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Determination of moxonidine (BDF 5895) in plasma by gas chromatography–negative ion chemical ionization mass spectrometry

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Abstract: For the measurement of the pharmacokinetic behaviour of moxonidine, 4-chloro-5-(2-imidazolin-2-yl-amino)-6-methoxy-2-methylpyrimidine, an extremely sensitive analytical method was needed. The GC–MS method developed is specific and reliably detects moxonidine plasma levels down to 40 pg ml^{-1}. Using negative ion chemical ionization (NICI) the M^- fragment of the ditrifluoromethyl benzamide derivative of moxonidine (*m/z* 721) and the $[M\text{-}HCl]^-$ fragment of the ditrifluoromethyl benzamide derivative of clonidine (internal standard, *m/z* 673) were monitored in the selected ion monitoring mode, ensuring a specific and sensitive detection of the compounds. The validation process carried out included assay precision, repeatability, linearity, accuracy, stability and estimation of the detection and determination limits. The plasma-level time-curves and pharmacokinetic parameters from two volunteers after oral administration of 0.2 mg moxonidine are presented and demonstrate the practicability of the method in, for example, clinical studies.

Keywords: *Moxonidine; NICI–MS; plasma; validation; pharmacokinetics.*

Introduction

Moxonidine, 4-chloro-5-(2-imidazolin-2-yl-amino)-6-methoxy-2-methylpyrimidine (**I**) (Fig. 1), is a new centrally-acting antihypertensive agent that reduces blood pressure by stimulating the central α2-adrenoceptor [1, 2]. For clinical studies and the evaluation of the pharmacokinetic behaviour a highly sensitive, specific and reliable assay is required. Because of the high affinity to the receptors and the low dosing (0.2 mg tablets), moxonidine plasma levels in the pg per ml range were expected. The NICI–MS was a sensitive method for quantification of drugs in biological fluids [3–6]. The analytical assay described here could be the method of choice for accurately evaluating the pharmacokinetic parameters of **I** after administration of different galenic formulations.

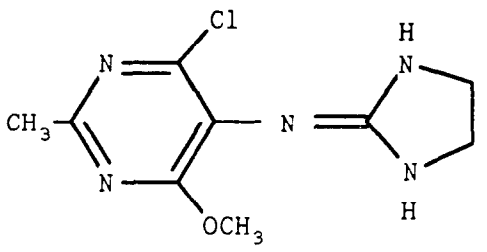

Figure 1
Structure of moxonidine.

Experimental

Reagents

Ethyl acetate, *n*-hexane and dichloromethane ($Chrom^{AR}$) were obtained from Promochem (Wesel, Germany). Ammonium chloride, ammonia solution 25% and anhydrous sodium sulphate, all analytical reagent grade, were supplied by E. Merck (Darmstadt, Germany). 3,5-Bis-(trifluoromethyl)benzoyl chloride (TFMBO) was supplied by Aldrich (Steinheim, Germany). Clonidine (**II**) was purchased from Sigma (Munich, Germany). All aqueous solutions were prepared with water purified on a Bion exchanger (Pierce, Rodgau, Germany). Drug-free human plasma was obtained from healthy volunteers. **I** (CH 91280 base) was synthesized in our chemical department. Ethyl acetate was dried over anhydrous sodium sulphate before use.

Apparatus

The GC–MS system consisted of a Model 5890 gas chromatograph (Hewlett–Packard,

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Bad Homburg, Germany) coupled via a transfer line to a Finnigan MAT SSQ-70 quadrupole mass spectrometer (Bremen, Germany). A 60 m DB-5 fused silica capillary column (0.1 μm film thickness, 0.25 mm i.d.) with helium as carrier gas and an inlet pressure of 2 bar was used. The injection port was set to 280°C, the injection volume was 1 μl and a split ratio of 1:4 was used. The initial oven temperature was 250°C for 1 min and then programmed with a rate of 10°C per min to 290°C which was maintained for 3 min. The end of the capillary was directly introduced into the ion source of the MS and the transfer line was set to 280°C.

The mass spectrometer was operated in the negative ion chemical ionization mode with an electron energy of 40 eV, an emission current of 200 μA and an ion source temperature of 150°C. Methane was used as reagent gas with a manifold pressure set to 0.01 mTorr. The determination was carried out in the SIM mode by monitoring *m/z* 673 and *m/z* 721 for **II** and **I**, respectively. The GC–MS was operated with the 'Instrument Control System' D8.4 and the 'Interactive Chemical Information System' 6.00 software programs.

Analytical procedure

For analysis, 1 ml of plasma was pipetted into a 12-ml screw-capped glass vessel, 50 μl of an aqueous internal standard solution (equivalent to 750 pg) were added and the samples were shaken on a vortex-mixer (10 s). A 200 μl volume of an ammonium chloride buffer (1 M, pH 9) and 3.5 ml of dichloromethane were added and the final solution was shaken for 15 min (250 strokes per min).

The mixture was centrifuged (2850*g*, 10 min) and the lower organic phase was transferred into an 8-ml screw-capped glass vessel. The solution was dried with 0.5 g anhydrous sodium sulphate by shaking on a vortex mixer for 10 s. The mixture was centrifuged (2850*g*, 10 min) and the upper phase was decanted into another 8-ml screw-capped glass vessel and evaporated to dryness.

To prevent adsorption, all glass vessels were rinsed with a 0.1% TFMBO in ethyl acetate solution for 30 min before use. Nevertheless, any glassware was used only once for the work-up procedure.

Derivatization procedure

The residue was dissolved in 50 μl of dry ethyl acetate and 10 μl of TFMBO were added. The vials were tightly capped and heated at 100°C for 1 h. After cooling, a sample volume of 1 μl was directly injected into the chromatograph. The retention times of **I** and **II** were 17.4 and 17.9 min, respectively.

Calculations

The peak areas for *m/z* 721 (**I**) and *m/z* 673 (**II**) were determined and their ratios calculated. The concentrations of the samples (in pg ml^{-1}) were taken from a calibration graph of peak-area ratios against varying amounts of **I**.

Results

Precision

The precision is described by the variation for samples each spiked with 750 pg **I** ml^{-1} after work-up. The mean relative standard deviation (RSD) for samples analysed on one day represents the within-assay variability and that for samples analysed on different days represents the between-assay variability. The RSDs obtained with the described method were 6.4% between assay and 2.5–7.1% within assay (Table 1).

Repeatability

The day-to-day repeatability was verified by preparing four calibration graphs at different days. Two analyses were performed for each concentration. Comparable results indicated that the analytical method was repeatable with the described precision.

Table 1
Precision of the moxonidine determination in plasma after spiking each with 750 pg ml^{-1} (n = 15)

Date	Amount found (pg ml^{-1})	Mean (pg ml^{-1})	SD (pg ml^{-1})	RSD (%)
11/30/89	786.3			
	853.0			
	814.7			
	787.1			
	763.1	800.8	34.4	4.3
12/04/89	746.3			
	795.6			
	651.6			
	741.6			
	744.5	735.9	52.2	7.1
12/05/89	738.0			
	700.8			
	730.9			
	727.4			
	751.3	729.7	18.6	2.5
	n = 15	755.5	48.2	6.4

Linearity

A concentration range of 100–4000 pg ml^{-1} was chosen for the spiked plasma samples, with individual concentrations of 100, 250, 500, 750, 1000, 1500, 2000, 3000 and 4000 pg ml^{-1}. Each analysis was performed four times. The parameters for the resulting calibration graph are given in Table 2. The results showed that the calibration graph was linear for the chosen concentration range.

Accuracy

The values for the spiked plasma samples were calculated from the calibration graph.

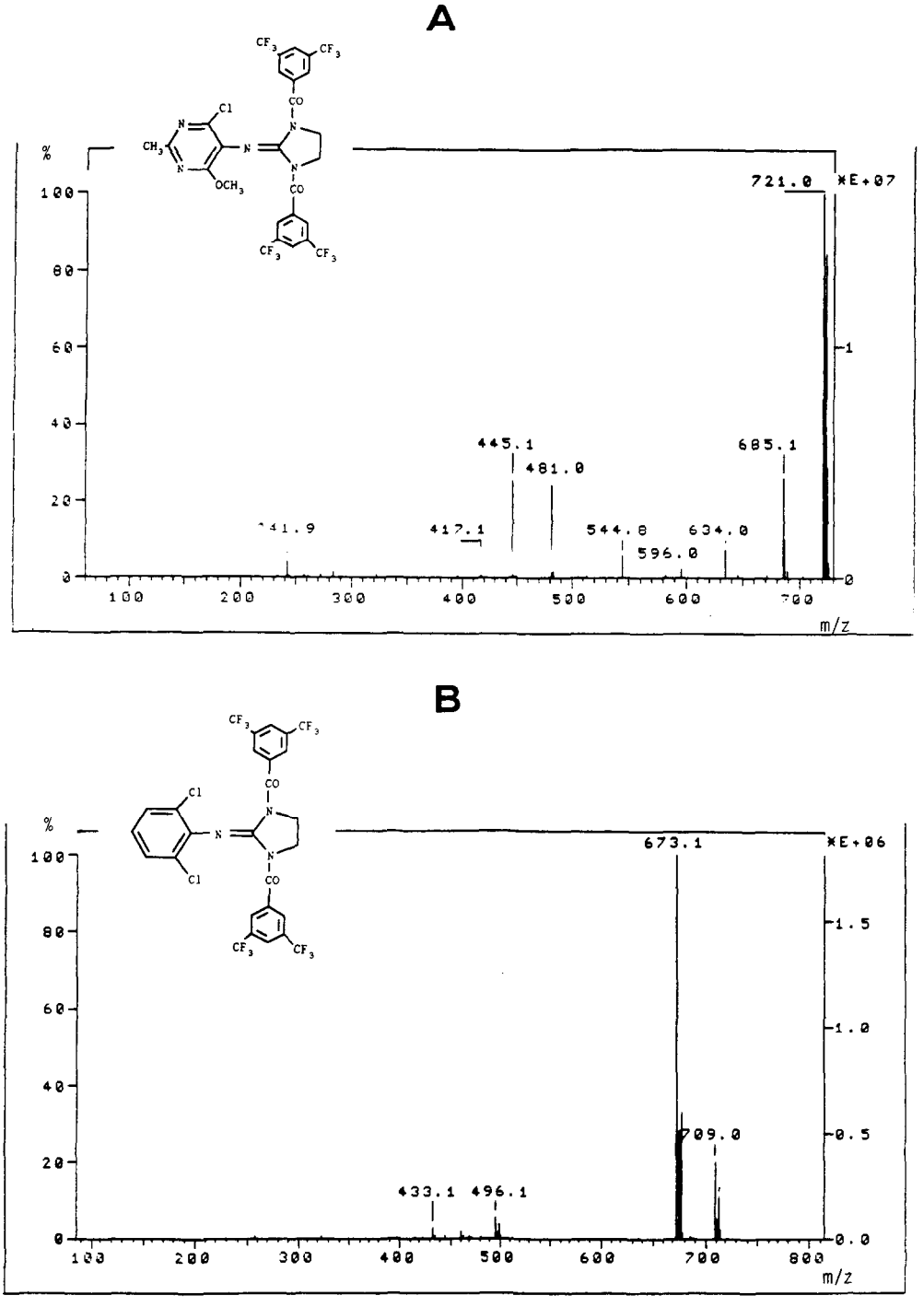

Figure 2
Mass spectra of the 3,5-bis-(trifluoromethyl)benzoyl chlorides of moxonidine (A) and clonidine (B).

Table 2
Accuracy and linearity of the moxonidine determination in plasma. Each result is the mean of four determinations

Concentration added (pg ml^{-1})	Concentration found (pg ml^{-1})	Deviation from amount added (%)
100	114.7 ± 29.2	+14.7
250	281.7 ± 16.9	+12.4
500	542.9 ± 50.8	+8.4
750	730.9 ± 53.0	−2.5
1000	897.6 ± 18.6	−10.3
1500	1500.6 ± 82.1	0.0
2000	2021.1 ± 112.9	+1.1
3000	3015.1 ± 111.1	+0.5
4000	3995.8 ± 273.4	−0.1
	χ_{median}	+0.5

Parameter	
Intercept at ordinate	−16.6
Slope	886.7
Correlation coefficient	0.9995

Four analyses were performed for each concentration (Table 2). For the mean deviation the median value was more useful, because of the greater deviations in the low concentration ranges. Furthermore, the slope of the calibration graph was a measure of the accuracy. The amounts determined were correct if the slope was close to unity. On plotting spiked amounts versus the determined values the calculated slope was 1.0000012. The accuracy of the method was therefore confirmed because of the small deviation (χ_{median} = 0.5%) and the closeness of the slope to unity.

Detection and determination limits

The above limits were determined following the procedure described in refs 7 and 8. The detection limit was found to be 40 pg ml^{-1} and the limit of determination 100 pg ml^{-1}.

Stability

The stability of stock solutions of **I** and **II** in methanol and water was studied over a period of 2 months. When stored in the cold (4°C), no decomposition was observed. The plasma samples were stored at −85°C. The same results were obtained over a period of 6 months with the accuracy given above.

Discussion

The mass spectra obtained for the TFMBO derivatives of **I** and **II** are shown in Fig. 2. In the mass spectra of **I** the base peak is *m/z* 721, corresponding to $[M]^-$, whereas the base peak of **II** is *m/z* 673, which corresponds to the $[M-HCl]^-$ fragment. After derivatization with TFMBO in dry ethyl acetate the disubstituted derivatives of **I** and **II** were obtained and underwent little fragmentation when methane was the reagent gas. Several investigations were carried out to optimize the derivatization conditions. The temperature varied from 60 to 120°C, the reaction time from 20 to 120 min and the TFMBO amount from 2 to 25 µl. Under the described reaction conditions of 10 µl TFMBO and 100°C for 1 h the derivatization was quantitative, yielding more than 99% of the disubstituted TFMBO derivatives, which was in good agreement with results previously described [9, 10]. However, when pentafluorobenzoyl chloride (PFBO) was used as the derivatization agent, monosubstituted derivatives of **I** and **II** were formed that underwent internal cyclization with separation of HF [11]. The derivatization procedure was not quantitative and resulted in different amounts of about 80–90% of the favoured product. Therefore, PFBO was not chosen as the derivatizing agent here, unlike another GC–MS method for the determination of **II** in body fluids [12].

The MS measurements were carried out in the SIM mode after NICI to obtain maximum sensitivity. In Fig. 3 examples of chromatograms of a blank plasma sample and a sample obtained 3 h after administration of a 0.2 mg moxonidine tablet are presented. The detection limit of 40 pg ml^{-1} enabled plasma concentrations to be measured for 12 h after dosing. The plasma-level time-curves for two volunteers receiving a 0.2 mg moxonidine dose are shown in Fig. 4. The pharmacokinetic parameters for the area under the curve (AUC), the elimination half-life ($t_{½}$), the maximum time (t_{max}) and maximum concentration (c_{max}) are summarized in Table 3 and correspond to previous results [13].

Table 3
Pharmacokinetic parameters of two volunteers after administration of a 0.2 mg moxonidine tablet

Parameter	Volunteer 1	Volunteer 2
$t_{½}$ (h)	2.33	1.89
C_{max} (pg ml^{-1})	1801.3	1668.1
t_{max} (h)	1.0	0.66
AUC (0 − t)	4217.4	4194.7

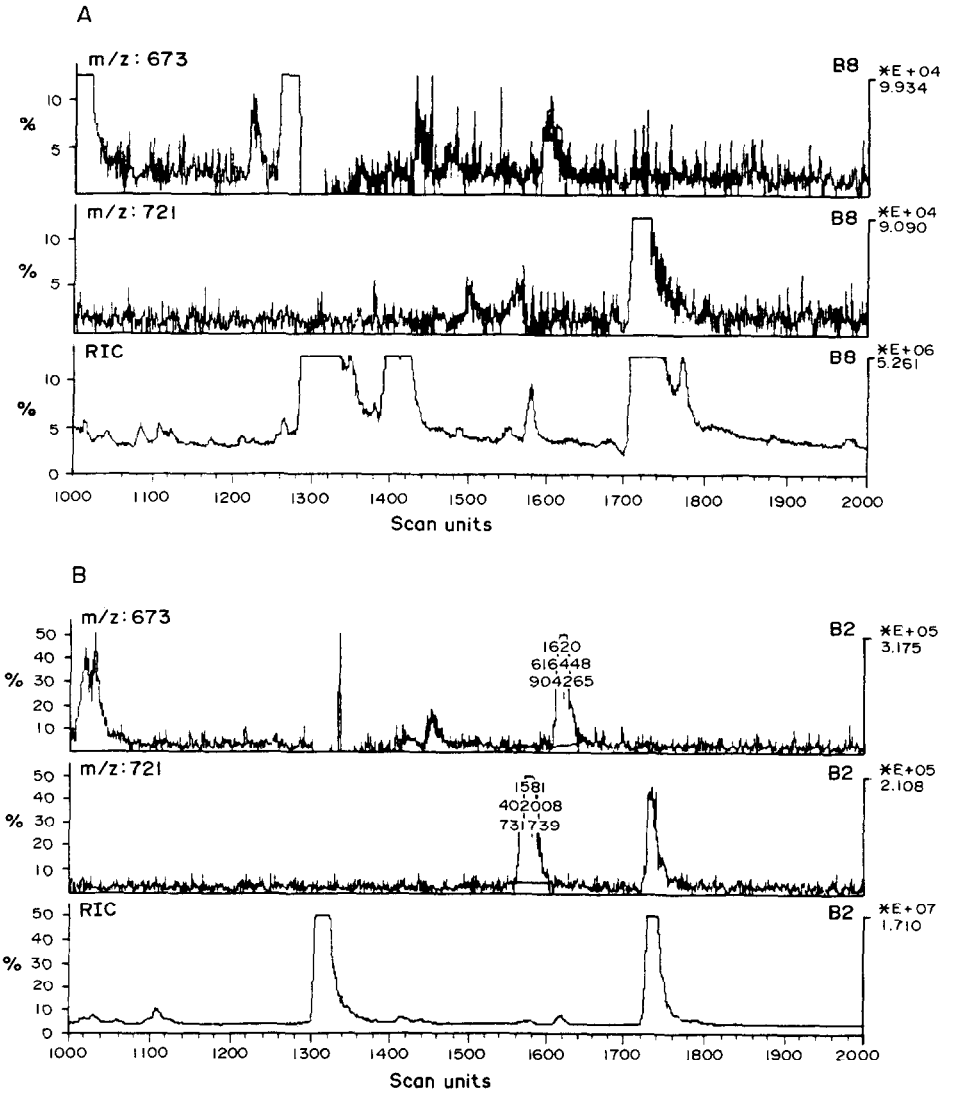

Figure 3
Typical mass chromatograms obtained from 1 ml of blank plasma (A) and a plasma sample obtained 3 h after administration of 0.2 mg moxonidine (514 pg moxonidine and 750 pg clonidine ml^{-1} plasma) (B) (RIC: reconstructed ion current).

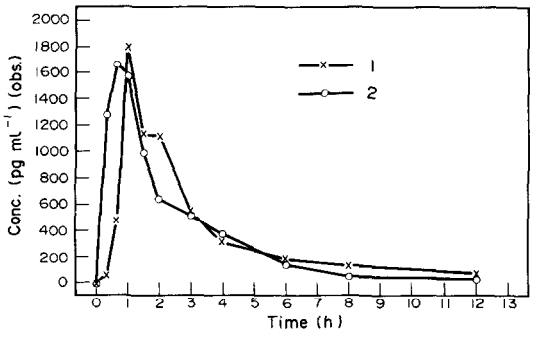

Figure 4
Plasma-level time-curves of two volunteers receiving a 0.2 mg moxonidine tablet.

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