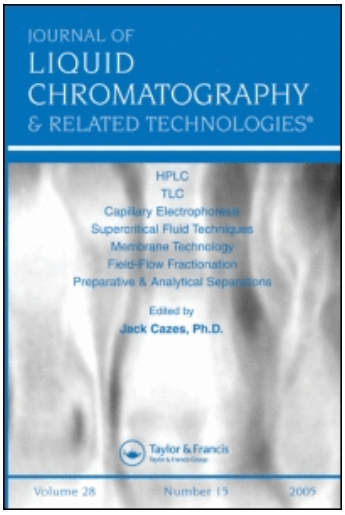

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Laila I. Abou-Basha[a]; Lobna F. Wahman[a]; Hassan Y. Aboul-Enein[b]
[a] National Organization for Drug Control and Research, Cairo, Egypt [b] Pharmaceutical Analysis Laboratory, Biological and Medical Research, Department (MBC 03-65), King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

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Simple HPLC Method for the Determination of Enalaprilat in Human Plasma

Laila I. Abou-Basha,[1] Lobna F. Wahman,[1] and Hassan Y. Aboul-Enein[2,*]

[1]National Organization for Drug Control and Research, Cairo, Egypt
[2]Pharmaceutical Analysis Laboratory, Biological and Medical Research, Department (MBC 03-65), King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

ABSTRACT

A simple and reliable isocratic reversed phase high performance liquid chromatography (HPLC) method for the determination of enalaprilat in human plasma is described. After plasma extraction, enalaprilat is analyzed using a μ Bondapak C18 column kept at 50°C. The mobile phase consists of 60% A (0.01 M KH_2PO_4, pH 4.0) and 40% B (80% of 50 : 50 $CH_3CN : CH_3OH$ + 20% A) v/v. Enalaprilat is monitored by UV detection at 205 nm. The method is quite simple and sufficiently sensitive, with a limit of quantitation of 5 ng/mL and limit of detection of 1 ng/mL;

*Correspondence: Hassan Y. Aboul-Enein, Pharmaceutical Analysis Laboratory, Biological and Medical Research, Department (MBC 03-65), King Faisal Specialist Hospital and Research Center, P.O. Box 3354, Riyadh 11211, Saudi Arabia; E-mail: enein@kfshrc.edu.sa.

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within day and between-days assays showed a relative standard deviation (RSD) below 15%.

Key Words: Enalaprilat; Angiotensin coverting enzyme inhibitor; HPLC; Plasma

INTRODUCTION

Enalapril, a pro-drug that requires de-esterification by the entrase enzymes in the hepatic system to provide an active metabolite enalaprilat; chemically known as 1-[N-[(S)-1-carboxyl-3-phenylpropyl]-*L*-adanyl]-*L*-proline,[1] an effective angiotensin converting enzyme (ACE) inhibitor,[2] is clinically used for the treatment of essential and renovascular hypertension.[3,4] Several methods has been reported in the literature for the analysis of enalaprilat in biological fluids including time-resolved fluoroimmunoassay,[5] and gas chromatography-mass spectrometry (GC-MS).[6,7] Worland and Jarrott described a radio-immunoassay for analysis of enalaprilat.[8] However, these methods requires expensive equipments and is also time consuming. This paper describes a simple, reliable, and rapid high performance liquid chromatographic assay for the analysis of enalaprilat in human plasma.

EXPERIMENTAL

Chemicals

Enalaprilat was kindly donated by Professor Hassan Y. Aboul-Enein. Vasotec® tablets containing 20 mg enalapril was obtained from Merck (West Point, PA). Potassium dihydrogen phosphate (E. Merck, Darmstadt, Germany), acetonitrile, methanol, and chloroform HPLC grade were purchased from BDH (Pool, England).

A single oral dose of 20 mg enalapril (Vasotec) was orally administered to 18 healthy male volunteers of an average age of 32.8 years. A written consent was signed by these volunteers for participation in this study, and approved by the ethics committee. A 5 mL of blood was withdrawn from these volunteers after one hour of enalapril administration. The blood was centrifuged to separate the plasma, which was kept at -20°C till the time of analysis.

Chromatography

The high performance liquid chromatography (HPLC) system consists of a quaternary SDS pump PU 4100 (Thermo Separation Products, USA), 7125

Model Injection, Philips Lambda Max 481 variable wave length detection set up at 205 nm and PU computing integrator.

The column used was a reversed-phase μ Bondapak C18 (30 cm × 3.9 mm i.d., particle size 10 μm) obtained from Waters Corporation (Millford, MA, USA).

Sample Preparation

Plasma (1 mL) was pipetted into a 15 mL glass extraction tubes and acidified with 1 M phosphoric acid. The samples were extracted with 7 mL of chloroform using a horizontal shaker for 15 min. After centrifugation at 1000 *g* for 5 min, the organic layer was separated in a clear tube. The remaining acidic phase was then re-extracted with 7 mL chloroform. The separated organic layer was added to the same tube and evaporated to dryness under a gentle stream of nitrogen. The residues were reconstituted in 500 μL of the mobile phase, and 50 μL was injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatograms

The chromatograms of enalaprilat from blank plasma spiked with enalaprilat and plasma from healthy volunteers dosed orally with 20 mg enalapril, are shown in Fig. 1. Enalaprilat has a retention time of 4.5 min under the chromatographic conditions described in the experimental section.

Linearity

The calibration curve of enalaprilat was constructed over the range of 10–80 ng/mL with a correlation coefficient 0.998 ($n = 6$). The lower limit of quantitation was 5 ng/mL.

Variability and Percentage Recovery

The intra-assay accuracy and precision were evaluated by analyzing six replicates of two different quality control levels, 5 ng/mL and 50 ng/mL enalaprilat, on the same day. The inter assay accuracy and precision were determined similarly, except that the analysis was carried out several times during a one week period.

The accuracy of the assay was calculated as the percentage deviation (DEV%) of the mean observed concentration from the nominal concentration of quality control levels. The precision was expressed as the relative standard

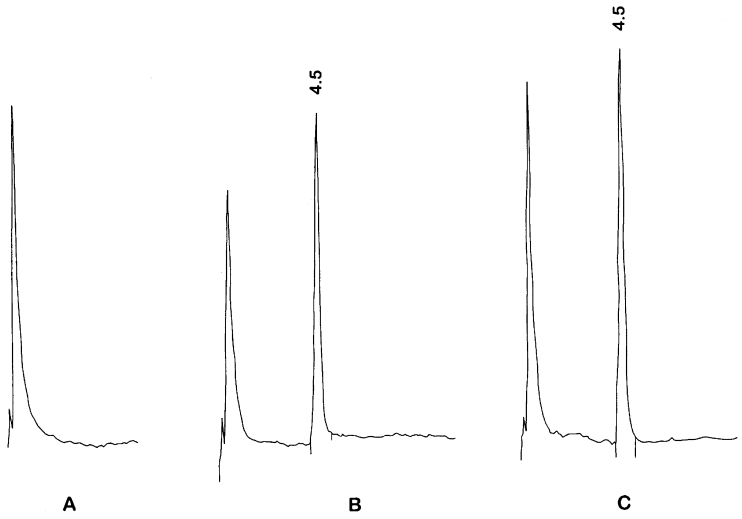

Figure 1. Chromatogram of: A. Blank Plasma, B. Blank plasma spiked with Enalaprilat (R_t 4.5 min), C. Plasma from drug dosed volunteer (20 mg enalapril, orally). Bondapak C18 (300 mm × 3.9 mm. 10μ particle size) preceded by guard column; mobile phase 60% A, 50% B. Flow rate: 1 mL/min, UV: 205 nm; sensitivity: 0.02 aufs: temp. 50°C; sample: 100 μL.

deviation (RSD%) of the observed concentration from the known concentration of quality levels. The results were shown in Table 1.

Recovery

Blank plasma is spiked by four concentrations 5, 10, 20, and 40 ng/mL. Plasma extraction is performed as described under sample preparation. The result is shown in Table 2.

Table 1. Accuracy and precision for enalaprilat assay.

	No	Mean	DEV%	RSD%
Intra-assay				
5	6	4.6	8	12.3
50	6	49	2	9.0
Inter-assay				
5	6	4.4	12	14.5
50	6	48	4	11.2

Table 2. Relative recovery of enalaprilat from plasma.

Enalaprilat spiked concentration (ng/mL)	Enalaprilat concentration found ($n = 6$)	Recovery (mean ± SD)%
5	3.4	68 ± 12.5
10	7.0	70 ± 10.5
20	14.6	73 ± 6.0
40	30.4	76 ± 4.5

CONCLUSIONS

The developed HPLC assay in this study is sensitive, rapid, simple, and reproducible, which makes it a potentially valuable tool in many applications such as drug level monitoring, drug–drug interaction, pharmacokinetic, and bioequivalence studies.

REFERENCES

1. Gross, D.M.; Sweet, C.S.; Ulm, E.H.; Backlund, E.P.; Morris, A.A.; Weitz, D.; Bohn, D.L.; Wenger, H.C.; Vassil, T.C.; Stone, C.A. J. Pharmacol. Exp. Ther. **1981**, *216*, 552–557.
2. Patchett, A.A.; Harris, E.; Tristram, E.W.; Wyvratt, M.J.; Wu, M.T.; Taub, D.; Peterson, E.R.; Ikeler, T.J.; ten Broeke, J.; Payne, L.G.; Ondeyka, D.L.; Thorsett, E.D.; Greenlee, W.J.; Lohr, N.S.; Hoffsommer, R.D.; Joshua, H.; Ruyle, W.V.; Rothrock, J.W.; Aster, S.D.; Maycock, A.L.; Robinson, F.M.; Hirschmann, R.; Sweet, C.S.; Ulm, E.H.; Gross, D.M.; Vassil, T.C.; Stone, C.A. Nature **1980**, *288*, 280–283.
3. Brunner, D.B.; Desponds, G.; Biollaz, J.; Kellar, I.; Ferber, F.; Gavras, H.R.; Brunner, H.R.; Schelling, J.L. Br. J. Clin. Pharmacol. **1981**, *11*, 461–467.
4. Hodsman, G.P.; Brown, J.J.; Dovies, D.L.; Fraser, R.; Lever, A.F.; Morton, J.J.; Murray, G.D.; Robertson, J.I. Br. Med. J. Clin. Res. Ed. **1982**, *285*, 1697–1699.
5. Yuan, A.S.; Gilbert, J.D. J. Pharm. Biomed. Anal. **1996**, *14*, 773–781.
6. Leis, H.J.; Gleispach, H.; Windischhofer, W. J. Mass. Spectrom. **1995**, *30*, 1447–1452.
7. Shioya, H.; Shimojo, M.; Kawahara, Y. Biomed. Chromatogr. **1992**, *6*, 59–62.
8. Worland, P.J.; Jarrott, B. J. Pharm. Sci. **1986**, *75*, 512–516.

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