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Application of sodium lauryl sulphate ion-pairing reversed-phase high-performance liquid chromatography in the separation of some isomeric and other closely related basic compounds in pharmaceutical analysis

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ABSTRACT

Ion-pair reversed-phase high-performance liquid chromatography proved to be useful for the separation of basic compounds of similar structure. Using suitable ion-pairing reagent concentration, pH and ionic strength values, good separations can be achieved. An ion-pair chromatographic study of a benzodioxanyl derivative, an aminopyrimidine derivative and a lysergic acid derivative was performed. Each of these compounds may be contaminated by a structural isomer produced as a by-product during the synthesis. By choosing the optimum experimental conditions these isomers can be separated from each other and from other related compounds and can be quantified in various substances and pharmaceutical products.

INTRODUCTION

Basic compounds of pharmaceutical interest are often contaminated by chemically closely related compounds, *e.g.*, isomers. As these compounds often have pharmaceutical properties different from those of the main component, their detection and quantification are important in pharmaceutical analysis.

Chromatographic separations of chemically similar compounds can be improved on the basis of differences in ion-pairing properties. By choosing appropriate pH values, concentration of the ion-pairing reagent and composition of the mobile phase, different separations can be optimized.

Several applications of alkyl sulphonates in the ion-pair separation of strongly basic compounds of pharmaceutical interest have been reported. Goldberg *et al.* [1] described a method for the optimization of the chromatography of some aromatic amines and tricyclic antidepressants. Kubo and Kinoshita [2] and Claes *et al.* [3] separated gentamycin-type antibiotics by alkyl sulphonate ion-pair chromatography. Adriamycin and related compounds have been chromatographed using sodium lauryl sulphate in phosphoric acid solution [4,5]. De Schutter *et al.* [6] separated 2-imidazoline drugs and Rustum [7] investigated ranitidin with the aid of different alkyl sulphonates.

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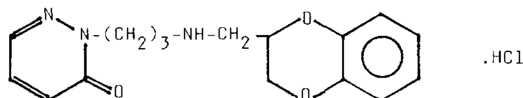
The aim of this work was to achieve some separations in pharmaceutical analysis. Using suitable experimental conditions, small amounts of isomers and related compounds were detectable and quantification could be effected.

EXPERIMENTAL

Materials

The compounds tested, which were synthesized in this institute, were as follows: (a) 2-[N-(2-benzo-1,4-dioxanylmethyl)amino-1-propyl]-3(2*H*)-pyridazinone hydrochloride; (b) 3-(2-benzo-1,4-dioxanylmethyl)amino-1-propyloxypyridazine hydrochloride; (c) 2-[N-(2-benzo-1,4-dioxanylmethyl)amino-1-propyl]-4,5-dihydro-3(2*H*)-pyridazinone hydrochloride [(b) and (c) are possible contaminants of (a)]; (d) 2-methyl-4-[(morpholinoethyl)amino]-6,7-dihydro-7(8*H*)-pyrimido[5,4-*b*]-1,4-oxazinone; (e) 2-methyl-8-[(morpholinoethyl)amino]-6,7-dihydro-7-pyrimido[5,4-*b*]-1,4-oxazinone; (f) 8*β*-[3(5)-methylcyclopentano[4,5(3,4)]pyrazol-1-ylmethylene-6-methylergol-9-ene; and (g) 8*β*-[3(5)-methylcyclopentano[4,5(3,4)]pyrazol-2-ylmethylene-6-methylergol-9-ene.



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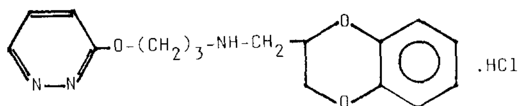

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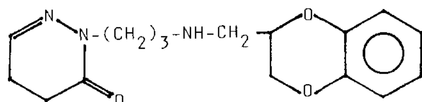

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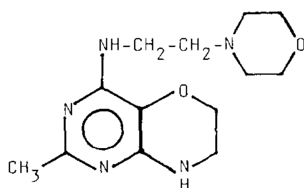

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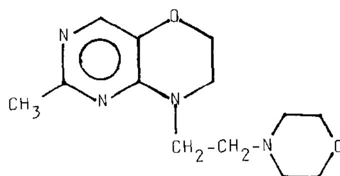

e

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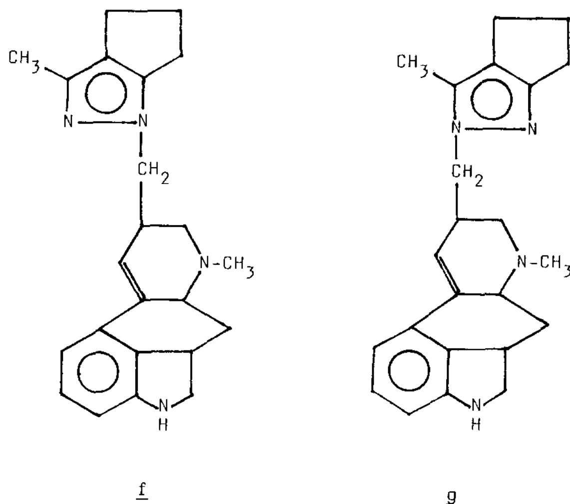

Acetonitrile (HPLC grade) was obtained from Interchemia (Budapest, Hungary) and sodium lauryl sulphate from Fluka (Buchs, Switzerland).

Equipment

High-performance liquid chromatography (HPLC) was carried out isocratically with an LKB liquid chromatograph. The peaks were detected with an LKB 2151 variable-wavelength monitor at 254 nm (compounds a, b and c), at 290 nm (compounds d and e) and at 320 nm (compounds f and g). Chromatograms were recorded on a Hewlett-Packard recording integrator.

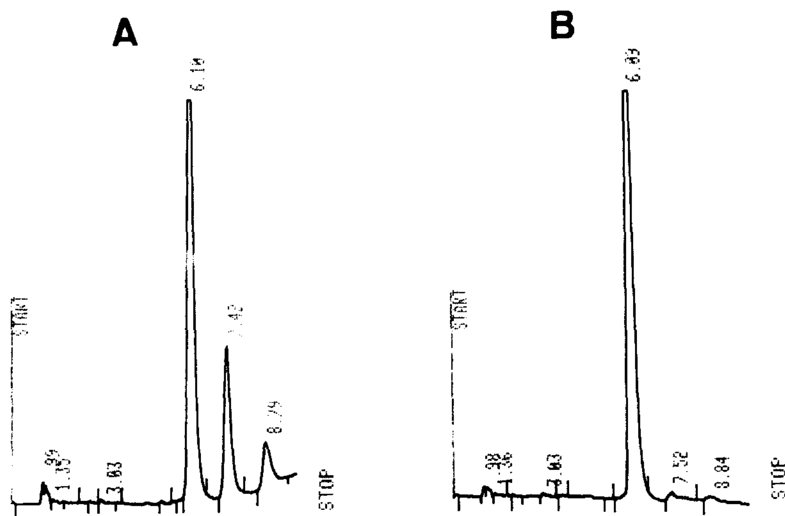

Fig. 1. Chromatograms of mixtures of compounds a, b and c in different proportions. Column: 200 × 4 mm I.D. Nucleosil C_{18} (5-μm). Mobile phase: 0.01 M sodium lauryl sulphate in 0.0066 M phosphate buffer (pH 7.4)–acetonitrile (30:70). Flow-rate: 1 ml/min. UV detection at 254 nm. Proportions: (A) a:b:c = 8:1:1; (B) a:b:c = 99:0.5:0.5. Retention times: a, 6.10; b, 7.42; c, 8.79 min.

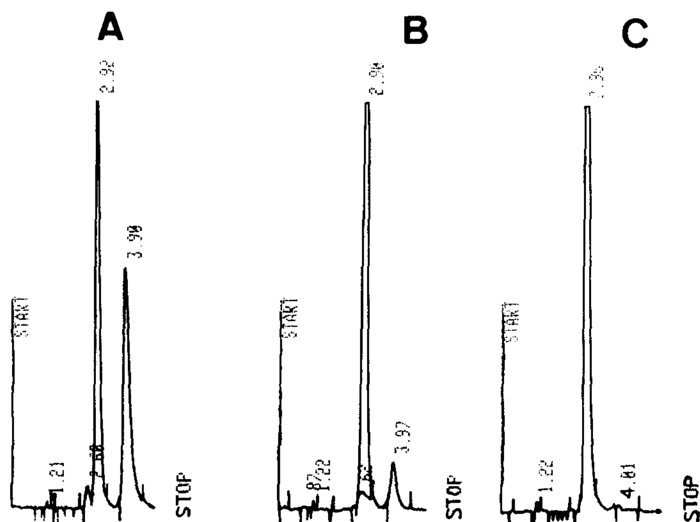

Fig. 2. Chromatograms of mixtures of compounds d and e in different proportions. Column: 200 × 4 mm I.D. Nucleosil C_{18} (5-μm). Mobile phase: 0.01 *M* sodium lauryl sulphate in 0.01 *M* phosphate buffer (pH 2)–acetonitrile (1:1). Flow-rate: 1 ml/min. UV detection at 290 nm. Proportions: (A) d:e = 1:1; (B) d:e = 9:1; (C) d:e = 99.5:0.5. Retention times: d, 2.92; e, 3.90 min.

Stationary and mobile phases

Nucleosil C_{18} (5-μm) columns (200 × 4 mm I.D.) were used (Bio-Separation Techniques, Budapest, Hungary). The mobile phases consisted of the mixtures of phosphate buffers (sodium lauryl sulphate dissolved in them) and acetonitrile. The flow-rate was 1 ml/min.

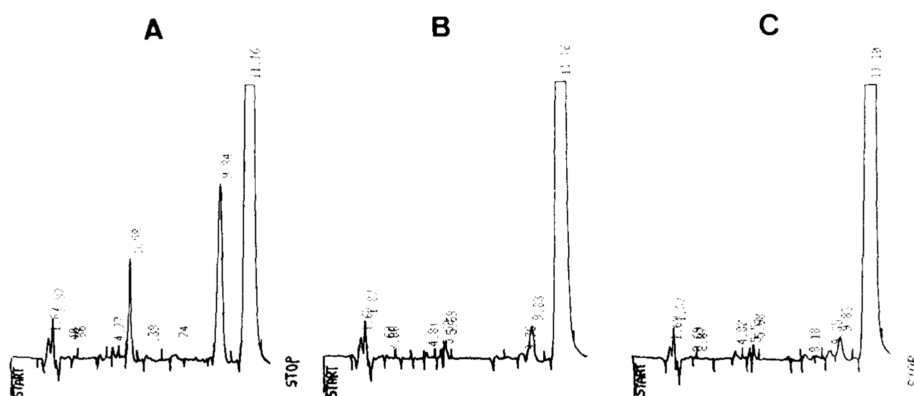

Fig. 3. Chromatograms of mixtures of compounds f and g in different proportions. Column: 200 × 4 mm I.D. Nucleosil C_{18} (5-μm). Mobile phase: 0.01 *M* sodium lauryl sulphate in 0.01 *M* phosphate buffer (pH 2)–acetonitrile (40:60). Flow-rate: 1 ml/min. UV detection at 320 nm. Proportions: (A) g:f = 9:1; (B) g:f = 99:1; (C) g:f = 99.5:0.5. Retention times: f, 9.84; g, 11.16 min.

RESULTS AND DISCUSSION

The substances considered are strongly basic compounds containing aliphatic or alicyclic secondary or tertiary amino groups. In a previous study [8] the separation of some compounds of this type using silica gel thin-layer chromatography in the presence of ammonia vapour was described; the separation of compounds a and c was poor. In our HPLC experiments the resolution of these compounds without an ion-pairing reagent was insufficient and therefore 0.01 *M* sodium lauryl sulphate was used to improve the separation.

Retention values were first tested in an acidic medium. Sodium lauryl sulphate was dissolved in 0.01 *M* (pH 2) phosphate buffer, as described for adriamycin [5], and the buffer containing sodium lauryl sulphate was mixed with acetonitrile (1:1, v/v). Compounds d and e were separated with this mobile phase, but compounds a and c remained unresolved and the peaks of compounds f and g were tailed because of the long retention times. The separation of compounds f and g was improved by increasing the acetonitrile concentration in the mobile phase. Compounds a, b and c were separated only around neutral pH of the buffer. At pH 7.4 and with a 70% acetonitrile concentration these three compounds were completely resolved.

Figs. 1–3 show the separation of mixtures of these compounds. It can be seen that a 0.5% isomer content was detectable and measurable in the presence of the main component and other contaminants of the latter. Quantification of the individual sample components was performed using the appropriate standards.

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