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Short Communication

Determination of clonidine in human plasma by gas chromatography–electron-impact mass spectrometry

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Abstract

A new and sensitive gas chromatographic–mass spectrometric (GC–MS) assay for routine analysis of clonidine in human plasma is described. Quantification is carried out with simple electron-impact ionization mass spectrometry and solid-phase cartridges for preliminary extraction from plasma. The pentafluorobenzyl derivative of clonidine yields an intense ion fragment at m/z 354, and the lower limit of detection is 0.025 ng/ml for a 1-ml plasma sample. The practical applicability of this method is demonstrated by determining plasma concentrations of clonidine in a clinical study of a new transdermal delivery system for clonidine.

1. Introduction

Clonidine, 2-[(2,6-dichlorophenyl)imino]imidazolidine, is a potent drug used in the treatment of essential (*i.e.* primary) hypertension. The hypotensive effect of clonidine is due to stimulation of α-2-adrenergic receptors in the central nervous system [1]. The orally administered therapeutic dose of clonidine is very low (50–200 μg/day), and the resulting plasma concentrations are in the ng/ml to pg/ml range [2]. However, many of the side effects associated with oral administration of clonidine have been related to its high peak plasma concentration [3]. Therefore, controlled release dosage forms such as transdermal therapeutic systems capable of minimizing marked fluctuation in plasma concentration and maintaining a steady state over a prolonged period of time have been developed [4]. We also have been developing a new transdermal delivery system for clonidine designed to reduce skin irritation. In order to determine with precision the pharmacokinetics of clonidine following application of this dosage form in therapeutic settings, and in order to analyze large numbers of samples during clinical studies extended over a long period of time, a sensitive and reliable method of determination is required.

A variety of sensitive methods for assaying clonidine has been reported, including radioimmunoassay (RIA) [5,6], gas chromatography with electron capture detection (GC-ECD) [7–9], and combined gas chromatography–mass spectrometry (GC–MS) [10–13]. The GC–MS method employing electron capture negative-ion chemical ionization (NICI) is the most specific and sensitive, and has been accepted as the method of choice [10,11].

Most of the procedures described previously

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have used conventional liquid–liquid extraction [10–13], which is time-consuming and lacks specificity. The insufficient purification of samples results in a decrease in the sensitivity of the GC–MS analysis. In addition, we suspect that these methods damage the columns and mass spectrometers since they involve direct injection into the GC–MS system of the active reagent remaining in the samples due to lack of purification following derivatization.

We therefore developed a new and sensitive gas chromatography–electron-impact ionization mass spectrometry (GC–EI-MS) analysis method suitable for routine analysis, which combined sample preparation by solid-phase extraction and purification following derivatization for the determination of clonidine in human plasma.

2. Experimental

2.1. Materials

Clonidine was obtained from Daiwa Pharmaceutical (Toyama, Japan) and the internal standard (I.S.) 2-[(2,4-dichlorophenyl)imino]-imidazolidine was supplied by Nitto Denko (Osaka, Japan). Pentafluorobenzyl bromide (PFBB) was purchased from GL Science (Tokyo, Japan). All other chemicals and solvents used were of analytical-reagent grade. Potassium carbonate was dried in an oven at 120°C for 2 h. Diethyl ether was refluxed with lithium aluminum hydride and distilled prior to use. Sep-Pak C_{18} cartridges (360 mg/cartridge, bed volume 1 ml) were purchased from Waters (Milford, MA, USA).

2.2. Instruments

The GC–MS system consisted of a Model 5890 Series-II gas chromatograph and a Model 5971A mass spectrometer (Hewlett-Packard, Fullerton, CA, USA) equipped with an Ultra 1 (Hewlett-Packard, Avondale, PA, USA) bonded phase fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness) connected directly to the mass spectrometer by a heated transfer line. The transfer line temperature was maintained at 280°C. Helium was used as the carrier gas at a column head pressure of 103 kPa, and the injector temperature was maintained at 250°C. The injector was operated in the splitless mode. The initial column temperature was set at 50°C, and 1 min after sample injection the temperature was increased at a rate of 40°C/min to 300°C.

The mass spectrometer was operated in the electron-impact mode with an electron energy of 70 eV, and with the ion-source temperature set at 180°C. Determination of clonidine was performed using the selected-ion monitoring (SIM) mode focused on the characteristic fragment ion of the PFB derivative of clonidine at m/z 354.

2.3. Extraction of plasma

A mixture of human plasma (1 ml) and 0.05 ml of 1 *M* potassium hydroxide was poured into a prewashed Sep-Pak C_{18} cartridge, and after washing with 10 ml of distilled water and 10 ml of water–methanol (9:1, v/v), elution was carried out with 3 ml of methanol–water (8:2, v/v). The elution mixture was concentrated to a volume of approximately 0.5 ml *in vacuo* under mild heating (40°C). Following the addition of 0.05 ml of 1 *M* potassium hydroxide, each sample was extracted twice with 2 ml diethyl ether by vortex-mixing. Following brief centrifugation (1800 *g*, 2 min), the organic layer was transferred to a Pyrex screw-cap tube and evaporated completely to dryness *in vacuo*.

2.4. Derivatization procedure

The derivatization procedure described by Edlund and Paalzow [7] was used, with slight modifications. To the residue were added 30 mg of dry potassium carbonate and 0.5 ml of acetone containing 4 ng of internal standard. Following vortex-mixing, 5 μl of PFBB was added to the mixture. The tube was tightly capped and heated at 70°C for 45 min in a heating block. After cooling, the organic solvent was transferred to another tube and evaporated *in vacuo*. The residue was transferred to a new tube with

0.2 *M* sulfuric acid (3 × 2 ml). To the acid solvent was added 2 ml of *n*-pentane, and the organic layer was discarded after vortex-mixing. The pentane wash was repeated three times, and following the addition of 0.6 ml of 1 *M* potassium hydroxide to the remaining aqueous layer, each sample was extracted three times with 1 ml of diethyl ether. The ether was evaporated *in vacuo* and the residue reconstituted in 50 μl of ethyl acetate. Samples of 2 μl were injected into the gas chromatograph–mass spectrometer.

2.5. Preparation of standard calibration curves

The calibration curve for clonidine was obtained by using 0.05, 0.1, 0.25, 0.5, and 1 ng added to 1 ml of human plasma (each point $n = 1$). Each sample was extracted and derivatized as described above. The calibration curve was obtained by an unweighted least-squares linear fitting of peak-area ratios *versus* the amounts of added clonidine.

2.6. Precision and accuracy

Within-day precision and accuracy were determined by assaying five preparations of 1.0 ml human plasma spiked with 0.05, 0.1, 0.25, 0.5, and 1 ng of clonidine. Day-to-day precision and accuracy were determined by assaying the spiked plasma for five days.

3. Results and discussion

Under the conditions described above, clonidine and the internal standard reacted with PFBB to yield PFB derivatives. The EI mass spectra of these PFB derivatives of clonidine and the internal standard are shown in Fig. 1. Clonidine and the internal standard showed a rather similar spectrum. They had a weak molecular ion at m/z 389, corresponding to $[M]^+$, and a very intense ion at m/z 354 resulting from the loss of a chlorine atom. These results agreed with the report of Edlund [8]. Therefore the fragment ion at m/z 354 was selected for determination of clonidine and the internal standard.

The recovery of clonidine from human plasma during the initial extraction procedure using Sep-Pak C_{18} cartridges and following liquid–liquid extraction with diethyl ether was found to be 95.3–95.8%; thus, the method used demonstrated considerable efficiency of extraction. A chromatogram obtained from human plasma using the SCAN operating mode over a prolonged period of time following sample injection is shown in Fig. 2. Few peaks due to other endogenous compounds were detected.

Chromatograms of extracted (A) blank plasma, (B) plasma spiked with 0.05 ng/ml, and (C) a plasma sample taken at 60 h after application of a transdermal delivery system containing 6 mg of clonidine, obtained using the SIM mode are shown in Fig. 3. There were no interfering peaks in the chromatograms with retention times close to those of clonidine and the internal standard. The lower detection limit of the present GC–MS assay was 0.025 ng/ml in plasma, with a signal-to-noise ratio of *ca.* 3.

The calibration curves obtained by plotting (Y) the peak-area ratios against (X) the known plasma concentrations of clonidine (ng/ml) were linear over the concentration range 0.05 to 1 ng/ml in plasma. The regression line was $Y = 0.263X - 0.004$; the correlation coefficient was 0.9993, and the value of the intercept was close to zero. The precision and accuracy results are shown in Tables 1 and 2.

The PFB derivative of clonidine yielded an intense ion fragment using this method, which proved to be of greater sensitivity than the EI ionization method reported previously [12].

A method used for drug monitoring in clinical studies not only requires sensitivity but also reliability, and the potential for stable application to large numbers of samples over a prolonged period of time. When large numbers of samples are tested, it is often found that fouling of the ion-source and the electron-multiplier caused by insufficient sample clean-up decreases the sensitivity of the analysis. The quality of the chromatograms obtained in this study using the SCAN mode suggests that purification of the samples is sufficient for routine work.

The method described here was applied to clinical studies of a new transdermal delivery

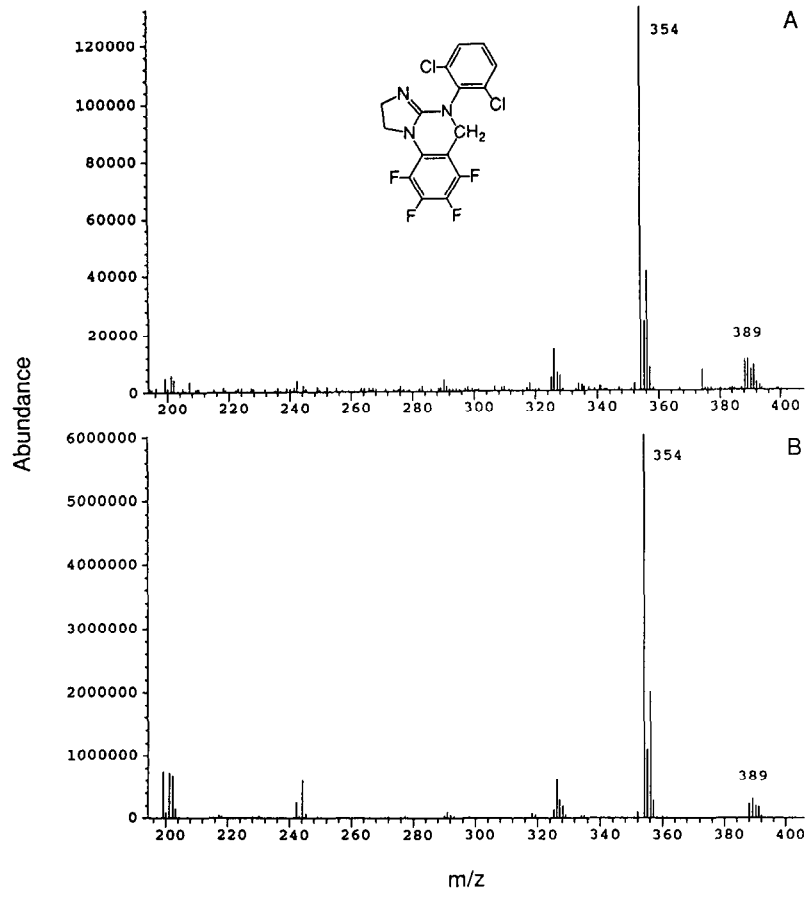

Fig. 1. The electron-impact mass spectra of PFB derivatives of (A) clonidine and (B) I.S.

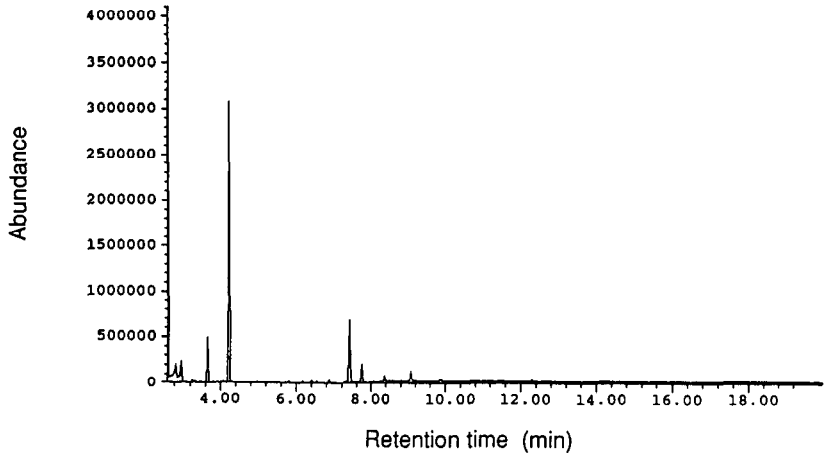

Fig. 2. The total-ion chromatogram obtained from blank plasma by the SCAN mode (m/z 60–600).

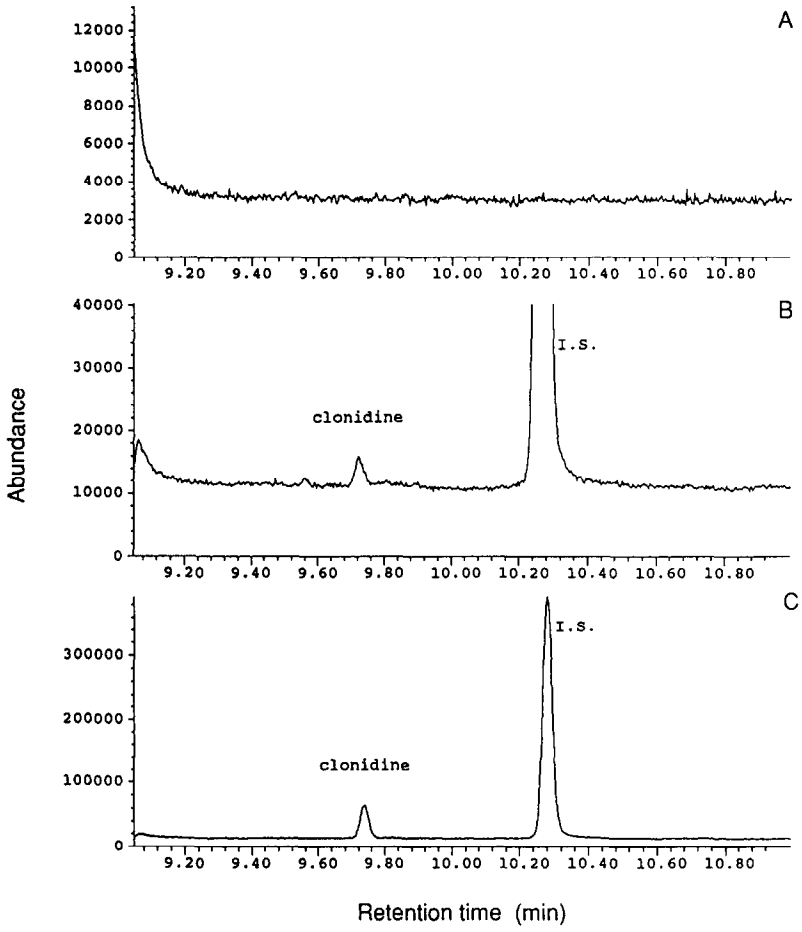

Fig. 3. The typical chromatograms obtained from 1 ml of (A) blank plasma, (B) plasma sample spiked with 0.05 ng/ml of clonidine, and (C) plasma sample taken at 60 h after application of a transdermal delivery system for clonidine to a human subject by selected-ion monitoring mode (m/z 354).

Table 1
Accuracy and within-day precision for the determination of clonidine in human plasma

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n = 5$) (ng/ml)	Accuracy (%)	C.V. (%)
0.050	0.056 ± 0.010	111.6	17.9
0.100	0.075 ± 0.013	74.8	17.3
0.250	0.215 ± 0.028	85.9	13.0
0.500	0.411 ± 0.032	82.2	7.8
1.000	0.934 ± 0.054	93.4	5.8

Table 2
Accuracy and day-to-day precision for the determination of clonidine in human plasma

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n = 5$) (ng/ml)	Accuracy (%)	C.V. (%)
0.050	0.057 ± 0.008	114.0	14.0
0.100	0.090 ± 0.009	90.2	10.0
0.250	0.209 ± 0.015	83.8	7.2
0.500	0.442 ± 0.030	88.5	6.8
1.000	0.980 ± 0.048	98.0	4.9

system for clonidine [14]. Results indicate that it is appropriate for pharmacokinetic studies of the new drug formulation of clonidine. The practical applicability of this method was demonstrated in a clinical study by testing 1000 samples of human plasma.

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