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Note

Use of an inexpensive mass-selective detector for the high-sensitivity gas chromatographic determination of nadolol in plasma

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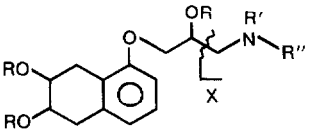
The application of mass spectrometry to body fluid analyses is becoming increasingly sophisticated based on a record of resolving complex analytical problems. High-resolution mass spectrometry (MS), MS—MS and new ionization methods are currently being investigated. The introduction of an inexpensive mass-selective detection (MSD) instrument interfaced to a capillary gas chromatographic (GC) unit represents another important development suited to most analytical problems. One such detector, the Hewlett-Packard 5970A,



		R	R'	R''	X
Nadolol	I	H	H	$C(CH_3)_3$	
	II	$Si(CH_3)_3$	H	$C(CH_3)_3$	86
Isotopomer	III	H	H	$C(CD_3)_3$	
	IV	$Si(CH_3)_3$	H	$C(CD_3)_3$	95
	V	H	CH_3	$C(CH_3)_3$	100

Fig. 1. Structures of nadolol (I), the internal standard, deuterated nadolol (III), the respective tri(trimethylsilyl) ethers (II and IV) and N-methylnadolol (V). X = the fragment ion monitored (SIM) mode.

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is a highly reliable electron-impact (EI) only unit optimized for capillary column GC. It has excellent ion transmission to its maximum 800 dalton mass range with an approximately ten-fold greater EI sensitivity than larger multi-ionization research-oriented mass spectrometers [1].

The assays of captopril [2, 3] and nadolol [4, 5] in body fluids were readily transferred from packed column to capillary column GC with an accompanying reduction in sample volume together with enhanced sensitivity. With the advent of the more sensitive MSD, it is no longer necessary to use chemical ionization for the measurement of S-methylcaptopril, an important metabolite of captopril [6].

As the ultimate test of MSD, a high-sensitivity method for nadolol (I, Fig. 1) was devised to measure plasma levels from the limit of determination of 1.9 pmol/ml (0.6 ng/ml) to 65 pmol/ml (20 ng/ml).

EXPERIMENTAL

Sample preparation

To each 3.0-ml plasma sample, 60 ng of internal standard (III, Fig. 1) were added, then the sample was adsorbed on a methanol (5 ml)—water (5 ml) activated 500-mg solid-phase extraction cartridge with cyanopropyl-bonded silica (Analytichem International, Harbor City, CA, U.S.A.) and washed with 10 ml of doubly distilled water followed by 5 ml of acetonitrile. The analyte was eluted with 2.5 ml of solution containing 0.7 ml of triethylamine and 0.06 ml of glacial acetic acid in 100 ml of methanol. After evaporation to dryness, the residue was dissolved in 1 ml of water and then processed on a DuPont PREP I automated sample processor with water-activated type W XAD-2 columns (DuPont Instruments, Wilmington, DE, U.S.A.). Methanol (15 ml) was placed in the right-hand solvent reservoir and 25 ml of water in the left-hand reservoir. Following program 13, supplied by the vendor, the purified sample was recovered in methanol, which was evaporated to dryness, under nitrogen, in a 1-ml reaction vial. After thorough drying under nitrogen in a desiccator, the sample was ready for trimethylsilylation and processing by GC—MSD. Calibration graphs were prepared by the addition of appropriate amounts of I, from 0 to 60 ng, and 60 ng of III to 3.0 ml of blank plasma and processing in the same manner as the samples.

The dried extracts were dissolved in 25 μl of Tri-Sil Z, (N-trimethylsilyl-imidazole) (Pierce, Rockford, IL, U.S.A.) and heated for 30 min at 60°C. With each sample set, a serum control sample containing 60 ng each of I and III, taken from a stock solution used throughout the study, was extracted for establishing the slope of the graph. A non-serum, unextracted control at the same concentration was used to establish recovery efficiencies and instrumental parameters.

Gas chromatography conditions

A Hewlett-Packard 5790A gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a capillary splitless injector was used. The fused-silica capillary column (15 m × 0.26 mm I.D., 0.15 μm film thickness) was coated with the bonded 1:1 methylphenylpolysiloxane phase DB-17 (J&W

Scientific, Rancho Cordova, CA, U.S.A.). The carrier gas was helium with an inlet pressure of 51 kPa (7.5 p.s.i.g.) with a 30 ml/min split flow and a 2 ml/min septum purge. The injector temperature was 270°C. The oven temperature was operated isothermally at 180°C for 1.0 min after injection and then programmed at 24°C/min to 280°C, with no final hold. Injections were made in the splitless mode, with the inlet purge turned on 1.0 min after injection.

Mass-selective detector conditions

A Hewlett-Packard 5970A mass-selective detector, operated in the selected-ion monitoring (SIM) mode, was used for the instrumental analyses. The filament emission current and electron energy are fixed at 0.22 mA and 70 eV, respectively. The ion source temperature was 200°C, inlet interface temperature 300°C and ion source pressure $2 \cdot 10^{-6}$—$5 \cdot 10^{-6}$ Torr, and the detector was turned on for 3.0—4.0 min after injection. The detector was calibrated daily with the Autotune software.

The 5970A detector data system controlled the selection of the ion windows, 86 ± 0.2 daltons for nadolol and 95 ± 0.2 daltons for internal standard III, using 0.9 dalton mass peak width resolution, and measured the areas of the GC peaks generated by the *m/z* 86 and 95 ions of II and IV, respectively.

RESULTS AND DISCUSSION

A method was required to measure nadolol in plasma at very low concentrations. Preliminary studies suggested that 3 ml of plasma would be required to have detection limits of subnanograms per millilitre. A two-step solid-phase extraction scheme was devised to obtain sufficiently clean extracts.

Nadolol can be adsorbed on a variety of solid phases, including C_2, C_8, C_{18}, phenyl and cyanopropyl extraction cartridges. Using [^{14}C]nadolol as a tracer, nadolol is quantitatively adsorbed from plasma on cyanopropyl-bonded silica, retained through the acetonitrile wash that removes other impurities, and

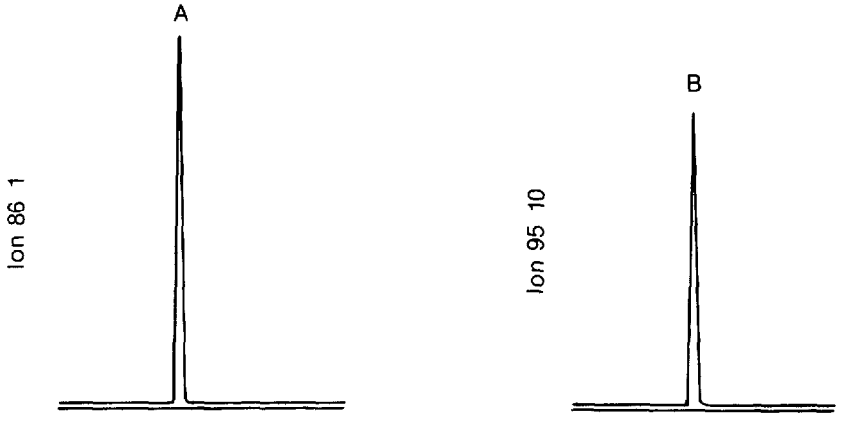

Fig. 2. Gas chromatograms of extract showing SIM profiles of (A) 20 ng of nadolol (*m/z* = 86.1 daltons, 3.49 min) and (B) 20 ng of internal standard (*m/z* = 95.1 daltons, 3.46 min) added to blank plasma.

completely displaced by the triethylamine–acetic acid–methanol solution. The residue is further purified and quantitatively recovered by the XAD-2 [5] provided by the DuPont PREP I automated sample processor.

In general, the higher the mass number monitored, e.g. the molecular ion in the case of captopril [2, 3], the less likelihood there is of interferences at the retention time of the analyte, but it is also desirable to monitor a high-intensity fragment ion to improve the quantitation. Under EI conditions II has an M^+ of very low abundance [4]. Decreasing the electron energy, an option currently not available with MSD, to reduce fragmentation also reduces the ionization efficiency of II, which results in an increased limit of detection. The *m/z* 86 ion represents approximately 43% of the total ionization of II. This fragment characterizes the *tert.*-butylamine structural feature of II, which is common to few β-adrenergic blockers, e.g., metoprolol, which are chromatographically resolvable from II, and uncommon in biological compounds. With the elimination of potential interferences through extraction and application of high-resolution capillary GC, MSD is suitable for quantitative measurements of a high-sensitivity low *m/z* ion peak such as the *m/z* 86 ion. A typical ion chromatogram is shown in Fig. 2.

At various times during the method development, the internal standard was either the isotopomer III or N-methylnadolol (V), more or less interchangeably. The N-methylnadolol internal standard, containing small amounts of analyte I, which contributes to a greater intercept, has a longer retention time. The deuterated isotopomer, which is similar to nadolol in extraction and response, was finally adopted as the internal standard, affording the added advantage of coincident measurement. Plasma blanks with internal standard III always had a non-zero value at the retention time of II, owing to the presence of trace amounts of I in III.

Although it facilitated the location of the analyte peak, the presence of small amounts of compound I affected the ultimate limit of quantitation. Extreme care was taken in order of injection to prevent high bias arising from earlier injections. A linear calibration graph with a slope of 1.20 and an intercept of 0.05 had a correlation coefficient of 0.9989 over the 0—20 ng/ml calibration range. The linear dynamic range was estbalished as 0.6—60 ng/ml.

Chromatographic data showed that the combined extraction and derivatization efficiency is greater than 80% at all plasma concentrations. This value is also

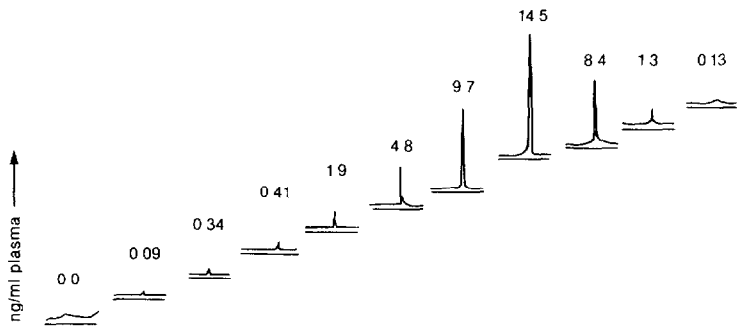

Fig. 3. Typical *m/z* 86 ion profiles for a subject's plasma extracts containing 0—14.5 ng/ml nadolol.

supported by liquid scintillation counting. A 1% change in the *m/z* 86 to 95 ion ratio is equivalent to 200 pg per millilitre of sample. The limit of detection (0.2 ng/ml) and limit of quantitation (0.6 ng/ml) were determined by the definitions accepted by IUPAC [7] and ascertained from eleven time-zero subjects. Six of the subjects had zero levels, two more 10 pg/ml, another two 70 pg/ml and one a level of 190 pg/ml. The *m/z* 86 ion profile for a subject at various intervals after dosing, shown in Fig. 3, demonstrates the sensitivity achievable. The data are shown as the amount found, even though some values are below the limit of quantitation.

CONCLUSIONS

A convenient subnanogram GC method is readily achievable using a low-cost mass-selective detector that competes with more expensive MS instruments in selectivity and sensitivity and with conventional non-selective GC detectors in sensitivity. The use of the highest sensitivity ions extends the application of MSD to a wider variety of drugs without the need to use other ionization methods. The high sensitivity and selectivity of detection allow for rapid method development and simpler sample work-up procedures which are more readily implemented during drug development and ultimately in therapeutic drug monitoring.

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REFERENCES

1 M. Jemal, E. Ivashkiv and A.I. Cohen, Biomed. Mass Spectrom., 12 (1985) 664.
2 E. Ivashkiv, D N. McKinstry and A.I. Cohen, J. Pharm. Sci., 73 (1984) 1113.
3 A.I. Cohen, R.G. Devlin, E. Ivashkiv, P.T. Funke and T. McCormick, J. Pharm. Sci., 71 (1982) 1251.
4 P.T. Funke, M.F. Malley, E. Ivashkiv and A.I. Cohen, J. Pharm Sci., 67 (1978) 653.
5 A.I. Cohen, R.G. Devlin, E. Ivashkiv, P.T. Funke and T. McCormick, J. Pharm. Sci., 73 (1984) 1571.
6 A.I. Cohen, E. Ivashkiv, T. McCormick and D.N. McKinstry, J. Pharm. Sci., 73 (1984) 1493.
7 IUPAC, Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry, Anal. Chem., 52 (1980) 2242.