

# Monitoring the Metabolism of Moexipril to Moexiprilat Using High-Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry

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## Abstract

High-performance liquid chromatography combined with a UV absorbance detector and electrospray ionization mass spectrometer is used for the simultaneous analysis of moexipril and moexiprilat in biological samples. Moexipril and moexiprilat are determined in samples metabolized by rat and human liver microsomal preparations, and also in rat urine. The calibration curve is linear in the ng/mL and µg/mL concentration range of the injected moexipril.

## Introduction

Moexipril hydrochloride [(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 hydrochloride] (Figure 1A) is a long-acting, non-sulfhydryl angiotensin-converting enzyme inhibitor (ACEI) used in the treatment of hypertension (1).

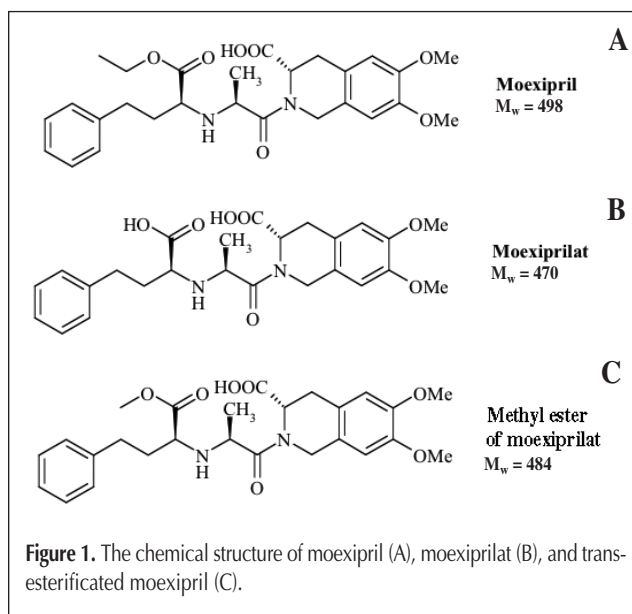
Moexipril can be preferentially used to lower blood pressure without a significant change in cardiac output and heart rate. Moexipril is one of the non-sulfhydryl ACEIs. Further ACEIs are benazepril, captopril, cilazapril, enalapril, fosinopril, imidapril, lisinopril, perindopril, quinapril, ramipril, spirapril, trandolapril, and zofenopril as indicated by the suffix “pril”. These drugs differ in their chemical structure and pharmacokinetics; in clinical use, they are probably interchangeable.

The overwhelming majority of ACEIs are prodrugs, so the active compound is generated by metabolic hydrolysis of the ethyl ester group. The alteration takes place mainly in the liver. These active metabolites are identifiable mainly by the suffix “prilat”. As such, the metabolic hydrolysis of moexipril yields moexiprilat (Figure 1B).

Pharmacokinetic studies generally require determination of both the parent compound and of its active metabolites.

This is the main reason why there is an effort to determine the ACEIs together with their respective “prilat”. Prieto et al. (2) analyzed cilazapril and cilazaprilat, and Gu et al. (3) simultaneously measured the level of enalapril and enalaprilat. The mass spectra of enalapril and enalaprilat differ by 28 amu. This difference corresponds to the loss of the ethyl ester during metabolic alteration.

Moexipril can be determined using both gas chromatography (GC) (4) and high-performance liquid chromatography (HPLC) (5). The GC analysis (4) consists of several steps, such as: (a) clean-up using Bond Elute C18, (b) methylation, (c) acid-base partition, and (d) converting both moexipril and moexiprilat to the corresponding trifluoroacetamides. GC separation can be monitored using negative-ion chemical ionization (NICI) mass spectrometry (MS) for the fragment ions of *m/z* 302 and 288 for moexipril and moexiprilat, respectively. The use of GC has been limited by the requirement of two-steps derivatization, that is, methylation and trifluoroacetylation.



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Following oral administration, mainly as an expression of the bioavailability, an essential portion of moexipril and moexiprilat was eliminated in the feces (52% moexiprilat and 1% moexipril), though only 13% of the dose was urinary eliminated (7% moexiprilat, 1% moexipril, and 5% other metabolites) (6). Another study (7) reported on an even higher ratio of moexiprilat being eliminated in feces. Intravenous administration shifted the elimination route of moexipril to the urine, as 66% of the intravenous-administered drug plus its metabolite, were eliminated in the urine (26% moexipril and 40% moexiprilat), though 20% of the dose was eliminated in the feces.

Reversed-phase chromatography (RPC) has eminently been used to separate biologically active compounds, such as drugs (8).

Moexipril hydrochloride and hydrochlorothiazide were simultaneously determined in tablets by Ertürk et al. (5) by isocratic RPC, using lisinopril as the internal standard and monitoring the eluate at 212 nm. The limit of quantitation for both moexipril and moexiprilat was 0.5 ng/mL, and linear calibration curves were obtained through the concentration range of 0.5–300 ng/mL in human plasma. The method has been utilized in various pharmacokinetic studies of human subjects.

This paper reports on a liquid chromatography–MS method developed for the simultaneous detection of moexipril and moexiprilat in microsomal preparations, and also in rat urine. Electrospray ionization (ESI) MS was used for determination.

## Experimental

### Solvents and chemicals

Solvents and chemicals were purchased from commercial sources at the highest available grade of purity. Moexipril was provided by the manufacturer Schwarz Pharma AG (40789, Monheim, Germany).

### Microsomal preparation

Human liver microsome (0.5 mg/mL) (Lot. No. 0410132, XenoTech, Lenexa, KS) and rat liver microsome (0.5 mg/mL) (Lot. No. 04.11.05, Gedeon Richter Ltd., Budapest, Hungary) were used.

Moexipril (50 μM) was incubated for 30 min with rat and human liver microsomes in 2.0 mL of incubation medium consisting of a tris-HCl buffer (0.12 mM, pH 7.4 at 37°C), MgCl<sub>2</sub> (5 mM), sodium pyrophosphate (6.25 mM), D-glucose 6-phosphate (5 mM), D-glucose 6-phosphate dehydrogenase (1 U/mL), and nicotinamide adenine dinucleotide phosphate (NADPH, reduced form) (0.5 mM). Control incubations were run with test compounds for 30 min in the incubation medium without NADPH (assessment of P450-independent metabolism) or for 30 min without microsomal proteins. Specific analytical conditions follow. Each experiment was run in duplicate.

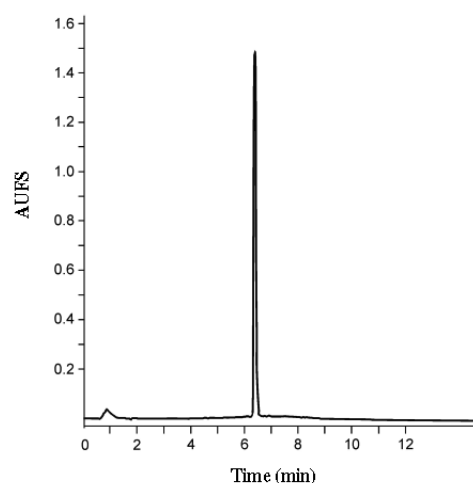
Three male Wistar rats (200–250 g) were subjected to oral treatment with moexipril hydrochloride with a dose of 0.1

mg/kg. Urine was collected for 4 h. The urine samples were subjected to a clean-up C18 cartridge, eluted using acetoni-

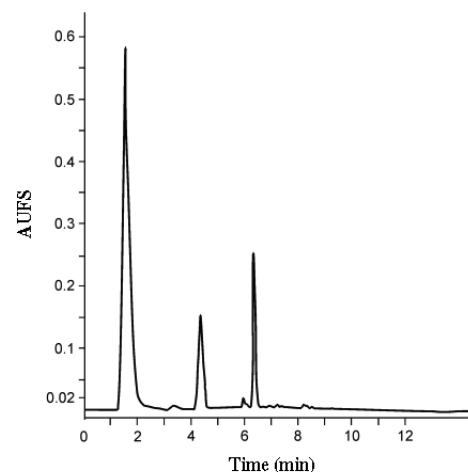
**Table I. Gradient Program Developed for the Simultaneous Analysis of Moexipril and Moexiprilat in Microsomal Preparations**

Time (min)	Mobile phase A*	Mobile phase B†
0	75	25
2	50	50
5	0	100
10	0	100
12	75	25
15	75	25

\* Methanol (10%)–buffer (90%) (1000 mL 0.1M ammonium acetate–2 mL 10% acetic acid).  
† Methanol (90%)–buffer (10%) (1000 mL 0.1M ammonium acetate–2 mL 10% acetic acid).



**Figure 2.** The chromatogram of 60 μL of 100 μM moexipril standard solution detected at 282 nm.



**Figure 3.** The HPLC chromatogram of microsomally metabolized sample (R2/30) detected at 282 nm.

tril-water (9:1), and aliquot was taken for the HPLC-MS analysis.

#### LC-MS method for the analysis of moexipril and moexiprilat in microsomal preparations

The HPLC separation was performed using a 3.9- × 150-mm column packed with Nova-Pack C-18, 4- $\mu$ m particles. A gradient elution was generated with several steps of the gradient, as given in Table I. The column and tray temperatures were held at 40°C, 60  $\mu$ L of sample was injected at a flow rate of 600  $\mu$ L/min, and the compounds were detected at 282 nm.

A Thermo Finnigan Surveyor HPLC was connected online to a Finnigan MAT 95 XP MS (Thermo Finnigan, Bremen, Germany) working in ESI mode. In the electrospray ion source the capillary voltage was 3.0 kV, and the temperature was held at 240°C.

A calibration curve was constructed for moexipril by the

injection of 60  $\mu$ L of sample aliquots with concentrations of 0.1, 1, 10, 50, and 100 $\mu$ M in triplicates (0.1 $\mu$ M corresponds to 53.5 ng/mL and 100 $\mu$ M corresponds to 53.5 $\mu$ g/mL concentration values) and detected at 282 nm. The data points resulted in a straight line with  $R^2 = 0.993$ . The signal-to-noise ratio was over 10, even for the lowest injected concentration. The chromatogram of 60  $\mu$ L of 100 $\mu$ M moexipril solution is shown in Figure 2.

#### LC-MS method for the analysis of moexipril and moexiprilat in rat urine

##### HPLC-MS

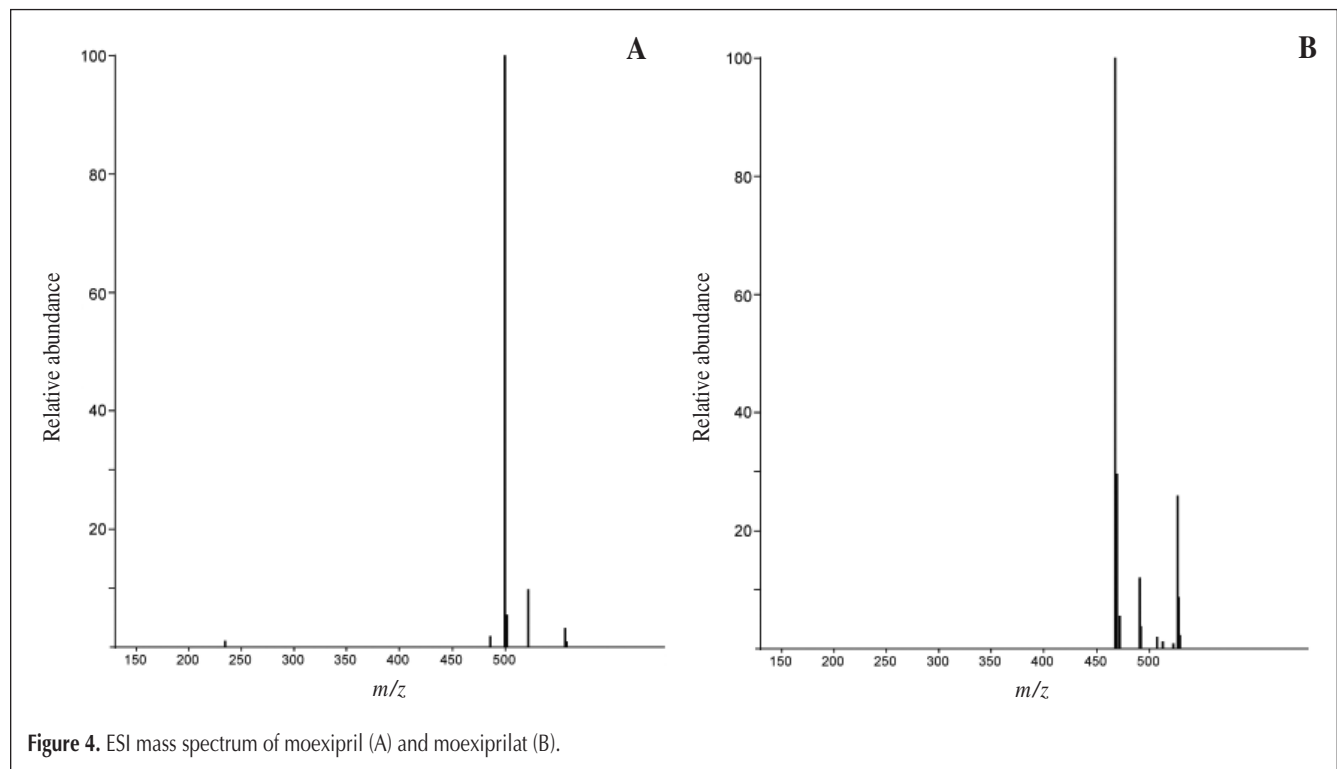
HPLC separation for MS was carried out using a 12-cm × 4-mm stainless steel column packed with 5  $\mu$ m Eurospher-100 C18 endcapped particles (Knauer, Berlin, Germany). The column temperature was held at 40°C. The mobile phase was acetonitrile-water (6:1) and also contained 0.1% formic acid.

A model HPLC-UV-MS 1100 instrument of Hewlett-Packard (Agilent, Waldbronn, Germany) was used for the measurements. The system contained a high-pressure gradient pump and a diode-array detector. The MS was used in positive atmospheric pressure chemical ionization mode. Injection volumes were either 1  $\mu$ L (for the moexipril standard sample) or 25  $\mu$ L (for the other samples). The vaporizer temperature, drying gas flow rate, and nebulizer pressure were 350°C, 5 L/min, and 40 psig, respectively. The level of moexipril and moexiprilat was monitored at 499 and 471 amu.

**Table II. Metabolism of Moexipril to Moexiprilat in Rat Liver Microsomal Preparations\***

Compounds	Retention time (min)	Samples					
		R1/0	R1/30	R2/0	R2/30	R3/0	R3/30
		Composition (%) <sup>†</sup>					
Moexipril	6.4	100	100	94.9	40.0	95.8	50.0
Moexiprilat	4.4	–	–	5.1	66.0	4.2	50.0

\* Abbreviations: rat liver microsomal preparation (R), test compound-incubation buffer-NADPH (R1), test compound-incubation buffer-rat liver microsomal preparation (R2), and test compound-incubation buffer-NADPH-rat liver microsomal preparation (R3) (0 = 0 min and /30 = 30 min).  
<sup>†</sup> Composition percentages are based on the UV peak areas of moexipril and moexiprilat, supposing identical UV response factors at the given wavelength.



**Figure 4.** ESI mass spectrum of moexipril (A) and moexiprilat (B).

**Table III. Metabolism of Moexipril to Moexiprilat in Human Microsomal Preparations\***

Compounds	Retention time (min)	Samples			
		H2/0	H2/30	H3/0	H3/30
Moexipril	6.4	100	93.8	98.5	97.1
Moexiprilat	4.4	–	6.2	1.5	2.9

\* Abbreviations: human liver microsomal preparation (H), test compound–incubation buffer–human liver microsomal preparation (H2), and test compound–incubation buffer–NADPH–human liver microsomal preparation (H3) (0 = 0 min and /30 = 30 min).

† Composition percentages are based on the UV peak areas of moexipril and moexiprilat, supposing identical UV response factors at the given wavelength.

## Results and Discussion

HPLC of the standard moexipril gave a single peak (Figure 2) eluted at 6.39 min. The experimental findings indicate that a rat liver microsomal preparation metabolizes approximately half of the parent compound in 30 min (Table II, samples R2/30 and R3/30). The HPLC chromatogram of sample R2/30 is shown in Figure 3.

The peak of moexipril eluted at 6.39 min, just as the moexipril standard. Its mass spectrum was also identical to that of the standard moexipril (not shown here). Another peak was eluted at 4.39 min; it was identified as moexiprilat, indicating the molecular weight to be 470. The mass spectra of standard moexipril and that of moexiprilat (from the microsomal preparation) are shown in Figures 4A and 4B. The definite decrease in the retention time mirrors an essential decrease of lipophilicity during the metabolic process. The applied gradient elution method made it possible to separate moexipril and moexiprilat from each other, and also from the buffer components detected at a retention time of 1.6 min. In the incubated mixtures the transesterified derivative of moexipril (a methyl ester of moexiprilat, Figure 1C) can also be detected at 5.98 min in the UV chromatogram. The signal-to-noise ratio was only 4.5. The ESI mass spectrum (not shown here) of the methyl ester gave the atomic mass unit of moexipril methyl ester as 484.

Table III gives data for the *in vitro* metabolism of moexipril using human liver microsomal preparation. In this case, moexipril metabolized to moexiprilat to a much lesser degree as compared with the rat liver microsome (Table II).

Both moexipril and moexiprilat could be detected in the urine samples of rats, 4 h after administration of moexipril. The detection could be done using UV absorbance at either 230 nm or 282 nm; however, an odd peak was present even in the control urine that interfered with the moexipril peak. It is the reason that selected ion monitoring [(M+1) at 499 and 471 amu] should be preferred to UV absorbance when moexipril and moexiprilat from samples of *in vivo* experiments are determined. Following oral administration, only 13% of the dose was eliminated in urine (2% moexipril and 11% moexiprilat).

## Conclusion

A simple LC–MS method was developed for the reliable detection of moexipril and moexiprilat in samples metabolized *in vitro* using rat and human liver microsomal preparations. The signal-to-noise ratio was well over the required 10 (47 and 78 for the moexiprilate and moexipril peaks, respectively) for the quantitative evaluation.

Weak metabolic stability was found when the moexipril was subjected to *in vitro* metabolism using rat liver microsomal preparation (intense NADPH-free metabolism). Microsomal treatment yields a few metabolites, and the methods can preferentially be used to scout the metabolites of *in vivo* treatments. HPLC–UV serves for quantitative evaluation of microsomal metabolism, and the identification of peaks is done on the basis of their mass spectra. The HPLC–MS–single ion monitoring method can be used for the determination of moexipril and moexiprilat from samples of *in vivo* experiments.

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