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Quantitative Determination of the Angiotensin-converting Enzyme Inhibitor Lisinopril in Human Plasma by Stable Isotope Dilution Gas Chromatography/Negative Ion Chemical Ionization Mass Spectrometry

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A simple, highly accurate and precise method for the quantitative measurement of the angiotensin-converting enzyme inhibitor lisinopril in human plasma is presented. The assay is based on gas chromatography/negative ion chemical ionization mass spectrometry. The preparation of stable isotope labelled lisinopril for use as an internal standard is described. The method involves solid phase extraction on C18 sorbent and derivatization to the methyl diester–trifluoroacetamide derivatives. The detection limit was found to be 50 pg and a lower limit of quantification was reached down to 0.5 ng/mL plasma. © **1998 John Wiley & Sons, Ltd.**

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Inhibitors of angiotensin-converting enzyme (ACE) have added a new dimension to the treatment of hypertension and congestive heart failure. Unlike its alanine analog enalapril, lisinopril (*N*-[1-carboxy-3-phenylpropyl-L-lysyl]proline) is not administered as a monoester prodrug, but used as the free dicarboxylic acid diamine in daily dosages of 10–80 mg.[1] Measurement of this drug in biological fluids is still a challenging task. Due to its amphoteric nature, extraction of the compound from plasma is difficult, and the low serum concentrations encountered (0.5–75 ng/mL) during routine clinical use require sensitive detection methods. There are not many methods described in the literature for the analytical determination of the drug. Pharmacokinetic data were exclusively obtained either indirectly by measurement of the ACE activity in the presence of lisinopril[2] or using an in-house radioimmunoassay[3–5] or fluoroimmunoassay[6,7] not readily available for all researchers. Some chromatographic methods have been developed for detection of the drug, but were only used in bulk drug analysis and for analysis of solid dosages formulations.[8–11] Recently, an assay using high-performance liquid chromatography was described and used for the measurement of lisinopril in urine.[12] The lower limit of quantitation, however, was reported to be 0.5 μg/mL, a concentration far beyond the requirements for pharmacokinetic plasma level determinations. We have recently elaborated methods for the quantitative measurement of other ACE inhibitors, captopril[13] and enalaprilate,[14] using negative ion chemical ionization mass spectrometry (NICI) and stable isotope labelled internal standards. Thereby the major goal was to enhance precision and specificity by the kind of standardization used and to employ the most sensitive detection mode available. In the case of lisinopril, application of this technique is seriously hampered by the fact that a suitable labelled lisinopril is not available, and the application of gas chromatography (GC) to the analysis of this highly thermolabile and ionic compound is more than doubtful.

It was therefore the aim of this study to provide an accurate and sensitive method for trace analysis of lisinopril in human plasma that can serve as a tool for pharmacokinetic investigations.

EXPERIMENTAL

Materials

Lisinopril was purchased from Sigma, Vienna. Diazomethane was generated from DIAZALD reagent, obtained through Aldrich, Vienna. Trifluoroacetic anhydride (TFAA) was also supplied by Aldrich, Vienna. Bond Elut C18 extraction cartridges were purchased from Varian, Vienna. Kiesegel 60, as well as all other solvents and reagents of analytical grade were from Merck, Darmstadt, FRG.

Gas chromatography/mass spectrometry

A CE 8000 Top gas chromatograph (GC) coupled to a Finnigan Voyager quadrupole mass spectrometer (MS) (ThermoQuest, Vienna) was used. The GC was equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from ThermoQuest. The injector was operated in the splitless mode at

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280 °C. Helium was used as a carrier gas. Initial column temperature was 150 °C for 1 min, followed by an increase of 40 °C per min to 320 °C followed by an isothermal hold until elution was complete. The mass spectrometer transfer line was kept at 325 °C. Negative ion chemical ionization was performed with methane as a moderating gas at an electron energy of 70 eV and an emission current of 0.135 A.

Derivatization

Methyl esters were formed with diazomethane (saturated solution in diethyl ether/methanol, 9:1, v/v) for 20 min at room temperature. Prior to addition of the reagent, the sample was dissolved in a small amount (50 μL) of methanol. Trifluoroacetamides (TFA) of the lisinopril methyl ester derivative were synthesized by reaction with 100 μL ethyl acetate and 50 μL TFAA at room temperature for 15 min.

Preparation of ^{18}O-labelled lisinopril

Lisinopril.2H_2O (approx. 10 mg) was dissolved in 400 μL of $H_2{}^{18}O$ (97,8%) and 10 μL of fuming HCl were added. The screw-capped vial was flushed with nitrogen, closed and kept at 75 °C for 2 weeks. After cooling, isotope distribution of the product was checked by GC/MS.

Plasma sample preparation

50 μL of a solution of the internal standard $^{18}O_4$-lisinopril (50 ng/50 μL methanol) were added to 1 mL of plasma, mixed throughly and diluted with 2 mL of 0.1 M HCl. The sample was applied to a Bond Elut C18 cartridge (200 mg, 3 cm) which had been preconditioned with methanol (2 × 2 mL) and 0.1 M HCl (3 × 3 mL). The adsorbed sample was washed with 0.1 M HCl (3 × 3 mL) and dichloromethane (3 × 3 mL). Elution was accomplished with 2 mL of methanol. The solvent was evaporated under nitrogen at 50 °C and the methyl diester prepared as described above. After evaporation of the reagent/solvent, the dry residue was trifluoroacetylated (see above). The samples were found to be sufficiently pure for analysis by GC/NICI-MS. After derivatization the dry residue was reconstituted in 70 μL of ethyl acetate, transferred to autosampler vials and stored at −20 °C until analysis. 6 μL were subjected to GC/NICI-MS measurement.

If a higher degree of sample purity is required, additional purification of the final derivative can be accomplished as follows: After formation of trifluoroacetamides, the dry residue is dissolved in 1 mL of ethyl acetate and applied onto a short column of Kieselgel 60 (300 mg), filled into a disposable pipet tip or luer-fitted column, which has been preconditioned by rinsing with 1 mL of ethyl acetate. The eluent (filtrate) is collected, together with an additional wash of 1 mL ethyl acetate, in a vacuum manifold by suction. The combined filtrates are dried under nitrogen, dissolved in 70 μL ethyl acetate and subjected to GC/MS analysis.

Analytical method validation

Calibration graphs were established in the range of 0.391 ng/mL plasma to 100 ng/mL plasma. The detection limit was estimated by derivatizing 1 μg of pure lisinopril sample and injecting diluted aliquots into the GC/MS system. Inter-assay variation was determined at 4 ng/mL plasma and 50 ng/mL plasma. Intra-assay precision was calculated at 4 ng/mL plasma and 17 ng/mL plasma, respectively. Freeze–thaw stability was confirmed by immediate analysis and after three freeze–thaw cycles.

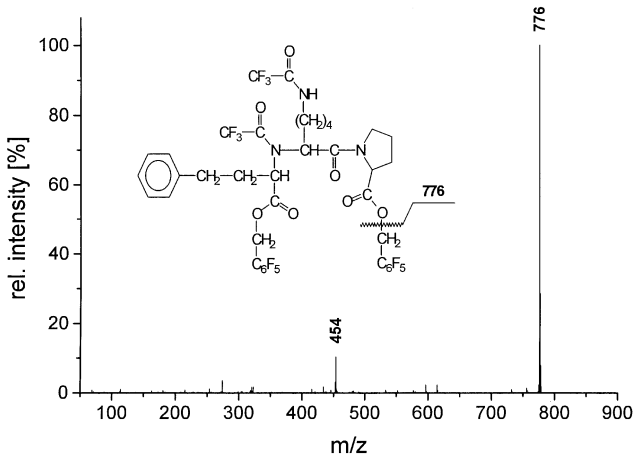

Figure 1. NICI mass spectrum of lisinopril PFB diester-bis(TFA) derivative.

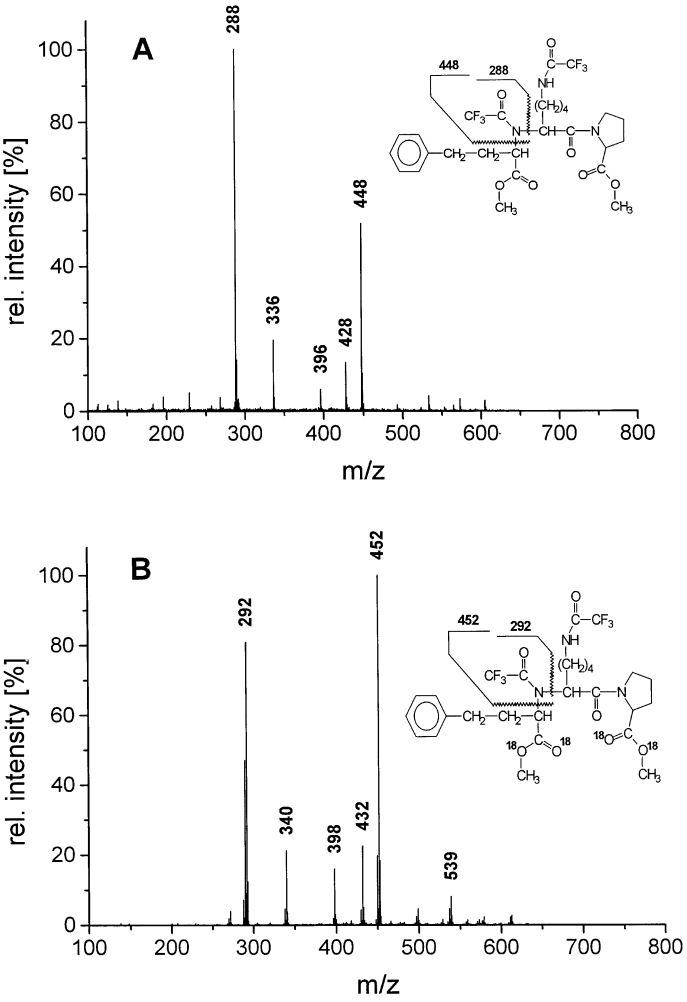

Figure 2. NICI mass spectra of native (a) and $^{18}O_4$-labeled (b) lisinopril dimethyl ester-bis(TFA) derivatives. Stable isotope labelling and derivatization were carried out as described under methods.

Table 1. Relative isotopic abundances of lisinopril after the ^{18}O-labelling procedure described under methods. The isotopic composition was determined by GC/NICI-MS of the dimethyl ester–bis(TFA) derivative, for the fragment ion at *m/z* 448

m/z	Isotopes	[%]
448	$^{16}O_2$	1.08
450	$^{16}O^{18}O$	15.07
452	$^{18}O_2$	83.85
Total		**100.00**

RESULTS AND DISCUSSION

Derivatization

Several derivatives of lisinopril have been prepared and checked for their potential use in quantitative GC/MS. As described earlier for enalaprilate[14] and captopril,[13] pentafluorobenzyl (PFB) ester derivatives have been proved to exhibit excellent electron capture response due to the formation of resonance-stabilized fragments under chemical ionization conditions. Thus, our first attempts were directed towards potential use of this derivative for lisinopril analysis. The NICI mass spectrum of lisinopril PFB diester-bis(TFA) derivative is shown in Fig. 1. As expected, there is excellent fragmentation behaviour for quantitative analytical applications since the monocarboxylate anion at *m/z* 776 is strikingly predominant. Despite of these ideal mass spectrometric properties the derivative could not be used. Derivatization with PFB-Br and diisopropyl ethylamine or potassium carbonate, as applied successfully to captopril and enalaprilate, leads to concomittant alkylation of the primary amine, yielding a mixture of more or less alkylated products with improper volatilities for GC/MS analysis. Trifluoroacetylation prior to the alkylation was not possible since rapid sample degradation occurred, presumably due to mixed anhydride formation and polymerization reactions. Thus, keeping electron capture response and volatility requirements in mind, acceptable results were obtained with the dimethyl ester-bis(TFA) derivative, whose NICI mass spectrum is given in Fig. 2(a). The mass spectrum is dominated by two fragment ions arising from cleavage of N-C bonds at one single trifluoroacetamide nitrogen. Thus, loss of the [1-methoxycarbonyl-3-phenyl]-propyl moiety yields the base peak at *m/z* 448, whereas cleavage of the other in-chain C-N bond produces the (*N*-[1-methoxycarbonyl-3-phenyl]propyl)trifluoroacetyl fragment at *m/z* 288. Because of the higher relative abundance and higher fragment ion mass, *m/z* 448 was chosen as the diagnostic fragment ion for quantitative measurements.

Preparation of ^{18}O-labelled lisinopril

In Fig. 2(b), the NICI mass spectrum of ^{18}O-labelled lisinopril dimethyl ester bis(TFA) is presented. Acid-catalysed ^{18}O-exchange reaction resulted in formation of a product suitable for internal standardization. The isotopic distribution of the compounds is shown in Table 1. The ^{18}O-labelling procedure expectedly leads to a fourfold labelled product by exchanging the oxygen atoms at both carboxylic acid functions. It is obvious for the two most abundant fragment ions that they carry only one of the two carboxylates and hence can only bear a twofold ^{18}O-label. In the case of the used fragment ion at *m/z* 448 the acid function of the proline moiety is shifted to *m/z* 452. Methyl ester formation with diazomethane proceeded smoothly and did not alter the isotopic composition. It is an ultimate criterion for the use as an internal standard in quantitative measurements, that the acquired isotope label remains stable throughout all procedures associated with sample preparation, clean-up and derivatization. In the case of $^{18}O_4$-lisinopril, addition of the standard to human plasma, either with or without added 0.1 M HCl, did not evoke any changes of the isotopic pattern.

Similarly, a plasma sample spiked with $^{18}O_4$-lisinopril retained the full label of the standard after 24 hours at room temperature. Thus, lisinopril is obviously a poor substrate for plasma esterases, which in certain cases may cause isotope back-exchange.[15] Using the $^{18}O_2$-label at *m/z* 452 as a standard for calibration, a minute amount of unlabeled lisinopril present (approx. 1%, see Table 1) has to be taken

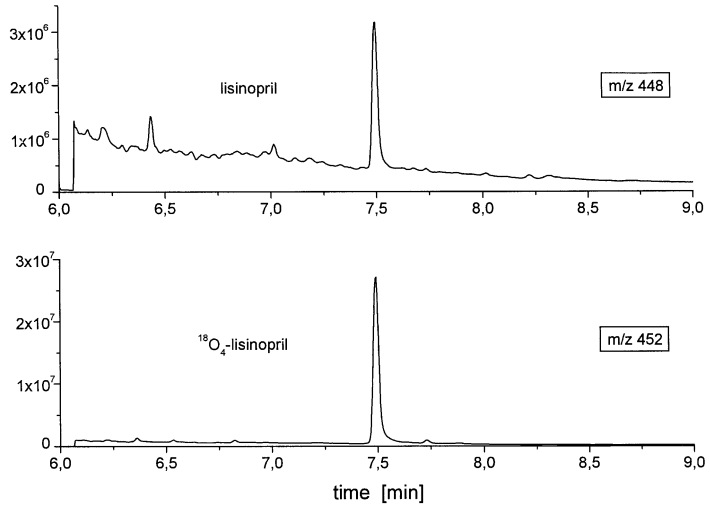

Figure 3. Typical SIR mass chromatogram of lisinopril dimethyl ester-bis(TFA) derivative obtained after analysis of a spiked plasma sample. The amount analysed corresponds to 4 ng/mL plasma.

into consideration. This is, however, easily compensated by the calibration curves established.

Sample preparation

Yield of solid phase extraction by Bond Elut C18 cartridges was 72%, as checked by addition of the internal standard to the spiked plasma samples after the extraction procedure. Derivatization reactions with diazomethane and TFA occurred quantitatively. The analytical procedure allows the use of polypropylene tubes throughout the sample preparation sequence, thus reducing the risk of cross contamination and adsorption on polar glass walls. The optional cleaning step of the final derivative on Kieselgel 60 provides certain flexibility in case matrices other than plasma are to be analysed.

A typical single-ion recording (SIR) mass chromatogram obtained after analysis of lisinopril in spiked plasma is given in Fig. 3.

Analytical method validation

The calibration graphs established were linear within the tested range of 0.391 ng/mL plasma and 100 ng/mL plasma ($r = 0.9987$). The limit of detection of the mass spectrometer was found to be 50 pg (actually injected) at a signal to noise ratio of at least 4:1. The lower limit of detection for the complete procedure was 500 pg/mL plasma, estimated by analyzing spiked plasma samples. The coefficients of inter-assay variation were estimated to be 4.01% (mean = 3.996, standard error of the mean (sem) = 0.159) at 4 ng/mL plasma, and 1.72% (mean = 48.955, sem = 0.842) at 50 ng/mL plasma ($n = 5$). The coefficients of intra-assay variations were found to be 1.08% (mean = 4.254, sem = 0.046) at 4 ng/mL plasma and 0.32% (mean = 17.105, sem = 0.054) at 17 ng/mL plasma ($n = 5$). Freeze–thaw stability tests (3 freeze–thaw cycles, $n = 3$) at 6 ng/mL plasma and 45 ng/mL plasma produced values of 99.4% and 96.9% of the original value, respectively. To our knowledge the method described is at present the only chromatographic assay suitable for the analysis of lisinopril in human plasma. The detection limit allows potential application to pharmacokinetic analysis, and a separate study is planned to conduct bioavailability measurements with this method.

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REFERENCES

1. S. G. Lancaster and P. A. Todd, *Drugs* **3,** 646 (1988).
2. J. A. Millar, F. H. M. Derkx, K. Mclean and J. L. Reid, *Br. J. Clin. Pharmacol.* **14,** 347 (1982).
3. P. J. Worland and B. Jarrot, *J. Pharm. Sci.* **75,** 512 (1986).
4. J. H. Lin, I. Chen, E. H. Ulm and D. E. Duggan, *Am. J. Pharmacol. Exp. Ther.* **16,** 392 (1988).
5. Y. Sun and F. A. O. Mendelsohn, *J. Cardiovasc. Pharmacol.* **18,** 478 (1991).
6. K. Shepley, M. L. Rocci, H. Patrick and P. Mojaverian, *J. Pharm. Biomed. Anal.* **6,** 241 (1988).
7. A. S. Yuan and J. D. Gilbert, *J. Pharm. Biomed. Anal.* **14,** 773 (1996).
8. D. Bonazzi, R. Gotti, V. Andrisano and V. Cavrini, *J. Pharm. Biomed. Anal.* **16,** 431 (1997).
9. R. T Sane, G. R. Valiyare, U. M. Deshmukh, S. R. Singh and R. Sodhi, *Indian Drugs* **29,** 558 (1992).
10. A. B. Avadhanulu and A. R. R. Pantulu, *Indian Drugs* **30,** 646 (1993).
11. X. Z. Qin, D. S. T. Nguyen and D. P. Ip, *J. Liquid Chromatogr.* **16,** 3713 (1993).
12. Y. C. Wong and B. G. Charles, *J. Chromatogr. B* **673,** 306 (1995).
13. H. J. Leis, M. Leis, W. Welz and E. Malle, *J. Chromatogr.* **529,** 299 (1990).
14. H. J. Leis, H. Gleispach and W. Windischhofer, *J. Mass Spectrom.* **30,** 1447 (1995).
15. H. J. Leis, E. Hohenester, E. Malle, B. Mayer and H. Gleispach, *Biomed. Environ. Mass Spectrom.* **14,** 617 (1987).

