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Development and Validation of RP-HPLC method for Simultaneous Determination of Hydrochlorothiazide and Eprosartan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

An accurate, highly sensitive, precise and reproducible isocratic RP-HPLC method was developed and subsequently validated for the simultaneous analysis of Hydrochlorothiazide and Eprosartan in bulk and tablet dosage forms. Method development was carried out on Agilent Eclipse XBD-C18 (5 μ m, 150mm \times 4.6mm I.D.) column. The mobile phase was a mixture of buffer (20mM KH₂PO₄) and methanol in the ratio of 80:20 v/v. The flow rate was set at 1.0 ml/min and UV detection at 225nm. The retention time of Hydrochlorothiazide and Eprosartan were found to be 3.34 min and 4.75 min respectively. Validation parameters such as linearity, accuracy, precision, and robustness, limit of detection (LOD) and limit of quantification (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. In the linearity study, the regression equations for Hydrochlorothiazide and Eprosartan were found to be $y=0.0123x + 0.0019$ and $y=0.0034x - 0.0163$. Correlation coefficient was 0.9984 and 0.9989 for Hydrochlorothiazide and Eprosartan respectively. The proposed method was successfully applied for the quantification of bulk and active pharmaceutical present in tablet dosage form.

Keywords: Hydrochlorothiazide, Eprosartan, RP-HPLC, Validation

INTRODUCTION

Hydrochlorothiazide (HCTZ) is a diuretic of the class of benzo-thiadiazine widely used in antihypertensive pharmaceutical formulations, alone or combination with other drugs. It decreases active sodium re-absorption and reduces peripheral vascular resistance. It is chemically known as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. HCTZ has been successfully used as single content or in association with other drugs in the treatment of hypertension. Its molecular weight is 297.7 [1,2]. The chemical structure of HCTZ is given in Figure 1;

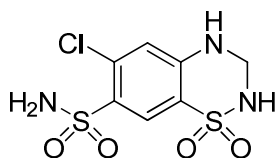


Figure1. The chemical structure of hydrochlorothiazide (HCTZ)

Eprosartan (EPRO) is an antihypertensive drug whose chemical name is 4-({2-butyl-5-[2-carboxy-2-(thiophen-2-ylmethyl) eth-1-en-1-yl]-1H-imidazol-1-yl} methyl) benzoic acid. Its chemical structure is as given in figure2 below. It is a new antihypertensive drug and acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. Firstly, it blocks the binding of angiotensin II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. This is followed by second step of inhibition of sympathetic norepinephrine production, which further reduces blood pressure [3, 4]

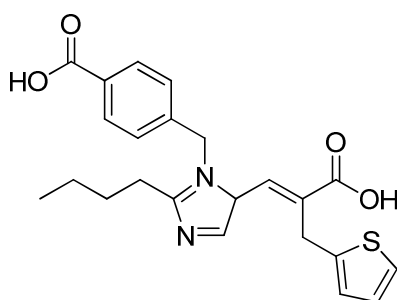


Figure 2. Chemical Structure of Eprosartan (EPRO)

Presently, there is a combined pharmaceutical formulation of Eprosartan mesylate(600mg) and Hydrochlorthiazide(25 mg) in market with trade name (Teveten® HCT) for the treatment of edema and hypertension. Thus, there is a need for the development of appropriate analytical method for the simultaneous determination of these drugs in different formulations. Literature survey showed that various analytical methods have been reported for quantification of each of these drugs as individual or in combination with other hypertensive drugs [5-13]. However, a very limited HPLC/ analytical methods were reported in literature for the simultaneous determination of of Eprosartan mesylate and Hydrochlorothiazide [14-16]. HPLC methods [16] reported so far in literature used at least three solvents for the mobile phase which is considered to be uneconomical and preparation of such mobile phase may be cumbersome or time consuming. From economic point of view and for the purpose of routine analysis, it was decided to develop a more economical HPLC method with simple mobile phase preparation for simultaneous estimation of HCTZ and EPRO. Thus, this paper reports an economical, simple and accurate RP-HPLC method for the simultaneous determination of HCTZ and EPRO in pure drug and solid dosage form.

MATERIALS AND METHODS

Chemicals and reagents

The pharmaceutical grade pure samples of Hydrochlorthiazide (99.28%) and Eprosartan mesylate (99.55%) were supplied by Hetero laboratories, Andrapredesh, India. Methanol HPLC grade solvent and all analytical grade solvents were purchased from Merck Ltd, Mumbai, India. Potassium dihydrogen phosphate was procured from Qualigens Fine Chemicals, Mumbai, India.

The HPLC grade water was obtained from a Milli-QRO water purification system, sonicated and used.

HPLC apparatus and conditions

Chromatography was performed using a JASCO HPLC 2080 model chromatograph (Japan) equipped with a PU-2080 isocratic delivery system (pump), UV-2075 detector (JASCO) with a Rheodyne 7725 injection valve with a 20 μ L loop volume. The analytical column was an Agilent xbd-reverse phase C18 column (150 \times 4.6mm I.D; particle size 5 μ m). Data acquisition and processing was performed using JASCO BORWIN software (Japan).

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile phase consisting of a mixture of Buffer solution (20m M potassium di-hydrogen orthophosphate): Methanol in the ratio of 80:20. The pH of buffer was 4.85 \pm 0.05 and was used as such without any adjustment. The mobile phase so prepared was filtered through 0.22 nylon membrane filter and degassed by sonication. The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1 mL / min and the detection was achieved at 225nm. The injection volume was 20 μ l (fixedloop).

Standard preparation

Standard stock solutions of 1 mg ml⁻¹ of HCTZ, and EPR were separately prepared by accurately weighing 100 mg of each of the standard drug into different 100 mL volumetric flasks. These were dissolved, sonicated and made up to the standard mark with mobile phase. A series of EPRO standard solutions in the concentration range of 20, 40, 60, 80 and 100 μ g/ml were prepared followed by a suitable dilution of stock solution with the mobile phase. Likewise, a series of solutions in the concentration range of 5, 10, 15, 20 and 30 μ g/ml were prepared for HTCZ.

The detection wavelength was fixed at 225 nm obtained from uv-overlay spectra of the two drugs. The standard calibration curves were constructed by plotting of graph of peak areas against the respective concentrations of standard drugs. The linear regression equations obtained are $y = 0.0123X + 0.0019$ ($R^2 = 0.9984$) and $y = 0.0034X - 0.0163$ ($R^2 = 0.9989$) for HCTZ and EPRO respectively. The typical chromatogram recorded for standards are as shown in Fig.3. The retention time of standard HCTZ and EPRO were found to be 3.34 and 4.75 min, respectively.

Assay procedure

Twenty tablets, each containing HCTZ (25 mg) and EPRO (600 mg) were weighed and finely powdered. Powder equivalent to approximately 25 mg hydrochlorothiazide and 600 mg eprosartan was weighed accurately, transferred to a 100 mL volumetric flask, and methanol (50 mL) was added. The solution was sonicated for 15 min, to ensure complete solubility of the drug. The excipients were separated by filtration. The mixture was then made up to 100 mL with diluents, thoroughly mixed and filtered through a 0.45- μ m pore-size, Nylon membrane filter. Further dilution of filtrate was made to obtain solutions different concentration. Thereafter, each concentration was injected five times into the column. From the peak area, the drug content in the tablets was qualified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution of HCTZ and EPRO in different mobile phases with various ratios of buffers and organic phase by using C18 column. The ideal mobile phase was found to be a mixture of buffer (20m M potassium dihydrogen orthophosphate, pH 4.75) and Methanol in ratio 80:20 v/v. This mobile phase used under isocratic elution gave a very satisfactory and good resolution of HCTZ and EPRO. Increasing or decreasing pH of mobile phase by ± 0.3 did not show significant change in retention time of each analyte. The retention time of HCTZ and EPRO on the analytical column was evaluated at a flow rate of 1.0 ml min^{-1} . The injection volume was $20 \mu\text{l}$. The retention time of standard and sample for HCTZ and EPRO were satisfactory with good resolution.

Validation

The Method was validated based on the International Conference on Harmonization (ICH) guidelines [17- 19]. The method validation parameters checked were specificity, linearity, accuracy, precision, limit of detection, limits of quantitation and robustness.

Linearity

The linearity for HPLC method was determined at five concentration levels ranging from 5-30 $\mu\text{g mL}^{-1}$ for HCTZ and 20-100 $\mu\text{g mL}^{-1}$ for EPRO. The calibration curve was constructed by plotting response factor (peak area) against concentration of drugs. The slope and intercept value for calibration curve were $y = 0.0123 X + 0.0019$ ($R^2 = 0.9984$) for HCTZ and $y = 0.0034 X - 0.0163$ ($R^2 = 0.9989$) for EPRO, where Y represents the peak area of analyte and X represents analyte concentration. The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of HCTZ and EPR are given in Figures 4 and 5 respectively.

Precision

The precisions of the analytical method were determined by repeatability (within-day) and Intermediate precision (between-day). Three different concentrations which were quality control samples (10, 20, 30 mg/mL) for HCTZ and (20, 40, 60) for EPRO were analyzed five times in one day for within-day precision and once daily for three days for between-day precision. The intraday and interday precision showed a coefficient of variation ranged from 0.64% to 0.94% and from 0.78% to 1.25% respectively for HCTZ. The coefficient of variation of intraday and interday precision for EPRO ranged from 0.65% to 0.98% and from 0.68% to 1.02% respectively. The results are shown in Table 2, and indicate that the method is precise.

Recovery

Recovery was determined by spiking the formulation with standards of each drug equivalent to 80,100, and 120 % of the amount originally present. % Recovery was calculated by comparing the area before and after the addition of the working standard. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in Table 3. The results of the recovery analysis were found to be 99.67 ± 0.21 to 100.11 ± 0.15 for HCTZ and 99.59 ± 0.31 to 100.16 ± 0.27 for EPRO, and reported in Table 3. The results of analysis showed that the amounts of drugs found were in good agreement with the label claim of the formulations.

Specificity and Selectivity:

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of either HCTZ or EPRO in sample solution. The sensitivity of measurement of HCTZ and EPRO was estimated as the limits of quantification (LOQ) and detection (LOD), which were calculated by use of the equations $LOD = 3 \times N/B$ and $LOQ = 3 \times N/B$, where N is the standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. The Limit of Detection (LOD) for HCTZ and EPRO was found to be $0.076 \mu\text{g mL}^{-1}$ and $0.063 \mu\text{g mL}^{-1}$ respectively. The Limit of Quantification (LOQ) was $0.232 \mu\text{g mL}^{-1}$ and $0.192 \mu\text{g mL}^{-1}$ for HCTZ and EPRO respectively reported Table 1.

Ruggedness and Robustness

Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

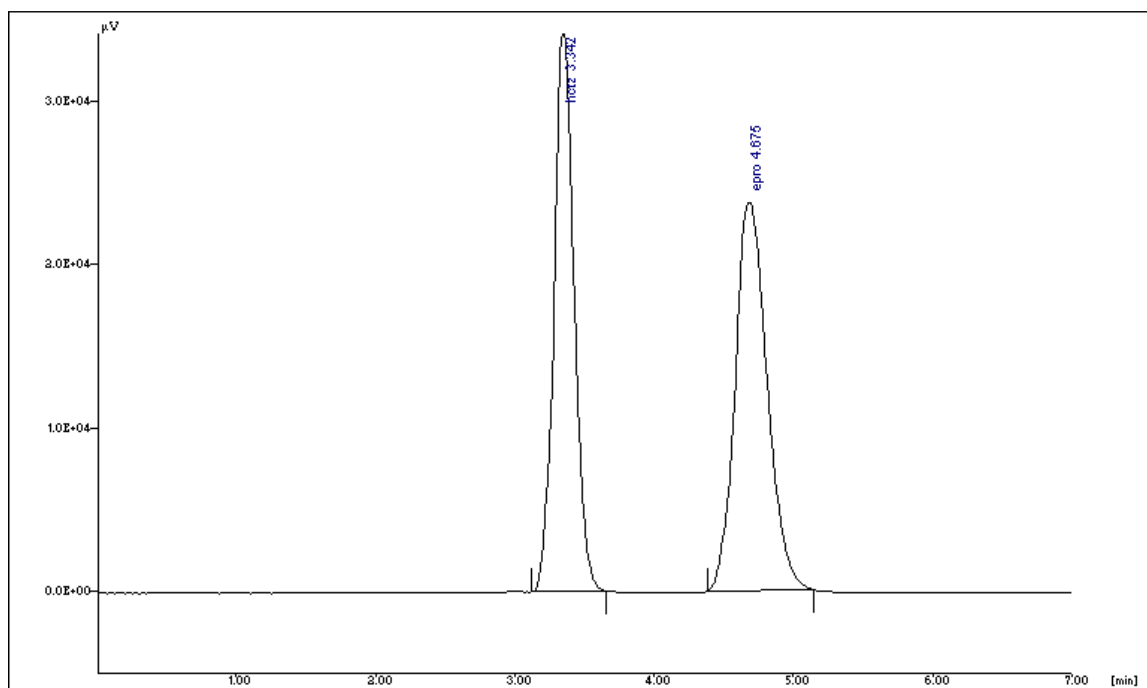


Figure3. A Typical Chromatogram of Hydrochlorothiazide and Eprosartan

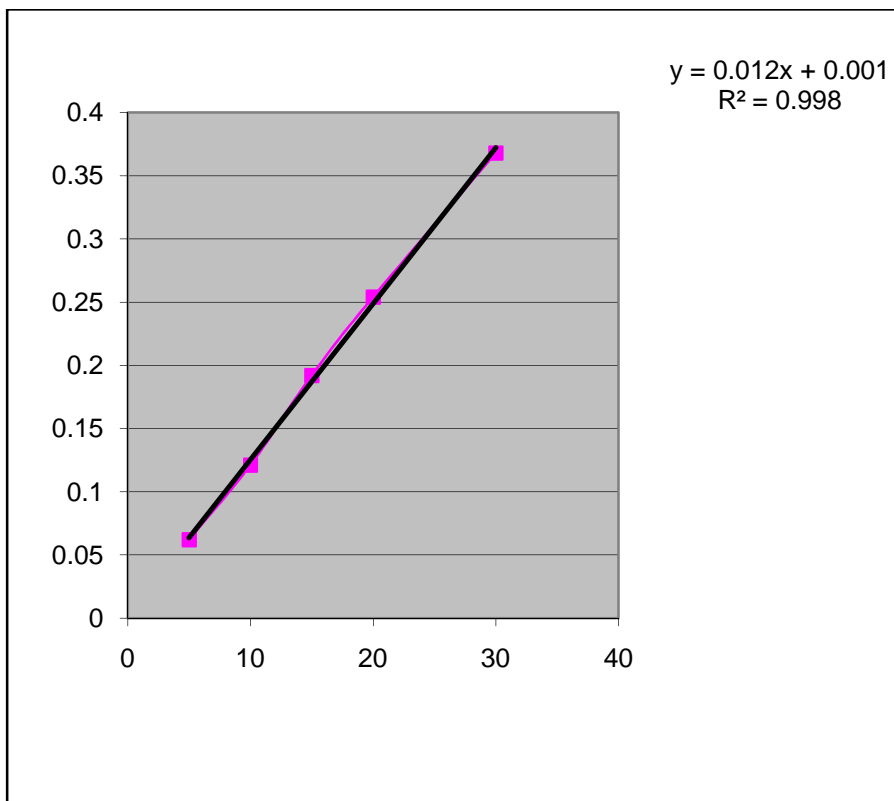


Figure4. Linearity curve for HCTZ

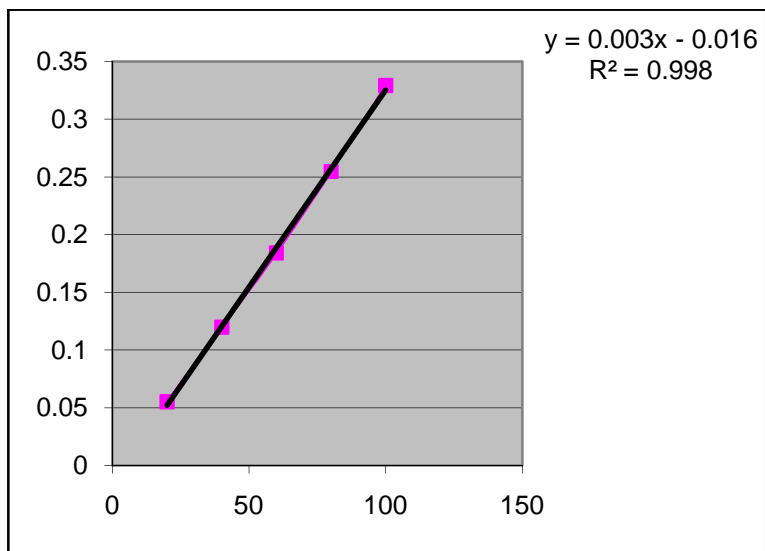


Figure4. Linearity Curve for EPRO

Table1. HPLC Parameters

Parameters	Drug	
	HCTZ	EPRO
Linearity range	5-30 $\mu\text{g mL}^{-1}$	20-100 $\mu\text{g mL}^{-1}$
Equation of regression	$y=0.0123x + 0.0019$	$y=0.0034x - 0.0163$.
Correlation coefficient(R^2)	0.9989	0.9989
Retention time	3.34	4.75
Resolution	0	5.13
Theoretical plate	4066	3106
Tailing factor	1.16	1.29
LOD.($\mu\text{g/mL}$)	0.076	0.063
LOQ.($\mu\text{g/mL}$)	0.232	0.192

Table 2. Precision of the Method

	CON. ($\mu\text{g/mL}$)	MEASURED CON.($\mu\text{g/mL}$) \pm SD		%CV	
		INTRADAY	INTERDAY	INTRADAY	INTERDAY
HCTZ	10	9.94 \pm 0.75	9.91 \pm 0.95	0.85	0.95
	20	20.01 \pm 0.6	19.96 \pm 0.86	0.68	1.25
	30	29.98 \pm 0.8	29.54 \pm 0.96	0.94	0.78
EPRO	20	19.98 \pm 0.8	19.88 \pm 0.56	0.89	0.68
	40	39.96 \pm 0.6	39.96 \pm 0.98	0.98	1.02
	60	60.01 \pm 0.9	59.98 \pm 0.68	0.65	0.85

Table3. Results of accuracy/Recovery studies

Analyte	Amount(%) of drug added to the analyte	Theoretical con.($\mu\text{g/mL}$)	Measured con. ($\mu\text{g/mL}$) \pm SD	% Recovery	%RSD
HCTZ	80%	12	11.96 \pm 0.21	99.67	0.36
	100%	15	14.99 \pm 0.47	99.93	0.54
	120%	18	18.02 \pm 0.15	100.11	0.37
EPRO	80%	64	63.83 \pm 0.39	99.73	0.55
	100%	80	80.13 \pm 0.27	100.16	0.34
	120%	96	95.61 \pm 0.31	99.59	0.41

CONCLUSION

An economical, simple, sensitive, precise and accurate method has been developed for the simultaneous determination of Hydrochlorothiazide and Eprosartan in solid dosage form. The simplicity in constitution of mobile phase and relatively cheap cost of the components of mobile phase coupled with its accuracy make this method the best choice in routine analysis of HCTZ and EPRO in pharmaceutical industry.

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