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Characterization of impurities in the bulk drug lisinopril by liquid chromatography/ion trap spectrometry*

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Abstract: Two trace impurities in the bulk drug lisinopril were detected by means of high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) with a simple and sensitive method suitable for HPLC/MS^n analysis. The fragmentation behavior of lisinopril and the impurities was investigated, and two unknown impurities were elucidated as 2-(6-amino-1-(1-carboxyethylamino)-1-oxohexan-2-ylamino)-4-phenylbutanoic acid and 6-amino-2-(1-carboxy-3-phenylpropylamino)-hexanoic acid on the basis of the multi-stage mass spectrometry and exact mass evidence. The proposed structures of the two unknown impurities were further confirmed by nuclear magnetic resonance (NMR) experiments after preparative isolation.

Key words: Lisinopril, Impurities, High-performance liquid chromatography (HPLC), Multi-stage mass spectrometry (MS^n)
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INTRODUCTION

Lisinopril, (1-[N-[(S)-1-carboxy-3-phenylpropyl]-L-lysyl]-L-proline dehydrate), is an angiotensin-converting enzyme (ACE) inhibitor, used for the treatment of hypertension, heart failure, and acute myocardial infarction (Eveson *et al*., 2007; Wang *et al*., 2006). A wide variety of separation and detection techniques have been applied to the analysis of lisinopril, such as gas chromatography coupled with mass spectrometry (GC/MS) (Leis *et al*., 1998; 1999), high-performance liquid chromatography (HPLC) (Wong and Charles, 1995; Beasley *et al*., 2005), and high-performance liquid chromatography coupled with mass spectrometry (HPLC/MS) (Shinde *et al*., 2007; Pei *et al*., 2006). The impurity profile of a drug substance is critical to its safety assessment and manufacturing process. For safety reasons, the impurities that exceed 0.1% in a drug must be identified prior to clinical trials (International Conference on Harmonization, 2003). This paper presents the identification of unknown impurities in trace level by on-line and off-line multi-stage mass spectrometry (MS^n) analyses. To ascertain the structures of the unknown impurities clearly, 1D and 2D nuclear magnetic resonance (NMR) techniques were applied after preparative isolation.

MATERIALS AND METHODS

Materials

The sample of lisinopril was obtained from Jianyuan Inc. (Hangzhou, China). Ammonium acetate and glacial acetic acid of analytical grade were purchased from Guangzhou Chemical Co. (Guangzhou, China) and Hangzhou Chemical Co. (Hangzhou, China), respectively. Acetonitrile of HPLC grade was obtained from Merck Co. (Darmstadt, Germany), and water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA).

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Analytical HPLC

Analytical HPLC was performed on an Agilent 1100 series HPLC equipped with a G1312A Binary pump and a G1314A variable wave detector (VWD). A model 7725 injection valve fitted with a 20 μl sample loop was used, along with an Agilent ChemStation data system. The separation was achieved on a Waters symmetry C18 column (4.6 mm×150 mm, 5 μm). The mobile phase consisted of a mixture of 5% (v/v) acetonitrile and 95% (v/v) aqueous buffer. The aqueous buffer was prepared by dissolving 20 mmol/L ammonium acetate in purified water adjusted with glacial acetic acid to pH 4.5. The flow rate was set at 1.0 ml/min, and the effluent was monitored at 210 nm.

Preparative HPLC

Preparation was conducted with a Waters 600 semi-preparative HPLC system on a Zorbax C18 column (9.4 mm×250 mm, 5 μm, Agilent, USA). The mobile phase consisted of methanol and water (5:95, v/v) at flow rate of 3.0 ml/min.

MS

HPLC/ESI-MS^n analyses were carried out on the Agilent HPLC system described above combined with a Bruker Esquire 3000^{plus} ion trap mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) which is equipped with an electrospray ionization (ESI) source. Instrument control and data acquisition were performed with Esquire 5.0 software. The samples were infused into the source chamber from the HPLC system with a T-junction delivering approximately 1/3 of the flow to the mass spectrometer. The ion source temperature was 250 °C and the ESI needle voltage was always set at 4.0 kV. Nitrogen was used as drying gas at a flow rate of 10 ml/min and the nebulizer gas at a back-pressure of 2.0685×10^5 Pa. Helium was introduced into the ion trap with an estimated pressure of 6.3×10^{-9} Pa to improve trapping efficiency, and act as the collision gas for both on-line and off-line MS^n experiments. The collision energy was set between 0.60 and 0.70 V to maximize the ion current in the spectra. The accurate MS experiments were performed on an Apex III 7.0 Tesla FTICR mass spectrometer (Bruker, Daltonic, Billerica, MA, USA) combined with an ESI source in the positive ion mode. Solution introduction was accomplished by using a Cole-Parmer syringe pump at a rate of 3 μl/min. Accurate mass measurements were performed using NaI as an external calibrant. XMASS software version 6.1.1 was used for instrument control, data acquisition and processing. The spray voltage was 4.5 kV. The temperature of the capillary was 250 °C. Nebulizing gas and drying gas (N_2) were set 2.41325×10^5 Pa and 30 units, respectively. Products ions were generated in the collision cell and argon was used as the collision gas.

NMR

NMR spectra were recorded with a Bruker Advance DMX 500 instrument (Bruker, Billerica, MA, USA) with a QNP probe head at ambient temperature. The data were acquired on Silicons Graphics O2 workstations by using XWINNMR version 2.1 (Bruker Analytik, GmbH, Germany).

RESULTS AND DISCUSSION

HPLC/MS analysis

During the routine impurity profiling of bulk lisinopril, two unknown trace impurities were detected with molecular masses of 308 and 379 by their positive ESI mass spectra (Fig.1).

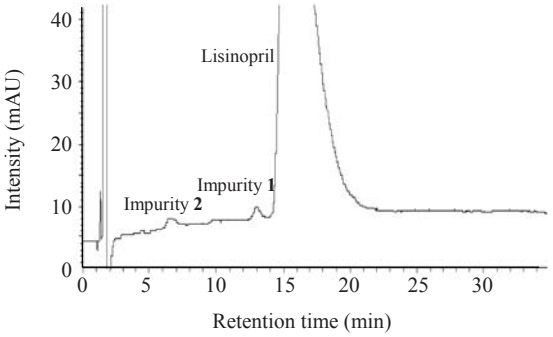

Fig.1 HPLC chromatogram of the bulk lisinopril

Identification of the unknown impurities

1. Impurity **1**

Impurity **1** had the molecule mass of 379. The collision-induced dissociation (CID) spectrum for the protonated impurity **1** was dominated by the ion at *m*/*z* 291 (Fig.2a), which was also observed in the product ion spectrum (Fig.2b) of protonated lisinopril. The MS^3 spectrum of the *m*/*z* 291 product ion of

impurity **1** was indistinguishable from that of lisinopril (Figs.3a and 3b). The dissociation spectra showed that the *m*/*z* 291 ion gave rise to a major dissociation product at *m*/*z* 245, which corresponded to the loss of HCOOH. No other significant fragmentation channels were observed. The same structure of the two ions at *m*/*z* 291 was further supported by the fragmentation experiments of the ion at *m*/*z* 245. When the respective ion of *m*/*z* 245 was further collisionally activated under the same condition, the product ion spectra (Figs.4a and 4b) were also very similar.

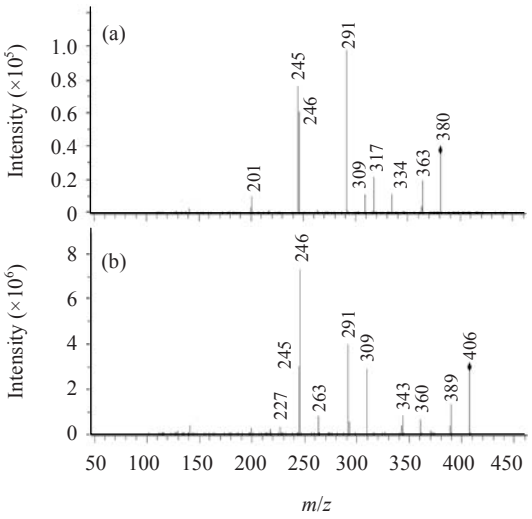

Fig.2 ESI/MS^2 spectra of protonated impurity 1 (a) and protonated lisinopril (b)

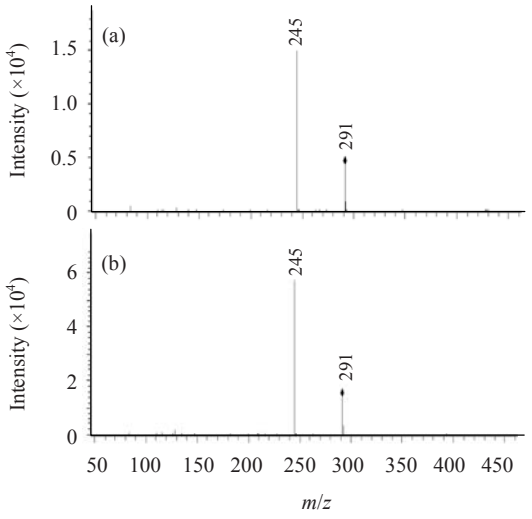

Fig.3 ESI/MS^3 spectra of *m*/*z* 291 from protonated impurity 1 (a) and protonated lisinopril (b)

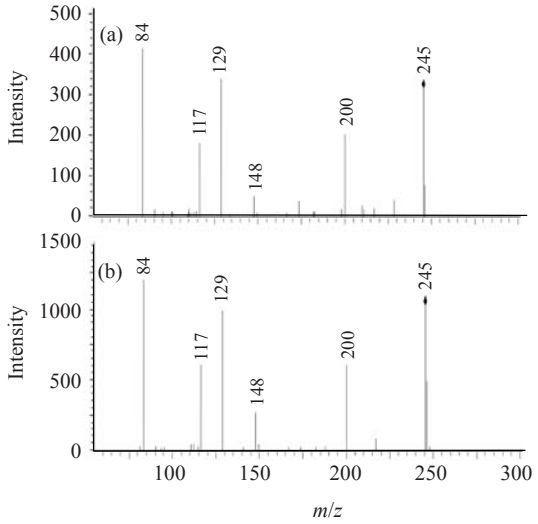

Fig.4 ESI/MS^4 spectra of *m*/*z* 245 from protonated impurity 1 (a) and protonated lisinopril (b)

Since the impurity was generated as a by-product when the bulk was synthesized, the results above suggest that the impurity **1** probably shared some common structural moieties with lisinopril. A detailed study of the fragmentation patterns of the parent drug served as a template to elucidate the structures of unknown impurities by comparison of their fragmentation pathways and neutral losses (Lee and Kerns, 1999). Analysis of product ion spectra of protonated lisinopril clearly indicated the precursor ion underwent a distinct loss of proline to form an abundant dipeptide N-terminal $\mathbf{b}_1$ ion at *m*/*z* 291, with subsequent loss of HCOOH to form the ion at *m*/*z* 245 (Roepstorff and Fohlman, 1984). The product ion at *m*/*z* 263 corresponded to the dipeptide N-terminal $\mathbf{a}_1$ ion with the combined loss of proline and CO, while ion at *m*/*z* 246 was formed from subsequent loss of NH_3. The product ion at *m*/*z* 309 was formed through a rearrangement (Florêncio *et al*., 1998; Hiserodt *et al*., 2007), with the elimination of dihydropyrrole and CO, and could be used to characterize the 6-amino-2-(1-carboxy-3-phenylpropyl-amino)-hexanoic acid structure. The product ion at *m*/*z* 227 was formed by the neutral loss of 2-amino-4-phenylbutanoic acid (179 Da). The CID spectrum of protonated lisinopril was similar to those obtained in previous studies (Burinsky and Sides, 2004; Pei *et al*., 2006).

The ion at *m*/*z* 309 from impurity **1** also suggested the probable structure of 6-amino-2-(1-carboxy-3-phenylpropylamino)-hexanoic acid. The

product ion at *m*/*z* 201 would arise from the same formation mechanism (loss of 2-amino-4-phenylbutanoic acid, 179 Da) as that responsible for the product ion at *m*/*z* 227 from lisinopril. The molecular formula for the impurity **1** ($C_{19}H_{29}N_3O_5$) was confirmed from the accurate mass (*m*/*z*) of the precursor ion in positive mode. These data suggest that the most plausible structure of impurity **1** would be 2-(6-amino-1-(1-carboxyethylamino)-1-oxohexan-2-ylamino)-4-phenylbutanoic acid (Fig.5), which was a by-product of the lisinopril synthesis. The proposed fragmentation mechanism for impurity **1** is shown in Fig.6. To confirm the fragmentation mechanism, we further studied the exact mass of these fragments using FT-ICRMS (fourier transform ion cyclotron resonance mass spectrometry) (Table 1). These mass values were determined in triplicate and were less than 2×10^{-6} from the calculated mass.

2. Impurity **2**

Impurity **2** exhibited an abundant ion at *m*/*z* 309 in the positive ion mass spectrum. The CID spectra for the protonated lisinopril also showed a fragment ion at *m*/*z* 309, a rearrangement ion which could be used to characterize the 6-amino-2-(1-carboxy-3-phenylpropylamino)-hexanoic acid structure unit. The structural relationships were investigated further by comparing the MS^2 and MS^3 CID spectra for the

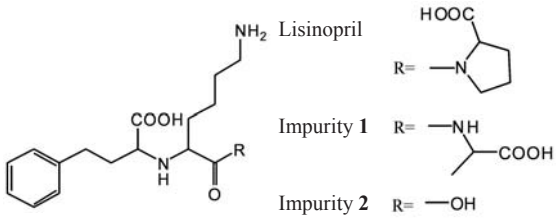

Fig.5 Structure of lisinopril and two unknown impurities

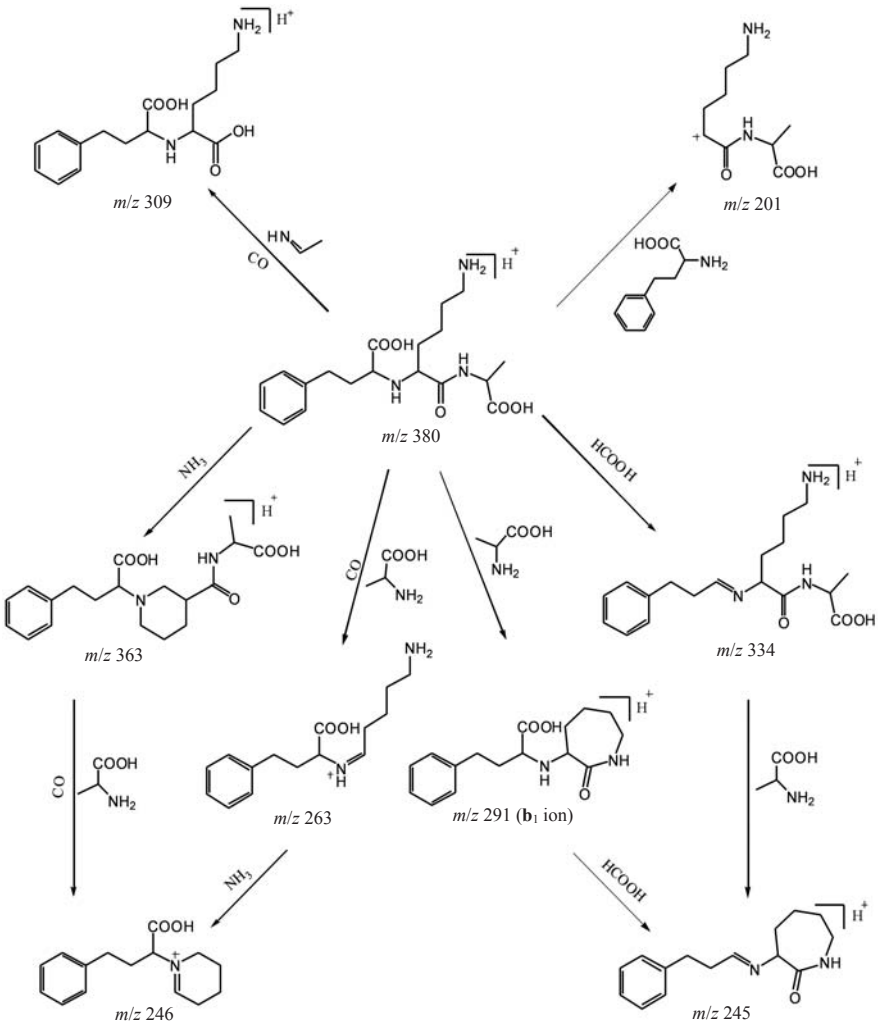

Fig.6 Proposed fragmentation pathway of impurity 1

Table 1 Summary of accurate mass measurements for the precursor and product ions of impurities

Precursor ions	Product ions	Measured mass	Calculated mass	Formula	Error ($\times10^{-6}$)
380 (Impurity **1**)	−	380.2181	380.2180	$C_{19}H_{30}N_3O_5^+$	0.26
	363	363.1920	363.1914	$C_{19}H_{27}N_2O_5^+$	1.65
	334	334.2128	334.2125	$C_{18}H_{28}N_3O_3^+$	0.90
	317	317.1859	317.1860	$C_{18}H_{25}N_2O_3^+$	−0.32
	309	309.1811	309.1809	$C_{16}H_{25}N_2O_4^+$	0.65
	291	291.1704	291.1703	$C_{16}H_{23}N_2O_3^+$	0.34
	246	246.1489	246.1489	$C_{15}H_{20}NO_2^+$	0
	245	245.1647	245.1648	$C_{15}H_{21}N_2O^+$	−0.41
	201	201.1234	201.1234	$C_9H_{17}N_2O_3^+$	0
309 (Impurity **2**)	—	309.1806	309.1809	$C_{16}H_{25}N_2O_4^+$	−0.97
	292	292.1542	292.1543	$C_{16}H_{22}NO_4^+$	−0.34
	291	291.1703	291.1703	$C_{16}H_{23}N_2O_3^+$	0
	246	246.1489	246.1489	$C_{15}H_{20}NO_2^+$	0
	245	245.1647	245.1648	$C_{15}H_{21}N_2O^+$	−0.41
	130	130.0863	130.0863	$C_6H_{12}NO_2^+$	0

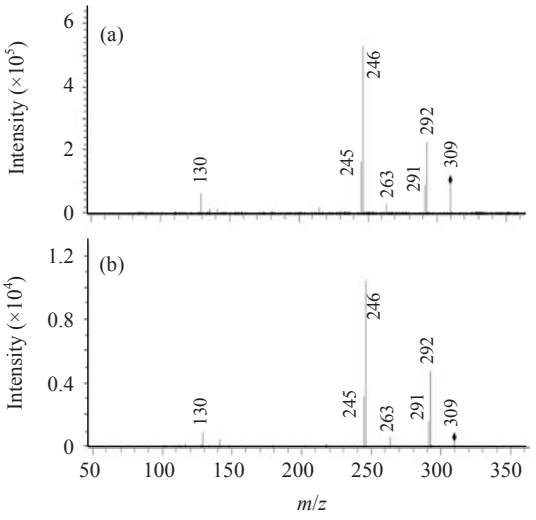

Fig.7 (a) ESI/MS^2 spectrum of *m*/*z* 309 from protonated impurity 2 and (b) ESI/MS^3 spectrum of *m*/*z* 309 from protonated lisinopril

protonated impurity **2** (Fig.7a) and the product ion of *m*/*z* 309 derived from the protonated lisinopril (Fig.7b). Analysis of the product ion spectrum of protonated impurity **2** clearly showed that its fragmentation pathway was indistinguishable from that of protonated lisinopril (*m*/*z* 309). The ion at *m*/*z* 292 suggested the loss of NH_3 from the ion of *m*/*z* 309, with subsequent loss of HCOOH to form the ion at *m*/*z* 246. The ion at *m*/*z* 291 was a $\mathbf{b}_1$ ion, which could lose HCOOH to form the fragment ion at *m*/*z* 245 (Pei *et al*., 2006). The ion at *m*/*z* 263 was thought to be formed by the direct elimination of HCOOH from *m*/*z* 309. Therefore, impurity **2** was proposed as 6-amino-2-(1-carboxy-3-phenylpropylamino)-hexanoic acid, which was further supported by off-line FT-ICRMS data (Table 1).

NMR analysis

The impurity **1** and impurity **2** were obtained by preparative chromatography and their structures were further confirmed by NMR experiments.

1. Selected data for impurity **1**

^{1}H-NMR (500 MHz, D_2O), δ: 1.26 (d, 3H), 1.48 (m, 2H), 1.64 (m, 2H), 1.91 (m, 2H), 2.10 (m, 2H), 2.69 (m, 2H), 2.96 (t, 2H), 3.36 (t, 1H), 3.83 (t, 1H), 4.10 (q, 1H), 7.26 (m, 5H); ^{13}C-NMR (125 MHz, D_2O), δ: 15.10, 19.27, 24.27, 27.96, 28.83, 30.14, 37.12, 49.48, 58.53, 59.83, 124.66~126.90 (5C), 138.51, 165.60, 171.05, 177.39.

2. Selected data for impurity **2**

^{1}H-NMR (500 MHz, D_2O), δ: 1.43 (m, 2H), 1.62 (m, 2H), 1.84 (m, 2H), 2.13 (m, 2H), 2.70 (m, 2H), 2.94 (t, 2H), 3.50 (t, 1H), 3.54 (t, 1H), 7.27 (m, 5H); ^{13}C-NMR (125 MHz, D_2O), δ: 19.59, 24.26, 27.66, 28.86, 30.31, 37.09, 60.63, 60.72, 124.58~126.89 (5C), 138.75, 171.41, 171.42.

CONCLUSION

An HPLC/MS^n method was developed for the identification of trace level impurities in the bulk drug lisinopril. In combination with the FT-ICRMS and NMR data, the structures of two unknown impurities were confirmed to be 2-(6-amino-1-(1-carboxyethyl-amino)-1-oxohexan-2-ylamino)-4-phenyl-butanoic acid and 6-amino-2-(1-carboxy-3-phenylpropylamino)-hexanoic acid.

References

Beasley, C.A., Shaw, J., Zhao, Z., Reed, R.A., 2005. Development and validation of a stability indicating HPLC method for determination of lisinopril, lisinopril degradation product and parabens in the lisinopril extemporaneous formulation. *J. Pharm. Biomed. Anal.*, **37**(3):559-567. [doi:10.1016/j.jpba.2004.11.021]

Burinsky, D.J., Sides, S.L., 2004. Mass spectral fragmentation reactions of angiotensin-converting enzyme (ACE) inhibitors. *J. Am. Soc. Mass Spectrom.*, **15**(9):1300-1314. [doi:10.1016/j.jasms.2004.05.010]

Eveson, D.J., Robinson, T.G., Potter, J.F., 2007. Lisinopril for the treatment of hypertension within the first 24 hours of acute ischemic stroke and follow-up. *Am. J. Hypertens.*, **20**(3):270-277. [doi:10.1016/j.amjhyper.2006.08.005]

Florêncio, M.H., Fernandez, M.T., Mira, M.L., Millar, A., Jennings, K.R., 1998. Electrospray mass spectrometry of angiotensin-converting enzyme inhibitors. *Rapid Commun. Mass Spectrom.*, **12**(23):1928-1932. [doi:10.1002/(SICI)1097-0231(19981215)12:23<1928::AID-RCM405>3.0.CO;2-W]

Hiserodt, R.D., Brown, S.M., Swijter, D.F.H., Hawkins, N., Mussinan, C.J., 2007. A study of $\mathbf{b}_1$+H_2O and $\mathbf{b}_1$-ions in the product ion spectra of dipeptides containing N-terminal basic amino acid residues. *J. Am. Soc. Mass Spectrom.*, **18**(8):1414-1422. [doi:10.1016/j.jasms.2007.04.018]

International Conference on Harmonization, 2003. ICH Harmonized Tripartite Guideline Topic Q3A(R): Impurities in New Drug Substances. Federal Register, US Department of Health and Human Services Food and Drug Administration, Vol. 68, p.6924-6925.

Lee, M.S., Kerns, E.H., 1999. LC/MS applications in drug development. *Mass Spectrom. Rev.*, **18**(3/4):187-279. [doi:10.1002/(SICI)1098-2787(1999)18:3/4<187::AID-MAS2>3.0.CO;2-K]

Leis, H.J., Fauler, G., Raspotnig, G., Windischhofer, W., 1998. Quantitative determination of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.*, **12**(21):1591-1594. [doi:10.1002/(SICI)1097-0231(19981115)12:21<1591::AID-RCM368>3.0.CO;2-C]

Leis, H.J., Fauler, G., Raspotnig, G., Windischhofer, W., 1999. An improved method for the measurement of the angiotensin-converting enzyme inhibitor lisinopril in human plasma by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.*, **13**(8):650-653. [doi:10.1002/(SICI)1097-0231(19990430)13:8<650::AID-RCM536>3.0.CO;2-X]

Pei, S., Wang, D., Sun, C., Pan, Y., 2006. Characterization of a novel trace-level impurity in lisinopril using multi-stage mass spectrometry. *Eur. J. Mass Spectrom.*, **12**(2):121-127. [doi:10.1255/ejms.801]

Roepstorff, P., Fohlman, J., 1984. Letter to the editors. *J. Biomed. Mass Sprctrom.*, **11**(11):601. [doi:10.1002/bms.1200111109]

Shinde, V., Trivedi, A., Upadhayay, P.R., Gupta, N.L., Kanase, D.G., Chikate, R., 2007. Identification of a new impurity in lisinopril. *J. Pharm. Biomed. Anal.*, **43**(1):381-386. [doi:10.1016/j.jpba.2006.06.046]

Wang, D.H., Pei, S.F., Zhou, M.H., Sun, C.R., Pan, Y.J., 2006. Characterization of a novel impurity in bulk drug of lisinopril by multidimensional NMR technique. *J. Zhejiang Univ. Sci. B*, **7**(4):310-313. [doi:10.1631/jzus.2006.B0310]

Wong, Y.C., Charles, B.G., 1995. Determination of the angiotensin-converting enzyme inhibitor lisinopril in urine using solid-phase extraction and reversed phase high-performance liquid chromatography. *J. Chromatogr. B.*, **673**(2):306-310. [doi:10.1016/0378-4347(95)00268-4]