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Short communication

Determination of the β-blocker carteolol in human plasma by a sensitive gas chromatographic–negative-ion chemical ionization high-resolution mass spectrometric method

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Abstract

A specific and sensitive gas chromatographic–high-resolution mass spectrometric method for the determination of 5-(3-*tert.*-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril (carteolol), which is a β-blocker giving depression of intraocular pressure, was developed to elucidate the pharmacokinetics of its ophthalmic application. Carteolol has been determined by high-performance liquid chromatography but with less satisfactory sensitivity. Carteolol was derivatized with pentafluorobenzoyl (PFB) amide followed by dimethylethylsilyl (DMES) ether, resulting in a high negative-ion current. The PFB–DMES derivative of carteolol was determined by the gas chromatography–negative-ion chemical ionization mass spectrometry (GC–NICI-MS) using selected-ion monitoring at low and high mass spectrometric resolution. The detection limit was less than 100 fg when the fragment ion was monitored at m/z 552.2067 in the NICI mode using methane as a reagent gas. The quantification limit of carteolol in human plasma with this method was less than 30 pg/ml. The proposed GC–MS method is considered to have sufficient specificity and sensitivity to study the pharmacokinetics of carteolol used as an ophthalmic solution.

1. Introduction

5 - (3 - *tert.* - Butylamino - 2 - hydroxy)propoxy-3,4-dihydrocarbostyril (carteolol) is a non-selective β-adrenoceptor agent with associated intrinsic sympathomimetic activity and without significant membrane-stabilizing activity [1–3]. Carteolol possesses a rapid onset time and long duration and gives a strong depression of intraocular pressure [4,5], and its ophthalmic solution is used for the treatment of glaucoma and for the depression of high intraocular pressure.

To investigate the pharmacokinetics in humans of carteolol administered as an ophthalmic solution, a highly sensitive and specific method is required. A quantification limit of less than 100 pg/ml is necessary to determine plasma concentrations of carteolol, which are expected to be at the subnanogram level, owing to its low clinical dose of 0.5–1 mg per person. Hence conventional methods, such as HPLC [6], might be insufficient with regard to sensitivity and/or selectivity.

To determine carteolol by a GC–MS method, it was derivatized with pentafluorobenzoyl (PFB) amide [7–9] followed by dimethylethylsilyl

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(DMES) ether [10–12], thereby giving a high negative-ion current. With this derivatization method, the detection limit for carteolol was found to be less than 100 fg when the fragment ion was monitored at m/z 552.2067 in the negative-ion chemical ionization (NICI) mode using methane as a reagent gas.

This paper reports a simple, rapid, sensitive and specific method for the determination of carteolol in human plasma by GC–NICI-MS using the PFB–DMES derivatization method.

2. Experimental

2.1. Materials

Carteolol and 5-(2-hydroxy-3-isopropylamino)propoxy-3,4-dihydrocarbostyril acidic maleate (OPC-1109, compound A) (Fig. 1) were synthesized at the 2nd Tokushima Factory of Otsuka Pharmaceutical. Pentafluorobenzoyl chloride (PFBCl) and dimethylethylsilylimidazole (DMESI) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Sep-Pak silica cartridges and Sep-Pak alumina N cartridges were obtained from Waters (Milford, MA, USA). Other reagents and solvents were of analytical-reagent grade.

2.2. Gas chromatography–mass fragmentography

The GC–MS system consisted of a Hewlett-Packard Model 5890 Series II gas chromatograph coupled to a JEOL JMS-SX102A mass spectrometer. A fused-silica capillary column coated with dimethylpolysiloxane (15 m × 0.25 mm I.D., film thickness 0.25 μm) was directly connected to the ion source of the mass spectrometer; the end of the column was 1.5 cm from the ion source centre. Helium was used as the carrier gas at an inlet pressure of 102 kPa for GC operation. The column temperature was maintained at 50°C for 0.7 min after a sample had been injected. The split valve of the split–splitless injector was closed before injection and opened for 0.7 min after injection. The column temperature was programmed at 55°C/min to 320°C and maintained at that temperature for 3 min. The injector, the interface oven and the transfer line were kept at 270, 300 and 300°C, respectively.

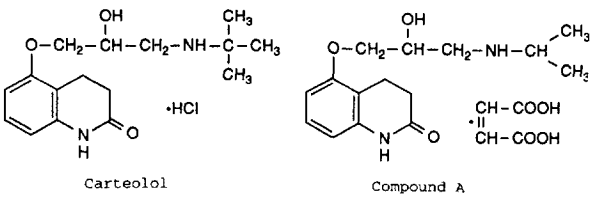

Fig. 1. Structures of carteolol and the internal standard (compound A).

The mass spectrometer was equipped with a pulsed NICI accessory and methane was used as the reagent gas at an ion source pressure of 0.01 Pa (the moderator secondary pressure was 0.8 kg/cm^2). The ionization current, ion source temperature, accelerating voltage and ionization energy were 0.3 mA, 270°C, −7 kV and 200 eV, respectively.

A JMA-DA7000 data system was used for high-resolution selected-ion monitoring (SIM). The mass spectral resolution in the high-resolution SIM mode was ca. 5000.

2.3. Sample preparation, derivatization and analysis

Blood samples were collected in disposable culture tubes and immediately centrifuged for 15 min at 2000 g to separate plasma. To each 1.0 ml of plasma, 50 μl of internal standard solution (500 pg of compound A per 50 μl in distilled water), 0.2 ml of 1 M sodium carbonate solution and 0.5 ml of saturated sodium chloride solution were added. The resulting solution was extracted with 4 ml of ethyl acetate for 10 min at room temperature, then the organic solvent was evaporated to dryness under reduced pressure. The residue was treated with 50 μl of diisopropylethylamine (DIPEA)–dichloromethane (1:10, v/v) and 50 μl of PFBCl–dichloromethane (1:20, v/v). The solution was allowed to stand at room temperature for 30 min. After evaporation under

reduced pressure, 200 μl of methanol were added to the residue and the methanol solution was allowed to stand at room temperature for 5 min.

A 100-μl volume of 0.5 *M* sodium carbonate solution was added to the methanol solution and after swirling it was allowed to stand at room temperature for 30 min. After evaporation of the solvents under reduced pressure, to the resulting residue was added 0.5 ml of distilled water, the mixture was extracted with 3 ml of ethyl acetate for 10 min at room temperature, then the organic solvent was evaporated to dryness under reduced pressure. The residue was dissolved in 1 ml of dichloromethane and applied to a Sep-Pak silica cartridge (prewashed with 5 ml of the same solvent). After washing with 5 ml of the same solvent, the PFB amide derivative was eluted with 4 ml of dichloromethane–methanol (10:1, v/v). The resulting eluate was evaporated to dryness under reduced pressure.

The PFB amide derivative obtained as described above was treated with 40 μl of distilled pyridine and 10 μl of DMESI at room temperature for 30 min, then 1 ml of dichloromethane was added. The dichloromethane solution was applied to a Sep-Pak alumina N cartridge (prewashed with 10 ml of acetone). The PFB amide–DMES ether derivative was eluted with 6 ml of acetone. After evaporation under reduced pressure, the residue was dissolved in 25 μl of dichloromethane and the solution was used as a sample solution.

2.4. Preparation of standard solutions

A solution of carteolol·HCl in distilled water was prepared in the concentration range 0.6–20 ng/ml as free base and 50 μl of the solution were added to 1 ml of blank plasma. These standard plasma specimens were also treated in the same manner as described for sample preparation, and the final solutions were used as standard solutions. A 1-μl volume of each solution was subjected to GC–NICI-MS analysis. A calibration graph was obtained with the standard solutions before the analyses of samples.

3. Results and discussion

3.1. Gas chromatography–mass spectrometry

The retention times of the PFB amide–DMES ether derivatives of carteolol and compound A were found to be 6.7 and 6.8 min, respectively, under the GC–NICI-MS conditions used.

Fig. 2 shows the mass spectra of the PFB amide–DMES ether derivatives obtained by GC–NICI-MS using methane as reagent gas. The PFB amide–DMES ether derivatives gave the characteristic fragment ion peak of $[M-20]^-$ due to the elimination of HF from the PFB moiety. Thus, the PFB amide–DMES ether derivatives of carteolol and compound A gave the corresponding prominent ions at *m/z* 552 and 538, respectively.

The sensitivities of the $[M-20]^-$ ions of the PFB–DMES derivatives of carteolol and compound A were influenced by the ion source temperature, and at 270°C the $[M-20]^-$ ions of these compounds showed higher peak intensities than those at 140–200°C. Therefore, carteolol and compound A were measured at 270°C, which

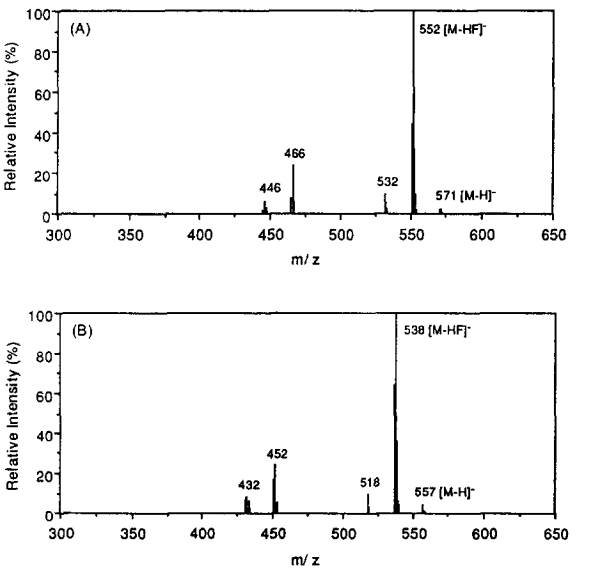

Fig. 2. NICI mass spectra for the PFB–DMES derivatives of (A) carteolol and (B) compound A.

is the ion source temperature for the GC–NICI-MS analysis.

The appearance of these prominent ions in the high-mass region is a great advantage for the determination of carteolol in human plasma by GC–MS with SIM, because the interfering peaks in the plasma are removed. The prominent peaks at m/z 552.2067 and 538.1911 for carteolol and compound A, respectively, were selected as monitoring ions for the determination of carteolol by SIM.

3.2. Representative SIM

GC–NICI-MS using SIM at low and high mass spectrometric resolution for the standard solution was carried out by monitoring the single ion at m/z 552.2067 (Fig. 3). When a low mass spectrometric resolution was used (Fig. 3B), several peaks of unknown origin were detected together with the peaks attributable to the PFB–DMES derivative of carteolol. However, at high mass spectrometric resolution (Fig. 3A), these interferences were removed and the signal-to-noise ratio was improved.

The detection limit of carteolol was found to be ca. 0.1 pg (at a signal-to-noise ratio of 7), as calculated from the peak of the PFB–DMES derivative of carteolol which appeared on the selected-ion recording (SIR), indicating that the proposed method is sensitive and specific. The SIRs of a human plasma sample spiked with carteolol or compound A at a concentration of 30 or 500 pg/ml, respectively, are shown in Fig. 4.

With the combined use of this purification procedure and high-resolution MS, the influence of impurities which have the same retention time as the PFB–DMES derivatives of carteolol and compound A was completely eliminated.

3.3. Calibration graph and recovery

A calibration graph was obtained using a series of mixtures of carteolol (30, 50, 100, 300, 500 and 1000 pg) and compound A (500 pg) in 1 ml of blank plasma, showing good linearity in the concentration range 30–1000 pg/ml. The linear regression for the calibration graph was found to be given by the equation $\log y = 0.9407 \log x - 2.4626$ (correlation coefficient = 0.9992), where y

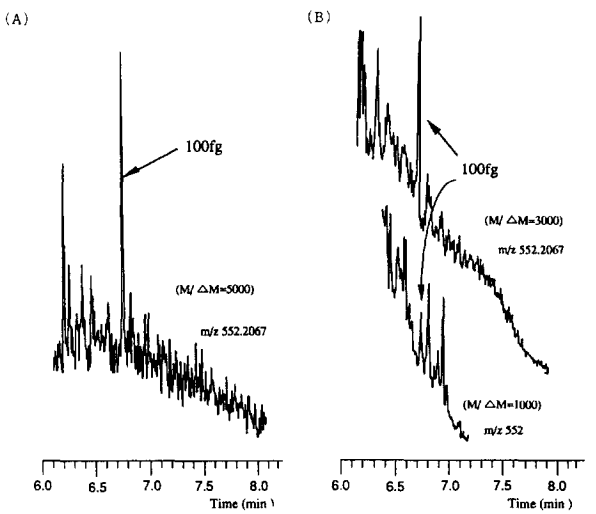

Fig. 3. (A) High- and (B) low-resolution selected-ion recordings for the PFB–DMES derivative of carteolol. Data were obtained after injection of 100 fg of carteolol.

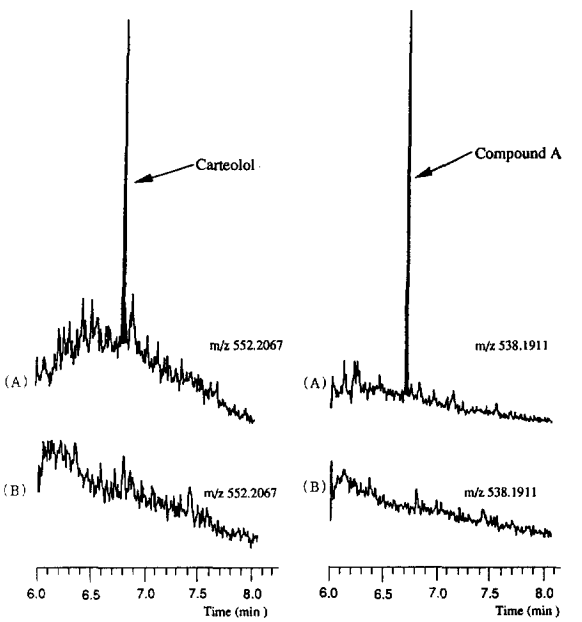

Fig. 4. Representative selected-ion recordings of extracts from (A) human plasma spiked with carteolol (30 pg/ml) and (B) human blank plasma.

Table 1
Accuracy and precision for the assay of carteolol at various concentrations in human plasma ($n = 3$)

Added concentration (pg/ml)	Concentration found (mean ± S.D.) (pg/ml)	Coefficient of variation (%)
30	30.8 ± 3.4	10.9
50	46.7 ± 1.0	2.2
100	94.1 ± 3.2	3.4
300	318.1 ± 9.0	2.8
500	504.3 ± 10.3	2.0
1000	982.9 ± 3.7	0.4

is the ratio of the peak area of carteolol to that of compound A and x is the concentration of carteolol in pg/ml.

The recoveries of carteolol and compound A following the extraction procedure were 10–11% in the concentration range 30–1000 pg/ml and 10% at a concentration of 500 pg/ml, respectively.

The accuracy and precision of the method using various concentrations of carteolol added to human plasma are summarized in Table 1. The quantification limit for carteolol was 30 pg/ml in plasma.

In conclusion, the combined use of the PFB–DMES derivatization and capillary GC–NICI-MS was found to be useful for determining trace amounts of carteolol using high-resolution SIM. The method may be useful for the study of the pharmacokinetics of carteolol used as an ophthalmic solution. We are now applying this method to the determination of carteolol in human plasma after instilling carteolol hydrochloride ophthalmic solution and the results will be reported elsewhere.

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