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Original Article

Simultaneous analysis of eprosartan and hydrochlorothiazide in tablets by high-performance liquid chromatography

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Abstract

Objective: A simple, precise and accurate isocratic reversed phase (RP) column high-performance liquid chromatographic (HPLC) method has been developed for simultaneous analysis of eprosartan (EPR) and hydrochlorothiazide (HCT) in tablet formulations. **Materials and Methods:** Isocratic RP-HPLC separation was achieved on phenomenex C18 column (250 x 4.6 mm i.d., 5 µm particle size) using mobile phase composed of 0.5% formic acid-methanol-acetonitrile [(80 : 25 : 20 v/v/v) pH, 2.80 ± 0.04] at a flow rate of 1.0 ml/min. The retention time for EPR and HCT was 7.69 ± 0.10 and 4.24 ± 0.09 minutes, respectively. The detection was performed at 272 nm. **Results:** The method was linear in the concentration range of 60-600 µg/ml for EPR and 2.5-25 µg/ml for HCT with a correlation coefficient of 0.9992 and 0.9997, respectively. The repeatability for six samples was 0.53 and 0.61 % RSD for EPR and HCT, respectively. The accuracy (recovery) was found to be in the range of 99.46 to 100.61% for EPR and 99.06 to 100.93% for HCT, respectively. **Conclusions:** The method was validated and successfully used for determination of the drugs in tablets.

Key words: Eprosartan, high-performance liquid chromatographic, hydrochlorothiazide, tablets

INTRODUCTION

Eprosartan (EPR) {(E)-3-[2-butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl]-2-[(2-thienyl) methyl] propenoic acid} is a highly selective, nonpeptide angiotensin-II antagonist [Figure 1]. The compound has been shown to inhibit angiotensin-II induced vasoconstriction in preclinical species and cause reductions in systolic and diastolic blood pressure at peak effect after dosing in clinical patients.[1] It is currently being developed for the treatment of hypertension as other compounds of the class angiotensin-II receptor antagonists (ARA-II).[2] Hydrochlorothiazide (HCT) {(6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide} is a diuretic drug [Figure 1].[3] The rationale behind this drug combination is that in treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy, oral administration of EPR with HCT has been found more effective than use of either drug alone.[4]

Literature survey revealed that different analytical methods are reported for EPR in pharmaceutical dosage forms and biological samples, which includes LC-MS-MS,[5] capillary zone electrophoresis,[6] micellar electrokinetic capillary chromatography[7] and capillary zone electrophoresis for ARA-II with HCT,[8] analysis of EPR in biological samples by HPLC,[9,10] chemometric method,[11] and densitometry with HCT.[12] There are several reports of the determination of HCT in combination with other ARA – II drugs, including use of HPLC, HPTLC and spectrophotometry.[13-16] So far, to our present knowledge, no validated HPLC method for the simultaneous determination of EPR and HCT in bulk drug and tablets was reported in literature. Therefore, the aim of the proposed method was to develop and validate HPLC method in accordance with International

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Conference on Harmonization (ICH) guidelines,[17] which can be used successfully to determine EPR and HCT in tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A Shimadzu (Columbia, MD) HPLC instrument (LC-10 ATvp) equipped with UV-Visible detector, manual injector of 20-µl loop and phenomenex (Torrence, CA) Luna C18 column (250 × 4.6 mm i.d., 5 µm particle size) was used; a weighing balance (Acculab ALC-210.4, India) and a sonicator (Enertech Fast clean, India) were used for the study.

Chemicals and reagents

EPR (Batch No. BK6-EP-080) and HCT (Batch No. RF0314) were obtained as gift samples from Dishmann pharmaceuticals Ltd. (Ahmedabad, India) and Unichem Laboratories Ltd. (Mumbai, India). EPR and HCT tablets (600 and 25 mg/tab) were procured from the market. Acetonitrile (HPLC grade, S. D. fine chemicals, Ahmedabad, India) methanol and water (HPLC grade, Finar chemicals Ltd., Ahmedabad, India), Formic acid (HPLC grade, Spectrochem Pvt Ltd., Mumbai, India) and nylon filter (Millipore Pvt. Ltd, Bangalore, India) were used for study.

Chromatographic conditions

HPLC was performed on a Phenomenex Luna C18 column (250 × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of 0.5% formic acid: methanol : acetonitrile [(80 : 25 : 20 v/v/v) pH, 2.80 ± 0.04]. The mobile phase was filtered through Nylon 0.45 µm, 47 mm membrane filter and was degassed before use. The flow rate was 1.0 ml/min. The determination was carried out at 272 nm and the injection volume was 20 µl. The total run time was 10 minutes.

Preparation of standard solution

Accurately weighed EPR standard (600 mg) and HCT standard (25 mg) was transferred into a 100-ml volumetric flask, and dissolved in and diluted to the mark with methanol to obtain standard stock solution (6000 µg/ml for EPR and 250 µg/ml for HCT). The stock solution was serially diluted with mobile phase to get linearity range of 60-600 µg/ml for EPR and 2.5-25 µg/ml for HCT.

Selection of wavelength

EPR and HCT stock solution (0.2 ml) was transferred into a 10 ml volumetric flask. The volume was adjusted to the mark with mobile phase and scanned over 200 to 400 nm. Wavelength 272 nm was selected for the study where HCT has good absorbance as EPR and HCT present in the tablets as ratio of 24: 1 [Figure 2].

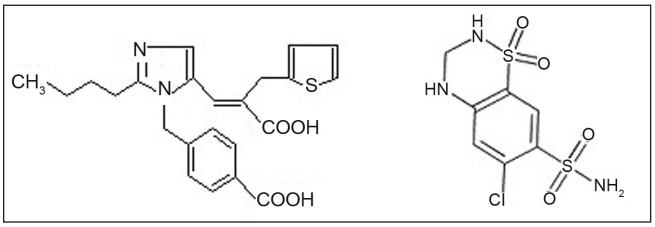

Figure 1: Structure of eprosartan and hydrochlorothiazide

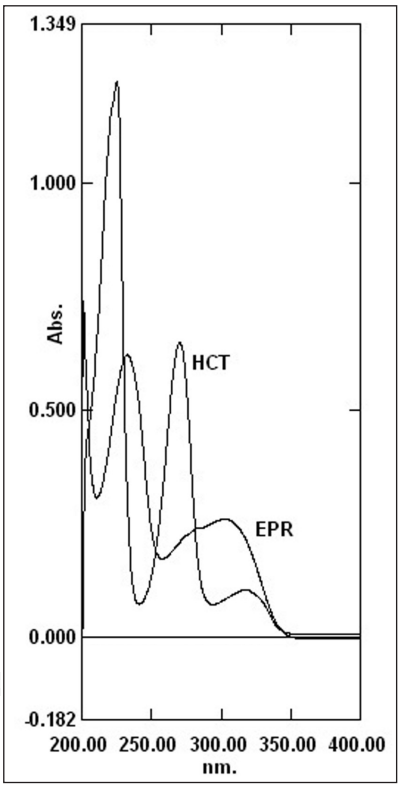

Figure 2: Overlay UV spectrum of EPR and HCT

Preparation of sample solution

Twenty tablets (each containing 600 mg of EPR and 25 mg HCT) were weighed accurately and powdered finely. The powder equivalent to 600 mg of EPR and 25 mg of HCT was transferred in a 100 ml volumetric flask; 60 ml methanol was added, sonicated for 20 minutes, and diluted up to the mark with methanol. The solution was filtered through 0.45 µm nylon filter paper. An aliquot (0.3ml) was transferred into a 10 ml volumetric flask and was diluted up to mark with mobile phase to obtain sample stock solution (180 and 7.5 µg/ml for EPR and HCT).

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Method validation

Linearity

Aliquots (0.1, 0.2, 0.3, 0.4, 0.5 and 1 ml) from the stock solution (equivalent to 60, 120, 180, 240, 300 and 600 µg/ml for EPR and 2.5, 5, 7.5, 10, 12.5 and 25 µg/ml) were transferred in a series of 10ml volumetric flasks and diluted to the mark with mobile phase. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions as described earlier. Calibration curve was constructed by plotting peak areas vs. concentrations, and the regression equation was calculated. Each response was average of five determinations.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding response three times on the same day and on three different days over a period of 1 week for three different concentrations of EPR (60, 120 and 180 µg/ml) and HCT (2.5, 5 and 7.5µg/ml). The results are reported in terms of relative standard deviation (RSD).

Method precision (repeatability)

The repeatability was checked by repeatedly injecting (n = 6) solutions of EPR (120 µg/ml) and HCT (5 µg/ml).

Accuracy

Accuracy was determined by calculating recovery of EPR and HCT by the standard addition method. Known amounts of sample solutions (120 µg/ml of EPR and 5 µg/ml of HCT) were spiked with three different concentrations of standard solutions (60, 120, 180 µg/ml for EPR and 2.5, 5, 7.5 µg/ml for HCT). Each solution was injected in triplicate and the percentage recovery was calculated by measuring peak areas and fitting these values into the regression equation of the calibration curves.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated using the following equations according to ICH guidelines.[17]

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where, σ is the standard deviation of the response and S is the standard deviation of slope of the regression equation.

System suitability test parameters

System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were asymmetry of the chromatographic peak resolution, theoretical plates and tailing factor.

Determination of eprosartan and hydrochlorothiazide in tablets

The validated method was used for the analysis of EPR and HCT in their combined tablets (Brand A).

RESULTS

We used several mobile phases in trying to accomplish good separation of EPR and HCT. Chromatographic conditions were optimized with a view to develop an assay method for EPR and HCT. The analytical conditions were selected after testing the different parameters such as organic solvents for mobile phase, mobile phase compositions, pH and other chromatographic conditions. Our preliminary trials using different combination of mobile phases of water with methanol and acetonitrile did not give good peak shape, optimum retention time and good resolution of peaks. Satisfactory results were obtained with the mobile phase consisting of 0.5% formic acid : methanol : acetonitrile (80 : 25 : 20 v/v/v, pH, 2.80 ± 0.04) [Figure 3]. The retention time of EPR

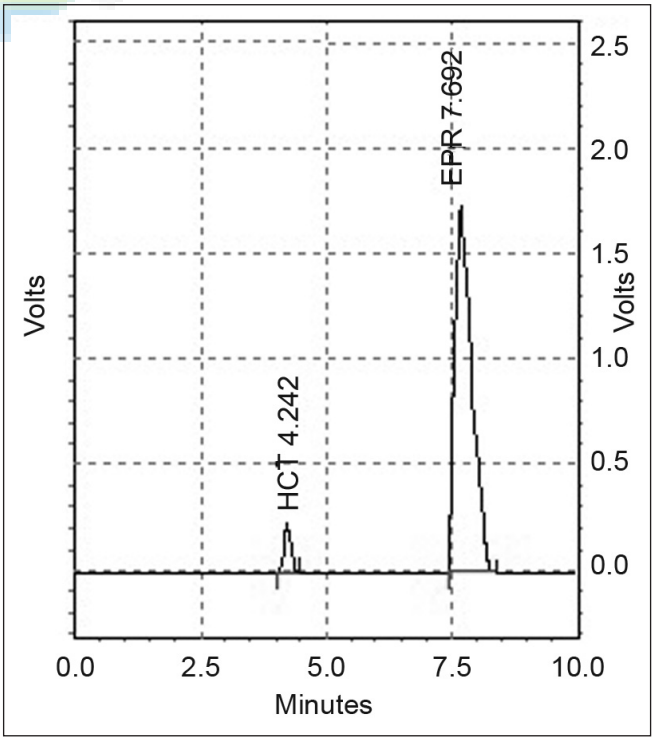

Figure 3: HPLC chromatogram of EPR and HCT (180 and 7.5 µg/ml; tablet dosage form at 272 nm)

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and HCT was 7.69 ± 0.10 and 4.24 ± 0.09 minutes, respectively.

Method validation

Linearity

Response to EPR and HCT was linear in the concentration ranges 60–600 µg/ml and 2.5–25 µg/ml, respectively. The regression equations for EPR and HCT (n = 6) were y = 35727x + 119429 and y 101589x + 27807 for EPR and HCT, respectively, where y is response and x the amount chromatographed. The correlation coefficients were 0.9992 and 0.9997 respectively, over these concentration ranges.

Table 1: Summary of validation parameters for the proposed method

Parameters	Eprosartan	Hydrochlorothiazide
Linearity (µg/ml)	60-600	2.5-25
LOD[a] (µg/ml)	0.0288	0.0139
LOQ[b] (µg/ml)	0.0872	0.0460
Repeatability (RSD[c], %, n = 6)	0.53	0.61
Prescision (RSD, %)		
Intraday (n = 3)	0.19-0.62	0.27-0.85
Interday (n = 3)	0.28-0.49	0.32-0.88

[a]LOD = Limit of detection, [b]LOQ = Limit of quantification, [c]RSD = Relative standard deviation, n = number of determinations

Sensitivity

The LOQ and LOD for EPR were 0.0288 and 0.0872 µg/ml, respectively. For HCT, the values were 0.0139 and 0.0460 µg/ml, respectively.

Precision and repeatability

The results for intraday& interday precision studies and repeatability are listed in Table 1.

Accuracy and system suitability parameters

The results for the accuracy study are shown in Table 2.The recovery was found in the range of 99.46 to 100.61% for EPR and 99.06 to 100.93% for HCT, indicating the method accuracy. System suitability parameters are listed in Table 3.

Determination of eprosartan and hydrochlorothiazide in combined tablets

The validated method was successfully applied to analysis of EPR and HCT in their combined tablets (Brand A). The results obtained for EPR and HCT were comparable with the corresponding labelled amounts [Table 4].

DISCUSSION

A new analytical method has been developed to determine EPR and HCT in their combined

Table 2: Accuracy data for analysis of EPR and HCT

Amount of sample taken (µg/ml) (A)		Amount of std. added (µg/ ml) (B)		Total amount (A + B)		Total amount found*		% Recovery ± % RSD	
EPR	HCT	EPR	HCT	EPR	HCT	EPR	HCT	EPR	HCT
120	5	60	2.5	180	7.5	179.03	7.489	99.46 ± 0.60	99.85 ± 0.57
120	5	120	5	240	10	239.89	10.093	99.95 ± 0.76	100.93 ± 0.72
120	5	180	7.5	300	12.5	301.82	12.382	100.61 ± 0.52	99.06 ± 0.84

*Average of three determinations, EPR = Eprosartan; HCT = Hydrochlorothiazide

Table 3: System-suitability test parameters for EPR and HCT

Drugs	Retention time*, min ± % RSD	Resolution* ± % RSD	Theoritical plates* ± % RSD	USP tailing factor* ± % RSD
EPR	7.69 ± 0.10	--	7386.88 ± 0.05	1.17 ± 0.08
HCT	4.24 ± 0.09	7.70 ± 0.06	5142.80 ± 0.13	1.27 ± 0.04

*Average of five determinations, EPR = Eprosartan; HCT = Hydrochlorothiazide; RSD = Relative standard deviation

Table 4: Analysis of eprosartan and hydrochlorothiazide in combined tablet dosage form

Tablet	Component	Label claim (mg)	Amount of drug found* (%) ± RSD (%)
Brand A	Eprosartan	600	100.68 ± 0.82
	Hydrochlorothiazide	25	99.45 ± 0.93

*Average of five determinations

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pharmaceutical dosage form. The developed method was proved to be simple, rapid, accurate and precise. There is no interference of any excipients in the determination of EPR and HCT in tablets and the method can be successfully applied for routine quality control analysis of EPR and HCT tablets.

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