Journal of Chromatography, 541 (1991) 474–479
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 050

Short Communication

Reversed-phase high-performance liquid chromatography–thermospray mass spectrometry of alprenolol and its ketoxime analogues

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(First received March 1st, 1990; revised manuscript received December 12th, 1990)

ABSTRACT

Thermospray mass spectrometric detection is applied for alprenolol and its isomeric (*E* and *Z*) ketoxime analogues separated by reversed-phase high-performance liquid chromatography. The thermospray process results in high-intensity $[M+H]^+$ ion formation. This method of detection provides high level of molecular specificity, and offers advantages for the identification of the stereoisomeric oximes due to the unique fragmentation pattern of the spectra.

INTRODUCTION

The general analytical aspects of drug delivery have been discussed recently [1]. There seems to be an increased demand to the sensitive and selective determination of low levels of compounds in the related studies. The ketoxime analogue (a mixture of *E* and *Z* isomers) of alprenolol, a potent β-adrenergic antagonist, has been designed to deliver the drug into the site of the action (*i.e.*, to the eye) via sequential bioactivation [2]. This new concept holds the promise for an improved glaucoma treatment. Monitoring the expected biotransformation in selected compartments of the eye usually requires the determination of very small amounts of compounds with high selectivity. Gas and liquid chromatography have been successfully applied to the analysis of β-blockers [3], although their routine detection methods often lack selectivity.

Retaining the apparent advantages of a chromatographic technique, a combination with mass spectrometry (MS) has been emerged as a promising way of providing additional selectivity to the detection of the separated compounds, and the sensitivity may als be increased. Gas chromatography–MS has been applied for selected β-blockers [4–7], and for some ketoxime analogues [8]. Derivatization is, however,

0021-9673/91/$03.50

necessary to permit gas chromatographic analysis by increasing the volatility and thermal stability of the compounds. This may be accompanied by unwanted side reactions for β-blockers [7], or preclude the separation of oxime isomers [8]. Labetalol has been analysed by coupling high-performance liquid chromatography (HPLC) with thermospray (TSP) MS [9], which eliminates the need for derivatization. In addition, the isomeric ketoxime analogues of several β-blockers have shown excellent separation using reversed-phase HPLC [10]. In the present paper, the development of an on-line reversed-phase HPLC–TSP-MS method will be reported and evaluated for alprenolol and its ketoxime analogues.

EXPERIMENTAL

Chemicals and solvents

The preparation of oximes has been described previously [11,12]. Alprenolol tartarate was obtained from Aldrich (Milwaukee, WI, U.S.A.). HPLC-grade ammonium acetate, triethylamine and acetonitrile were supplied by Fisher Scientific (Fair Lawn, NJ, U.S.A.). Water was purified by reverse osmosis and ion exchange.

High-performance liquid chromatography and mass spectrometry

The mass spectrometer was an MS80RFA (Kratos Analytical, Manchester, U.K.) double focusing instrument, operated at 4 kV accelerating voltage and at a nominal resolution of 1000. The experiments were performed with the manufacturer's thermospray option. The HPLC apparatus consisted of a Spectroflow 400 solvent delivery system, a Spectroflow 430 gradient former and a Spectroflow 480 injector module equipped with a Rheodyne 7125 valve and a 20-μl sample loop. A 5 cm × 4.6 mm I.D. Supelcosil LC-8-DB column with a Supelguard cartridge (Supelco, Bellefonte, PA, U.S.A.) was used for separation. The aqueous buffer solution applied as mobile phase component contained 0.1 *M* ammonium acetate and 0.02 *M* acetic acid, as well as 0.02% (v/v) triethyamine (pH 4.54). At 1.0 ml/min flow-rate and with 25% acetonitrile in the mobile phase, optimal thermospray conditions were achieved by setting the probe, vaporizer, source and jet temperatures to 135, 165, 175 and 220°C, respectively. These parameters were readjusted upon changing mobile phase compositions; higher percentages of organic modifier required proportionally lower temperature settings, and *vice versa* [13].

The mass spectrometer was scanned repetitively (at 4.0 kV accelerating voltage) from *m/z* 800 to 100 using a scan speed of 3 s/mass decade, under the control of the DS90 data system. The nominal mass resolution was set to 1000. Mass scale calibration was established using the positive ions of polyethylene oxide oligomers (PEG 600) produced under TSP conditions; the calibrated mass range extended from *m/z* 167 to 653.

Desorption chemical ionization (CI) mass spectra were obtained with the same instrument. The samples (*ca.* 0.1 μg) were supplied onto the platinum-iridium coil of the probe using the thermospray solvent (25% acetonitrile in ammonium acetate buffer, as described above) which was then gently evaporated with a heat-gun. The probe was inserted into the electron impact (EI)/CI source of the mass spectrometer. The following conditions were applied: reagent gas, ammonia; electron energy, 40 eV; emission current, 500 μA; source temperature 220°C; source pressure, *ca.* 10^{-5} Torr

(measured with the source vacuum gauge). Mass spectra were recorded at 4.0 kV accelerating potential by scanning the mass range of m/z 700 to 100 (calibrated with the positive ions of perfluorokerosene obtained under EI conditions). The instrument was operated at a scan rate of 1 s/decade, while the direct exposure probe was inserted into the heated ion source. The CI spectrum recorded at the apex of the total ion current (TIC) chromatogram was evaluated.

RESULTS AND DISCUSSION

Adapting an existing HPLC method [10] to TSP-MS detection requires the substitution of the phosphate buffer in the mobile phase with a suitable one containing volatile salts. Ammonium acetate is preferred for its high ionization efficiency [13]. We have applied its plain 0.1 *M* solution, but it has been realized that the mobile phase tolerates additive used for setting the pH (acetic acid) and that for improving the chromatographic separation (triethylamine) without noticeably influencing the process of TSP ionization. This is not surprising, since it is generally thought that ions

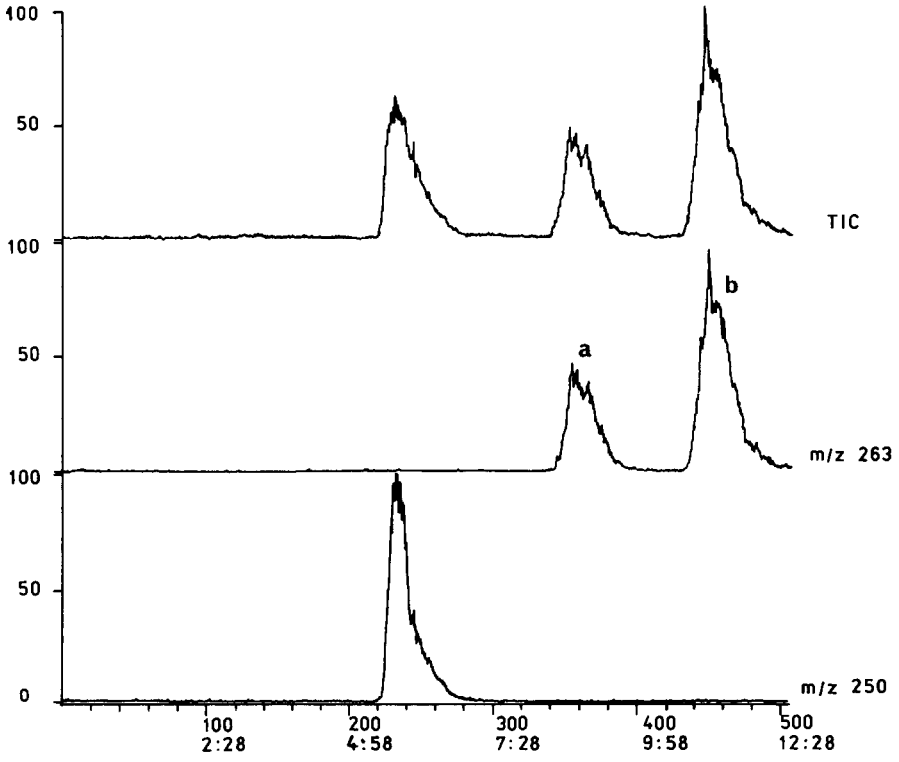

Fig. 1. Reconstructed total-ion current (TIC) and selected-ion chromatograms of 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanol (alprenolol, $[M + H]^+$ at m/z 250) and 1-(isopropylamino)-3-(2-allylphenoxy)-2-propanone oxime (alprenolone oxime, $[M + H]^+$ at m/z 263) obtained by HPLC–TSP-MS. Column: Supelcosil LC-8-DB (5 cm × 4.6 mm I.D.), mobile phase: acetonitrile–0.1 *M* ammonium acetate buffer (25:75, v/v) (see Experimental), 1.0 ml/min flow-rate. Ordinate: intensity (%); abscissa: top scale, scan No., bottom scale, time in min:s.

leaving the TSP source obey the rules of gas-phase thermochemistry [14]. Triethylamine, a strong base, has been ineffective for thermospray ionization [13] (lack of protonated molecular ion formation), while acetic acid (applied in molar ratio representing only one fifth of the amount of ammonium acetate) merely affects dissociation equilibria in the solution. Thus, both are compatible with the TSP solvent.

The TSP ionization of the title compounds results in high-intensity $[M + H]^+$ ion formation. In fact, this is the only ion detectable for alprenolol. Loss of water from the aryloxyalkylaminoalcohol due to thermolysis in the ion source, as described for labetalol [9], is absent. Fig. 1 shows selected-ion chromatograms for alprenolone oxime isomers ($[M + H]^+$ at m/z 263) and alprenolol ($[M + H]^+$ at m/z 250), together with the reconstructed TIC chromatogram. In addition, the TSP mass spectra of the oxime isomers feature fragmentation related to the steric position of the oxime hydroxyl, as exemplified in Fig. 2. The loss of the alkyl group from the nitrogen (m/z 221) is noteworthy. This process is very pronounced when the oxime hydroxyl is oriented toward the aryloxy group (Z isomer). On the other hand, the cleavage of the

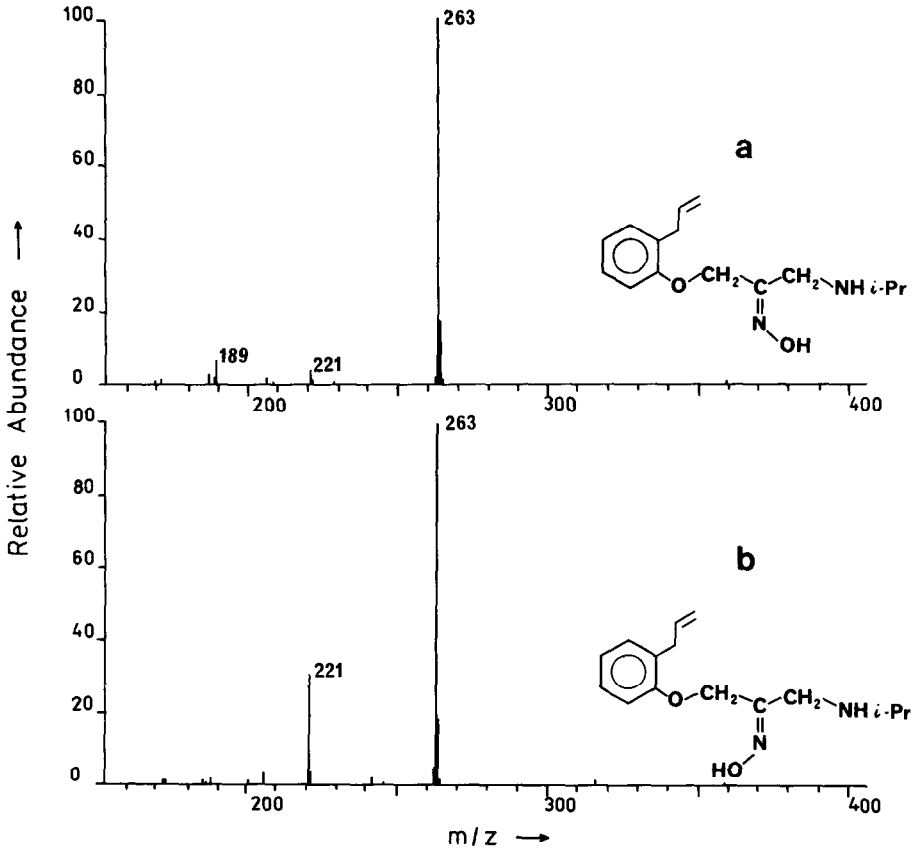

Fig. 2. Thermospray mass spectra of alprenolone oxime isomers. (a) E isomer, (b) Z isomer. i-Pr = Isopropyl.

carbon–carbon bond at the alkylamino side of the ketoxime resulting in *m/z* 189 appears to be characteristic exclusively to the *E* isomer.

The possible reasons for fragmentation in TSP is usually thermolysis and collision-induced dissociation. It is difficult to speculate on the origin of the above process, since experiments involving variation of the thermospray conditions (temperature settings, buffer concentrations, etc.) were inconclusive. The ionization processes operating under thermospray conditions are believed to be very similar to those prevailing during chemical ionization [15]. Gas-phase ionization, by NH_4^+ from the ammonium acetate buffer, appears to be predominant in TSP. We have, therefore, compared desorption CI mass spectra (with ammonia reagent gas) to those obtained by TSP ionization. We attempted to imitate TSP conditions by depositing the sample onto the direct exposure probe by dissolving it in the mobile phase used for HPLC–TSP-MS. The probe was then introduced into the heated ion source and exposed to the CI plasma. The spectrum obtained from the pure *Z* oxime isomer is shown in Fig. 3. Surprisingly, no common fragment ions with the corresponding TSP spectrum (Fig. 2b) was found. From the $[M + H]^+$ ion, loss of water (*m/z* 245) results in the most prominent fragment in the CI spectrum. Preliminary experiments on the collision-induced dissociation of the protonated alprenolone oxime isomers (*m/z* 263) have given neither *m/z* 221 nor *m/z* 189 as daughter ions. There has to be a process of yet unknown origin, perhaps specific thermolytic reaction, peculiar to thermospray ionization that results in the appearance of those ions. Nevertheless, the latter characteristically reflect the steric position of the oxime hydroxyl.

In quantitative terms, HPLC–TSP-MS is able to detect 10–50 ng of alprenolol or alprenolone oxime in full-scan mode, while the limit may reach several hundred pg by selected-ion monitoring. However, HPLC with UV spectrophotometric detection [10] gives better sensitivities (3 ng for alprenolol, with 3:1 signal-to-noise ratio at 272 nm) than full-scan TSP-MS. The inherent instability of the thermospray jet, which is observed as "spiking" on the peak profile in the reconstructed TIC chromatogram [16], dictates that deuterated or ^{13}C internal standards be employed for accurate determination of these compounds.

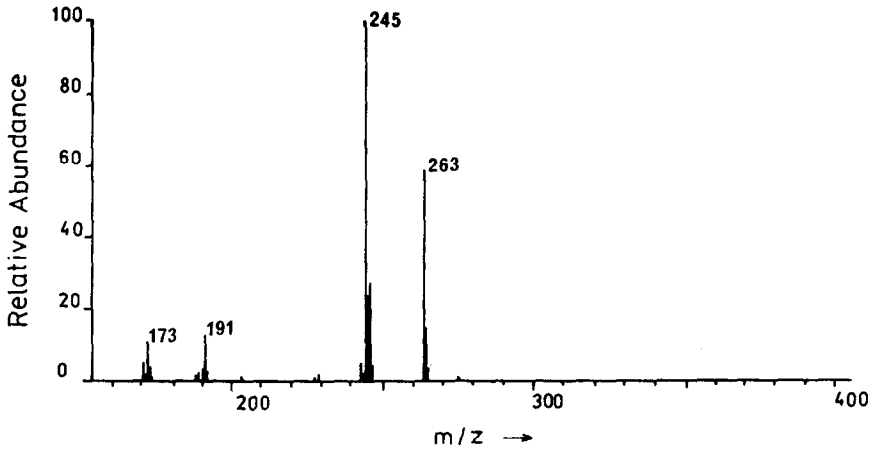

Fig. 3. Desorption chemical ionization mass spectrum alprenolone (*Z*-) oxime.

In conclusion, on-line HPLC–TSP-MS provides a high level of molecular specificity and increased detection sensitivity for the β-adrenergic antagonist alprenolol, and for its ketoxime analogues. The method also offers advantages with respect of the identification of stereoisomeric oximes due to the unique fragmentation pattern of the TSP spectra obtained from the chromatographically separated compounds.

ACKNOWLEDGEMENT

Partial support of the work by Xenon Vision (Alachua, FL, U.S.A.) is gratefully acknowledged.

REFERENCES

1 L. A. Sternson and T. Malefyt, in R. T. Borchardt, A. J. Repta and V. J. Stella (Editors), *Directed Drug Delivery —A Multidisciplinary Approach*, Humana Press, Clifton, NJ, 1984, p. 291.
2 N. Bodor and L. Prokai, *Pharm. Res.*, 7 (1990) 723.
3 C. L. Davies, *J. Chromatogr.*, 531 (1990) 131.
4 P. Hermann, J. Fraisse, J. Allen, P. L. Morselli and J. P. Tenot, *Biomed. Mass Spectrom.*, 11 (1984) 29.
5 C. Y. Sum and A. Yacobi, *J. Pharm. Sci.*, 73 (1984) 1177.
6 C. R. Lee, A. C. Coste and J. Allen, *Biomed. Environ. Mass Spectrom.*, 16 (1988) 387.
7 M. S. Leloux, E. D. DeJong and R. A. A. Maes, *J. Chromatogr.*, 488 (1989) 357.
8 L. Prokai and N. Bodor, in *Proceedings of the 37th ASMS Conference on Mass Spectrometry and Allied Topics, May 21–26, 1989, Miami Beach, FL*, American Society for Mass Spectrometry, East Lansing, MI, p. 1354.
9 M. S. Lant, J. Oxford and L. E. Martin, *J. Chromatogr.*, 394 (1987) 223.
10 L. Prokai, A. Simay and N. Bodor, *J. Chromatogr.*, 541 (1991) 469.
11 N. Bodor, A. Elkoussi, M. Kano and T. Nakamura, *J. Med. Chem.*, 31 (1988) 100.
12 A. Simay, L. Prokai and N. Bodor, *Tetrahedron*, 45 (1989) 4102.
13 R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991.
14 K. B. Tomer and C. E. Parker, *J. Chromatogr.*, 492 (1989) 189.
15 R. W. Smith, C. E. Parker, D. M. Johnson and M. M. Bursey, *J. Chromatogr.*, 394 (1987) 261.
16 G. Schmelzeisen-Redeker, M. A. McDowall, U. Giessmann, K. Levsen and F. W. Röllgen, *J. Chromatogr.*, 323 (1985) 127.