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Journal of Chromatography A, 712 (1995) 55–60

JOURNAL OF CHROMATOGRAPHY A

Estimation of ranolazine and eleven Phase I metabolites in human plasma by liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry with selected-ion monitoring

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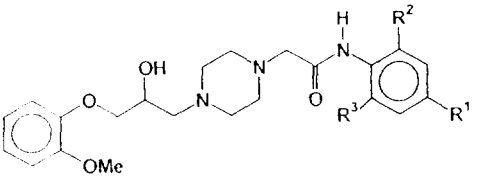
Abstract

The estimation of ranolazine, a novel piperazine derivative, and eleven of its Phase I metabolites has been undertaken by liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry (LC–APCI-MS). Plasma samples, taken on day 5 of a multiple-dose study, were extracted by solid-phase extraction (SPE) and analysed, using a gradient HPLC system coupled to the APCI source of a Finnigan MAT TSQ 700 mass spectrometer. Metabolites were analysed in selected-ion monitoring (SIM) mode, using an instrument control language (ICL) procedure. The LC–MS combination allowed resolution of all eleven metabolites, including four hydroxylated metabolites and five unresolved components. The results from the linear regression showed good correlation ($r^2 > 0.980$) for all the metabolites. Plasma concentrations indicated that three metabolites were present at levels higher than 10% of the parent compound.

1. Introduction

The introduction of APCI mass spectrometers as routine, robust, and very sensitive analytical instruments has led to a much wider use of mass spectrometry in drug metabolism departments throughout the pharmaceutical industry. These instruments have been used to develop very specific and sensitive assay methods [1,2], as well as for the characterisation of drug and metabolites [3,4].

Ranolazine (RS-43285; Fig. 1) is a novel piperazine derivative that has been indicated in the treatment of both angina and intermittent claudication [5,6]. Previous in vitro and in vivo studies had allowed the identification of the extensive metabolic pathway for ranolazine. These proposed metabolites were synthesised to



	R1	R2	R3
RS-43285	H	Me	Me
RS-87986	Et	H	H

Fig. 1. Chemical structures of ranolazine and RS-87986.

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0021-9673/95/$09.50
SSDI 0021-9673(95)00475-0

allow confirmation of retention times and mass spectra. Plasma samples, taken on day 5, of a multi-dose clinical study were initially analysed by LC–MS to provide qualitative metabolite identification information. In this study semi-quantitation of the observed metabolites was undertaken to provide preliminary concentration data to determine which metabolites were at levels greater than 10% of parent drug for which a fully validated method would subsequently be developed.

2. Experimental

2.1. Chemicals

All chemical used were of analytical grade or better and were supplied by Fisons (Loughborough, UK), BDH (Poole, Dorset, UK) and Aldrich (Gillingham, Dorset, UK).

RS-87986 (internal standard; Fig. 1), RS-43285 and its metabolites were supplied by Recherche Syntex France (RSF) (Leuville, France), or the Institute of Organic Chemistry (IOC) (Palo Alto, CA, USA).

2.2. Sample extraction

Human plasma (1 ml), spiked with RS-87986 (25 μl, 15 μg/ml), was thoroughly mixed and loaded onto a Bond-Elut (C_{18}, 3 ml) SPE cartridge (supplied by Crawford Scientific, Strathaven, UK.) conditioned by successively washing with methanol (3 ml), distilled water (3 ml) and sodium hydroxide (0.1 *M*, 0.5 ml). The plasma was allowed to elute under gravity. The SPE cartridge was washed with distilled water (2 ml), dried with positive pressure, and transferred to a clean culture tube. The absorbed components were eluted by washing the cartridge with methanol (2 ml) followed by methanolic ammonium acetate (0.1 *M*, 2 ml). The combined extract was evaporated to dryness under a stream of nitrogen at 40°C, and the residue reconstituted in distilled water (300 μl) by whirl-mixing before centrifuging for 5 min at 3000 rpm. The supernatant was transferred to a 300-μl autosampler microvial for analysis.

For the analysis of ranolazine 0.1 ml of plasma was spiked with RS-87986 (25 μl, 15 μg/ml) and extracted as described above.

2.3. Calibration curves

Calibration curves, from 20 to 1000 ng/ml, were constructed by spiking varying volumes of stock solutions (4 μg/ml and 20 μg/ml) containing the eleven metabolites into control human plasma (1 ml in duplicate). RS-87986 (25 μl, 15 μg/ml) was added and the samples extracted as described above.

A calibration curve for ranolazine, over the range 0.5–5 μg/ml, was constructed by spiking varying volumes of a stock solution (10 μg/ml) into control human plasma (0.1 ml in duplicate). RS-87986 (25 μl, 15 μg/ml) was added and the samples extracted as described above.

2.4. HPLC conditions

Aliquots (200 μl) of the plasma extracts were injected using an ISS-100 autosampler (Perkin-Elmer, Beaconsfield, UK) and chromatographed on a Waters 600MS HPLC system (Waters Associates, Watford, UK) at 1 ml/min using an Ultrasphere ODS (150 × 4.6 mm I.D.) HPLC column (Beckman Instruments UK) maintained at 50°C in a column oven.

For the analysis of the metabolites a gradient system was developed in order to resolve four hydroxylated metabolites. Solvent A was composed of aqueous ammonium acetate (20 m*M*) and trifluoroacetic acid (TFA, 0.12%) at pH 3.0, and Solvent B was methanolic ammonium acetate (20 m*M*) and TFA (0.12%). The HPLC gradient was linearly increased from 10 to 55% B in 30 min, then linearly from 55 to 100% B in 3 min, held at 100% B for 2 min before returning to 10% B in 1 min. The column was allowed to re-equilibrate for 4 min before injection of the next sample.

For the analysis of ranolazine an isocratic mobile phase of A–B (40:60, v/v) was used.

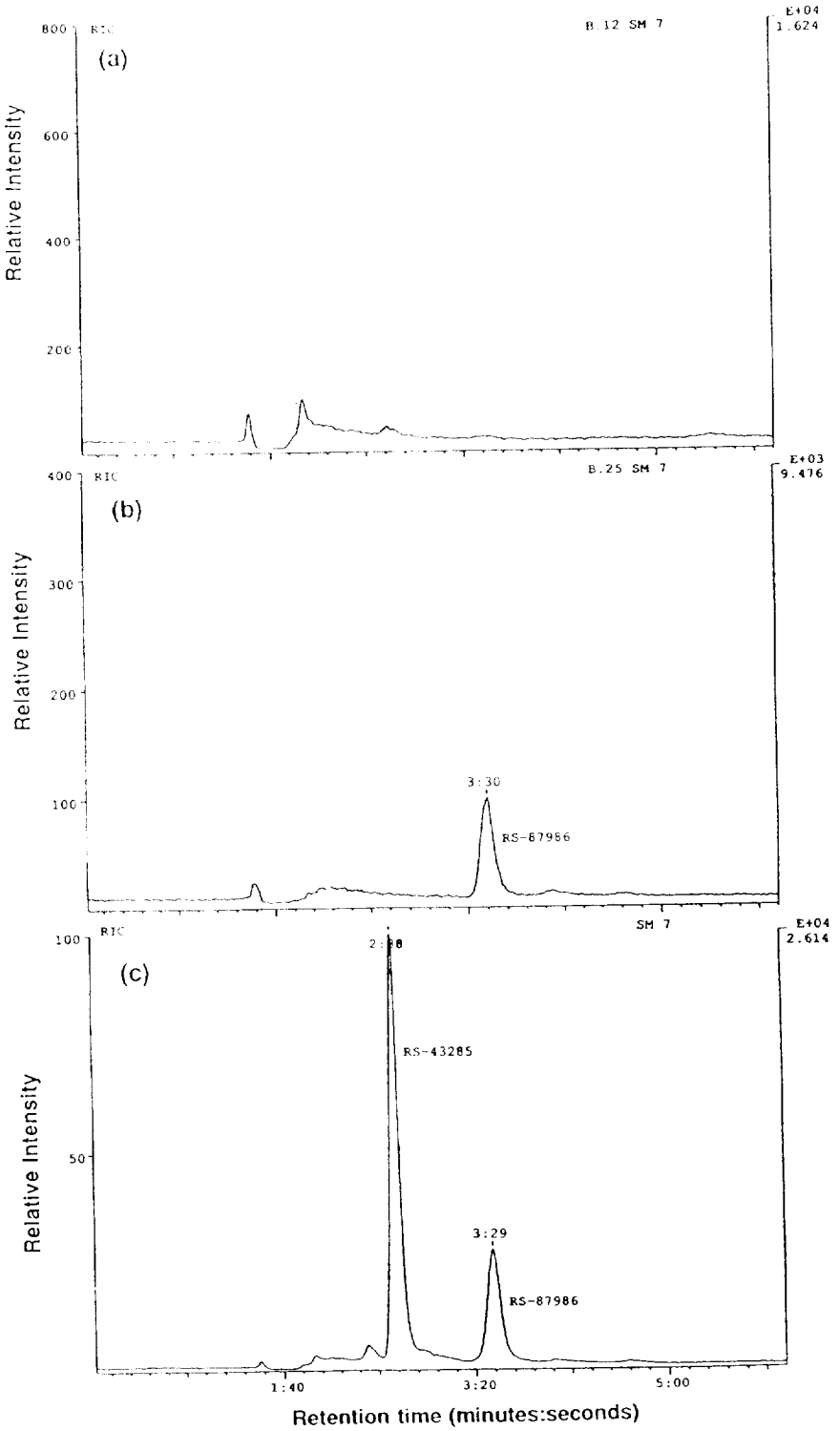

Fig. 2. SIM chromatograms for the estimation of ranolazine. (a) Control human plasma, (b) pre-dose (day 1), (c) 1 h post-dose (day 5).

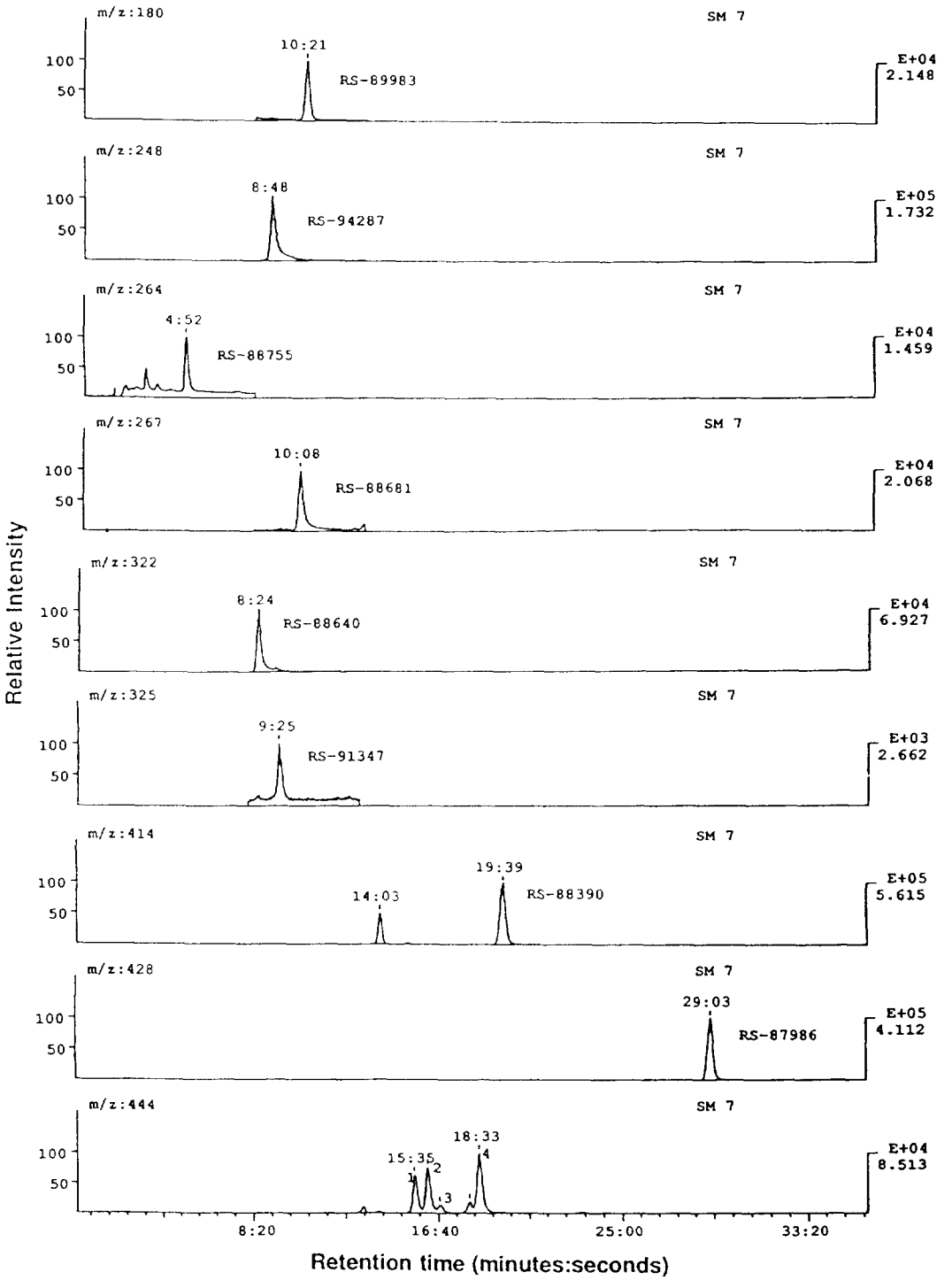

Fig. 3. SIM chromatograms for the estimation of ranolazine metabolites on day 5 (1 h post-dose). Peaks labelled 1–4 are identified as RS-88772, RS-88597, RS-88835 and RS-89961 respectively. The additional peak observed in the m/z 414 trace (14:03 min:s) is a glucuronide metabolite.

2.5. Mass spectrometric conditions

The eluate from the HPLC column was plumbed directly into the APCI probe assembly of a Finnigan MAT APCI source which was fitted to a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT, Hemel Hempstead, UK). The HPLC flow was nebulised using N_2 as both a sheath gas, at a pressure of $3.1 \cdot 10^5$ Pa, and an auxillary gas, at a flow-rate of 2 l/min. The APCI vapouriser and capillary heaters were held at 500°C and 250°C respectively to assist in the desolvation and declustering of the HPLC solvent. A corona discharge of 5 μA was also applied to assist in ionisation. Capillary, tube lens and octapole voltages of respectively 40 V, 100 V and −3.0 V were also applied to assist in the focusing of ions into the mass spectrometer.

Analysis in the mass spectrometer was carried out in positive ion mode with unit mass resolution. The mass spectrometer was operated in the selected-ion monitoring (SIM) mode using an ICL procedure written to allow the mass spectrometer to switch between the metabolite masses at specific retention times, and a 100-ms dwell-time for each mass was used. Ranolazine and RS-87986 have the same molecular mass (M_r 427) and therefore the mass spectrometer was operated in SIM for m/z 428 $[M + H]^+$ continually.

Peak areas for all components were automatically integrated using CHRO software on the TSQ 700 and peak-area ratios (area of drug or metabolite/area RS-87986) were plotted versus concentration using unweighted linear regression. From the calibration lines obtained concentrations of unknown samples were determined by interpolation.

3. Results

SIM chromatograms are shown for the analysis of ranolazine in Fig. 2. SIM chromatograms for the analysis of the metabolites are shown in Fig. 3 and show the HPLC separation obtained for four hydroxylated metabolites (m/z 444) and the

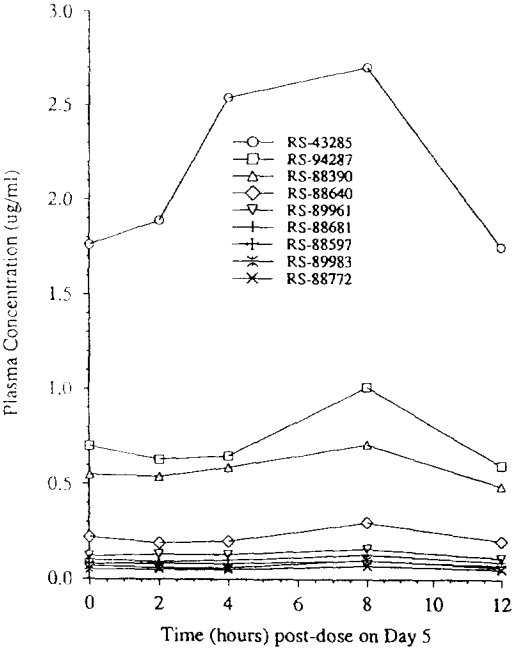

Fig. 4. Plasma concentration profiles of ranolazine and eight Phase I metabolites on day 5.

mass resolution of five co-eluting components (8:24–10:21). For the linear regression of ranolazine an r^2 value of 0.996 was obtained; r^2 values of between 0.980 and 0.999 were obtained for the linear regression of the metabolites. The plasma concentration profiles for ranolazine and the metabolites are shown in Fig. 4 and indicated that three of the metabolites (RS-94287, RS-88390 and RS-88640) were at levels greater than 10% of parent drug. Three of the metabolites (RS-88755, RS-88835 and RS-91347) gave levels below the limit of quantitation of this method and are not shown in Fig. 4. The plasma concentrations of ranolazine and RS-88390, obtained by this method, were compared with results previously obtained using a validated HPLC–UV method and were found to give a mean inter-assay variation of 18.5% for ranolazine and 13.1% for RS-88390.

4. Conclusions

The results of this experiment indicate the tremendous selectivity and specificity that can be

achieved by the use of LC–MS techniques. The quantitation, by mass resolution, of the five components with retention times between 8:24 and 10:21 (min:s) would not be possible using this HPLC system with UV detection. Likewise, the quantitation of the four hydroxylated components would not have been possible without the separation by HPLC. Excellent r^2 values were also obtained for the linear regression considering the differences in the metabolite structures and the use of only one internal standard, but may also in part be due to the stability of the signal obtained by APCI. The presence of only three metabolites at levels greater than 10% of the parent drug also allows the possibility of modifying the HPLC conditions to give a shorter run time thus increasing the sample throughput for future studies. The simplicity of the SPE methodology may also allow its transfer to automated sample processors, i.e. ASPEC.

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