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Simultaneous Determination and Validation of Enalapril Maleate, Hydrochlorothiazide and Paracetamol in Combined Tablet Dosage Form Using RP-HPLC Method

NITIN DUBEY*, ANKIT JAIN, JINENDRA SARDIYA and DINESH K. JAIN

College of Pharmacy, IPS Academy, Indore-452 012, India

*Corresponding author: E-mail: nitindubeympharm@yahoo.com

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A simple, accurate and precise RP-HPLC method has been developed for the simultaneous estimation of enalapril maleate, paracetamol and hydrochlorothiazide in commercially available tablet formulations. The chromatographic separation was achieved on Luna C_8 column using mobile phase acetonitrile:phosphate buffer:triethylamine (20:79.1:0.1 % v/v/v) (pH 4 ± 0.1) as mobile phase at a flow rate of 1.0 mL/min. Quantitation was carried out at λ_{max} of 215, 245 and 270 nm for enalapril maleate, paracetamol and hydrochlorothiazide, respectively. The method was validated as per ICH guidelines. The method may be applied for the routine simultaneous estimation of the above mentioned drugs in tablet dosage form.

Key Words: RP-HPLC, Enalapril maleate, Paracetamol, Hydrochlorothiazide, Tablets.

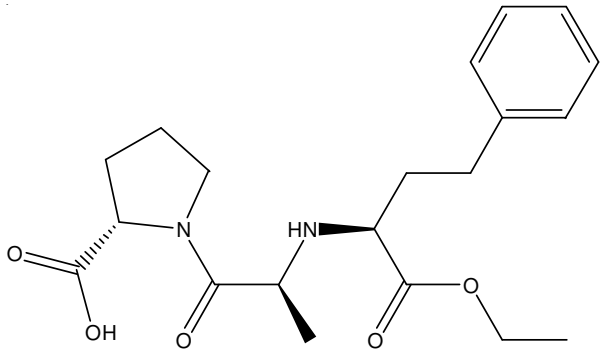
INTRODUCTION

Enalapril maleate is (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}propanoyl]pyrrolidine-2-carboxylic acid maleate inhibits angiotensin-converting enzyme (ACE) in human subjects and animals. Angiotensin-converting enzyme is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex[1].

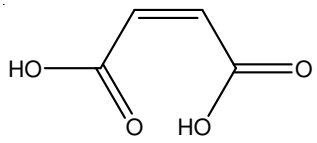
Paracetamol is N-(4-hydroxyphenyl) acetamide widely used analgesic and antipyretic agent. Its activity is attributed to the inhibition of the cyclo-oxygenase enzyme, prostaglandin synthesis in the central nervous system and its direct activity on the hypothalamus. Paracetamol produces analgesia by elevation of the pain threshold and antipyretic activity is mediated through inhibition of cyclooxygenase enzyme in central nervous system and inhibition of the hypothalamic heat regulating center, respectively[2].

Hydrochlorothiazide is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance[3]. The structures of drugs are shown in Fig. 1.

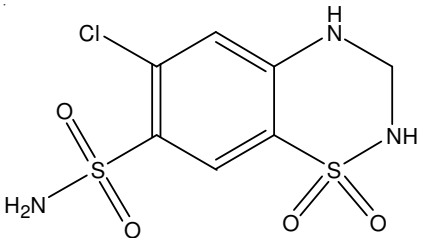
Assay of enalapril maleate in bulk and in dosage form is official in Indian Pharmacopoeia (2007) and British Pharma-



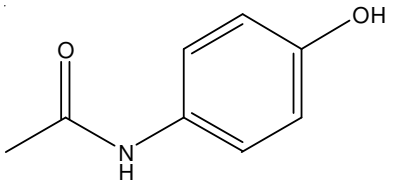
Enalapril



Maleate



Hydrochlorothiazide



Paracetamol

Fig. 1. Chemical structures of enalapril maleate, hydrochlorothiazide and paracetamol

copoeia (2004). The determination of enalapril maleate has been reported to becarried out by HPLC, first derivative UV and spectrophotometrically, alone or in combination with other drugs[4].

Assay of paracetamol in bulk and in dosage form is official in Indian Pharmacopoeia (2007) and British Pharmacopoeia (2004). Several analytical methods have been reported for the determination of paracetamol in dosage form, in biological fluids and in urine using HPLC, LC/MS[5-8].

Assay of hydrochlorothiazide in bulk and in dosage form is official in Indian Pharmacopoeia (2007) and British Pharmacopoeia (2004). Several analytical procedures have been described for the individual determination of hydrochlorothiazide, most frequently by using spectrophotometric methods and jointly with other drugs, using HPLC procedures[9-11].

This paper describes a novel method for the simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol in tablets dosage form. The procedure, based on the use of reversed-phase high-performance liquid chromatography, is simple rapid and provides accurate and precise results. The proposed methods were optimized and validated according to current International Conference on Harmonization (ICH) guidelines[12].

EXPERIMENTAL

Enalapril maleate and paracetamol were provided by Schon (Indore, India) and hydrochlorothiazide was obtained from Ipca Laboratories (Ratlam, India). All the chemicals and reagents used were of HPLC grade and purchased from Merck Ltd. (Mumbai, India). The percentage purity of enalapril maleate, hydrochlorothiazide and paracetamol were found to be 99.45, 99.21 and 99.50 %, respectively.

Chromatographic system and conditions: The LC system consisted of (Shimadzu LC $10AT_{VP}$) gradient pump with universal loop injector (Rheodyne 7725i) of 20 µL injection capacity, photodiode array detector (PDA) SPD-10 A_{VP} and Phenomenex Luna C_8 (25 cm × 5 µm × 4.6 mm i.d.) column at 1.0 mL/min flow rate controlled by a PC work station equipped with software CLASS-V_P (software M 10 version 1.6).

The mobile phase consisted of a mixture of acetonitrile: phosphate buffer:triethylamine (pH 4.0 ± 0.1) (20:79.1:0.1 % v/v/v). Mobile phase was filtered prior to use through a 0.45 µm membrane filter.

Standard stock solutions: The equivalent of 10 mg each of enalapril maleate, 5 mg of paracetamol and hydrochlorothiazide were accurately weighed, different dilutions were prepared for each drug having concentration from 10, 20, 30, 40 and 50 µg/mL for enalapril maleate, 5, 10, 15, 20 and 25 µg/mL for paracetamol and hydrochlorothiazide with mobile phase. 20 µL of these solutions were injected into the LC system with the help of Hamilton syringe. The chromatograms were recorded at λ_{max} of 215, 245 and 270 nm for enalapril maleate, paracetamol and hydrochlorothiazide, respectively. Peak area was used to prepare calibration curve against their respective concentrations.

Analysis of tablets: As the result of mixed standard analysis was found satisfactory, the method was applied for the quantitative study of all the two drugs in commercially available tablet. 20 tablet of invozide were weighed and powder equivalent to 10 mg of enalapril maleate was taken in 100 mL volumetric flask and dissolved in 10 mL of mobile phase (acetonitrile:phosphate buffer:triethylamine 20:79.1:0.1 % v/v/v) (pH 4 ± 0.1) with vigorous shaking for 5 min. The supernatant liquid was transferred to 100 mL of volumetric flask through a whatman # 41 filter paper. The residue was washed twice with solvent and the combined filtrate was suitably diluted. Five replicates of sample solutions were prepared and 20 µL of each replicates were injected. The concentrations of these drugs were extrapolated from their respective calibration curves by using the area.

Recovery study: To check the accuracy of the developed method, recovery study was carried out in triplicate as per ICH guideline. Standard solutions of all the three drugs were added equivalent to 80, 100 and 120 % of target drug concentration. Precision of the method was checked using three replicates over three concentration levels of within range expressed as % RSD values.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for enalapril maleate, paracetamol and hydrochlorothiazide. Mobile phase containg acetonitrile:phosphate buffer:triethylamine (20:79.1:0.1 % v/v/v, pH 4 ± 0.1) was selected as optimal for obtaining well-defined and resolved peaks. The quantitation was carried out at λ_{max} of 215, 245 and 270 nm for enalapril maleate, paracetamol and hydrochlorothiazide, respectively (Fig. 2). Table-1 summarizes linearity range limit of detection (LOD), limit of quantitation (LOQ) and λ_{max} values for all three drugs and system suitability parameters for the RP-HPLC method.

TABLE-1
VALIDATION AND SYSTEM SUITABILITY PARAMETERS FOR RP-HPLC METHOD

Parameters	ENAL	PARA	HCTZ
Linearity range(µg/mL)	10-100	5-100	5-100
λ_{max} (nm)	215	245	270
Limit of detection(µg/mL)	0.71	0.31	0.23
Limit of quantitation(µg/mL)	2.16	0.96	0.71
Theoretical platenumber	2720	5246	7921
Retention time(min)	2.83	4.65	8.44
HETP*	0.0919	0.0476	0.0315
Tailingfactor	1.32	1.48	1.24
Capacity factor(k')	_	0.62	1.68
Resolution	_	7.59	10.08

*HETP=Height equivalent to theoretical plate (cm); ENAL = enalapril maleate, PARA = paracetamol, HCTZ = hydrochlorothiazide.

TABLE-2
RESULTS OF COMMERCIAL TABLET ANALYSIS AND STATISTICAL VALIDATION RECOVERY STUDY

Particulars	Conc. of drug added(%)	ENAL[§] conc. found(%)**	PARA[§] conc. found (%)**	HCTZ[§] conc. found(%)**
Recovery study	80	100.42 ± 0.35	98.84 ± 0.08	99.36 ± 0.04
	100	100.37 ± 0.29	98.84 ± 0.08	98.76 ± 0.38
	120	102.26 ± 0.31	99.33 ± 0.19	99.1 ± 0.35
Commercial tablet analysis		100.37 ± 0.29*	98.84 ± 0.082*	98.76 ± 0.38*

[§]30 µg/mL for ENAL, 15 µg/mL for PARA and 15 µg/mL for HCTZ, respectively(%RSD, n=5). [a]Mean ± RSD (n=3); ENAL = enalapril maleate, PARA = paracetamol, HCTZ = hydrochlorothiazide.

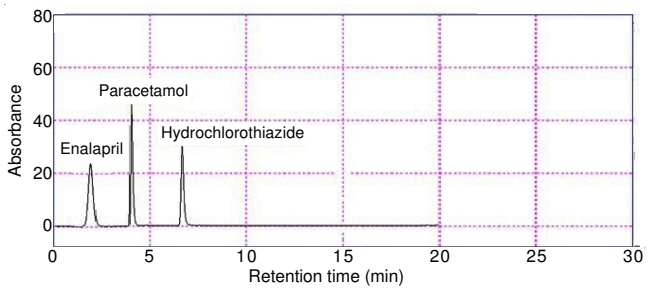


Fig. 2. Chromatograms of 10 µg/mL enalapril maleate, 5 µg/mL paracetamol and 5 µg/mL hydrochlorothiazide at 215, 245 and 270 nm, respectively in acetonitrile:phosphate:triethylamine buffer (pH 4 ± 0.1) (20:79.1:0.1 % v/v/v) as mobile phase

The proposed methods were also evaluated in the assay of commercially available tablets containing enalapril maleate, paracetamol and hydrochlorothiazide. Five replicates determination were performed on the accurately weighed amounts of tablets (Table-2). Linearity range was found to be in the range of 10-100, 5-100 and 5-100 µg/mL with LOQ of 2.16, 0.96 and 0.71 for enalapril maleate, paracetamol and hydrochlorothiazide, respectively, the recovery study showed a n acceptable range of variation below RSD of 2. The solution was found to be stable in precision study for long period of time.

Conclusion

The validated RP-HPLC method developed here proved to be simple, fast, accurate, precise and sensitive. The developed method was validated as per ICH guidelines. Thus it can be used for routine analysis of enalapril maleate, paracetamol and hydrochlorothiazide in combined tablet dosage form.

ACKNOWLEDGEMENTS

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