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LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY OF TRACE COMPOUNDS WITH A MOVING-BELT INTERFACE AND MULTI-DIMENSIONAL CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method with on-line mass spectrometric detection is described for the structural analysis of a number of synthetic impurities, present at trace levels in almitrine. To obtain mass spectra with various ionization methods and high-resolution mass measurements, a moving-belt liquid chromatograph–mass spectrometer interface is used. A two-column switching system allows the injection of large amounts of almitrine, from which the trace compounds are trapped on a second column, while discarding the major component. This permits the introduction of the impurities into the mass spectrometer by elution of the second column, without the risk of introducing too large an amount of the major compound into the mass spectrometer. The mass spectra thus obtained are of sufficient quality to permit a correct structural assignement of the impurities.

INTRODUCTION

The physico-chemical characterization of an active compound in a drug includes the structural identification of synthetic impurities. As the major part of the chromatographic separations is performed with liquid chromatography, combined liquid chromatography–mass spectrometry (LC–MS) is often the method of choice for a first structural determination of these impurities.

The amount of synthetic impurities in a compound used as a drug is often very low, usually of the order of 0.01–0.5%. As it is undesirable to introduce large amounts of the major component into the LC–MS system, column-switching techniques can be used effectively to separate and accumulate sufficient amounts of minor constituents on a second column[1–5]. The moving-belt interface offers the advantage that the mass spectrometric conditions are much less influenced by changes in flow and/or solvent composition than, for instance, the thermospray interface[6–10].

Generally, in handling problems of structural identification of unknown compounds ionization by electron impact (EI) is often the first technique to be used, because in this mode the fragmentation pattern provides the major part of the structural information. At some stage chemical ionization and/or fast atom bombardment can be used to acquire information on molecular weight. The moving-belt LC–MS

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interface in combination with a double-focusing mass spectrometer offers the advantage of a variety of ionization methods which can be used[11–15], with the possibility of performing high-resolution measurements for accurate mass determinations[16].

During an analysis of almitrine (Vectarion)[17–19], reversed-phase LC was used to separate a number of minor impurities (<0.1%) from the main component. To permit structural analysis of these compounds by mass spectrometry, a two-dimensional LC system was used, allowing the injection of large amounts of the compound without disturbing the performance of the moving-belt LC–MS system.

The first column was used to discard the major part of the main compound (S 2620) while the second column in the LC system was used to collect, by repeated injections, sufficient amounts of the impurities. On elution of the second column, EI and chemical ionization (CI) were used for structural analysis. The chromatographic separation on the two columns was monitored by two UV detectors in series with the LC system.

EXPERIMENTAL

Chromatography

LC was performed with two Waters 590 solvent delivery systems and a Rheodyne 7125 injection valve. The two columns were of stainless steel with Nucleosil $5C_{18}$ (150 × 4.7 mm I.D., first column) and Nucleosil $3C_{18}$ stationary phases (70 × 4.7 mm I.D., second column). The mobile phase for both columns was methanol–water–trifluoroacetic acid (60:40:0.1, v/v/v), at a flow-rate of 1.0 ml/min in the first stage of the analysis (elution of the first column) and 0.5 ml/min for the subsequent elution of the second column, on-line with the mass spectrometer. The switching between the various elution modes was made by three- and six-way valves (Chromatem SSI, Rheodyne 7000). The elution of the columns was monitored by (1) a Waters M 440 and (2) a Shimadzu SPD-6A variable-wavelength UV detector set at 254 nm.

Moving-belt LC–MS system

A VG Analytical LC moving-belt interface equipped with a spray deposition probe and a Kapton belt was coupled to a VG Analytical 70-250 S double-focusing mass spectrometer. The interface conditions were a spray probe temperature of 280°C, auxiliary gas (nitrogen) at 70 kPa and a belt speed of 0.8 cm/s.

The EI mass spectra were taken at 70 eV ionization energy and 0.1 mA ionization current. The CI mass spectra were obtained with an ionization energy of 125 eV and an emision current of 1.0 mA. The source temperatures were 180 and 140°C in the EI and CI mode, respectively. The mass range was 600–60 amu at a scan speed of 1.0 s/decade. The CI reagent gas was ammonia at approximately 0.5 Torr ion source pressure. High-resolution mass measurements were performed at a resolving power of 7500.

RESULTS AND DISCUSSION

Fig. 1 shows the LC–UV analysis of almitrine obtained after an injection of 100 μg on to a one-column system. In addition to the main compound (S 2620), there are six impurities, present at levels varying from 0.1 to 0.01%. Based on LC analyses of

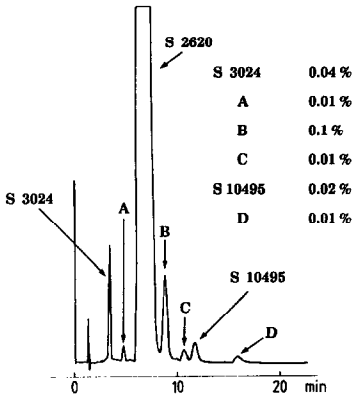
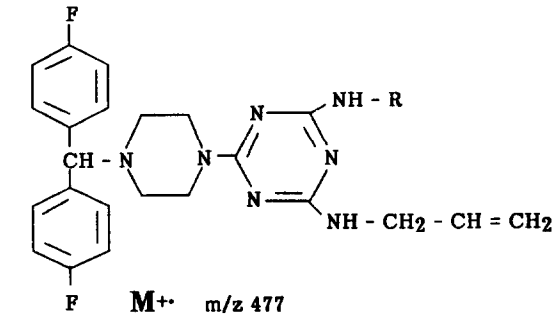
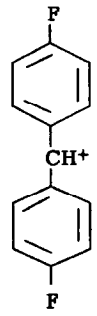


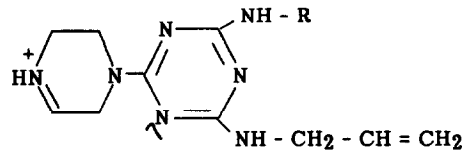
Fig. 1. Liquid chromatogram (UV, 254 nm) showing S 2620 and six minor impurities.





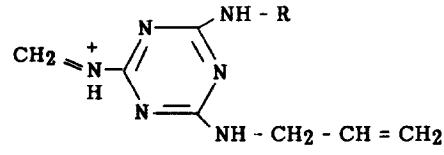
m/z 203

III



m/z 274

I



m/z 219

II

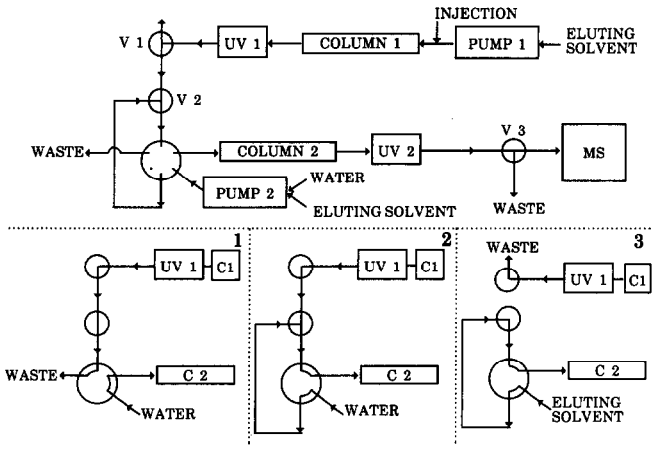
S 2620 R = - CH_2 - CH = CH_2
S 3024 R = - H
S 10495 ortho para isomer of **S 2620**

Scheme 1. Structure and fragmentations (EI) of almitrine (S 2620) and two known impurities. *m/z* values for S 2620.

some reference compounds, the structures of two of the impurities were known (Scheme 1). On-line LC–MS analysis showed that, in order to obtain mass spectra of sufficient quality to permit structural identification (*i.e.*, EI, CI and high-resolution spectra), an injection of at least 500 μg of almitrine was necessary. As it is impossible to introduce 500 μg of a compound into the mass spectrometer, a column-switching procedure was set up to discard the major part of the principal component eluting from the first column, while trapping the minor compounds on the second column for later LC–MS analysis.

Switching technique

Before performing the actual injection, the first column is conditioned with the elution solvent system, while the second column is flushed with water (Scheme 2, 1). After the injection the minor components (as indicated by the first UV detector) are concentrated on to the head of the second column, while diluting the elution solvent with water, to diminish its eluting capacity (2).



Scheme 2. Overall schematic view of the two-dimensional LC system. (1) Initial conditioning and disposal of major compound; (2) concentration of impurities on the second column; (3) elution of the second column.

When the major compound (S 2620) starts to elute from the first column, the system is switched to its first configuration (1), *i.e.*, the compound is led to waste while the second column is flushed with water. After elution of the main component, the system is switched back to the second configuration to trap the impurities eluting after S 2620.

The third configuration is used to elute the compounds which are trapped and concentrated on the second column, and to feed them through the LC–MS interface into the mass spectrometer. The first column remains under eluting conditions (3). The elution process can be monitored by the second UV detector. In the event that it is undesirable to lead the eluted compounds into the MS, a valve can be used to divert the eluate to waste.

Compound A

The mass spectrum of compound A, obtained after four injections of 500 μg, is similar to that of S 2620 (Table II), but with two differences: the molecular ion has shifted to *m/z* 489 and the *m/z* 203 ion, $[(F - C_6H_4)_2 - CH]^+$ (Scheme 1, fragment III), can now be found at *m/z* 215. The molecular ion was confirmed to be at *m/z* 489 from the CI/NH_3 mass spectrum (MH^+, *m/z* 490). High-resolution mass measurement showed the mass of the molecular ion to be 489.2676 ($C_{27}H_{32}N_7FO$ requires 489.2652), while the accurate mass for the fragment at *m/z* 215 was 215.0873 ($C_{14}H_{12}FO$ requires 215.0872). The conclusion is therefore that in compound A one of the two fluorine atoms on the diphenylmethyl moiety has been replaced by OCH_3.

Compound B

Whereas the LC–MS analysis of compound A was relatively easy, as it eluted before the main compound, the trapping of compound B, eluting just after S 2620, was more difficult because of the large amount of S 2620 eluting just before compound B. The UV trace shows the result of the elution of the second column after a single injection of 0.5 mg of almitrine (Fig. 2). The presence of S 2620 shows that this compound undergoes tailing to some extent when injected in these amounts. However, the impurity B/S 2620 ratio has increased sufficiently to obtain an interpretable mass spectrum (Fig. 3).

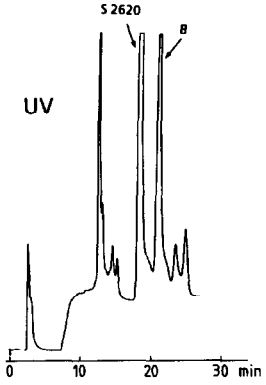


Fig. 2. Liquid chromatogram (UV, 254 nm) obtained after trapping of compound B and elution of the second column.

The fragments corresponding to the bisallylaminotriazine part of the molecule (*m/z* 477, 274, 219) (Scheme 1) are all shifted two mass units upwards, which led us to suspect that one of the allylamino groups is replaced by a propylamine. CI/NH_3 mass spectral analysis produced a protonated molecule at *m/z* 480, thus confirming a molecular weight of 479. The presence of one allylamino and one propylamino group was shown by high-resolution mass measurements of the molecular ion and the major fragments (Table I).

The chromatographic separation is satisfactory, as indicated by the ion chromatogram traces (Fig. 4). Synthesis of a reference compound confirmed that the identification of compound B was correct.

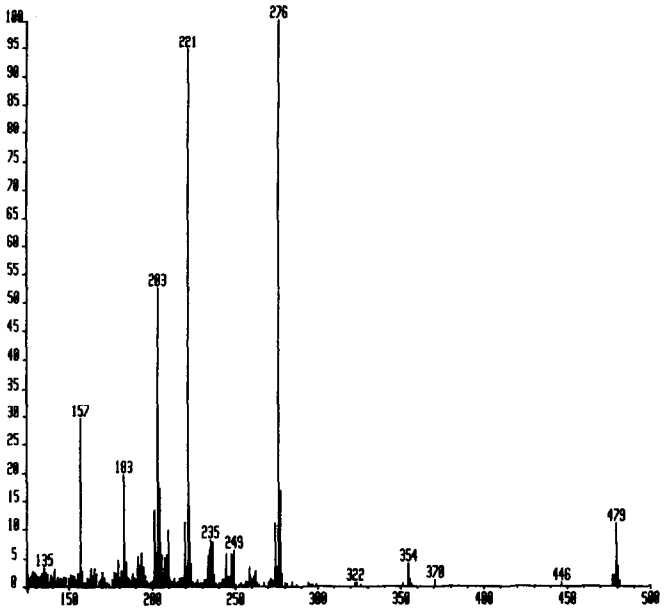


Fig. 3. Mass spectrum (EI, 70 eV) of compound B.

Compound C

Impurity C represents only 0.01% in the initial compound. Consequently, only one injection of 0.5 mg is insufficient to produce a reliable mass spectrum. Therefore, four repeated injections were made, while trapping the impurity C and S 10495 (which has a known structure) on the second column. Once again there is an important amount of S 2620 present owing to tailing of the major compound (Fig. 5).

The mass spectra of S 2620, impurity C and S 10495 are virtually identical (Table II), so that impurity C can be identificed as another isomer of S 2620. By synthesis the compound was confirmed to be the *meta–para* isomer of S 2620. No loss of chromatographic integrity was observed, as indicated by comparison of the UV and reconstructed ion chromatogram traces (Fig. 6).

Finally, peak D, isolated via a slightly modified procedure because of the longer retention time, produced a mass spectrum which again showed only minor differences

TABLE I

HIGH RESOLUTION MASS MEASUREMENT OF COMPOUND B

Found	*Theoretical*		*Deviation (ppm)*
479.2600	$C_{26}H_{31}N_7F_2$	= 479.2609	−1.9
276.1931	$C_{13}H_{22}N_7$	= 276.1937	−2.2
221.1521	$C_{10}H_{17}N_6$	= 221.1514	3.2
203.0663	$C_{13}H_9F_2$	= 203.0672	−4.4

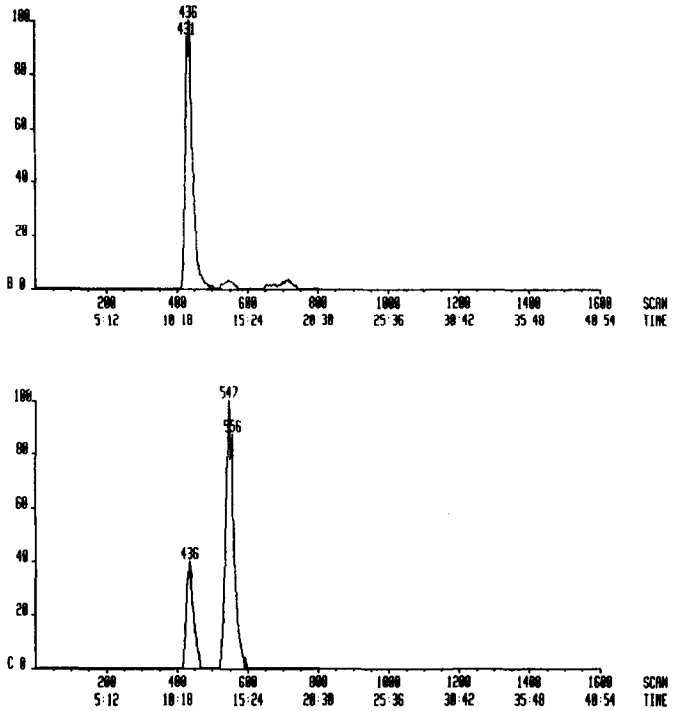


Fig. 4. Reconstructed ion chromatogram of *m/z* 477 (top) and *m/z* 479 (bottom) of compounds B and S 2620.

in the relative abundances of the molecular ion and the important fragments found in the mass spectrum of S 2620 (Table II). Synthesis of some reference compounds identified compound D as another isomer of S 2620, having the two fluorine atoms at the *ortho* and *meta* positions.

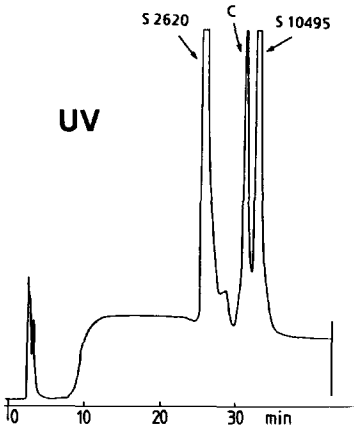


Fig. 5. Liquid chromatogram (UV, 254 nm) obtained after trapping of compounds C and S 10495 and elution of the second column.

TABLE II

MASS SPECTRA (EI, 70 eV) OF S 2620 AND IMPURITIES

Compound	$[M]^{+\cdot}$	*Fragments (Scheme 1)*		
		I	*II*	*III*
S 2620	477 (10)	274 (95)	219 (100)	203 (45)
S 3024	437 (10)	234 (60)	179 (100)	203 (50)
A	489 (15)	274 (70)	219 (100)	215 (90)
B	479 (15)	276 (100)	221 (95)	203 (55)
C	477 (15)	274 (100)	219 (100)	203 (40)
S 10495	477 (20)	274 (90)	219 (100)	203 (40)
D	477 (25)	274 (100)	219 (100)	203 (65)

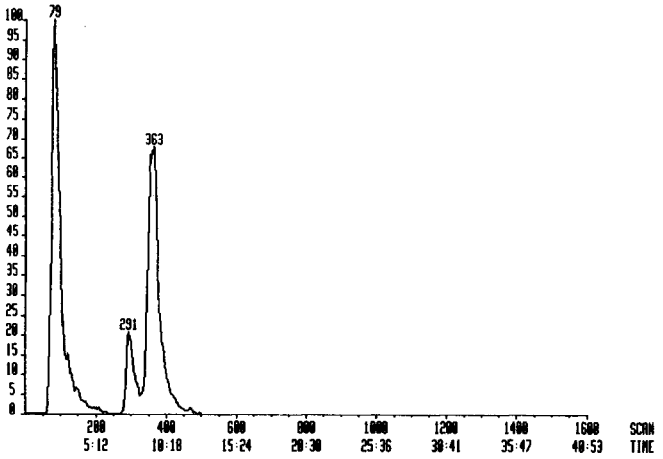


Fig. 6. Reconstructed total ion chromatogram of compounds S 2620, C and S 10495.

CONCLUSION

The method has proved itself useful in the structural analysis of unknown compounds present at trace levels in a major component. The advantage of the moving-belt LC–MS interface in this particular instance has been the possibility of using different ionization techniques (EI and CI) to assist in the structural determination of unknown compounds during on-line LC–MS.

Another useful application that has been tested is to use the two-column LC system to change the elution solvent to make it more compatible with the LC–MS system, such as removal of (phosphate) buffers and avoiding excess amounts of water[20,21].

REFERENCES

1 J. F. K. Huber, R. van der Linden, E. Ecker and M. Oreans, *J. Chromatogr.*, 83 (1973) 267.
2 F. Erni, H. P. Keller, C. Morin and M. Schmitt, *J. Chromatogr.*, 204 (1981) 65.
3 C. C. Honegger, W. Krenger, H. Langemann and A. Kempf, *J. Chromatogr.*, 381 (1986) 249.
4 C. Rodriguez, N. Bertrand, C. Julien, G. Cuisinaud and J. Sassard, *Int. J. Environ. Anal. Chem.*, 25 (1986) 235.
5 L. C. Verhagen, J. Strating and U. R. Tjaden, *J. Chromatogr.*, 393 (1987) 85.
6 T. R. Covey, J. B. Crowther, E. A. Dewey and J. D. Henion, *Anal. Chem.*, 57 (1985) 474.
7 R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991.
8 I. G. Beattie, D. E. Games, J. R. Startin and J. Gilbert, *Biomed. Mass Spectrom.*, 12 (1985) 616.
9 D. J. Liberato and A. L. Yergey, *Anal. Chem.*, 58 (1986) 6.
10 C. Lindberg and J. Paulson, *J. Chromatogr.*, 394 (1987) 117.
11 T. P. Fan, E. D. Hardin and M. L. Vestal, *Anal. Chem.*, 56 (1984) 1870.
12 J. van der Greef, A. C. Tas, M. C. ten Noever de Brauw, M. Höhn, G. Meijerhoff and U. Rapp. *J. Chromatogr.*, 323 (1985) 81.
13 J. G. Stroh, J. C. Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, K. L. Rinehart, Jr. and I. A. S. Lewis, *Anal. Chem.*, 57 (1985) 985.
14 J. G. Stroh, K. L. Rinehart, Jr., J. C. Cook, M. Suzuki and T. Arai, *J. Am. Chem. Soc.*, 108 (1986) 858.
15 W. Luijten, G. Damien, B. Marchand and J. Capart, *Biomed. Environ. Mass Spectrom.*, 16 (1988) 93.
16 J. van der Greef, A. C. Tas, L. M. Nijssen, J. Jetten and M. Höhn, *J. Chromatogr.*, 394 (1987) 77.
17 Y. Castaing, G. Manier, N. Varene and H. Guenard, *Bull. Eur. Physiopath. Resp.*, 17 (1981) 917.
18 G. Simmoneau, A. Denjean, B. Raffestin, O. Meyniard, S. Laurent, M. Meignan and A. Harf, *Am. Rev. Respir. Dis.*, 123 (1981) 88.
19 F. Arnaud, A. Bertrand, J. Charpin, J. Chrétien, G. Decroix, F. Guerrin, J. C. Kalb, J. Lissac, F. B. Michel, P. Moere, B. Paramelle, R. Pariente, M. Perrin-Fayole, J. Rochemaure and C. Voisin, *Bull. Eur. Physiopath. Resp.*, 18 (1982) 373.
20 G. M. Kresbach, T. R. Baker, R. J. Nelson, J. Wronka, B. L. Karger and P. Vouros, *J. Chromatogr.*, 394 (1987) 89.
21 C. Lindberg, J. Paulson and S. Edsbäcker, *Biomed. Environ. Mass Spectrom.*, 14 (1987) 535.