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Short communication

Improved method for the separation of ranitidine and its metabolites based on supercritical fluid chromatography

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Abstract

The analysis by supercritical fluid chromatography (SFC) of ranitidine and its metabolites isolated from biological fluids is demonstrated. Chromatography was performed using a cyanopropyl column (100 × 4.6 mm I.D.) with supercritical carbon dioxide modified by a mixture of methanol–methylamine–water as the mobile phase. Separation of ranitidine from its acidic and basic metabolites is achieved in under 10 min. The investigation demonstrates the suitability of SFC for the analysis of polar drug compounds from biological matrices.

1. Introduction

Ranitidine hydrochloride is a H_2 receptor antagonist used as an inhibitor of gastric secretion in the treatment of patients who have peptic ulceration. Metabolism studies in rat, dog, rabbit and marmoset have shown that ranitidine (I) forms four metabolites, namely: ranitidine N-oxide (II), ranitidine S-oxide (III), desmethyl ranitidine (IV) and the 5-substituted-2-furan carboxylic acid (V) [1,2] as shown in Fig. 1. A number of methods have been described for the determination of ranitidine in drug substances and biological fluids [3–15]. However, none of the previously reported LC methods enable the simultaneous separation of ranitidine and all of its known metabolites.

Supercritical fluid chromatography (SFC) is a technique with superior kinetics to those of liquid chromatography and is capable of separation efficiencies approaching those of gas chromatography. Furthermore when used with polar mobile phases there are indications that SFC may be used for the assay of polar analytes with no on-column decomposition [16,17].

Accordingly it was decided to investigate the possibility of using packed-column SFC for the simultaneous analysis of ranitidine and its acidic and basic metabolites.

2. Experimental

SFC was performed on 100 × 4.6 mm I.D. stainless-steel columns packed with either 3- or 5-μm cyanopropyl or aminopropyl silica (Phase Separations, Queensferry, UK). Columns were heated in a Model TC1900 oven (ICI Scientific Instruments, Dingley, Australia) at temperatures between 50 and 80°C. The mobile phase con-

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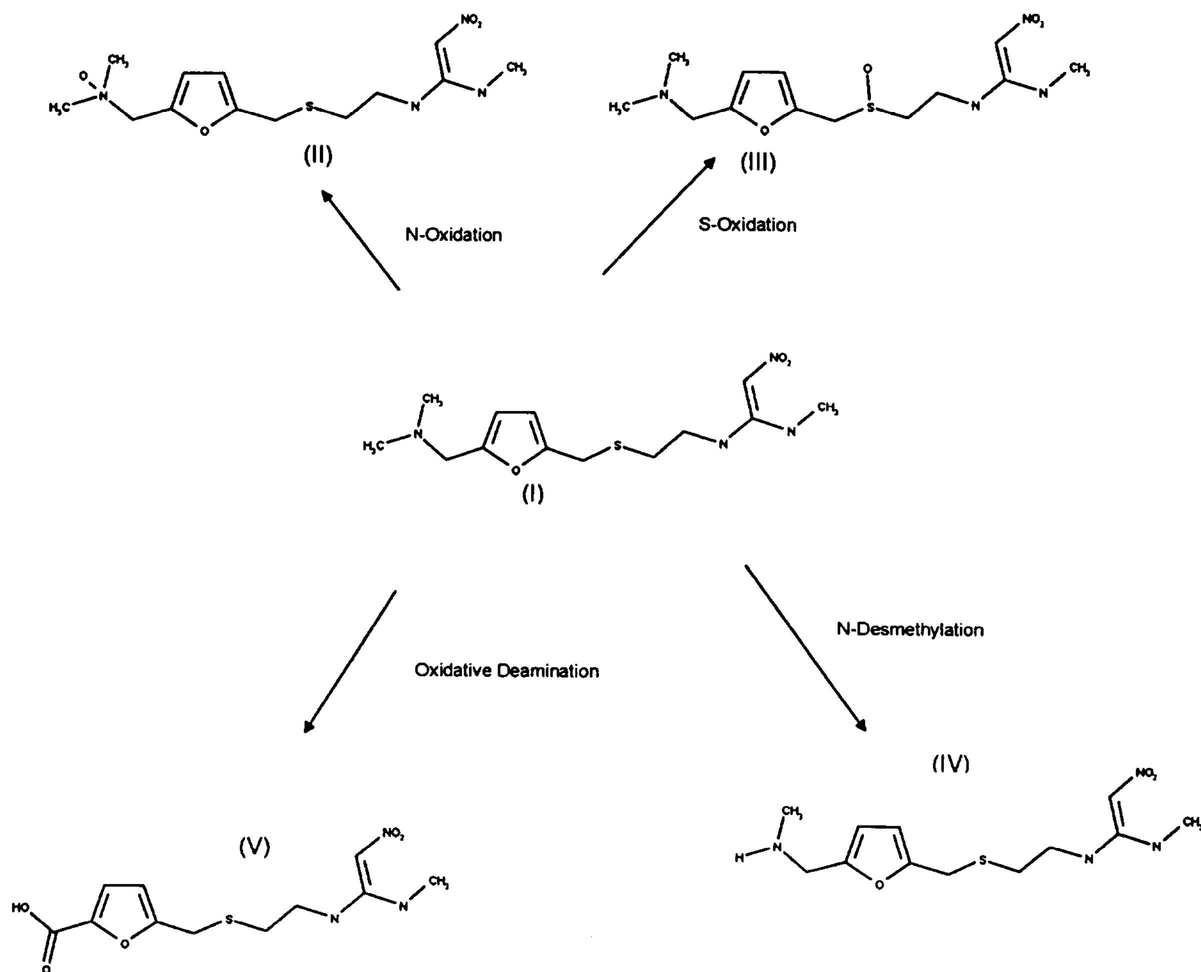

Fig. 1. The metabolism of ranitidine.

sisted of mixtures of carbon dioxide (British Oxygen Gases, London, UK), methanol, methylamine and water pumped through the system by Model 302 and 303 piston pumps (Gilson, Middleton, WI, USA) controlled by means of an Apple II GS microcomputer. The pumphead was cooled to −15°C by means of a RTE-4 refrigerated bath cooler (Jencons, Leighton Buzzard, UK) to facilitate the filling of the pump with liquid mobile phase. Samples were introduced by means of a Rheodyne Model 7125 injector fitted with a 10-μl loop. The column effluent was monitored by a Model 757 variable-wavelength UV detector (Kratos Analytical, Ramsey, NJ, USA). A Tescom back pressure regulator (Tescom Instruments, Elk River, MI, USA) was used to maintain supercritical conditions.

2.1. Reagents and materials

HPLC-grade methanol was obtained from BDH (Poole, UK) whilst 40% (v/v) aqueous methylamine was obtained from Aldrich.

Ranitidine hydrochloride, ranitidine N-oxide, ranitidine S-oxide, desmethyl ranitidine and the furoic acid analogue were synthesised in the Chemistry Division of Glaxo Research and Development, Ware, UK.

3. Results and discussion

The initial attempt to separate the metabolites of ranitidine was performed using an aminopropyl bonded phase with supercritical carbon dioxide modified with methanol as the mobile phase. Ranitidine, ranitidine S-oxide, ranitidine N-oxide and desmethyl ranitidine as can be seen in Fig. 2 were found to be separated within 9 min; however, there were signs of peak tailing in the case of desmethyl ranitidine. The furoic acid analogue was not eluted even with high pressures and modifier concentrations indicating irreversible adsorption onto the basic stationary phase.

Clearly a different strategy was required to facilitate a simultaneous separation of the acidic and basic metabolites. In the first instance, the stationary phase was changed to cyanopropyl silica which is a non-basic sorbent that is less likely to retain acidic or basic analytes irreversibly. The cyanopropyl silica proved to be an ideal stationary phase for the furoic acid metabolite which was eluted in less than 2 min at 53°C. The basic components, however, were strongly retained by the stationary phase, especially ranitidine and ranitidine N-oxide, which were retained for over 30 min even using high pressures and a 20% methanol concentration.

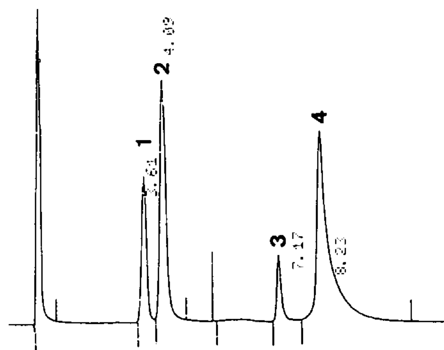

Fig. 2. The separation of ranitidine (1), ranitidine S-oxide (2), ranitidine N-oxide (3) and desmethyl ranitidine (4) on an aminopropyl column by SFC. Column, 10 × 0.46 cm, 5-μm aminopropyl bonded silica; inlet pressure, 3600 p.s.i.; mobile phase, carbon dioxide–methanol, a linear gradient of 15 to 20% methanol and a flow-rate of 3 to 4 ml/min over 10 min; temperature, 53°C. Values at peaks are retention times in min.

Janicot et al. [18] have successfully separated the alkaloids narcotine, papaverine, thebaine, ethylmorphine, codeine, morphine and cryptopine from a poppy straw extract by SFC using a mixture of methanol, aliphatic amine and water as eluent along with an aminopropyl bonded silica column. They suggested that the amine effectively precluded analyte retention by residual silanols whilst the water enhanced the alkaloid–amine–aminopropyl chain interactions. Thus if an amine, which competes well with an eluite for residual silanols, is included in the mobile phase at a sufficiently high concentration; retention of the eluite will occur only by a solvophobic mechanism [19]. Since the basicities of the alkaloids examined by Janicot et al. were not too dissimilar to the basic ranitidine metabolites it was decided to test the applicability of methanol, methylamine and water as modifiers for carbon dioxide for the analysis of ranitidine. The concentration of methylamine in the modifier was found to be critical, the greater the concentration of methylamine the longer the retention of the analytes, also above a 3% amine level difficulties were encountered due to column blockage. Below 1% the amine gave rise to negligible selectivity between ranitidine and ranitidine S-oxide as can be seen from the data in Table 1.

Accordingly a 1% methylamine level was chosen as the standard modifier concentration for all subsequent investigations. Methylamine was introduced as a 40% aqueous solution, the water was obviously important because little selectivity was observed when the same concentration of amine was added by means of a 33% solution in methylated spirits. Janicot et al. used an aminopropyl bonded silica to separate the basic alkaloids. However, with ranitidine and its basic metabolites ample retention could already be achieved using a cyanopropyl bonded phase. Thus by reducing the silanol interactions for ranitidine and ranitidine N-oxide with the amine modifier all five compounds might be expected to be separated and in fact this was found to be the case as shown in Fig. 3.

The experiments with model compounds indicate the potential of SFC in drug metabolism

Table 1

Effect of the methylamine concentration (in the modifier) on the capacity ratios of ranitidine and its metabolites

Component	Capacity ratio			
	Methylamine concentration in the modifier (%)			
	0.5	1	3	4
I	2.83	2.90	3.09	3.08
III	2.83	3.15	3.57	3.64
IV	3.91	4.38	4.69	4.85
V	4.79	4.97	6.41	6.85
II	7.98	9.94	10.79	10.84

Column, 10 × 0.46 cm 3 μm cyanopropyl bonded silica; pressure inlet, 4000 p.s.i. (1 p.s.i. = 6894.76 Pa); mobile phase composition, carbon dioxide–(methanol + 0.5–4% methylamine + 1% water) (80:20, v/v); flow-rate, 2.5 ml/min; temperature, 71°C.

studies. Not only was the resolution achieved superior to that obtained by LC but also the speed of analysis was improved. Accordingly it was decided to test the method using biological extracts containing drug and metabolites.

The metabolism of drugs in the body occurs largely in the liver. A convenient method of monitoring drug metabolism in vitro involves using isolated hepatocytes. In this procedure a liver is removed from an animal, the hepatocytes, which contain all the detoxifying liver enzymes necessary for metabolism to occur, are isolated and incubated with the target drug, in this instance ranitidine [20]. The drug and its metabolites were recovered by solid-phase extraction (SPE), as described previously [16], and submitted for SFC. Typical results are shown in Fig. 4, which indicate that the metabolism of the ranitidine has occurred in the presence of both guinea pig and rat hepatocytes. As can be seen both species produce little or no furoic acid metabolite and the guinea pig produces a higher proportion of ranitidine N-oxide relative to the rat.

In a further experiment ranitidine metabolites present in rat urine, isolated by SPE, were analysed by SFC. The results obtained as shown

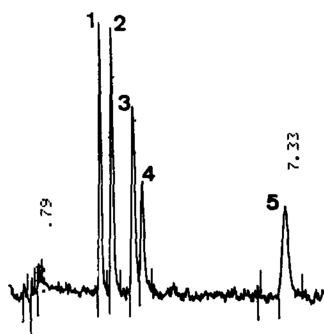

Fig. 3. Isocratic elution of ranitidine and its metabolites by SFC. Column, 10 × 0.46 cm, 3-μm cyanopropyl bonded silica; inlet pressure, 3200 p.s.i.; mobile phase, carbon dioxide–methanol–40% aqueous methylamine–water (80:19.6:0.2:0.2); flow-rate, 3 ml/min; temperature, 71°C. Peaks: 1 = I; 2 = III; 3 = IV; 4 = V; 5 = II.

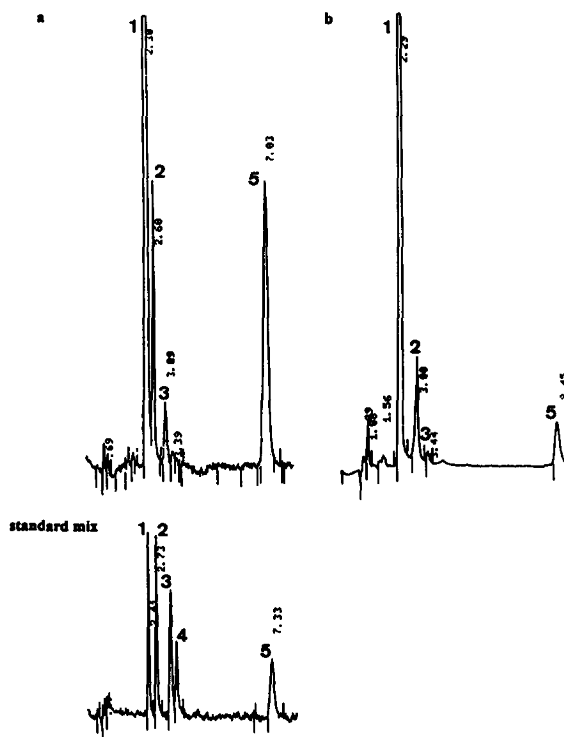

Fig. 4. SFC analysis of ranitidine from (a) rat and (b) guinea pig hepatocytes. Conditions and peak numbering as in Fig. 3.

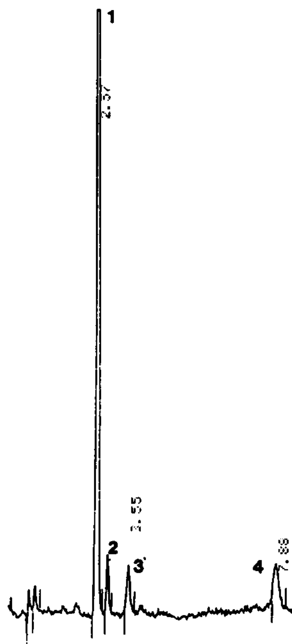

Fig. 5. SFC separation of ranitidine and its metabolites from rat urine. Conditions as in Fig. 3. Peaks: 1 = I; 2 = III; 3 = IV; 4 = II.

in Fig. 5 again emphasise the potential of SFC in this context.

4. Conclusions

Studies involving model compounds and biological samples reveal the potential of SFC for the analysis of drugs and their phase 1 metabolites.

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