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Journal of Chromatography B, 670 (1995) 81–89

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Simultaneous determination of moexipril and moexiprilat, its active metabolite, in human plasma by gas chromatography–negative-ion chemical ionization mass spectrometry

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First received 30 November 1994; revised manuscript received 14 March 1995; accepted 15 March 1995

Abstract

A sensitive and selective method for the simultaneous determination of moexipril and moexiprilat, its active metabolite, in human plasma is described using quinapril and quinaprilat as internal standards. The analytes are isolated from human plasma by means of Bond Elut C_{18} cartridges, methylated with diazomethane, purified by acid-base partitioning and converted to the corresponding trifluoroacetamides using trifluoroacetic anhydride. Moexipril and moexiprilat were analysed by gas chromatography–negative-ion chemical ionization mass spectrometry (GC–NICI-MS) at the fragment ions *m/z* 302 and *m/z* 288, respectively. The lower limit of quantitation both for moexipril and moexiprilat is 0.5 ng/ml of human plasma. Linear calibration curves are obtained over the concentration range 0.5–300 ng/ml of human plasma. In any case the imprecision and the inaccuracy are $< 15\%$. The present method has been successfully applied to various pharmacokinetic studies in human subjects and patients.

1. Introduction

Moexipril · HCl, (3*S*) - 2 - [(2*S*) - 2 - {[(1*S*) - 1 - (ethoxycarbonyl) - 3 - phenylpropyl]amino} - 1 - oxopropyl] - 6,7 - dimethoxy - 1,2,3,4 - tetrahydroisoquinoline - 3 - carboxylic acid, HCl (Fig. 1) is a new potent, orally active non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor under development at Schwarz Pharma for the treatment of hypertension and congestive heart failure. In biological systems it is rapidly deesterified by esterases resulting in its active metabolite moexiprilat (Fig. 1) [1].

Quantitation of other ACE inhibitors has been conducted by high-performance liquid chromatography (HPLC) [2], gas chromatography (GC) [3–5], gas chromatography–mass spectrometry (GC–MS) [6,7] and gas chromatography–negative-ion chemical ionization (GC–NICI-MS) [8–10]. A sensitive method is required to evaluate reliable pharmacokinetic parameters even after administration of a 15-mg dose of moexipril · HCl. The present paper describes the development and the validation of a sensitive and specific method for the simultaneous determi-

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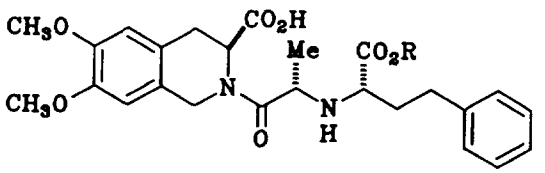
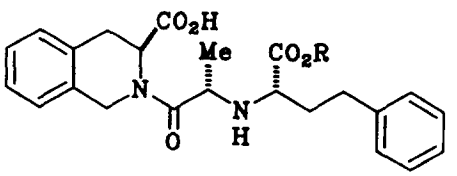
0378-4347/95/$09.50
SSDI 0378-4347(95)00135-2



Moexipril: R = C_2H_5

Moexiprilat: R = H



Quinapril: R = C_2H_5

Quinaprilat: R = H

Fig. 1. Chemical structure of moexipril, moexiprilat, quinapril and quinaprilat.

nation of moexipril and moexiprilat in human plasma by GC–NICI-MS.

2. Experimental

2.1. Chemicals and reagents

Moexipril · HCl, moexiprilat dihydrate and the internal standards quinapril · HCl and quinaprilat monohydrate (Fig. 1) were synthesized by Warner-Lambert (Ann Arbor, MI, USA). Methanol, hexane, dichloromethane and ethyl acetate were purchased from Promochem (Wesel, Germany), ethanol, *n*-butanol, hydrochloric acid and dry chemicals from Merck (Darmstadt, Germany), trifluoroacetic anhydride (TFAA) from Fluka (Neu-Ulm, Germany) and N-methyl-N-nitroso-*p*-toluenesulfonamide (Diazald) from Aldrich (Steinheim, Germany). All chemicals were of the highest grade available and used without further purification. Diazomethane was prepared with a Diazald Generator Kit supplied by Aldrich.

Bond Elut C_{18} cartridges and the VacElut were obtained from ICT (Frankfurt, Germany). Water was purified with the NANOpure system delivered by Werner (B.-Gladbach-2, Germany).

2.2. Chromatographic system and detection

Analyses were performed on a Finnigan SP 5100 MS (Finnigan MAT, Bremen, Germany) equipped with a HP 5890 Series II GC, a HP 7673A autosampler (Hewlett-Packard, Palo Alto, CA, USA) and a temperature-programmable split/splitless injector (Gerstel, Mülheim/Ruhr, Germany). A fused-silica capillary column (DB-1, 10 m × 0.32 mm I.D., 0.25 μm film thickness) from Fisons Scientific (Mainz, Germany) was inserted directly into the ion source. Helium was used as carrier gas at an inlet pressure of 13.8 kPa. The injector temperature was programmed from 70°C to 300°C at 10°C/s (held for 5 min) and the oven temperature from 150°C (held for 0.1 min) to 300°C at 25°C/min (held for 1.5 min). The transfer line, auxiliary heater and the ion source were kept at 270°C, 270°C and 250°C, respectively. The MS was operated in the NICI mode with an emission current of 75 μA and an electron energy of 70 eV. Methane was used as reagent gas with an ion source pressure of 400 Pa measured at the end of the Pirani gauge line.

2.3. Standard and working solutions

For the preparation of the stock solutions moexipril · HCl, moexiprilat, quinapril and quinaprilat were dissolved in 2 ml of methanol and diluted with water to obtain concentrations of 100 ng/μl. All working solutions containing both moexipril and moexiprilat or quinapril and quinaprilat were prepared using these stock solutions further diluted with water in order to get concentrations of 10 ng/μl, 1 ng/μl and 0.1 ng/μl for the preparation of the calibration curves. The working solutions were stored at 4°C and prepared every 4 weeks.

2.4. Sample preparation

Bond Elut C_{18} cartridges were preconditioned by sequential treatment with 6 ml of methanol and 6 ml of water. Human plasma unknown or standard (1 ml) containing the internal standards were acidified with 1 ml of 0.1 mol l^{-1} of hydrochloric acid and transferred to the preconditioned Bond Elut C_{18} cartridges. After successive washing with two column volumes of water and hexane the cartridges were dried for 10 min at 80 kPa. The analytes were eluted with two 2-ml volumes of dichloromethane–methanol (2:1, v/v). The eluates were evaporated to dryness at room temperature.

The residues were dissolved in 100 μl of methanol, methylated with about 0.5 ml of ethereal diazomethane for 5 min and evaporated to dryness at room temperature.

The samples were acidified with 1 ml of 0.1 mol l^{-1} of hydrochloric acid, shaken with 5 ml of hexane for 5 min and centrifuged at 1000 *g* for 5 min. The organic layer was discarded. The aqueous solution was basified with 1 ml of 0.5 mol l^{-1} of sodium carbonate buffer (pH 9.5), shaken with 5 ml of hexane–*n*-butanol (98:2, v/v) for 5 min and centrifuged at 1000 *g* for 5 min. The organic layer was transferred to a centrifuge tube and evaporated to dryness at room temperature.

The residues were dissolved in 250 μl of ethyl acetate. After the addition of 1 ml of 5% (v/v) TFAA in hexane, the mixtures were incubated at 60°C for 30 min, evaporated to dryness, redissolved in 100 μl of methanol, vortexed and again evaporated to dryness.

The samples were dissolved in 100 μl of hexane–ethyl acetate (10:1, v/v) and 1 μl was injected into the GC–MS system.

2.5. Calibration

Calibration curves were generated daily by adding known amounts of moexipril·HCl, moexiprilat and the internal standards to 1 ml of blank human plasma in the range 0.5–300 ng. Quantitation was achieved by calculating the peak area ratio of moexipril to quinapril and of moexiprilat to quinaprilat versus the concentration of the corresponding analyte followed by linear regression analysis with $1/x$ weighting.

3. Results

3.1. Plasma clean-up: solid-phase extraction

As a result of the amphoteric properties of the analytes, they cannot be extracted from plasma into organic solvents. Therefore, the analytes were isolated from human plasma using a solid-phase extraction technique on Bond Elut C_{18} cartridges.

3.2. Derivatization–purification

In order to be suitable for GC–NICI-MS analysis, the functionalities of the analytes had to be derivatized. The carboxylic acids were methylated with diazomethane and the free amino group was trifluoroacetylated with TFAA in order to insert a volatile electron-capturing group. Because of the free amino group the analytes could be purified by acid-base partitioning after the methylation with diazomethane.

3.3. GC–NICI-MS

Under the given conditions the derivatized moexipril, moexiprilat, quinapril and quinaprilat had retention times of about 7.32 min, 7.25 min, 6.20 min and 6.15 min, respectively. The analytes are well separated from each other and from endogenous material. Typical GC–MS chromatograms of blank human plasma, spiked with 0.5 or 100 ng of moexipril and moexiprilat in comparison to blank human plasma processed with this method are shown in Figs. 2 and 3, respectively. The full scan NICI mass spectra of the derivatized analytes are shown in Fig. 4.

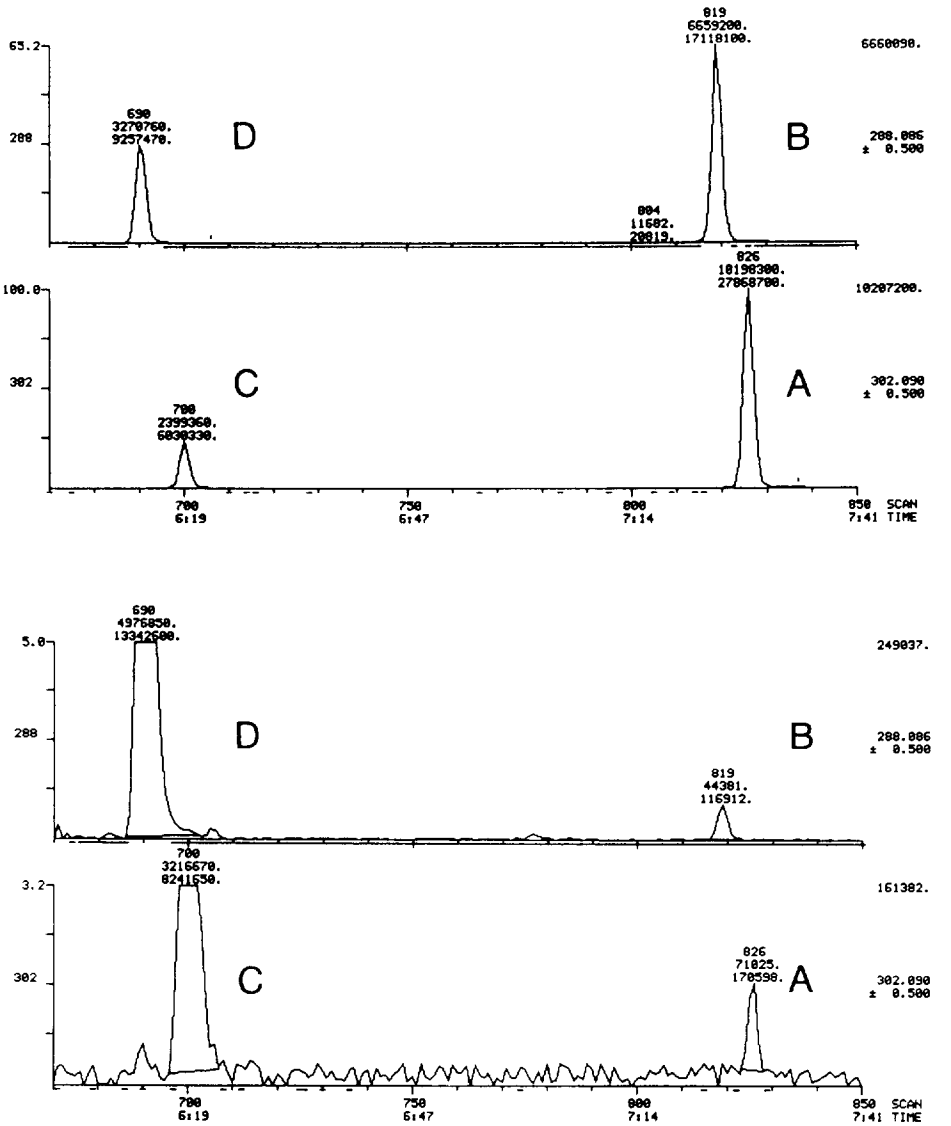


Fig. 2. MID chromatograms of human plasma samples: (top) blank human plasma spiked with 100 ng of moexipril/moexiprilat; (bottom) blank human plasma spiked with 0.5 ng of moexipril/moexiprilat; scale of the chromatogram enlarged by a factor of 20; (A) moexipril, (B) moexiprilat, (C) quinapril, and (D) quinaprilat.

Under optimized conditions the base peak $[M-306]^-$ carries more than 50% of the total ion current. Detection and quantitation of the samples were performed by multiple ion detection (MID) at the fragment ions m/z 302 for moexipril and quinapril and m/z 288 for moexiprilat and quinaprilat.

3.4. Linearity, sensitivity

Linear calibration curves were obtained for moexipril and moexiprilat over the concentration range 0.5–300 ng/ml of human plasma. A typical calibration curve of moexipril was described by the equation $y = 0.0449x + 0.0012$, $r = 0.9997$.

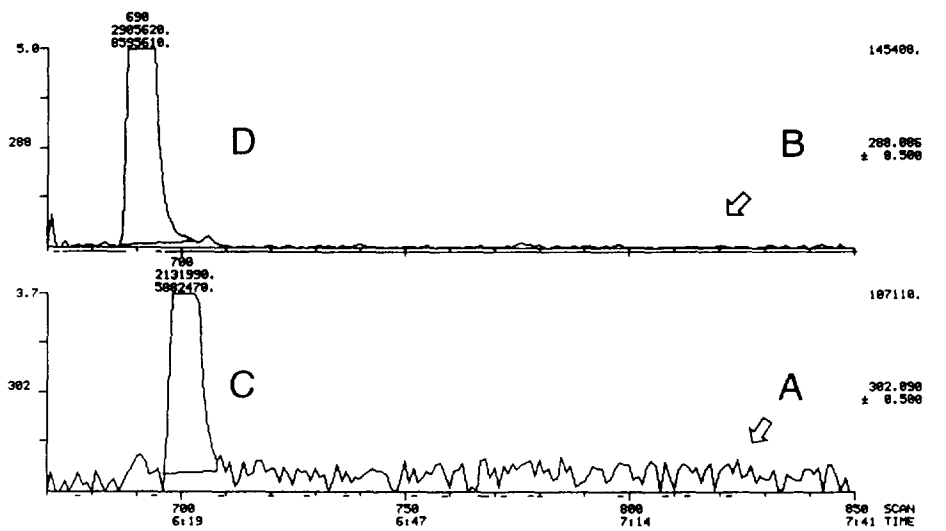


Fig. 3. MID chromatogram of blank human plasma; scale of the chromatogram enlarged by a factor of 20; (A) moexipril, (B) moexiprilat, (C) quinapril, and (D) quinaprilat.

The corresponding equation for the determination of moexiprilat was $y = 0.0171x + 0.0023$, $r = 0.9996$. The quantitation limit of the assay was 0.5 ng/ml both for moexipril and moexiprilat at a signal-to-noise ratio $S/N = 7.5:1$.

3.5. Reproducibility

The imprecision and the inaccuracy of the method were determined by analysis of blank human plasma spiked with moexipril and moexiprilat at different concentrations in the range 0.5–300 ng. All intra- and inter-assay data are summarized in Tables 1–4. In any case, both the imprecision and the inaccuracy were $< 15\%$ and showed good reproducibility.

3.6. Recovery

As the derivatized analytes are not available in their pure form, only the recovery of moexipril and moexiprilat from human plasma could be determined. Assuming a 100% yield during the derivatization and the purification steps, the recovery was calculated by analysing the parent compounds directly and after isolating on Bond Elut C_{18} cartridges resulting in 80–100% over the concentration range measured.

3.7. Application of the method

The method was used to determine plasma concentrations after oral administration of 15 mg moexipril · HCl in order to investigate the pharmacokinetics of moexipril and moexiprilat in human subjects with normal renal function and in patients with various degrees of renal impairment. Representative MID chromatograms of two postdose samples are shown in Fig. 5. A characteristic plasma concentration–time profile of moexipril and moexiprilat of one human subject (age 34 years; body weight 74.5 kg; creatinine clearance 116 ml min^{-1}; normal renal function) is shown in Fig. 6. In the case of moexipril a maximum plasma level of 52.2 ng/ml was obtained 1 h after administration. Plasma levels of moexiprilat above the limit of quantitation were detectable until 96 h after administration. The imprecision and the inaccuracy data of the calibration curves and of the quality control samples processed by this method and calculated

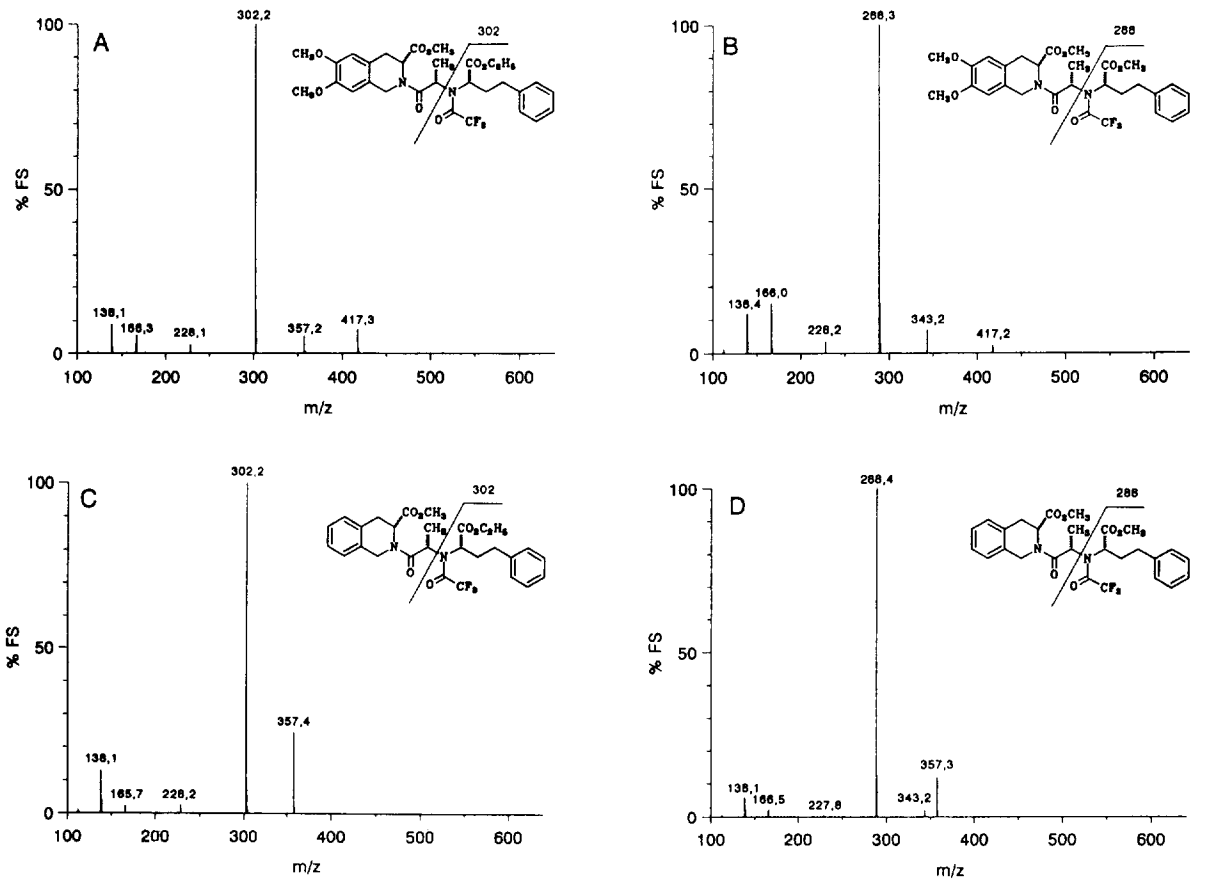


Fig. 4. Full scan NICI mass spectra of the methyl-trifluoroacetyl derivatives of moexipril (A) and moexiprilat (B), quinapril (C) and quinaprilat (D).

Table 1
Inter-assay data of moexipril ($n = 5$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.51 ± 0.04	2.0	8.6
1.0	1.01 ± 0.13	1.1	12.8
5.0	4.96 ± 0.30	−0.8	6.0
25	23.88 ± 1.15	−4.5	4.8
50	49.25 ± 3.94	−1.5	8.0
100	95.40 ± 6.13	−4.6	6.4
200	193.97 ± 7.77	−3.0	4.0
300	292.95 ± 6.73	−2.4	2.3

[a] Mean value ± standard deviation.

Table 2
Inter-assay data of moexiprilat ($n = 5$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.53 ± 0.06	6.0	11.5
1.0	1.01 ± 0.12	1.0	11.4
5.0	4.75 ± 0.39	−5.0	8.2
25	23.79 ± 1.32	−4.8	5.6
50	47.71 ± 1.48	−4.6	3.1
100	95.62 ± 7.14	−4.4	7.5
200	199.00 ± 6.18	−0.5	3.1
300	298.36 ± 28.68	−0.6	9.6

[a] Mean value ± standard deviation.

Table 3
Intra-assay data of moexipril ($n = 6$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.56 ± 0.88	12.0	14.1
2.0	2.09 ± 0.09	4.5	4.1
10	9.46 ± 0.78	−5.4	8.3
50	45.94 ± 3.25	−8.1	7.1
100	104.21 ± 4.31	4.2	4.1
250	255.09 ± 11.21	2.0	4.4

[a] Mean value ± standard deviation.

Table 4
Intra-assay data of moexiprilat ($n = 6$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.56 ± 0.07	12.0	11.6
2.0	2.17 ± 0.07	8.5	3.2
10	10.72 ± 1.01	7.2	9.5
50	45.61 ± 2.39	−8.7	5.3
100	98.23 ± 3.27	−1.8	3.3
250	274.19 ± 19.37	7.1	9.7

[a] Mean value ± standard deviation.

Table 5
Imprecision and inaccuracy data of the calibration curves of moexipril ($n = 21$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.53 ± 0.06	6.0	11.5
1.0	0.99 ± 0.10	−1.0	9.6
5.0	4.81 ± 0.31	−3.8	6.4
25	24.74 ± 1.39	−1.0	5.6
50	49.48 ± 2.20	−1.0	4.5
100	100.90 ± 2.09	0.9	2.1

[a] Mean value ± standard deviation.

Table 6
Imprecision and inaccuracy data of the calibration curves of moexiprilat ($n = 21$)

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
0.5	0.53 ± 0.05	6.0	10.0
1.0	1.02 ± 0.07	2.0	7.2
5.0	4.80 ± 0.37	−4.0	8.0
25	24.82 ± 1.34	−0.7	5.6
50	49.59 ± 2.53	−0.8	5.3
100	100.80 ± 5.42	0.8	5.6

[a] Mean value ± standard deviation.

Table 7
Imprecision and inaccuracy of the quality control samples of moexipril

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
1.0 ($n = 29$)	0.98 ± 0.08	−2.0	8.3
2.0 ($n = 5$)	1.80 ± 0.08	−10.0	4.5
5.0 ($n = 8$)	4.75 ± 0.27	−5.0	5.6
25.0 ($n = 18$)	24.43 ± 1.69	−2.3	6.9

[a] Mean value ± standard deviation.

Table 8
Imprecision and inaccuracy of the quality control samples of moexiprilat

Added (ng/ml)	Found[a] (ng/ml)	Inaccuracy (%)	Imprecision (%)
1.0 ($n = 20$)	1.02 ± 0.09	+2.0	9.8
2.0 ($n = 4$)	1.80 ± 0.09	−10.0	5.0
5.0 ($n = 7$)	4.70 ± 0.35	−6.0	7.5
25.0 ($n = 18$)	24.34 ± 1.25	−2.6	5.4

[a] Mean value ± standard deviation.

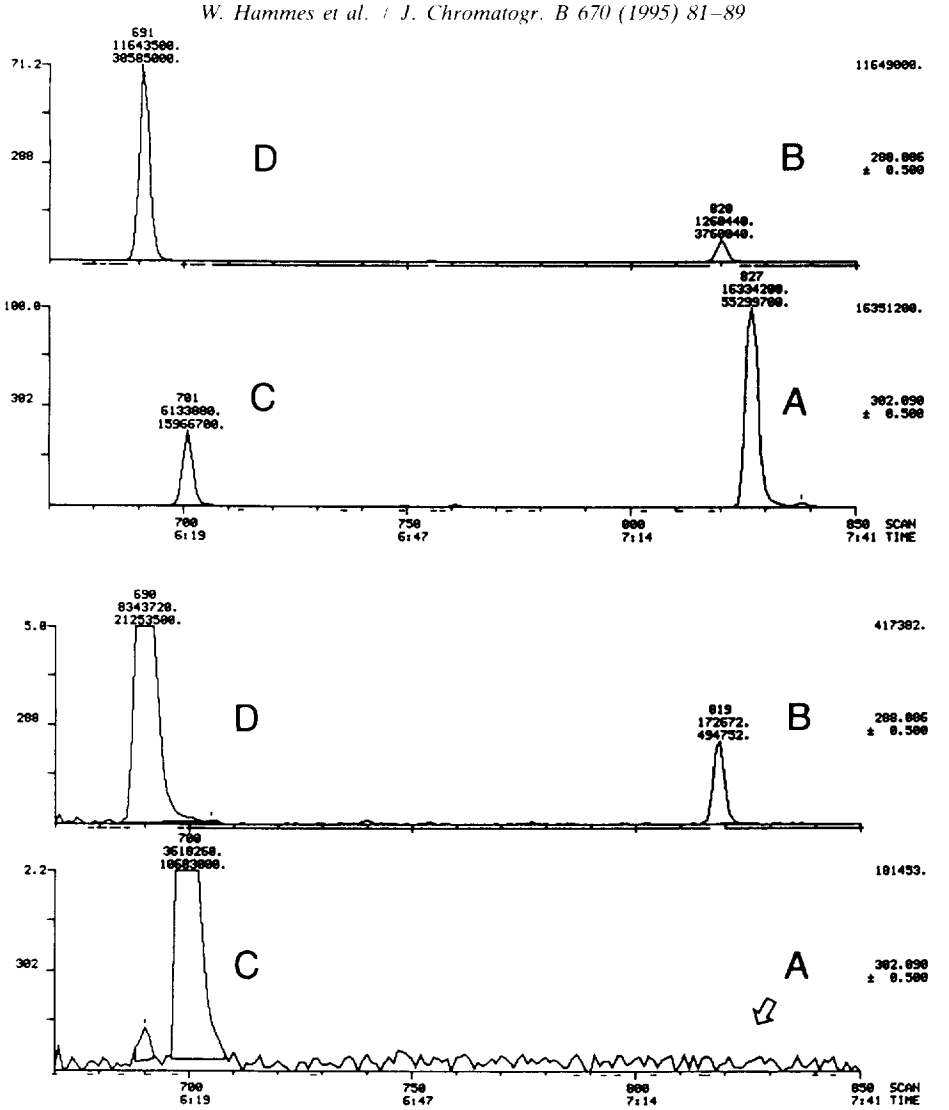


Fig. 5. MID chromatograms of human plasma samples after oral administration of 15 mg moexipril·HCl; (top) plasma sample taken 1 h after administration, sample containing 52.2 ng/ml moexipril and 27.0 ng/ml moexiprilat; (bottom) plasma sample taken 96 h after administration, sample containing 1.2 ng/ml moexiprilat; scale of the chromatogram enlarged by a factor of 20; (A) moexipril, (B) moexiprilat, (C) quinapril, and (D) quinaprilat.

by the corresponding calibration curve are given in Tables 5–8.

4. Conclusions

A selective and sensitive method for the simultaneous determination of moexipril and moexiprilat in human plasma was developed. Detection was achieved by GC–NICI-MS resulting in a limit of quantitation of 0.5 ng/ml. The assay is suitable for the quantitative determination of moexipril and moexiprilat in plasma samples collected in various clinical pharmacokinetic studies.

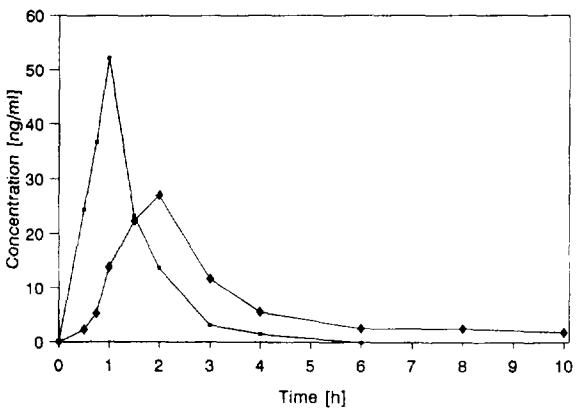


Fig. 6. Representative plasma concentration–time profile of moexipril (■) and moexiprilat (◆) of one human subject after oral application of 15 mg moexipril · HCl.

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