicate analyses of three samples containing 1.9 and 15 m*M* CSA.

CSA concentration (mM)	*Standard deviation of peak-area ratios*	*Relative standard deviation (%)*
1	0.00605	2.55
9	0.0471	2.39
15	0.0677	1.95

surements. Under the analytical conditions, the limit of detection (corresponding to a signal-to-noise ratio of *ca.* 4:1, with an injection volume of 10 μl) was 0.2 μg (absolute amount).

RESULTS AND DISCUSSION

Influence of optical isomers

To determine the possible influence of rotatory power on determinations, we prepared by accurate weighing and dilution three solutions of 10-camphorsulphonic acid [(+), (−) and racemic (±)] and two solutions with sodium camphorsulphonates from natural and synthetic camphor. The comparison between capacity factors (k') and peak areas showed total similarity between the responses of the various optical antipodes (enantiomers) and mixtures. Therefore, we shall henceforth refer to camphorsulphonates and 10-camphorsulphonic acid without specifying the rotatory power.

Influence of pH

CSA is a strong acid, fully dissociated even at pH < 3, and therefore the effect of pH on separation can be neglected. We chose the mobile phase pH so that phenethylamine would be ionized enough (into phenethylammonium) to favour ion-pair formation without damage to the column. Hence, in accordance with Bidlingmeyer[13], it was deemed preferable to adjust the pH of the water solvent before mixing it with the organic solvent. The acid used was perchloric acid, which improves peak shapes[1]. Fig. 1 is an illustration of the influence of pH on ion-pair formation. Here the solute and the UV-absorbing counter ion have opposite charges and the solute peak is positive after the "system peak", well known in this chromatographic system. This is in accordance with principles presented in several papers[10–15,17,18,20].

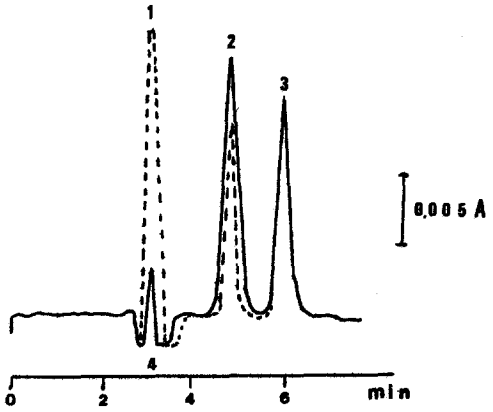

Fig. 1. Influence of pH on ion-pair formation of CSA with 2.5 m*M* phenethylamine. 1 = Perchlorate system peak; 2 = CSA; 3 = internal standard (phenol); 4 = phenethylamine system peak. Dashed line, pH = 5; solid line, pH = 3.

TABLE III

RESULTS FOR 10-CAMPHORSULPHONATES IN VARIOUS COMMERCIAL DOSAGE FORMS

Results are averages of three consecutive analyses, expressed as 10-camphorsulphonic acid

Commercial formulation	*Salt*	*CSA content*		*Percentage of label claim determined*
		Manufacturer's label claim	*Found (mg)*	
Tablet A	Codeine	10.92 mg/tablet	10.89	99.7
Suppository B	Na	18.27 mg/suppos.	18.29	100.1
Syrup C	Codeine Ethylmorphine Ephedrine Piperazine	4.85 mg/ml	4.89	100.8
Syrup D	Na Thiamine	13.55 mg/ml	13.38	98.7
Oral drops E	Piperazine	102.0 mg/ml	105.5	103.4
Injection F	Piperazine	102.13 mg/ml	102.21	100.1

Influence of other variables on the capacity factor

As generally occurs in reversed-phase chromatography, with increasing methanol content the capacity factor k' decreases. Sachok et al.[14], Denkert *et al.*[17] and others[15,16] have thoroughly investigated the influence of counter-ion concentration. Here, when the phenethylamine concentration changes from 1 to 3 mM, the capacity factor increases from 1.74 to 2.24. We chose a phenethylamine concentration of 2.5 mM because good separations were obtained in a reasonable time. It will be noted that the capacity factor is not influenced by the nature of the camphorsulphonate cation.

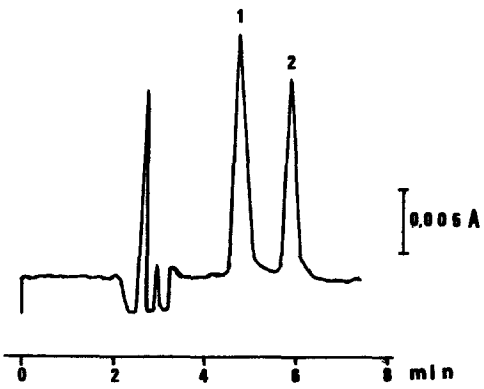

Fig. 2. Example of separation of camphorsulphonate as an ion pair with phenethylammonium in a commercial formulation (injection F). 1 = CSA; 2 = internal standard (phenol).

Applications

The method was applied to six commercial pharmaceutical formulations: two solid forms (tablets A, suppository B) and four liquid forms (syrups C, and D, oral drops E and injection F). The results (means of three replicates) are reported in Table III; they show a good correlation between the labelled and found amounts of CSA. An example a chromatogram is presented in Fig. 2.

CONCLUSIONS

Ion-pair RP-HPLC with a UV-absorbing counter ion can be applied to salts of 10-camphorsulphonic acid in commercial pharmaceutical preparations without interfering with the nature of the cation. Separation can be carried out by simply dissolution in the mobile phase for solid forms, or by simple dilution with mobile phase for liquid forms. Because camphorsulphonates are highly water soluble, this simple method is limited to formulations with a camphorsulphonate concentration sufficient to produce by dilution a solution in the range 1–15 m*M*.

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