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QUALITATIVE AND QUANTITATIVE ANALYSIS OF RANITIDINE AND ITS METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

M. S. LANT, L. E. MARTIN* and J. OXFORD
Biochemical Pharmacology Division, Glaxo Group Research Limited, Ware, Herts. SG12 0DJ (U.K.)

SUMMARY

Reversed-phase high-performance liquid chromatography systems for the separation of ranitidine and its metabolites ranitidine-N-oxide, ranitidine-S-oxide, and desmethylranitidine have been developed for use in high-performance liquid chromatography–mass spectrometry.

A direct liquid introduction–high-performance liquid chromatography–mass spectrometry system to analyse qualitatively and quantitatively solutions containing ranitidine and its metabolites by reversed-phase chromatography is described.

A sample of urine collected from a subject given an oral dose of 75 mg of ranitidine and 75 mg of tris-deuterated ranitidine was analysed by this system. Ranitidine and its metabolites were identified by the ion doublets in the mass spectra which were 3 a.m.u. apart.

INTRODUCTION

The analysis of ranitidine and three of its metabolites, ranitidine-N-oxide, ranitidine-S-oxide and desmethylranitidine, using a liquid chromatograph coupled to a mass spectrometer via a moving-belt interface has been described[1,2]. It was found that the drug and its metabolites were thermally degraded under the conditions required to evaporate the reversed-phase high-performance liquid chromatography (HPLC) eluent from the belt. To overcome this, a normal-phase HPLC–mass spectrometry (MS) system was developed. This system has limited sensitivity because only 10 μl of urine could be injected on column without affecting the chromatography. The ability to use a reversed-phase system would have increased the sensitivity because larger volumes of urine could be injected.

Systems have been described for directly introducing a reversed-phase HPLC eluent into the source of a quadrupole mass spectrometer operating in the chemical ionisation mode[3]. These systems have been shown to cause less destruction of thermally labile molecules[4]. The use of a reversed-phase HPLC system coupled via a direct liquid introduction (DLI) probe into a HP 5987A quadrupole mass spectrometer for the identification and quantification of ranitidine and its metabolites is described.

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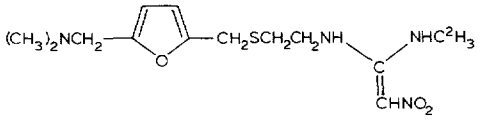
EXPERIMENTAL

Reagents and materials

All reagents were of analytical grade. The solvents used for HPLC were filtered through a 0.2-μm pore size filter (Millipore, Bedford, MA, U.S.A.) to remove particulate matter and then continuously purged with helium. Ranitidine hydrochloride, ranitidine-N-oxide, ranitidine-S-oxide, desmethylranitidine hydrochloride and tris-deuterated ranitidine hydrochloride ([$^2H_3$]ranitidine) (I) were synthesised in the Chemistry Division of Glaxo Group Research Limited, Ware, U.K.



(I)

High-performance liquid chromatography

A Hewlett-Packard 1090 liquid chromatograph was equipped with a binary pumping system, automatic injector and a filter photometric detector. A stainless-steel column, 200 × 2 mm I.D., which was packed with 5 μm Hypersil ODS, was fitted. One of the following solvent systems was used for the analyses: System A, acetonitrile–water (50:50) at flow-rates of 200, 300 and 500 μl/min; System B: acetonitrile–0.05 *M* ammonium acetate (60:40) at a flow-rate of 150 μl/min; System C: acetonitrile–0.05 *M* ammonium acetate under a gradient elution of 10% to 40% acetonitrile in 10 min at a flow-rate of 300 μl/min.

The column efficiency was determined with benzophenone using HPLC System A. The eluent was monitored at 250 nm.

DLI–HPLC–MS interface

The outlet from the HPLC column was connected to a DLI–HPLC–MS probe with stainless-steel tubing (100 cm × 0.1 mm I.D.). Two DLI probes were used. One was the standard probe supplied with the instrument (Hewlett-Packard, Palo Alto, CA, U.S.A.) and the other was a low dead volume probe with stainless-steel transfer lines of 0.1-mm I.D. A needle valve was used to provide a small back-pressure, and *ca.* 10% of the HPLC eluent entered the mass spectrometer ion source. An extended desolvation chamber without a cartridge heater was used in these experiments and has previously been described[5].

Mass spectrometry

A Hewlett-Packard 5987A GC/MS/DS mass spectrometer was fitted with a DLI interface and a short-fingered liquid nitrogen cooled cryopump. Data were acquired and processed using the standard software supplied by Hewlett-Packard.

Chemical ionization (CI) operating parameters of the mass spectrometer for DLI–HPLC–MS were as follows: emission current, 300 μA; electron energy, 200 eV; source temperature, 200°C; ion source pressure *ca.* 2.0 Torr (as measured at the CI-GC–MS interface); electron multiplier voltage, 2500.

Scanning data were acquired between masses 200 to 350 to avoid the high background resulting from solvent cluster ions.

Selected ion monitoring (SIM) data for ranitidine and [$^2H_3$]ranitidine were obtained by monitoring the ions *m/z* 315 and *m/z* 318, respectively. A 300-msec dwell period was used for each ion.

Preparation and analysis of calibration standards

The internal standard solution was prepared by dissolving 8 mg of [$^2H_3$]ranitidine hydrochloride in 10 ml of distilled water. This was diluted 100-fold to give a concentration of 8 μg/ml. Ranitidine hydrochloride (11.5 mg) was dissolved in 10 ml of distilled water giving a concentration of 1.15 mg/ml. This was diluted ten-fold to give a concentration of 115 μg/ml. This solution was further diluted to give a series of standard solutions in the range 0–40 μg/ml. The internal standard solution (500 μl, 4 μg) was added to 500 μl of each of the ranitidine standard solutions to give calibration standards in the range 0–20 μg ranitidine/4 μg [$^2H_3$]ranitidine/ml. Each standard (5 μl) was analysed by DLI–HPLC–MS using HPLC System B. The intensities of the ions *m/z* 315 and 318 were recorded.

The ratio of the peak areas of the ions *m/z* 315: *m/z* 318 for each standard was plotted against the ranitidine concentration in the sample, and a calibration line determined by the method of least squares regression analysis.

Qualitative DLI–HPLC–MS studies

Standard solution of ranitidine and its metabolites. An aqueous solution containing 125 μg/ml each of ranitidine, ranitidine-N-oxide, ranitidine-S-oxide and desmethylranitidine was prepared; 20 μl were analysed by DLI–HPLC–MS using HPLC System C.

Identification of ranitidine and its metabolites in urine. A male volunteer was given an oral dose of 75 mg of ranitidine and 75 mg of [$^2H_3$]ranitidine. Urine was collected at 2-h intervals up to 8 h after dosing and stored at −15°C before analysis; 20 μl of the 4–6 h urine sample was analysed by DLI–HPLC–MS using HPLC System C.

RESULTS

Optimisation of instrumental parameters

To optimise the source parameters, a solution of the compound under investigation was introduced continuously into the mass spectrometer. The source pressure was measured and optimised by adjustment of the jet length and position of the probe in the desolvation chamber. To improve the ease of optimising the pressure an extended desolvation chamber similar to that described by Sugnaux *et al.*[5] was fitted. For gradient elution the source pressure was optimised using the solvent composition which corresponded to that at the mid-point of the gradient.

The efficiency, as shown in Table I, of 2 mm I.D. HPLC columns at flow-rates less than 500 μl/min should be maintained by the DLI probe. The standard Hewlett-Packard probe, which has an internal volume of *ca.* 40 μl, caused significant extra-column band broadening, and a decrease in overall efficiency. When a more recently available low dead volume probe, which has an internal volume of *ca.* 10 μl, was used the column efficiency was maintained (Table I).

TABLE I

COMPARISON OF COLUMN EFFICIENCY USING A UV DETECTOR AND THE MASS SPECTROMETER

Analyte, benzophenone; eluent, System A.

Detector	*Flow-rate (μl/min)*	*Theoretical plates, experiment number*		
		1	*2*	*3*
UV	200	11,638	11,595	12,079
	300	9653	9980	9541
	500	8824	8600	
Mass spectrometer fitted with standard DLI probe	200	–	–	
	300	5883	5500	
	500	6584	5796	
Mass spectrometer fitted with low volume DLI probe	200	11,165	11,138	11,239
	300	9912	9000	9094
	500	7960	7891	

Qualitative and quantitative analysis of ranitidine

The maximum source temperature which could be used for the analysis of ranitidine without causing major fragmentation of the molecule was 200°C. The mass spectrum (Fig. 1) obtained by DLI–HPLC–MS analysis of ranitidine showed the $[M + H]^+$ ion, *m/z* 315, as base peak. The reconstructed TIC from this analysis (Fig. 2a) showed evidence of peak tailing and the mass chromatograms of the ions *m/z* 281 and *m/z* 315 (Fig. 2b) did not coincide. Mass spectra determined on the leading edge top of peak and tailing edge of the chromatogram showed that the ratio of the abundancies of *m/z* 281 and *m/z* 315 increased across the peak (Table II).

A complete mass spectrum was obtained on 100 ng of ranitidine injected on column. When the SIM technique was used and the $[M + H]^+$ ion *m/z* 315 monitored the lower limit of detection was 5 ng injected on column (Fig. 3).

Quantitative DLI–HPLC–MS analysis of solutions of ranitidine was carried

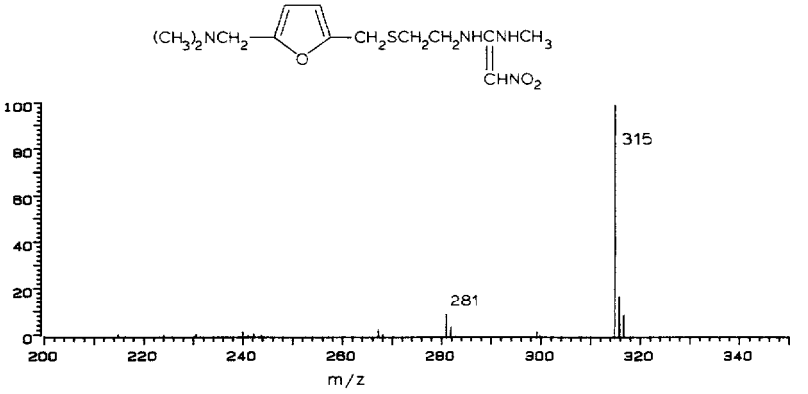


Fig. 1. Mass spectrum of ranitidine obtained by DLI–HPLC–MS using HPLC System C.

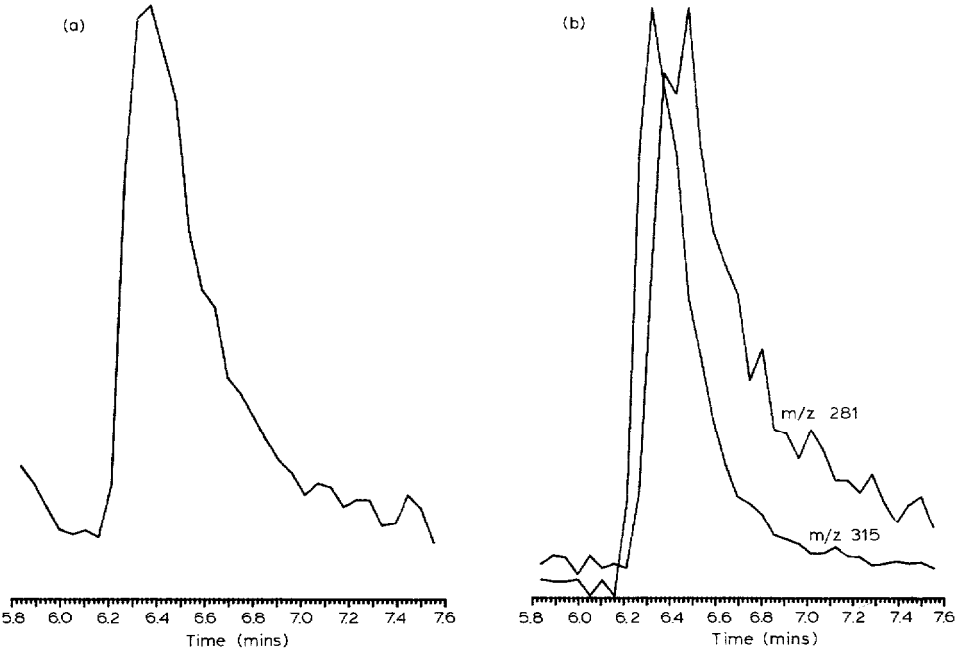


Fig. 2. (a) Reconstructed TIC of the DLI–HPLC–MS analysis of ranitidine, using HPLC System C; (b) Mass chromatograms of the ions *m/z* 281 and 315 derived from ranitidine.

out using [$^2H_3$]ranitidine as an internal standard. There was a linear relationship between the area ratio of the ions *m/z* 315 and 318 over the range 5–100 ng of ranitidine injected on column (Fig. 4).

Qualitative analysis of ranitidine and its metabolites in aqueous and biological samples

Ranitidine and its metabolites, ranitidine-S-oxide, ranitidine-N-oxide and desmethylranitidine, were separated on a reversed-phase system by gradient elution. The reconstructed TIC (Fig. 5a) showed the chromatogram of the four compounds. Mass spectra from each of the metabolites showed abundant $[M + H]^+$ ions (Fig. 5b, c, d). Only in the mass spectrum of ranitidine-N-oxide was any significant fragmentation observed: the base peak *m/z* 315 was derived by loss of an oxygen atom from *m/z* 331 the $[M + H]^+$ ion.

Fig. 6 shows the reconstructed TIC resulting from DLI–HPLC–MS analysis

TABLE II

VARIATION OF THE RATIO OF THE ABUNDANCIES OF THE IONS *m/z* 281 AND *m/z* 315 AS RANITIDINE ELUTES INTO THE ION SOURCE

Position on chromatographic peak, (Fig. 2a)	*Ratio of abundance of m/z 281 to abundance of m/z 315*
Leading edge	0.1
Peak top	0.3
Tailing edge	1.4

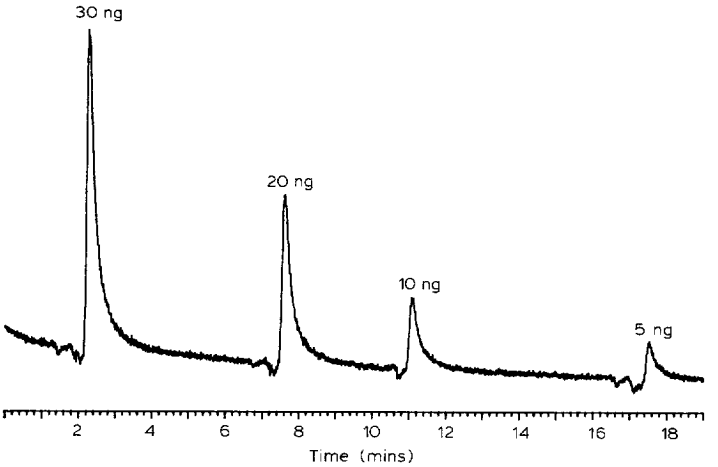


Fig. 3. DLI–HPLC–MS analysis of ranitidine, using HPLC System B, by SIM of the ion m/z 315.

of the 4–6 h urine sample obtained from a subject who received orally a 1:1 mixture of ranitidine and [$^2H_3$]ranitidine. This sample contained 155 μg/ml ranitidine, 33 μg/ml ranitidine-N-oxide, 7.3 μg/ml ranitidine-S-oxide and 7.9 μg/ml desmethylranitidine, as determined by HPLC[6]. The mass spectrum of the major peak (Fig. 7a) showed the ion doublet m/z 315, 318 derived from ranitidine and [$^2H_3$]ranitidine. The retention times of the three metabolites were determined from mass chromatogram of their respective protonated molecular ions. Their mass spectra, shown in Figs. 7b–d all show $[M + H]^+$ ion doublets derived from the metabolites and their deuterated analogues. Under the chromatographic conditions, the minor peak derived from desmethylranitidine was poorly resolved from the major peak derived from ranitidine. This is the reason why the ion doublet derived from ranitidine and its deuterated analogue is present in the mass spectrum of desmethylranitidine. The latter was identified by the ion doublet at m/z 301, 304.

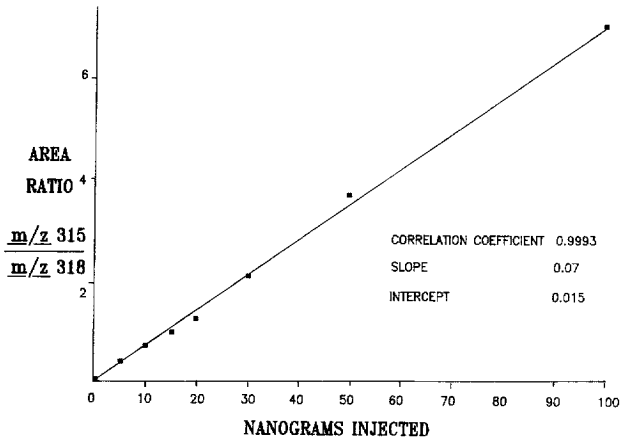


Fig. 4. The calibration graph obtained for ranitidine with [$^2H_3$]ranitidine as internal standard.

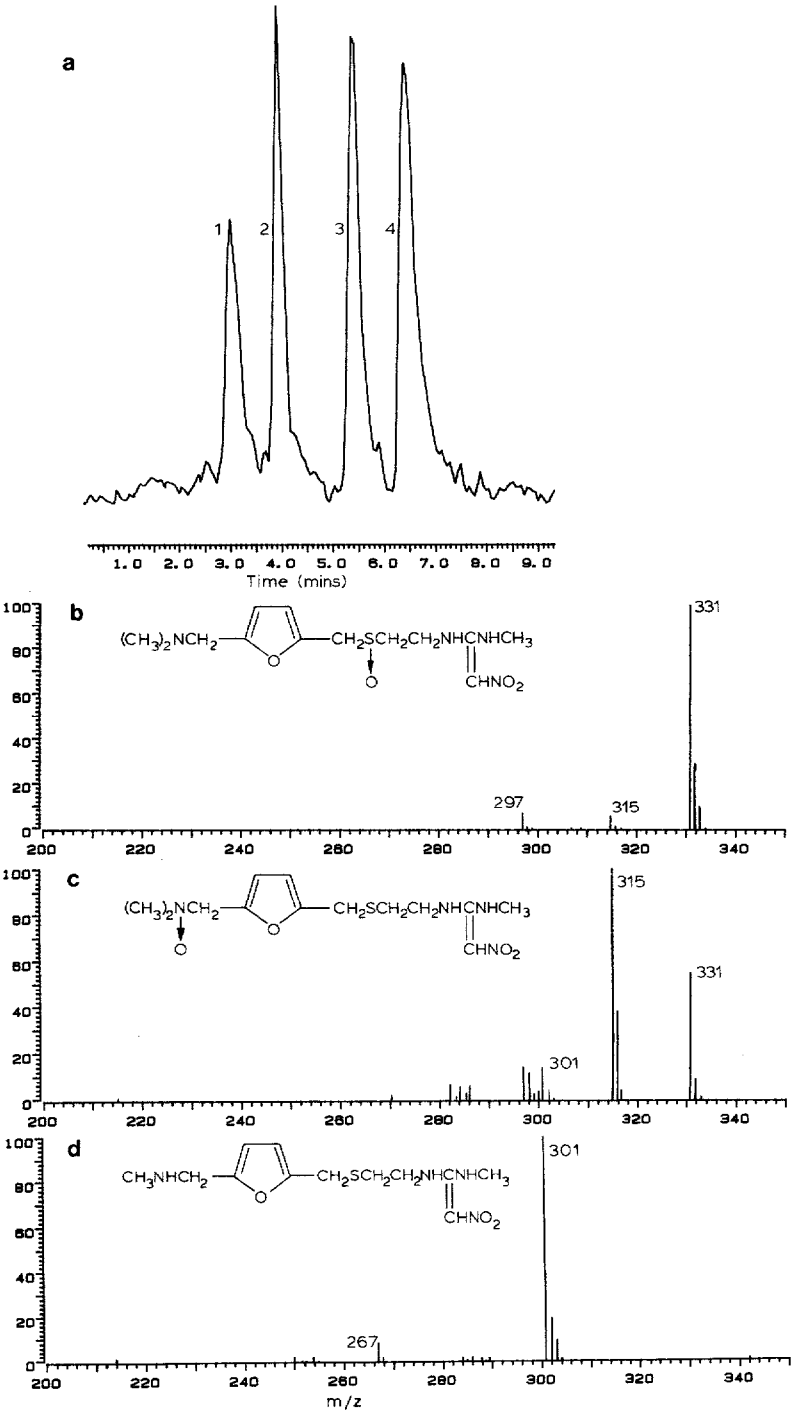


Fig. 5. DLI-HPLC-MS analysis of ranitidine and its metabolites using HPLC System C. (a) Reconstructed TIC; mass spectra of (b) peak 1, ranitidine-S-oxide; (c) peak 2, ranitidine-N-oxide; (d) peak 3, desmethylranitidine. Peak 4 is derived from ranitidine.

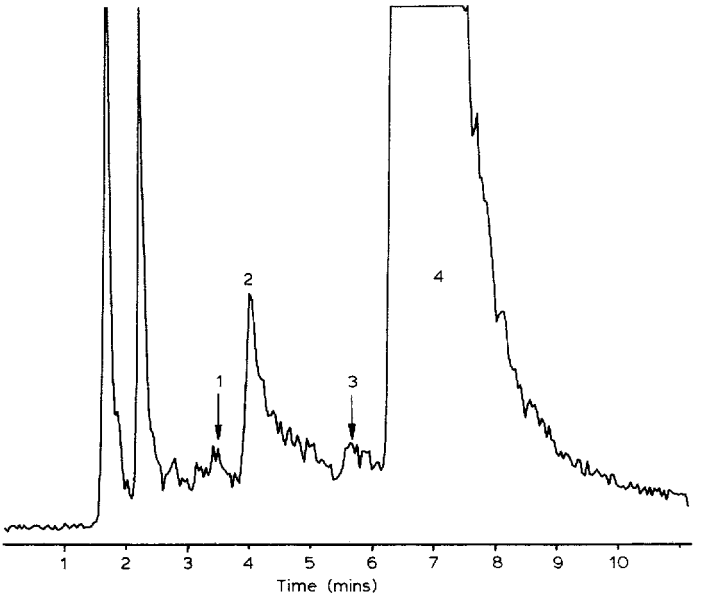


Fig. 6. Reconstructed TIC of the DLI–HPLC–MS analysis, using HPLC System C, of the 4–6 h urine from a subject who was given a 1:1 mixture of ranitidine and [$^2H_3$]ranitidine. Peaks: 1 = ranitidine-S-oxide/[$^2H_3$]ranitidine-S-oxide; 2 = ranitidine-N-oxide/[$^2H_3$]ranitidine-N-oxide; 3 = desmethylranitidine/[$^2H_3$]desmethylranitidine; 4 = ranitidine/[$^2H_3$]ranitidine.

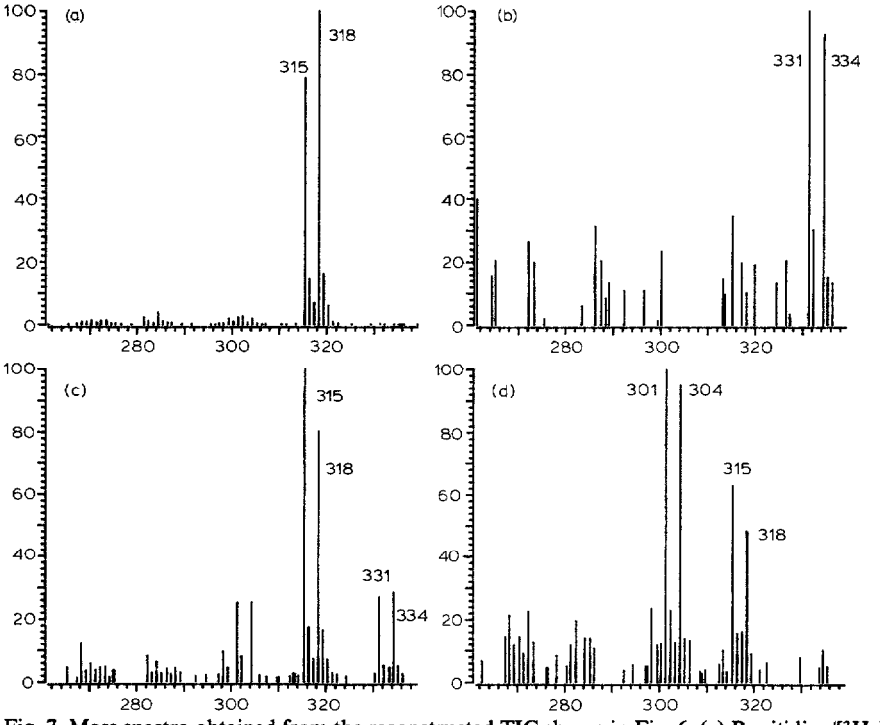


Fig. 7. Mass spectra obtained from the reconstructed TIC shown in Fig. 6. (a) Ranitidine/[$^2H_3$]ranitidine; (b) ranitidine-S-oxide/[$^2H_3$]ranitidine-S-oxide; (c) ranitidine-N-oxide/[$^2H_3$]ranitidine-N-oxide; (d) desmethylranitidine/[$^2H_3$]desmethylranitidine.

DISCUSSION

Voyksner *et al.* have described the use of solvent cluster ions as a solute-independent method of tuning source pressure[7]. They showed that selected cluster ions maximised in abundance with the protonated molecular ions of some solute molecules at optimum pressure. However, this effect was not observed for the protonated molecular ion of ranitidine and this method of pressure optimisation could not be used.

The sensitivity for the qualitative and quantitative analysis of drugs and metabolites using DLI–HPLC–MS is limited because the operating conditions of the mass spectrometer are affected if more than *ca.* 30 μl of eluent are introduced into the source. The use of a 2 mm I.D. column enabled lower flow-rates to be used and therefore a greater proportion of the HPLC eluent could be split into the source. At low flow-rates the effect of extra column band broadening introduced by dead volume in the DLI–HPLC–MS becomes significant. To minimise this a low dead volume DLI probe was used.

The mass spectra obtained from ranitidine and its metabolites using DLI–HPLC–MS with reversed-phase chromatography showed abundant $[M + H]^+$ ions. This was in marked contrast to those obtained using the moving-belt interface, which showed that extensive thermal fragmentation of these molecules was occurring[1,2]. The DLI mass spectrum of ranitidine showed an increase in the ratio of the abundancies of the ions *m/z* 281 and 315 across the chromatographic peak. The reason for this was probably that ranitidine was deposited on the walls of the ion source and the desolvation chamber. During subsequent desorption the ranitidine was thermally degraded. If this phenomenon is observed when analysing solutions containing unknown compounds, it may be concluded that two components were present.

Preliminary quantitative analyses of ranitidine showed that a stable isotopically labelled internal standard was required to compensate for changes in ionisation efficiencies between samples. This variation in efficiency was measured by determining the coefficient of variation of the peak area of the ion *m/z* 318 derived from $[^2H_3]$ranitidine in each of the calibration standards. It was found to be 29%. This value was greater than could be attributed to variations in dilution or injection and could only be attributed to variations in ionisation efficiency occurring within the source.

The limit of detection of ranitidine using DLI–HPLC–MS and SIM of the ion *m/z* 315, was 5 ng injected on column. With reversed-phase eluents up to 100 μl of a biological sample can be injected on column and this can be increased to 1 ml if a pre-column is used. Therefore DLI–HPLC–MS should offer a significant improvement in sensitivity for quantitative measurement of ranitidine over that obtained with the moving-belt interface[2].

The identification of drugs and metabolites in biological samples by mass spectrometry is aided by co-administering a 1:1 mixture of the drug and its stable isotopically labelled analogue[8]. This technique in combination with DLI–HPLC–MS was used to characterise ranitidine and its metabolites in a urine sample from a subject who received orally a 1:1 mixture of ranitidine and $[^2H_3]$ranitidine. The mass spectra of the metabolites of ranitidine were characterised by ion doublets 3 a.m.u. apart.

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