Original Article

DEVELOPMENT AND VALIDATION OF EPROSARTAN MESYLATE AND HYDROCHLOROTHIAZIDE IN PURE AND IN FIXED DOSE COMBINATION BY UV SPECTROPHOTOMETRY

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Abstract

A simple efficient, precise and accurate Simultaneous equation method have been developed for the Simultaneous estimation of Eprosartan Mesylate and Hydrochlorothiazide in pure and in fixed dose combinations. In this method, UV Spectra of Eprosartan Mesylate and Hydrochlorothiazide were overlained. The linearity ranges for Eprosartan Mesylate and Hydrochlorothiazide were 6-36µg/ml and 1-10µg/ml, respectively. The proposed procedures were successfully applied for the simultaneous determination of both drugs in the laboratory prepared mixtures and in commercial tablet preparations. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 99.36 ± 0.701 and 98.9 ± 0.728 for Eprosartan Mesylate and Hydrochlorothiazide, respectively. The proposed procedure is rapid, simple, require no preliminary separation steps and can be used for routine analysis of both drugs in quality control laboratories. The results of analysis have been validated statistically and by recovery studies confirmed the accuracy of the proposed method.

Key words: Eprosartan Mesylate, Hydrochlorothiazide, Simultaneous equation method, Method Validation.

Introduction

Eprosartan Mesylate (EPM)[1 – 2] a new drug and it is used as anti hypertensive agent which is chemically mono methane sulfonate of (E) – 2 – butyl – 1 - (p-carboxybenzyl) – α – 2 – thienyl methyl imidazole – 5 – acrylic acid. (Fig.1). EPM is not official in any pharmacopoeia. EPM, a potent vasoconstrictor, is the principal pressor agent of rennin - angiotensin system. Hydrochlorothiazide (HCT)[3] is used as anti hypertensive agent which is chemically 6 - chloro - 1, 1 – dioxo - 3, 4 – dihydro - 2H -1, 2, 4 - benzothiadiazine -7 – sulfonamide (fig 2). Hydrochlorothiazide belongs to the thiazide class of diuretics, acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. This increases the osmolarity in the lumen, causing less water to be reabsorbed from the collecting ducts. This leads to increased urinary output. . HCT is official in I.P[4]., B.P[5]. and U.S.P[6].

Literature survey revealed that SPE – HPLC – UV [7 – 8] and LC – MS – MS [9] methods were reported for the estimation of EPM in plasma. HPLC [10], HPTLC and spectroscopic [11] methods have been reported for the determination of HCT in combination with other drugs. HPTLC [12] and capillary electrophoresis [13] methods were reported for the estimation of EPM and HCT in combined tablet dosage forms.

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However, there is no UV spectrophotometric method has been reported for the estimation of EPM and HCT in combination. Hence the present work aims to develop a simple, precise, accurate and validated UV spectrophotometric method (simultaneous equation method)[14] for the estimation of EPM and HCT in pure and in fixed dose combination. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) [15 – 16] guidelines for the determination of EPM and HCT in pure and in fixed dose combination.

Fig.1. Chemical structure of EPM

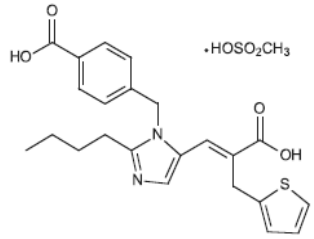
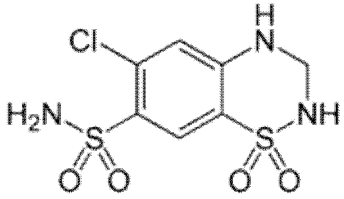


Fig.2. Chemical structure of HCT



Experimental

Materials

EPM and HCT were gift samples from Sairam Organics Pvt. Ltd., Hyderabad, India. The commercial fixed dose combination product Teveten HCT containing 600 mg of EPM and 25 mg of HCT (Solvay Pharmaceuticals, Mumbai, India) was procured from the local market. 0.1M Sodium hydroxide AR grade (Qualigens India Pvt. Ltd., Mumbai, India) was used as solvent in this study.

Equipments

Shimadzu UV- 1700 UV-Visible spectrophotometer with 1cm matched quartz cells was used for the measurement of absorbance. Shimadzu-AX-200 electronic balance was used for weighing the samples. Class 'A' volumetric glasswares were used.

Procedure

Preparation of standard stock solution

Accurately, 60 mg of EPM and 20 mg of HCT were weighed separately and transferred in to two different 100ml volumetric flasks. Each drug was dissolved in 0.1 M sodium hydroxide and made up to the mark with 0.1 M sodium hydroxide. The standard stock solutions contain 600 μg/ml of EPM and 200 μg/ml of HCT. These solutions were further diluted separately to obtain (10 μg/ml) of each drug individually.

Study of spectra and selection of wavelengths

Each standard solution was scanned between the range 200 – 400 nm in 1cm cell against blank. After examining the overlain spectra, two drugs have different λ max and both the drugs showed the absorbance at each other' s λ max. The wavelengths selected for the analysis of EPM was 294.2 nm where HCT has absorbance and the wavelength selected for the analysis of HCT was 274.5 nm where the EPM has absorbance.

Preparation of calibration graph

1.0 – 6.0 ml of standard stock solution of EPM and 0.5 – 5.0 ml standard stock solution of HCT were transferred into a series of six 100 ml volumetric flasks separately and made up to mark with 0.1M sodium hydroxide. The absorbance of different concentration solutions was measured at 294.2 nm and 274.5 nm against blank. The calibration curve was plotted using concentration against absorbance. The solutions were found to be linear with the concentration range of 6 – 36 μg/ml of EPM and 1 – 10 μg/ml of HCT.

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated from the data obtained from the linearity studies (ICH guidelines). The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, LOD and LOQ were calculated as follows,

$$3.3\,\sigma/S \qquad \& \qquad 10\,\sigma/S$$

Where, σ = standard deviation of response
S = average of slope

Application of the proposed procedures for the simultaneous determination of EPM and HCT in laboratory prepared mixtures

Different mixtures of the two drugs were prepared by transferring different volumes of EPM and HCT from working solutions into 100ml volumetric flasks and diluting to volume with 0.1 M sodium hydroxide. The concentrations of both EPM and HCT were determined by measuring the absorbance of the prepared mixtures at 294.2 nm and 274.5 nm. From these absorbance values, the concentrations of EPM and HCT were determined using Simultaneous equation method.

Application of the proposed procedure for the determination of dosage form

Twenty tablets were weighed accurately and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of tablet powder equivalent to 60 mg of EPM was weighed and transferred into 100ml volumetric flask and added a minimum quantity of 0.1 M Sodium hydroxide to dissolve the substance and made up to the volume with the same (600μg/ml). The solution was sonicated for 15 minutes and centrifuged for 15 minutes at 100 rpm. The supernatant liquid was separated and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 4ml to 100ml with 0.1 M sodium hydroxide to obtain 24 μg/ml solution of EPM which also contains 1 μg/ ml of HCT theoretically. The absorbance was measured at their selected wavelengths and the concentrations of two drugs were drugs were determined as described for synthetic mixture. The procedure was repeated for six times.

Recovery studies

The accuracy of the proposed method was confirmed by recovery studies. To the pre analyzed formulation a known amount of raw material was added and it can be analyzed by proposed method.

To an accurately weighed quantity of the tablet powder equivalent to 60 mg of EPM, 7.5 mg, 15 mg and 22.5 mg of EPM and 2.5 mg, 5 mg and 7.5 mg of HCT raw materials were added into a series of 100ml volumetric flasks. Then the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. The procedure was repeated for three times for each concentration.

Results and Discussion

Selection of solvent for analysis

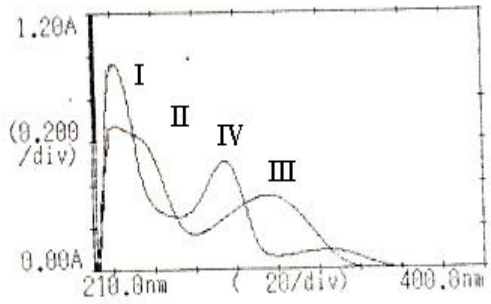
The UV spectra of EPM and HCT, obtained from different solutions (methanol, 2 - propanol, Distilled water, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide) were studied. The drugs were insoluble in distilled water and in 0.1 M hydrochloric acid. In methanol and 2 – propanol, the stability of EPM is less. In 0.1M sodium hydroxide EPM is

slightly soluble and HCT is sparingly soluble. The stability of both the drugs was found to be 24 hours and six hours for EPM and HCT, respectively. At the end of these studies, 0.1 M sodium hydroxide was chosen, because of the time gain while preparing solutions and cost saving by eliminating the purchase and disposal of organic solvents.

Vierodt's method of simultaneous equation

The overlain spectra of EPM and HCT show overlap, that prevents the use of direct absorbance measurements for determination of both the drugs in their mixtures. Figure 3 shows that the λ max for EPM at 294.2 nm and for HCT at 274.5 nm.

Fig.3
Overlain spectrum of EPM and HCT containing 10µg/ ml concentration of both the drugs in 0.1M sodium hydroxide



[I is HCT; II is EPM; III λ max of EPM (294.2 nm) and IV is the λ max of HCT (274.5 nm)]

They were linear in concentration range of 6 – 36 µg /ml and 1 - 10 µg /ml for EPM and HCT, respectively. The r^2 -values were found to be 0.99989 and 0.99978 for EPM and 0.99994 and 0.99980 for HCT at 294.2 nm and 274.5 nm, respectively. As per ICH guidelines, LOD and LOQ can be determined using visual evaluation, signal to noise ratio or from slope of linearity plot and standard deviation. Visual evaluation may be used in non instrumental methods and signal to noise ratio is normally possible with chromatographic methods. Hence, the method based on determination of slope of linearity plot and standard deviation of y intercept of linearity was used for the determination of LOD and LOQ. The calibration curves for EPM and HCT at 294.2 nm and 274.5 nm are shown in figure 4 and 5, respectively.

The LOD for EPM and HCT was found to be 0.4142 µg /ml and 0.5119 µg /ml, 0.2579 µg /ml and 0.1337 µg /ml at 294.2 nm and 274.5 nm, respectively. The LOQ at 294. 2 nm and 274.5 nm were found to be 1.2552 µg /ml and 1.5495 µg /ml for EPM and 0.7805 µg /ml and 0.4052 µg /ml for HCT, respectively.The optical characteristics such as correlation coefficient, slope, intercept, LOD, LOQ, Molar absorpitivity and Sandells sensitivity were calculated and are shown (Table 1). To study the mutual interference, if any, In the simultaneous estimation of EPM and HCT, synthetic mixtures containing various proportions of EPM and HCT were prepared and the contents were estimated by the proposed method. The percentage recovery varied from 99.18% to 101.52% for EPM and 98.58% to 102.27% for HCT indicating that no mutual interference up to the ratio of 28:10 for both the drugs (Table 2). The stability of the solutions was determined by measuring the absorbance at 294.2 nm and 274.5 nm for EPM and HCT, respectively at periodic intervals. From the stability studies the drugs were stable up to 24 hours and 6 hours for EPM and HCT, respectively.

Fig.4.
Calibration curve of EPM in 0.1 M sodium hydroxide at 294.2 nm and 274.5 nm.

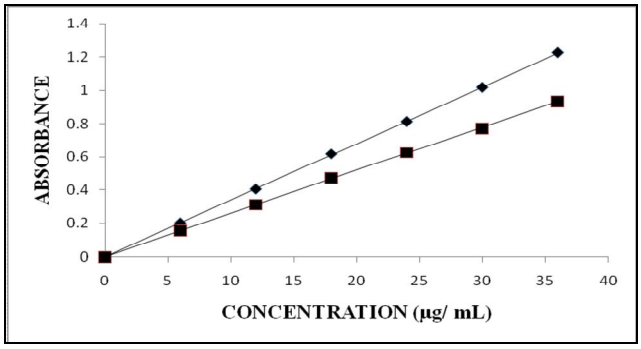
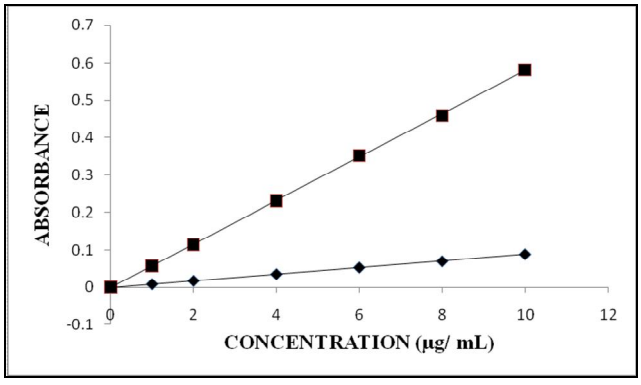


Fig.5.
Calibration curve of HCT in 0.1 M sodium hydroxide at 294.2 nm and 274.5 nm



Commercial formulation containing EPM and HCT were analysed by proposed method. Six replicate analysis of formulation were carried out and the mean EPM content was 596.31 mg/tablet and the mean content of HCT was 25.20 mg/tablet. The corresponding standard deviation was found to be 0.7582 for EPM and 0.5352 for HCT indicating that the method has required precision. The results of analysis of formulation are shown in Table 3.

Table 1:
Optical Characteristics of EPM AND HCT

PARAMETERS	EPM		HCT	
	At 294.2 nm	At 274.5 nm	At 294.2 nm	At 274.5 nm
Beers law limit (µg/ mL)	6 – 36	6 – 36	1 – 10	1 – 10
Molar absorptivity (L mol^{-1} cm^{-1})	17927.34	13316.14	2525.03	16793.49
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.02920	0.0395	0.1236	0.0176
Correlation coefficient (r)	0.99989	0.99978	0.99994	0.99980
Regression equation (Y = mx + c)	*Y = 0.0343x + 0.0017*	*Y=0.0254x- 0.000028*	*Y = 0.0085 x + 0.00036*	*Y=0.0565 x + 0.00035*
Slope (m) *Intercept (c)* *LOD (µg/ml)* *LOD (µg/ml)* *Standard Error*	0.0343 0.0017 0.4142 1.2552 0.000568	0.0254 *- 0.000028* *0.5119* *1.5495* *0.00153*	0.0085 0.00036 0.2579 0.7805 0.00013	0.0565 0.00035 0.1337 0.4052 0.0008

* Mean of six observations

Table 2:
Analysis of Synthetic Mixture of EPM and HCT

Sample No	Concentration of EPM (µg/ mL)		%Recovery*	Concentration of HCT (µg/ mL)		% Recovery*
	Theoretical	Experimental*		Theoretical	Experimental*	
1	10	9.919	99.183	1	0.9984	99.844
2	12	11.928	99.396	2	2.0375	101.87
3	14	14.082	100.588	3	2.9575	98.582
4	16	15.966	99.786	4	4.0359	100.897
5	18	18.013	100.069	5	5.0403	100.804
6	20	20.039	100.191	6	5.8946	98.243
7	22	22.34	101.523	7	6.9451	99.216
8	24	23.947	99.779	8	8.1820	102.275
9	26	26.036	100.141	9	9.1316	101.462
10	28	28.507	100.181	10	10.151	101.508

*Mean of Three observations

Table 3:
Analysis of Formulation

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average* (%)	S.D.*	% R.S.D.*	S.E.*
EPR	1 2 3 4 5 6	600 600 600 600 600 600	600.99 598.80 589.73 591.53 599.19 597.65	100.17 99.80 98.30 98.59 99.86 99.61	99.39	0.7582	0.7628	0.3096
HCT	1 2 3 4 5 6	25 25 25 25 25 25	25.30 25.54 24.81 24.97 25.65 24.92	101.19 102.18 99.21 99.89 102.58 99.67	100.78	0.5352	0.5311	0.2185

** Mean of six observations*

Table 4:
Intermediate Precision and Ruggedness of the Method

PARAMETERS	PERCENTAGE OBTAINED		% RSD	
	EPM	HCT	EPM	HCT
1) Intraday*	100.45 ± 0.1346	100.93 ± 1.4146	0.1340	1.4016
2) Inter day*	99.80 ± 1.6506	99.08 ± 1.2708	1.6539	1.2826
Different Analysts**				
i) Analyst I	100.64 ± 0.4424	100.17 ± 1.3471	0.4396	1.3481
ii) Analyst II	100.29 ± 0.4342	101.29 ± 1.8430	0.4329	1.8192
Different instruments**				
i) Instrument I	100.19 ± 1.0061	99.69 ± 0.9833	1.0041	0.9864
ii) Instrument II	99.64 ± 0.9624	100.62 ±0.8162	0.9658	0.8112

* Mean of three observations & ** Mean of six observations

Table 5:
Recovery Studies

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)*	Amount estimate (µg/ ml)*	Amount recovered(µg/ ml)*	% Recovery*	S.D*	% R.S.D*	S.E.*
EPM	1 2 3	23.86 23.86 23.86	3 6 9	26.93 29.84 33.13	3.065 5.975 9.269 Mean	102.17 99.58 102.95 101.57	1.6162	1.5912	0.9331
HCT	1 2 3	1.0081 1.0081 1.0081	1 2 3	2.016 3.026 4.015	1.008 2.018 3.007 Mean	100.80 100.90 100.23 100.64	0.3614	0.3592	0.2087

* Mean of Three Observations

Further, the precision was confirmed by intermediate precision. The analysis of formulation was carried out for three times in the same day and on three successive days. The % RSD values for inter day and intraday analysis of formulation was found to be less than 2% .The ruggedness was confirmed by different analysts and different instruments. The % RSD values for different analysts and different instruments were found to be less than 2%. The results for intermediate precision and ruggedness are shown in table 4.

The accuracy of method was confirmed by recovery studies. To the pre analyzed formulation a known quantity of raw material was added in different concentrations. The amount of drug recovered was calculated and the percentage recovery was found to be in the range of 99.58% - 102.95% for EPM, 100.23 – 100.90% for HCT. The procedure was repeated for three times for each concentration and the % RSD values were calculated. The low %RSD values ensure that the excipients used in formulation are not interfering in the analysis of EPM and HCT. This is shown in table 5.

Conclusion

The proposed method based on Vierodt's simultaneous equation method can be used for the simultaneous determination of EPM and HCT either in their binary mixture or in combined tablet dosage form. HPTLC and Capillary electrophoretic techniques were already reported for the simultaneous estimation of EPM and HCT in tablet formulations. However they require highly sophisticated instruments, costly solvents, time consuming when compared to UV Spectrophophotometric method. Also, the better precision and accuracy was achieved than the reported methods. Thus, the proposed method is precise, accurate, and simple to perform. Also, no separation step is required. It is rapid and does not require any expensive or sophisticated apparatus, in contrast with chromatographic and capillary electrophoretic techniques. Hence, the proposed method UV Spectrophotometric method can be effectively used for the routine analysis of EPM and HCT bulk and in combined tablet dosage form.

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References

1. Budavari, The Merck Index – An Encyclopedia of Chemicals, Drugs and Biologicals, 14th edn, Merck Research Laboratories, Whitehouse Station, New Jersey,2006, pp. 621 – 622.
2. http://en.wikipedia.org/wiki/Eprosartan Mesylate.
3. http://en.wikipedia.org/wiki/Hydrochlorothiazide.
4. Indian Pharmacopoeia, Vol. II, Government of India, Ministry of wealth and family welfare. New Delhi: The Controller of Publication, 2007, pp. 1194 – 1196.
5. British Pharmacopoeia, Vol. I, Department of Health and Social Services for Northen Ireland, London: Stationery Publication, 1993, pp. 330.
6. The United State Pharmacopoeia 32 and National Formulary 27, Vol.II, Asian ed. Rockville MD: United State Pharmacopoeial Convention Inc, 2009, pp. 2566 – 2568.
7. Ferreiros N, Iriarte G, Alonso RM and Jimenez RM, *Talanta,* 69(3), 2006, 747 – 756.
8. Ferreiros N, Iriarte G, Alonso RM, Jimenez RM and Ortiz E, *J.Chromatogr. A,* 1119(1 – 2), 2006, 309 – 314.
9. Fei Liu, Yu Xu, Shu Gao, Jundong Zhang and Qingxiang Guo, *J.Pharm.Biomed. Anal. ,*44(5), 2007, 1187 – 1191.
10. Farthing, Itaf Fakhry, Elizabeth B.D, Ripley and Domenic Sica, *J. Pharm. Biomed. Anal.,* 17(8), 1998, 1455-1459.
11. El – Gindy A, Ashour A, Abdel – Fattah L and Shabana M M, *J. Pharm.Biomed.Anal.*, 25 (5 - 6), 2001, 923 – 931.
12. Hillaert S and Van den Bossche, *J. Pharm.Biomed.Anal.*, 31, 2003, 329 – 339.
13. Beckett and Stenlake J B. Practical Pharmaceutical Chemistry. Edn 4. Part 2, CBS Publishers and Distributors, New Delhi, 2007, pp 284 – 288.
14. ICH guidelines Q2A. Text on validation of analytical procedures: Methodology. In the proceedings of International Conference on Harmonization, Geneva, March 1994, pp 1 – 5.
15. ICH guidelines Q2B. Text on Validation of analytical procedures: Methodology. In the proceedings of International Conference on Harmonization, Geneva, March, 1996, pp. 1 – 8.