BIOMEDICAL CHROMATOGRAPHY, VOL. **12**, 164–166 (1998)

Narrow-bore Reversed-phase Liquid Chromatography of Metronidazole Benzoate and its Hydrolysis Products

W. R. G. Baeyens,[1]* G. Van der Weken,[1] A. Van Overbeke,[1] S. De Meyer,[1] A. M. García Campaña,[2] P. Kiekens,[3] J. P. Remon[3] and C. Dewaele[4]

[1]Laboratory of Drug Quality Control, Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium
[2]Faculty of Sciences, Department of Analytical Chemistry, University of Granada, Avda. Fuentenueva s/n, E-18071 Granada, Spain
[3]Laboratory of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium
[4]BIO-RAD RSL, Begoniastraat 5, B-9810 Nazareth, Belgium

Biomed. Chromatogr. **12**, 164–166 (1998)

INTRODUCTION

Metronidazole benzoate, 2-(2-methyl-5-nitroimidazole-1-ethanol benzoate), is the benzoyl ester of metronidazole, an antibacterial agent used against a wide range of anaerobic bacteria and protozoa including trichomoniasis and amoebiasis, vaginosis and gingivitis. Because of the bitter taste of the metronidazole free base in oral liquid dosage forms the ester is generally preferred.

In order to determine the stability of this drug in a suspension, an HPLC method was developed for a simultaneous quantification of metronidazole benzoate and its hydrolysis products metronidazole and benzoic acid (Fig. 1). Several methods have been described for the quantification of metronidazole with eluents containing a small amount of organic modifier. From the literature dealing with the ester (Sa'sa' *et al.*, 1986; Mathew *et al.*, 1994; Pashankov and Kostova, 1987) only the latter includes a simultaneous determination of the hydrolysis products.

EXPERIMENTAL

Chromatography. Pump, Varian 9010; injector, Rheodyne 7125 SL 20 μL; columns BIO-SIL C18-5S, 250 × 3.2 mm (flow, 0.5 mL/min), 250 × 4.6 mm (flow 1.0 mL/min); column temperature, room temperature or water-bath at 35°C; mobile phase 70 A (6 mM sodium dodecyl sulphate (SDS), 2.5 mM phosphoric acid brought to pH 3.5 and 10% acetonitrile (v/v)), and 30 B (acetonitrile) (SDS and acetonitrile from Panreac Quimica SA, Spain); U.V. Detector, Hewlett-Packard 1050 (254 nm) equipped with Vectra QS/16S for data handling.

Sample analysis. (See also "Results") About 0.8 g (accurately weighed) of the suspension containing 6.4% (w/w) metronidazole benzoate was transferred to a 50.0 mL flask. A volume (10.0 mL) of the internal standard (ethylparaben, concentration 0.5 mg/mL in methanol) and methanol (35 mL) were added and after sonication for 10 min the solution was made up to volume with the same solvent. The solution was filtered through a PTFE 25 mm 0.45 μm syringe filter (Chromacol, England). A dilution of the methanolic solution (1 + 3) was made with buffer A and 20 μL was injected into the chromatograph.

* Correspondence to: W. R. G. Baeyens, Laboratory of Drug Quality Control, Faculty of Pharmaceutical Sciences, University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium.

RESULTS

An HPLC method for metronidazole benzoate and its hydrolysis products was developed, aiming at a simultaneous analysis of sodium benzoate, used as a preservative, in a pharmaceutical suspension. The chromatographic behaviour of methyl paraben and propylparaben as potential preservatives is also considered.

No isocratic mobile phase composition (water–acetonitrile mixtures) could be proposed providing acceptable k'-values for most polar analytes (metronidazole, benzoic acid, parabens). Therefore, the introduction of an ion-pairing reagent so as to enhance the retention behaviour of the poorly retained metronidazole molecule was considered. Based on the results of Pashankov and Kostova, the difference in effects between nitric and phosphoric acids was investigated in the presence of 4 mM sodium dodecyl sulfate (SDS) brought to pH 2.0 in the presence of varying amounts of acetonitrile. The addition of potassium nitrate to the eluent was omitted because of lack of its influence and because of pH adjustment difficulties. As identical retention behaviour was noticed, phosphoric acid was subsequently chosen for further use in the mobile phases.

The influence of SDS concentration (2–16 mM) in phosphate buffer 0.01 M pH 2.0 and of acetonitrile was investigated. The k' factor was highest using 2 mM SDS and decreased with higher SDS concentrations; however no satisfactory separation of metronidazole, benzoic acid and methylparaben was obtained. When changing the pH (from pH 2.0 to 4.0) the retention of metronidazole and metronidazole benzoate decreased, as expected. At a pH-value of about 3.5, a good separation was obtained not

CCC 0269–3879/98/030164–03 $17.50

Received 12 June 1997
Accepted 19 June 1997

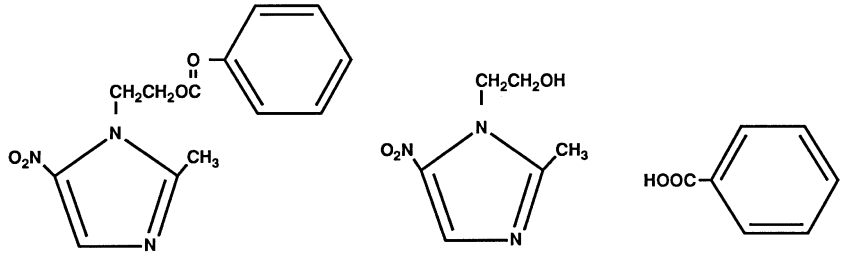

Figure 1. Structure of metronidazole benzoate, metronidazole and benzoic acid.

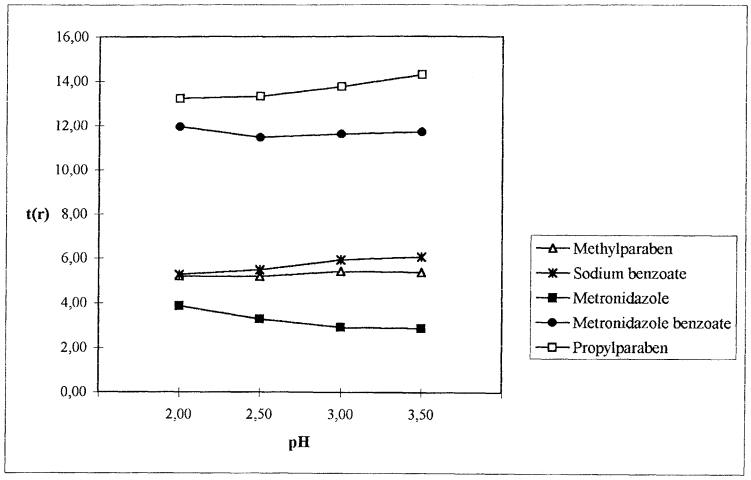

Figure 2. Influence of pH on separation. Column 3.2 i.d. × 250 mm, temperature 35°C, flow 0.5 mL/min, mobile phase 70 A (0.01 M phosphoric acid 6 mM SDS brought to pH + 10% acetonitrile (v/v)) - 30 B (acetonitrile).

taking in account the methylparaben and propylparaben chromatographic pattern. When using other ion-pairing reagents no retention changes were observed except with a mobile phase containing octanesulfonic acid (sodium salt, 20 mM) at pH 2.0. An excellent separation between metronidazole and the co-eluting methylparaben and benzoic acid was obtained.

Applying a concentration of 6 mM SDS and of 37%

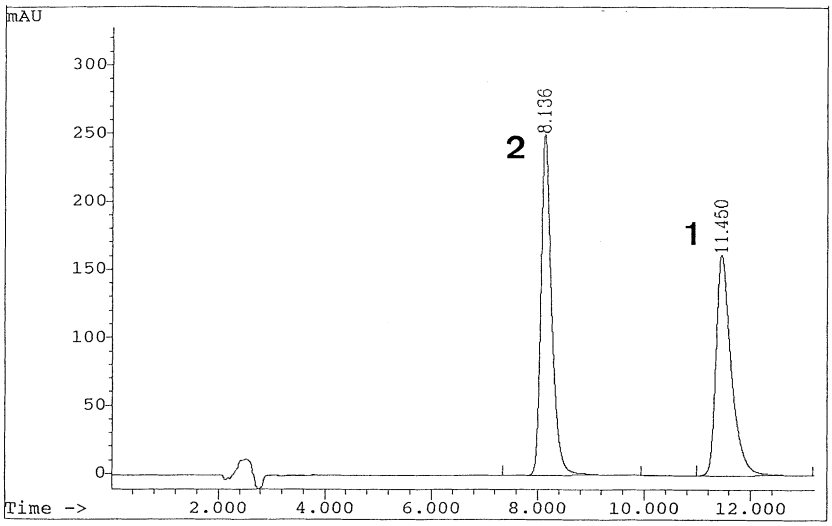

Figure 3. Determination of metronidazole benzoate (0.2 mg/mL) (1) with internal standard ethylparaben (2). Column 3.2 i.d. × 250 mm, temperature 35°C, flow 0.5 mL/min, mobile phase: see Experimental.

acetonitrile (v/v) the influence of pH was investigated for a column temperature of 35°C (Fig. 2). At a pH of 3.5 excellent separation was obtained. The influence of SDS (4 to 10 mM) at a pH-value of 3.4 was checked, resulting as well in a slight retention decrease of metronidazole benzoate.

Linearity was controlled on both reversed-phase columns with different internal column diameters. The linearity of metronidazole in the range of 0.1 to 0.4 mg/mL was controlled with solutions made in pH 3.5 buffer. Good linearity was observed on both columns (3.2 mm i.d. $r = 0.99996$; 4.6 mm i.d., $r = 0.99999$). For the 3.2 mm i.d. column there was a gain of about 230% in terms of peak height and of about 200% for peak-area calculations, besides the (more than important) saving of 50% of mobile fase consumption.

Linearity of metronidazole benzoate (10–40 mg/100 mL) was controlled, using ethylparaben as an internal standard, the latter eluting in between sodium benzoate and metronidazole benzoate. Before injection, the methanolic stock solutions were diluted with buffer A (1 + 3) (3.2 i.d. × 250 mm, $r = 0.99989$; 4.6 i.d. × 250 mm, $r = 0.99999$).

In the determination of metronidazole benzoate there was a gain in sensivity of about 200% when using areas, for peak heights only of 180%.

A typical narrow-bore chromatogram is given in Fig. 3.

CONCLUSIONS

Replacing a conventional HPLC column (i.d. 4.6 mm) by a narrow-bore analogue (i.d. 3.2 mm) brings along an improvement not only in terms of sensitivity but also a considerable reduction of solvent consumption (50%) and thus of solvent waste. The applied method confirmed the described system in that the introduction of an ion-pairing reagent in the acetonitrile-containing mobile phase for the enhancement of the metronidazole k' value is most indicated for the simultaneous determination of the benzoate ester together with its hydrolysis products.

REFERENCES

Mathew, M., Das Gupta, V. and Bethea, C. (1994). *J. Clin. Pharm. Therapeutics,* **19,** 31–34.

Pashankov, P. P. and Kostova, L. L. (1987). *J. Chromatogr.* **394,** 382–387.

Sa'sa', S. I., Khalil, H. S. and Jalal, I. M. (1986). *J. Liquid Chromatogr.* **9**(16), 3617–3631.

