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Note

Reversed-phase high-performance liquid chromatography of metronidazole benzoate in suspension dosage form

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Metronidazole is a drug widely used in the treatment of anaerobic infections but with restricted application to paediatrics because of its bitter taste. Therefore, its derivative metronidazole benzoate is used for these purposes as a suspension dosage form. The most probable degradation products of metronidazole benzoate in such a drug formulation are benzoic acid and metronidazole. Hence, their presence can serve as evidence of instability of the drug.

Spectrophotometry[1] and differential-pulse polarography[2] have been utilized for the determination of metronidazole benzoate in suspension forms, but these methods are time-consuming and non-specific. Metronidazole alone has been determined by gas chromatography, while benzoic acid was separately evaluated by non-aqueous titration of the drug[3]. These methods are not directly applicable to analysis of the final suspension dosage form. Previously[4] it was shown that the determination of both the impurities could be effected on a reversed-phase column. In spite of the high selectivity, the elution of the strongly retained metronidazole benzoate was not possible and regeneration of the column had to be performed after each analysis.

Reversed-phase high-performance liquid chromatography (RP-HPLC) for monitoring of metronidazole either in biological fluids or in dosage forms has been extensively used (see, *e.g.*, refs. 5 and 6) with buffered aqueous mobile phases containing a small amount of organic modifier. The acid–base properties of metronidazole were not interpreted in these works and the chromatographic systems proposed were not suitable for simultaneous analysis of metronidazole benzoate and metronidazole.

In the present study two reversed-phase chromatographic systems were developed on acid-base considerations for simultaneous analysis of metronidazole benzoate, admixtures of benzoic acid and metronidazole and a pair of preservatives (methyl- and propylparaben), being the most important components of a metronidazole benzoate suspension dosage form.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer Model Series 4 liquid chromatograph with a Rheodyne Model 7125-075 syringe-loading sample injector and a Perkin-Elmer Model LC-85B vari-

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able-wavelength detector were used. The detector was operated at 254 nm. A Shimadzu computing integrator Chromatopac Model C-R3A was employed with a BASIC program for statistical evaluation of the chromatographic constants.

The potentiometric titrations and pc_H^* measurements were performed as previously described[7] using a Radiometer PHM 64 digital pH meter at about 22–25°C.

Columns

The chromatographic experiments were carried out with a C_{18} reversed-phase Perkin-Elmer analytical column (250 mm × 4 mm I.D.), mean particle size 10 μm, under isocratic elution conditions. A guard column (50 mm × 2 mm I.D.) filled with 30–38 μm Co:Pell ODS (Whatman, U.K.) was attached to the main column.

Reagents and sample preparation

Methanol and acetonitrile (Merck, F.R.G.) were used without further purification for aqueous–organic solvents. The buffer salts, dipotassium hydrogenphosphate, sodium formate, sodium acetate, orthophosphoric acid, acetic acid, nitric acid and potassium nitrate were of analytical reagent grade. Sodium dodecyl hydrogensulphate was from Merck (F.R.G.). The standard solutions were prepared from analytes of pharmacopoeial purity, except for metronidazole benzoate which was in correspondence to an analytical certificate (Trans-Medica, F.R.G.).

About 2.0 g (accurately weighed) of the suspension sample ("Metronidazole suspension", Bulgaria)* containing 3.5% (w/w) metronidazole benzoate were transferred to a 100.0-ml volumetric flask. An 80-ml volume of methanol–water (80:20) was added. The contents were ultrasonicated for a few minutes then made up to the mark with the solvent. A portion of the mixture obtained was centrifuged at *ca.* 900 *g* for 10 min. Aliquots (10 μl) from the upper clear layer were chromatographed.

A standard solution containing 0.5% (w/w) of both metronidazole and benzoic acid was prepared, and quantitation by the external standard method was performed.

Chromatographic procedures

Solutions of 0.005 *M* buffer salts and 0.05 *M* potassium nitrate were prepared in methanol–water (60:40, v/v). Solutions of *X M* potassium nitrate and *Y M* sodium dodecyl hydrogensulfate, where $X + Y = 0.04$ *M*, and 0.01 *M* nitric acid at a constant ionic strength of 0.05 *M* and $pc_H^* = 2.0$ were prepared in methanol–water (60:40) or in acetonitrile–water (35:65) for ion-pair chromatographic experiments.

The mobile phase hold-up time was determined as described[7].

RESULTS AND DISCUSSION

The dependence of the capacity factors, k', on the composition of either water–methanol (Fig. 1A) or water–acetonitrile (Fig. 1B) mixtures has been determined for all the substances investigated: metronidazole, benzoic acid, methylparaben, propylparaben and metronidazole benzoate. It was concluded that there is no isocratic mobile phase composition at which a good retention for the most polar analytes (metronidazole and benzoic acid) and an adequate separation of all the

* "Klion-suspension" (Hungary) is an identical dosage form.

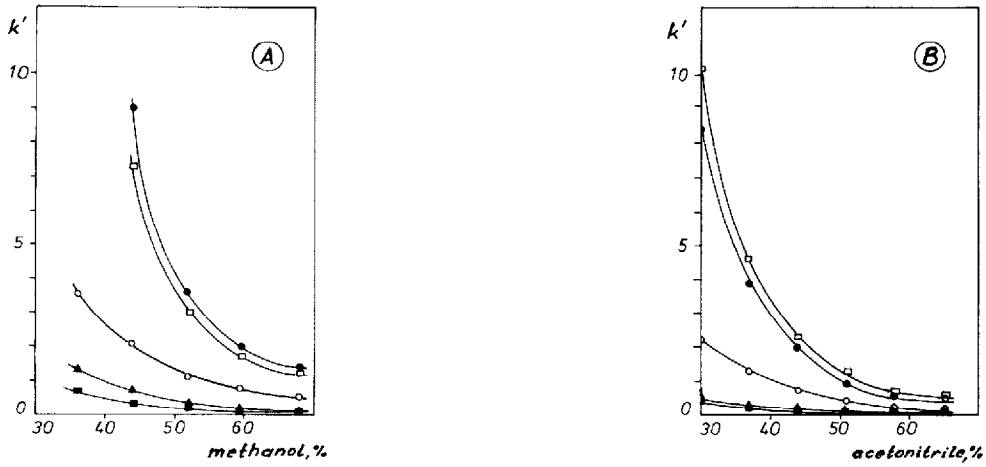

Fig. 1. Dependences of the capacity factors, k', on solvent composition: (A) methanol–water, (B) acetonitrile–water mixtures. Solutes: metronidazole (■); benzoic acid (▲); methylparaben (○); metronidazole benzoate (□) and propylparaben (●).

components could be achieved. Furthermore, a poor peak shape for benzoic acid was observed with the methanolic mobile phases. The resolution of the pair benzoic acid/metronidazole in acetonitrilic eluents was not satisfactory, and that of the pair metronidazole benzoate/propylparaben was not sufficient for quantitation purposes because of the high mass ratio for these components in the dosage form.

Three of the above mentioned substances show acid–base properties: metronidazole and metronidazole benzoate are protonated acids, BH^+, and benzoic acid is an uncharged acid, HA. Thus, the following approaches were preferred.

A. A simple pH-control approach

Fig. 2 shows the dependence of k' on pc_H^* in methanolic mobile phases. The retention of the fully protonated acids, BH^+, is not possible because of the instability of the silica-based columns when the mobile phase pH is lower than 2. With the computer program, the dissociation constants pK_a^* and one of the capacity factors of

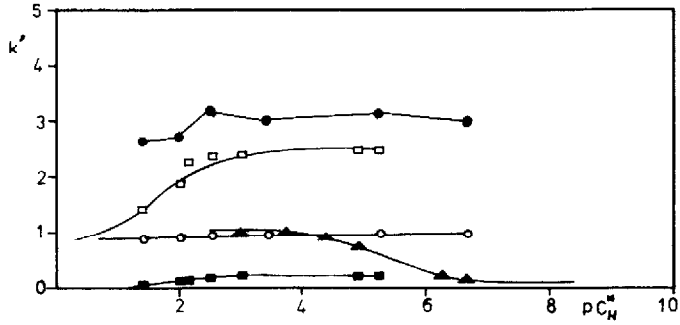

Fig. 2. Plots of k' *vs.* pc_H^*. Mobile phases: 0.005 *M* buffers and 0.05 *M* potassium nitrate in methanol–water (60:40). Solutes as in Fig. 1.

TABLE I

CAPACITY FACTORS AND pK_a^* VALUES OF BENZOIC ACID, METRONIDAZOLE AND METRONIDAZOLE BENZOATE

Mobile phases: 0.005 *M* buffers and 0.05 *M* potassium nitrate in methanol–water (60:40).

Compound	*Experimental values*		*Calculated values*			pK_a^*
	k'_0	k'_{-1}	k'_0	k'_1	pK_a^*	
Metronidazole	0.23	–	–	0.0 (±0.03)**	2.11 (±0.12)	2.55* (ref. 9)
Metronidazole benzoate	2.47	–	–	0.80 (±0.07)	1.59 (±0.06)	–
Benzoic acid	–	0.05	0.92 (±0.06)	–	5.49 (±0.05) 5.27§ (±0.01)	5.54*** (ref. 10)

* Determined spectrophotometrically in water.
** Confidence interval ($p = 0.95$).
*** Determined potentiometrically in methanol–water (60:40).
§ Determined potentiometrically in this work.

the respective species were calculated* and the theoretical curves were drawn. A good agreement between experimental and theoretical data was observed, hence, the retention change of the monoprotolytes in such buffered eluents is as predicted by the theory[7,8]. The chromatographic constants are presented in Table I. It is seen that the pK_a^* values obtained and those cited are in good agreement.

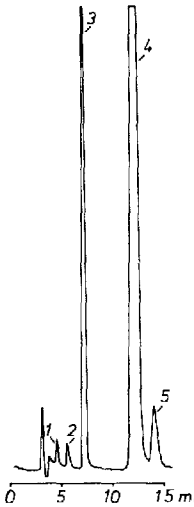

Fig. 3. Sample chromatogram ("Metronidazole suspension"). Mobile phase: 0.005 *M* acetate buffer and 0.05 *M* potassium nitrate in methanol–water (60:40), $pc_H^* = 5.2$, flow-rate 1 ml/min. Peaks: 1 = metronidazole; 2 = benzoic acid; 3 = methylparaben; 4 = metronidazole benzoate and 5 = propylparaben.

* The program required one of the two constants, k'_0 for uncharged species B and HA or k'_1 for BH^+, k'_{-1} for A^-, respectively, to be determined experimentally.

Based on the constants and capacity factors determined for all substances from Fig. 2, a mobile phase having pc_H^* about 5.5 was chosen as the most appropriate one. The separation of the sample solution using such an eluent is shown in Fig. 3.

*B. An ion-pair liquid chromatographic approach**

The results obtained by approach A show that metronidazole and metronidazole benzoate are relatively strong acids, being of the type BH^+. Therefore, one can use an ion-pair reagent to enhance the retention of the poorly retained metronidazole. The dependence of k' on the counter ion (dodecyl hydrogensulphate) concentration in both the methanol- and acetonitrile-containing mobile phases is presented in Fig. 4. The counter ion concentration was varied at a constant pH ($pc_H^* = 2.0$). With methanolic mobile phases (Fig. 4A), metronidazole is more strongly retained but benzoic acid and methylparaben are not resolved —the well known drawback of this chromatographic technique. A change in the elution order of propylparaben and metronidazole benzoate was observed at about 4 mM dodecyl hydrogensulphate.

For better selectivity towards these two pairs, acetonitrile (being more selective towards parabens[11] was used instead of methanol as an organic modifier (Fig. 4B). A good retention for metronidazole ($k' \approx 1$) and a separation of the pair methylparaben/benzoic acid (resolution, $R_s \approx 1$) for quantitation purposes was achieved at a concentration 4 mM dodecyl hydrogensulphate, although the capacity factors (and analysis time) were increased two-fold. The separation of all substances was possible over a wide range of a counter-ion concentrations. The chromatographic system is more selective towards BH^+ type compounds and can be used when any doubts exist about peak purity or identity for metronidazole.

A statistical evaluation by the least-squares method (multiple standard mode) in the region of 80–120% (w/w) of each compound (declared content) was performed by means of the two chromatographic methods. Linear detector responses were observed and the sensitivity of both methods was estimated as equivalent to three times the baseline noise, *i.e., ca.* 0.1% for both benzoic acid and metronidazole. The relative standard deviation did not exceed 2% in the course of a study of drug stability and the contents of metronidazole and benzoic acid were less than 0.5% (usually 0.3%).

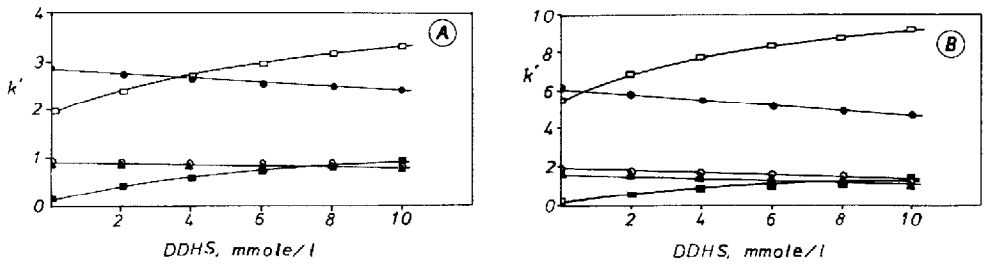

Fig. 4. Ion-pair chromatography. Plots of k' *vs.* dodecyl hydrogensulphate (DDHS) concentration. Mobile phases: 0.01 M nitric acid, potassium nitrate and DDHS at constant ionic strength $I = 0.05$ M and $pc_H^* = 2.0$ in (A) methanol–water (60:40), (B) acetonitrile–water (35:65). Solutes as in Fig. 1.

* More preciselly, this is ion-pair liquid chromatography with pH control. Some ionogenic substances which do not possess acid–base properties can form ion-pairs, too, and no pH control is needed.

REFERENCES

1 K. P. R. Chowdary and K. T. R. Kumar, *Indian J. Pharm. Sci.*, 45 (1983) 182.
2 S. Dalkara, H. Ong, J. Braun and R. Plourde, *Anal. Lett.*, 17 (1984) 793.
3 S. Görög, M. Fütó and A. Laukó, *Acta Pharm. Hung.*, 46 (1976) 113.
4 L. Kostova and P. Pashankov, *Izv.-Durzh. Inst. Kontrol Lek. Sredstva*, 18 (1985) 82.
5 J. C. Jensen and R. Gugler, *J. Chromatogr.*, 277 (1983) 381.
6 V. D. Gupta, *J. Pharm. Sci.*, 73 (1984) 1331.
7 P. P. Pashankov, P. S. Zikolov and O. B. Budevsky, *J. Chromatogr.*, 209 (1981) 149.
8 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
9 V. A. Palm (Editor), *Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions*, Vol. II(1), VINITI, Moscow, 1976, p. 221.
10 B. L. Karger, J. N. LePage and N. Tanaka, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography–Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980, p. 138.
11 S. R. Bakalyar, R. McIlwrick and E. Roggendorf, *J. Chromatogr.*, 142 (1977) 353.