Journal of Chromatography, 562 (1991) 39–45
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5563

Use of particle beam liquid chromatography–electron impact mass spectrometry for structure elucidation of oxodipine and three of its metabolites

C. JULIEN-LAROSE*, P. VOIRIN, C. MAS-CHAMBERLIN and A. DUFOUR

Département de Biopharmacie, Laboratoires Delagrange, 1 Avenue Pierre Brossolette, 91380 Chilly Mazarin (France)

ABSTRACT

In order to test the analytical capabilities of the particle beam liquid chromatograph–mass spectrometer interface in structural identification in drug metabolism, a liquid chromatographic–mass spectrometric (LC–MS) method using this new technique was developed for oxodipine and some of its expected metabolites. After two extraction steps at pH 9 and 1.5, the separation of the compounds, which have a wide polarity range, was carried out by an isocratic high-performance liquid chromatographic method with a 25-cm cyano-bonded column. The compounds were eluted with hexane–methanol–methylene chloride (76:12:12). Mass spectra were recorded after electron impact ionization (75 eV) with a source temperature of 150°C. Under these conditions, comparison of the spectra with those obtained after gas chromatography or with a direct introduction probe showed identical fragmentation patterns when a sufficient amount of product was injected for LC–MS analysis.

INTRODUCTION

The inapplicability of gas chromatography–mass spectrometry (GC–MS) to polar or thermally labile compounds led to the development of several on-line interfaces for combining liquid chromatography and mass spectrometry (LC–MS). The two main techniques available are direct liquid introduction (DLI) [1] and thermospray (TSP) [2,3]. These two systems operate by using soft ionization and consequently cannot generate electron impact (EI) ionization mass spectra.

Recently, a particle beam LC–MS interface, which is an improved monodispersal aerosol generation interface, was developed [4,5]. The most important feature of this system is the capability of producing EI mass spectra with normal- or reversed-phase high-performance liquid chromatography (HPLC), the only restriction on the mobile phase being the use of volatile buffers in the aqueous phase. Hence this system seems very attractive for structural identification in metabolism studies and its analytical capabilities were tested for this particular application by analysing urine spiked with oxodipine, a new calcium antagonist, and some of its expected metabolites (Fig. 1).

0378-4347/91/$03.50

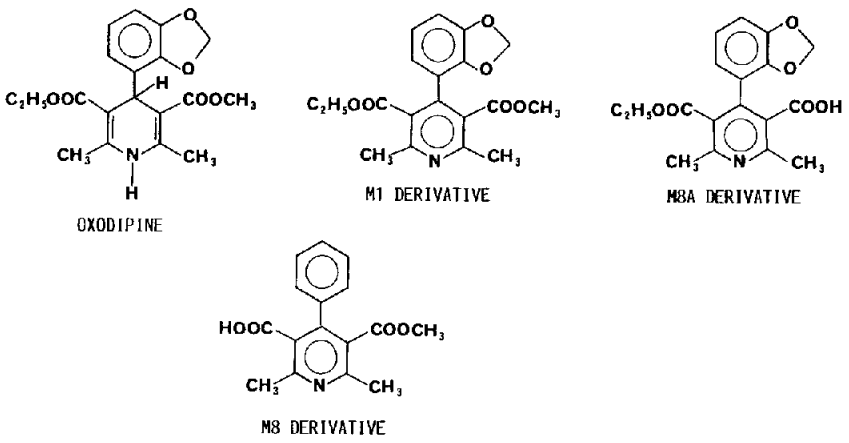

Fig. 1. Structures of oxodipine and its expected metabolites.

EXPERIMENTAL

A Hewlett-Packard Model 1050 liquid chromatograph and a Model 5988 mass spectrometer were used.

Particle beam interface

In this system, a nebulizer generates an aerosol from the HPLC effluent. As the aerosol passes through a desolvation chamber (at 200 Torr and 45°C), the volatile components such as the mobile phase are vaporized while less volatile compounds such as analytes condense to form submicrometre particles. This mixture enters a two-stage separator in which the vapour molecules of relatively low momentum are pumped away while the particles of higher momentum pass through the transfer probe into the ion source and are then ionized.

Liquid chromatography–mass spectrometry

A Hypersil 5-μm cyano-bonded column (250 mm × 4.6 mm I.D.) from SFCC (Neuilly-Plaisance, France) was used. The mobile phase was hexane–methanol–methylene chloride (76:12:12) at a flow rate of 0.2 ml/min. Detection was carried out by mass spectrometry in the EI mode (75 eV) with a source temperature of 150°C.

Chemicals and reagents

Oxodipine and M1, M8 and M8A, metabolites corresponding to the structures given in Fig. 1, were obtained from IQB (Madrid, Spain).

All solvents were used without prior distillation. Methanol and ethyl acetate (RS grade for fluorimetry) were purchased from Carlo Erba (Milan, Italy), hex-

ane (HPLC grade) from Fisons (Loughborough, U.K.), methylene chloride (HPLC grade) from Rathburn (Walkerburn, U.K.) and 0.1 *M* sodium hydroxide (Titrisol grade) from Merck (Darmstadt, F.R.G.). Hydrochloric acid (6 *M*) was obtained by dilution of 12 *M* hydrochloric acid (Merck) with water purified with a Millipore Milli-Q system.

Standard solutions

Stock standard solutions (400 μg/ml) of oxodipine and M1 metabolite were prepared by dissolving 0.8 mg of product in 2 ml of mobile phase. Stock standard solutions (800 μg/ml) of M8 and M8A metabolites were prepared by dissolving 2 mg of product in 2 ml of mobile phase. All these solutions were stored at 4°C.

Extraction procedure

A 1-ml volume of urine was spiked with 20 μg of oxodipine and M1 metabolite and 50 μg of M8 and M8A metabolites. The urine was made alkaline with 100 μl of 0.1 *M* sodium hydroxide solution (pH 9) and extracted with 2 × 5 ml of ethyl acetate. After agitation (25 min) and centrifugation (5 min at 1000 *g*), the two organic phases (4.5 ml each) were pooled. The remaining aqueous phase was then acidified with 50 μl of 6 *M* hydrochloric acid (pH = 1.5) and re-extracted twice with 5 ml of ethyl acetate. All the organic phases (first and second extractions) were mixed together and evaporated under a stream of nitrogen. The dry residue was dissolved in 100 μl of mobile phase and 50 μl were analysed by LC–MS.

RESULTS AND DISCUSSION

LC–MS profiles

Typical profiles of blank urine and urine spiked with oxodipine and M1, M8 and M8A metabolites obtained by LC–EI-MS are presented in Fig. 2. The retention times were 20.9, 27.7, 29.2 and 31.2 min for M1, oxodipine, M8A and M8, respectively, with a good separation.

The extraction recovery was calculated by external standardization to be 80% for oxodipine and 90% for the other products.

The comparison between the two traces in Fig. 2 shows that after liquid extraction, no interference is found in the blank sample at the retention times of the compounds. The good signal-to-noise ratio obtained for the amounts injected (10 μg of oxodipine and M1, 25 μg of M8 and M8A) demonstrates that the same analysis could have been performed with half the amounts of the compounds.

Mass spectral characterization in spiked urine

Oxodipine and M1 metabolite. The EI mass spectra obtained for these two compounds are shown in Fig. 3. Mass spectra were recorded in the mass range 100–400 a.m.u. in order to avoid the contribution of low-mass ions due to the residual mobile phase passing through the interface. The oxodipine spectrum

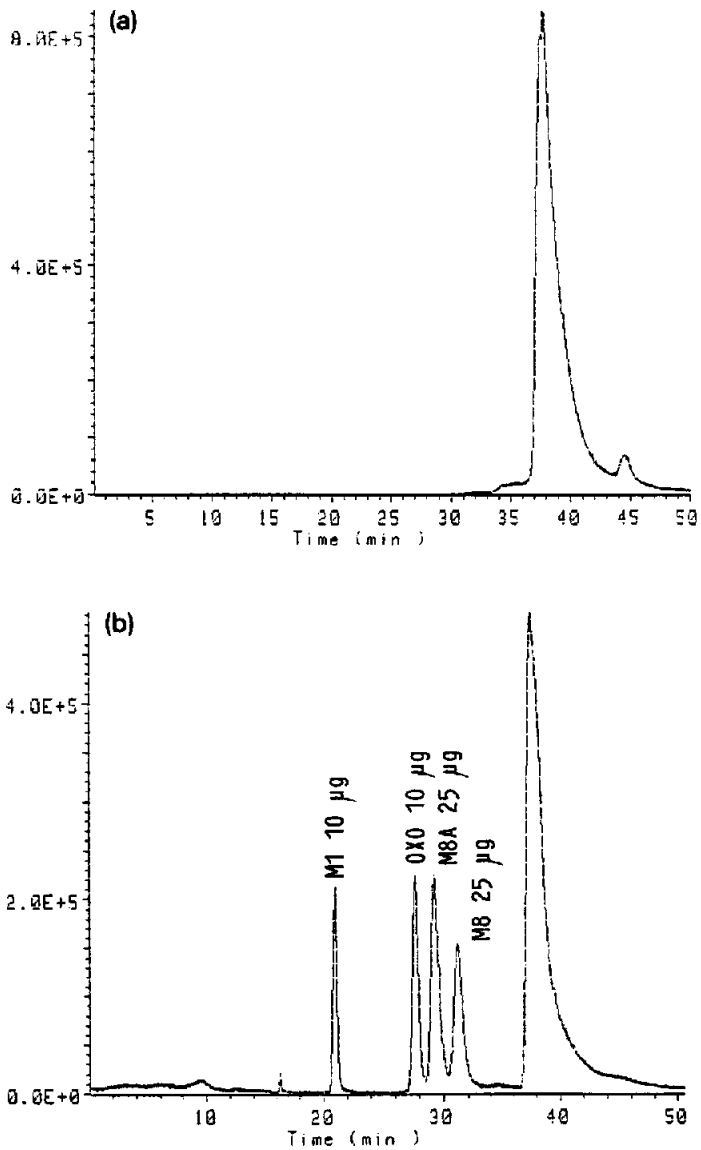

Fig. 2. Total ion current of urinary extract obtained by LC–EI-MS for (a) a blank sample and (b) spiked urine.

exhibits a molecular ion at *m/z* 359 with a low relative abundance (about 20%), the base peak at *m/z* 238 corresponding to the ion with the dihydropyridine moiety. The other main intense fragments at *m/z* 330, 286 and *m/z* 210 correspond to $[M^+ - C_2H_5]$, $[M^+ - COOC_2H_5]$ and $[238 - C_2H_4]$, respectively. The spectrum of the M1 metabolite shows different features: it has few fragmentations

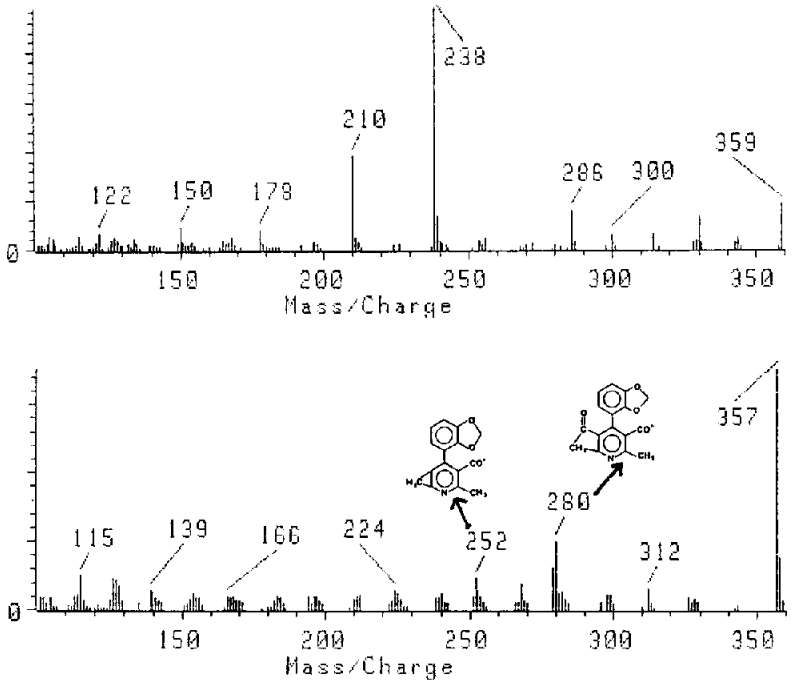

Fig. 3. Mass spectra of oxodipine and M1 metabolite obtained by LC–EI-MS.

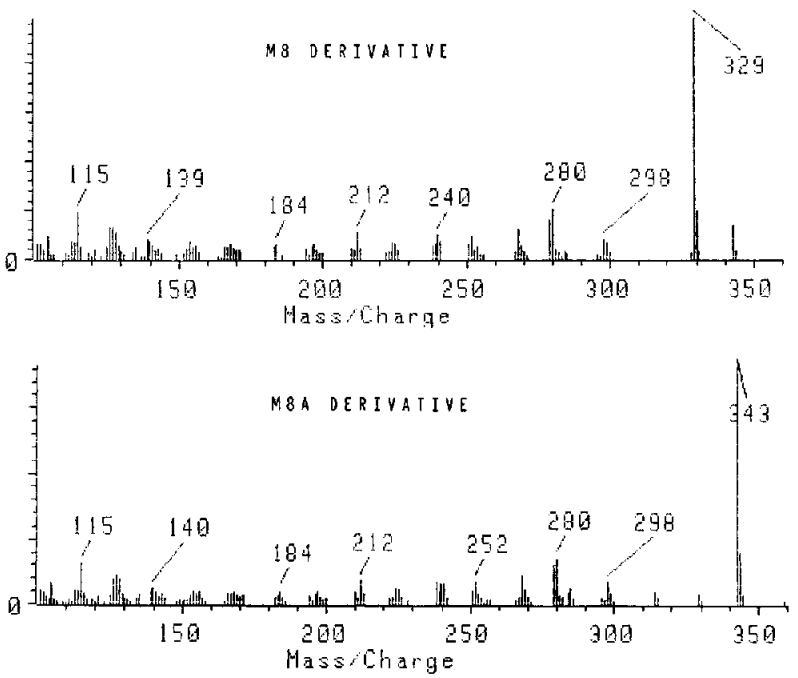

Fig. 4. Mass spectra of M8 and M8A metabolites obtained by LC–EI-MS.

and the base peak at *m/z* 357 is the molecular ion. The two main fragments at *m/z* 280 (40%) and 252 (20%) correspond to the structures shown.

M8 and M8A metabolites. The EI mass spectra obtained for these two acids are presented in Fig. 4. The molecular ions at *m/z* 343 and 329 corresponding to M8A and M8 are the base peaks of the spectra. Except for these ions, the two spectra show low-intensity ions, the main ones being found at *m/z* 298, 280 and 252. The ion at *m/z* 298 corresponds to the loss of OCH_3 and OC_2H_5 from the molecular ion for M8 and M8A, respectively. The ions at *m/z* 280 and 252 are as described for the M1 metabolite. In this context, it is noteworthy that the relative abundances of the molecular ion and of the base peak in the spectra are a good criterion for evidence of a pyridine or dihydropyridine structure in an unknown metabolite.

Comparison of LC–EI and classical mass spectra

The comparison of LC–EI and GC–EI mass spectra for oxodipine and M1 metabolite presented in Fig. 5 shows that similar fragmentation patterns were obtained with the two methods but LC–MS analysis required an amount fifteen times greater than for GC–MS. The inability to achieve the analysis of acids by GC–MS without prior derivatization necessitated the comparison of the LC–EI mass spectrum of metabolite M8 with the direct introduction probe (DIP) EI mass spectra and the results are presented in Fig. 6. The fragmentation patterns obtained for each compound are identical with the two introduction techniques. It is important to note that the amounts of compound needed to record good EI mass spectra are very similar for LC–MS and DIP–MS analysis.

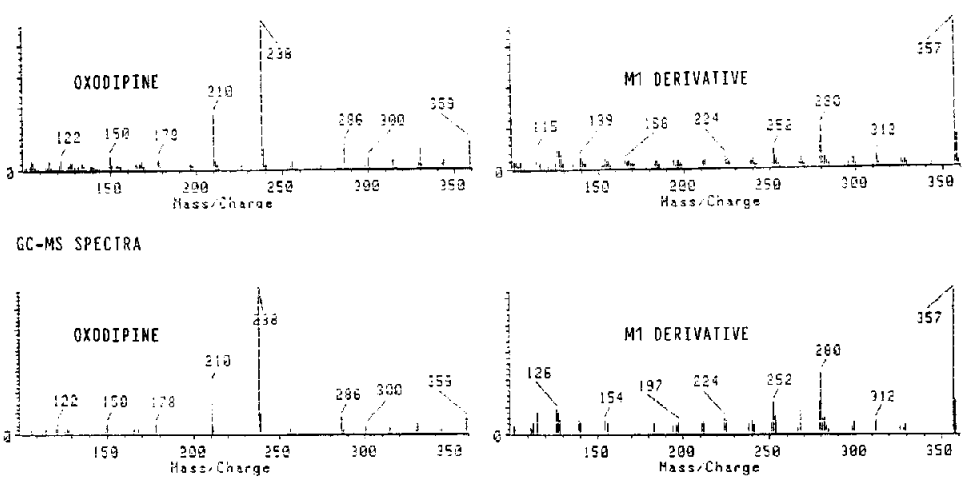

Fig. 5. Comparison of EI mass spectra obtained by LC–MS and GC–MS for oxodipine and M1 metabolite.

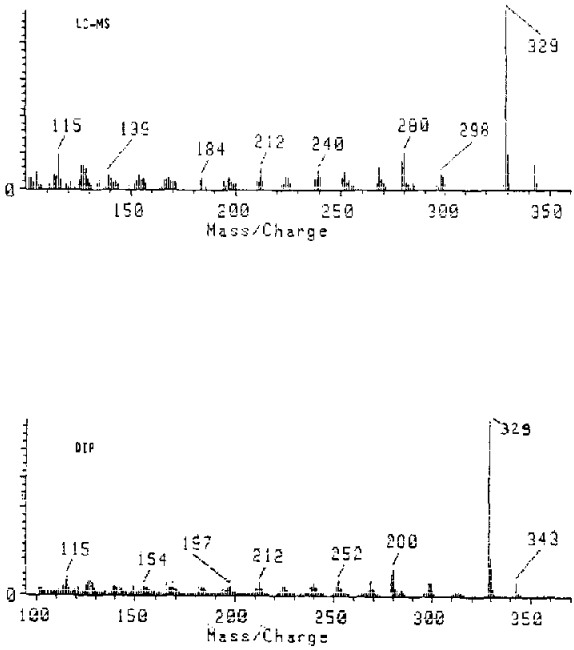

Fig. 6. Comparison of EI mass spectra obtained by LC–MS and DIP–MS for M8 metabolite.

CONCLUSION

The use of a particle beam LC-MS interface with normal-phase HPLC allows (i) specific EI mass spectra having an identical fragmentation pattern with those obtained by classical GC–MS to be generated, (ii) EI mass spectra to be obtained, without derivatization, for polar compounds with characteristic ions leading to structural identification and (iii) the routine performance, with biological samples, of the separation and identification of compounds with a wide polarity range.

Wider applications, particularly with a reversed mobile phase with buffer, must be studied in order to validate the use of this technique in the drug metabolism field.

REFERENCES

1 P. J. Arpino, *J. Chromatogr.*, 323 (1985) 3.
2 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
3 M. L. Vestal, in A. L. Burlingame and N. Castagnoli, Jr. (Editors), *Mass Spectrometry in the Health and Life Sciences*, Elsevier, Amsterdam, 1985, pp. 99–118.
4 R. C. Willoughby and R. F. Browner, *Anal. Chem.*, 56 (1984) 2626.
5 P. C. Winkler, D. D. Perkins, W. K. Williams and R. F. Browner, *Anal. Chem.*, 60 (1988) 489.