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Research Article

DEVELOPMENT AND VALIDATION OF HPLC-UV METHOD FOR THE ESTIMATION OF EPROSARTAN IN HUMAN PLASMA

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ABSTRACT

A Simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of eprosartan from human plasma. The drug was extracted with a mixture of 0.05M sodium hydroxide and ethyl acetate. Eprosartan was measured in plasma using a validated a HPLC method with UV detector at 235nm chromatographic peaks were separated on 5µm intensil, C18 column (4.6x250mmx5µm) using 60:40 v/v Phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of eprosartan and internal standard were approximately 5.3±0.05 min and 9.1± 0.03 min respectively. The mean recovery from human plasma was found to be above 88%. The method was linear over the concentration range of 300 to 20,000ng/ml with coefficient of correlation (r^2) 0.9983. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Key words: Eprosartan, Liquid-Liquid Extraction, HPLC-UV Method, Validation.

INTRODUCTION

Eprosartan (fig-1) is an angiotensin II receptor antagonist used for the treatment of high blood pressure. It acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotensin II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic norepinephrine production, further reducing blood pressure.A recent advance in the treatment of hypertension and heart failure is the development of a new class of pharmacologic agents, angiotensin II receptor antagonists, which block the effects of the renin-angiotensin system directly at the angiotensin II receptor level. Angiotensin II, the primary mediator of the renin-angiotensin system, plays a major role in the regulation of fluid and electrolyte balance, blood pressure, and blood volume[1]. Eprosartan (Teveten (SK&F 108566)), a new nonpeptide, nonbiphenyl, nontetrazole orally active angiotensin II receptor antagonist that is highly selective for the AT_1 receptor subtype[2]. Plasma samples were assayed for eprosartan concentrations using reversed-phase HPLC with UV detection[3]. Three methods have been reported for the determination of eprosartan from plasma .The first two methods described by R.M.Alonso and **coworkers** was based on use of HPLC with solid phase extraction[4-5]. These two methods were tedious and time consuming and having lot of solvent preparation steps. While the third method reported by Xue-ning li and coworkers was based on HPLC with mass spectrophotometric detection requires expertise[6]. Ferreirós et al, a method For the quantitation of angiotensin II receptor antagonists (ARA-II) in human plasma. using liquid-chromatography (LC)-electrospray ionization tandem mass spectrometry (MS/MS). This method has been developed with respect to simple sample clean-up and investigation of ion suppression effects[7]. HU Patel et al, reported Simultaneous analysis of eprosartan and hydrochlorthiazide in tablets by HPTLC with ultraviolet absorption[8]. Hence, an attempt was made to develop and optimize an alternative, simple, rigid and sensitive HPLC method for the estimation of Eprosartan from plasma which can be applied to pharmacokinetic study. The results of the analysis were validated by statistical method and recovery studies.

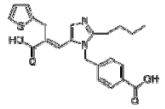

Fig. 1: Chemical structure of Eprosartan

MATERIALS AND METHODS

Eprosartan and losartan were purchased from Pei Li Pharmaceutical Ind.co. Ltd, Taiwan, HPLC grade acetonitrile, ethylacetate, methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grade Potassium Dihydrogen Phosphate and Sodium Hydroxide were purchased from SD fine chemicals, Mumbai, India. Pooled drug free expired human plasma was purchased from Red Cross Society, Warangal.

Chromatographic conditions

The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. The wavelength of detection as set at 235nm. Separation was carried out on inertsil C18 column(4.6x250mmx5µm) using 60:40v/v phosphate buffer pH4, Acetonitrile as mobile phase at a flow rate of 1 ml/min.The mobile phase filtered through nylon milli pore(0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultrasonicator prior to use. Chromatography was carried out at room temperature 25^0c and maintains the column temperature at 32^0c.

Preparation of standard solutions

Stock solutions of eprosartan (0.5mg/ml) and losartan (1mg/ml) internal standard were prepared in methanol. Further dilutions were carried out in methanol. Calibration standards were prepared freshly by spiking drug free plasma with eprosartan stock solution to give the concentrations of 300,600,2000,3000,5000,10000,15000 and 20,000ng/ml.

Quality control standards

Lowest quality control standards, Median quality control standards and highest quality control standards were prepared by spiking drug free plasma with eprosartan to give solution containing 900, 10,000 and 18,000ng/ml respectively. They were stored at -20^0c till the time analyzed.

Sample preparation method

To 250µl of plasma, 50µl of losartan (50µg/ml) and 100µl buffer (0.05M NaOH, pH: 4) and vortexed. The drug was extracted with 2.5ml of ethyl acetate followed by centrifugation at 2000 rpm/min on a cooling centrifuge for 15min at 4^0c. The organic phase was withdrawn and dried using lyophiliser. To the residue 300µl of mobile phase was added and respective samples were injected into column.

Validation

Specificity

A solution containing 300ng/ml was injected on to the column under optimized chromatographic conditions to show the separation of eprosartan from the impurities from the plasma. The specificity of the method was checked for the interference from plasma.

Linearity

Spiked concentrations were plotted against peak area ratios of eprosartan to internal stds and the best fit line was calculated .Wide range calibration was determined by solutions containing 300ng/ml to 20,000ng/ml.

Recovery studies

The % mean recoveries were determined by measuring the responses of the extracted plasma Quality control samples at HQC, MQC and LQC against unextracted Quality control samples at HQC, MQC and LQC.

Limit of quantification

To estimate the LOQ, a drug free blank plasma sample was extracted and injected ten times and analyzed as described under optimized chromatographic conditions .The noise level was then determined, the limit of quantification for eprosartan was determined.(signal to noise ratio=10).

Precision and accuracy

Intraday precision and accuracy was determined by analyzing quality control standards (900.10000,18000ng/ml) and LLOQ Quality control standards (300ng/ml) five times a day randomly, interday precision and accuracy was determined from the analysis of each quality control stds (900,10000,18000ng/ml) and LLOQC standards(300ng/ml) once on each of five different days.

Stability studies

The stability of eprosartan was determined by measuring concentration change in control sample overtime. The plasma control samples were stored in eppendrorff tubes at -20^{0}c. Stability was tested by subjecting the plasma controls to three freeze thaw cycles and stored for 24hrs at room temperature.

RESULTS AND DISCUSSION

Under the chromatographic conditions employed, the sample showed sharp peaks of drug & internal standard with good resolution. The retention time of the drug was found to be 5.3 +0.05 min and the retention time of internal standard was 9.1±0.03 min (figure-2). The method developed was validated for specificity, accuracy & precision, linearity and stability as per USFDA guidance[9]. The results of validating parameters are given below.

Specificity of the method was proven by the absence of the peaks near the reaction time of drug as well as the internal standard (figure-3). The calibration function (peak area ratio Vs Concentration) was linear over working range of 300 to 20,000ng/ml with eight point calibration used for quantification by linear regression. The regression equation for the analysis was Y=5.32e-0.05x-4.82e-0.003 with coefficient of correction (r^2) = 0.9979 (figure-4). The % mean recovery for eprosartan in LQC, MQC and HQC was 85.69%, 80.56% and 81.58% respectively (Table-1).

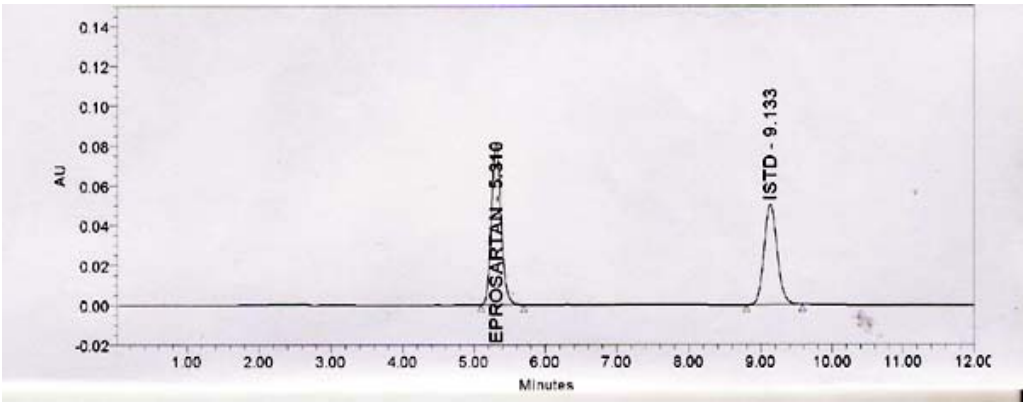

Fig. 2: Retention times of aqueous mixture consists of Eprosartan (5µg/ml) and losartan (5µg/ml)

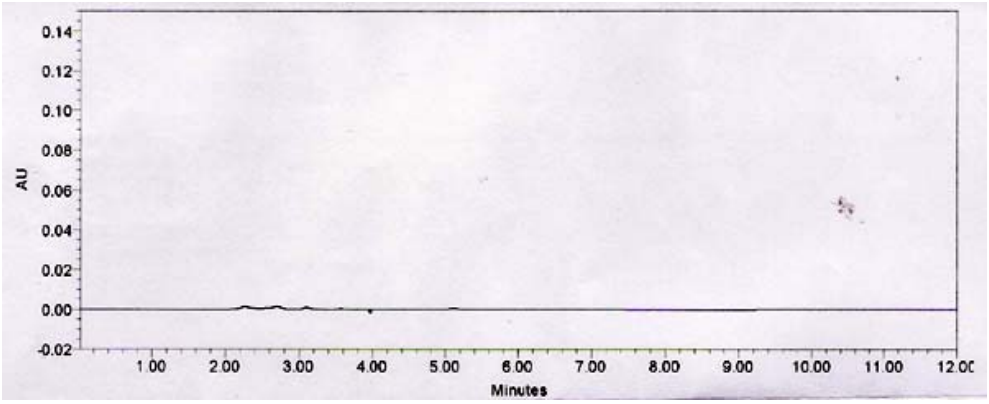

Fig. 3: Blank plasma sample showing no interference at the RT of eprosartan and losartan.

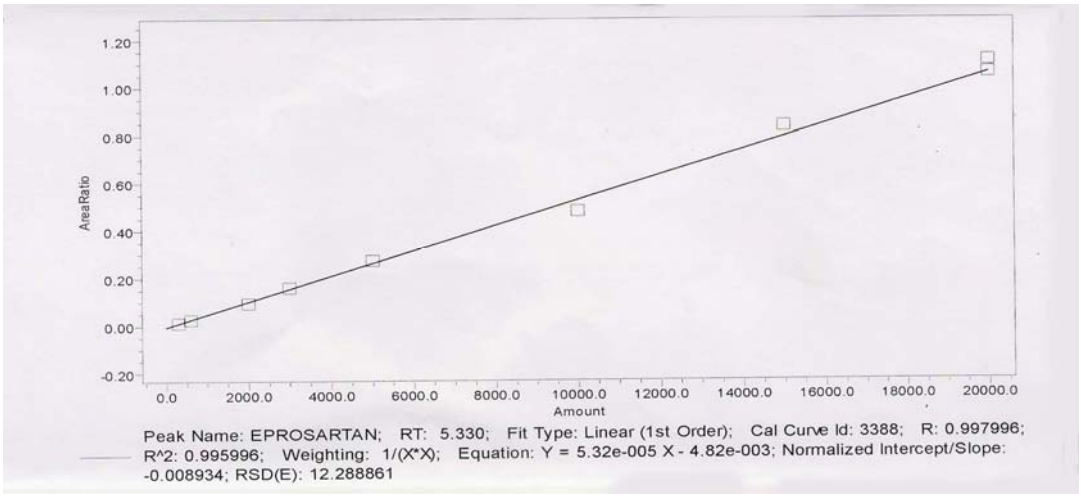

Fig. 4: Spiked concentrations (300 to 20,000ng/ml) were plotted against peak area ratio vs concentration with eight point calibration used for quantification by linear regression.

Table 1: Recovery - Eprosartan

ID	LQC			MQC			HQC		
	Unextracted	Extracted	%Recovery	Unextracted	Extracted	% Recovery	Unextracted	Extracted	%Recovery
	(area ratio)	(area ratio)		(area ratio)	(area ratio)		(area ratio)	(area ratio)	
1	0.117	0.093	79.49	1.5	1.314	87.60	2.644	2.173	82.19
2	0.116	0.097	83.62	1.494	1.193	79.85	2.628	2.143	81.54
3	0.123	0.109	88.62	1.48	1.135	76.69	2.619	2.139	81.67
4	0.124	0.106	85.48	1.486	1.162	78.20	2.627	2.144	81.61
5	0.122	0.107	87.70	1.475	1.182	80.14	2.634	2.132	80.94
6	0.121	0.108	89.26	1.478	1.196	80.92	2.627	2.142	81.54
Mean	0.121	0.103	85.69	1.486	1.197	80.56	2.630	2.146	81.58
±SD	0.003	0.007		0.010	0.062		0.008	0.014	
%CV	2.71	6.44		0.66	5.15		0.32	0.66	

The intraday and interday precision and Accuracy of the method was found to be o.66 to 6.44% and 87.84 to 100.21% respectively for the quality control samples. This is within the acceptance limits of precision is 15% and accuracy is 85 to 115% (Table-2). The limit of Quantification was found to be 300ng/ml. at such concentration the inter day precision was 2.38 and the accuracy was 100.7%. Which are within the acceptance limits of precision is 20% and accuracy is 80 to 120% (Table-3).

Table 2: Precision and accuracy of quality control standards

Batch ID	QC ID	LQC	MQC	HQC	Batch ID	QC ID	LQC	MQC	HQC
	Actual conc.(ng/mL)	900	10000	18000		Actual conc.(ng/mL)	900	10000	18000
	1	844.815	9983.803	18165.32		1	781.366	9796.083	18126.138
	2	866.674	9914.829	17842.11		2	787.398	9626.214	17794.238
	3	854.908	9980.013	17731.98		3	801.554	9846.318	17719.688
	4	842.138	9751.858	18068.13		4	785.871	9801.081	18077.791
	5	844.796	9795.174	18385.31		5	796.793	9706.783	18405.275
	Mean	858.889	9904.28	18038.570		Mean	790.596	9755.296	18024.626
Intraday	± SD	0.003	97.013	259.695	Interday	± SD	8.310	88.120	275.722
	% CV	3.22	0.98	1.44		% CV	1.05	0.90	1.53
	% Accuracy	95.43	99.04	100.21		% Accuracy	87.84	97.55	100.14

Table 3: Precision and accuracy of LLOQC standard

Parameters	LLOQC
Actual conc.(ng/mL)	300
`1	299.796
2	314.703
3	298.651
4	299.378
5	297.353
Mean	301.9762
±SD	7.175
%CV	2.38
% Accuracy	100.7

Table 4: Freeze-thaw stability of quality control standards

Freeze – thaw III Cycles		
QC ID	**LQC**	**HQC**
Actual conc.(ng/mL)	900	18000
1	912.497	19513.2
2	909.335	18580.37
3	890.052	18551.21
4	919.715	18237.61
5	867.634	18148.88
6	884.137	18521.43
Mean	897.228	18592.117
± SD	19.920	485.250
% CV	2.22	2.61
% Accuracy	99.69	103.29

Stability was assessed by comparing against the freshly thawed quality control samples. The %mean stability for HQC and LQC were 103.29 and 99.69 respectively, which is within the acceptance limits of 85 to 115%. Plasma Quality control samples of Eprosartan were found to be stable for at least one month (Table-4).

Eprosartan is soluble in methanol. Hence standard solutions were prepared in methanol. The proportion of acetonitrile in the mobile phase was optimized to 40% and 60% mobile phase was made up of with phosphate buffer (pH: 4). A slight increase and decrease in concentration of acetonitrile and pH by 2% does not affect the reaction times. The extraction of eprosartan was based on liquid-liquid extraction technique. Various solvent systems were tried for recovery studies. The maximum recovery was obtained with a mixture of phosphate buffer (pH 4) and ethyl acetate. Five drugs were attempted for selection as internal standard. The other drugs tried were found to be overlapping with reaction time of eprosartan under the optimized chromatographic conditions.

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