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Original Research Article

Simultaneous analysis of eprosartan and hydrochlorothiazide in tablet formulation by High- Performance thin layer chromatography with ultraviolet absorption densitometry

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ABSTRACT

A new, simple, accurate, and precise high-performance thin-layer chromatographic method has been established for simultaneous analysis of Eprosartan and Hydrochlorothiazide from a tablet formulation. Standard and sample solutions of Eprosartan and Hydrochlorothiazide were applied to precoated 250 μm layer of silica gel G 60 F_{254} and the plates were developed with Chloroform: Acetonitrile: Glacial Acetic Acid (7:3:1,v/v/v) as mobile phase. Detection and evaluation of densitograms was performed densitometrically at 254 nm. The linear range was 200-700 ng/band with the retention factors of Eprosartan and Hydrochlorothiazide were 0.26± 0.02 and 0.44±0.02, respectively. The method was validated and successfully used for analysis of the drugs in tablets.

1. Introduction

Eprosartan (EPS), (E)-3-[2-butyl-1-[(4 carboxyphenyl)methyl]-1H-imidazol-5-yl]-2-[(2 thienyl)methyl]propenoic acid (Figure 1)^{A}, is a highly selective, non-peptide angiotensin-II antagonist. It has been shown to inhibit angiotensin-II induced vasoconstriction and to reduce systolic and diastolic blood pressure.[1] Hydrochlorothiazide (HYT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide (Figure 1)^{B} is a diuretic drug.[2] The rationale behind use of this drug combination is that in treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy. Oral administration of EPS with HYT has been found to be more effective than use of either drug alone.[3]

Other work dealing with analysis of EPS and other drugs in pharmaceuticals and biological samples includes use of LC–MS–MS,[4] capillary zone electrophoresis,[5] and micellar electrokinetic capillary chromatography.[6] Analysis of EPS in biological samples by HPLC–UV[7] and a chemometric method for optimization of solid-phase extraction HPLC–UV[8] of EPS in plasma have also been reported. There are several reports of the analysis of HYT

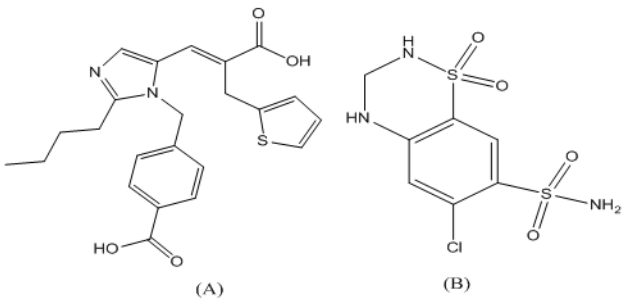

Fig. 1: The structures of (A) Eprosartan and (B) Hydrochlorthiazide

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individually or in combination with other drugs, including use of HPLC, HPTLC, spectrophotometry, and non-aqueous potentiometric titration.[9–12] As far as we are aware, no validated HPTLC method for analysis of EPS and HYT in bulk and in tablets has been reported in the literature. The purpose of this research was to establish such a method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines and the directives for good laboratory practice,[13–17] to use the method for analysis of the drug content of tablets.

2. Materials and Methods

2.1. Materials

EPS and HYT were received as a gift sample from Abbott Laboratories. Methanol (HPLC grade) was purchased from MERCK Specialities Private Limited. AR grade Ethyl acetate, Ammonia solution, Chloroform, Acetonitrile and Glacial Acetic Acid were purchased from ANALAB fine chemicals, Mumbai. The tablet formulation containing 600 mg of EPS and 25 mg of HYT (Teveten HCT) was purchased from local market.

2.2. Instrumentation and chromatographic condition

Chromatography was performed on 20 cm × 10 cm aluminum-backed TLC plates, coated with 250 μm layer of silica gel G 60 F_{254} (E. Merck, Darmstadt, Germany supplied by Anchrom Technologists, Mumbai). The solubility of EPS and HYT were checked in different solvents and methanol was selected as the solvent for dissolving both the drugs. Samples were applied to the plates as 8 mm bands, by means of 25 microlitre Linomat V applicator (Camag, Muttenz Switzerland) equipped with a Hamilton syringe (Bonaduz., Switzerland). The slit dimensions were 6.00 mm × 0.30 mm and the scanning speed was 20 mm $s^{-\cdot 1}$ A constant application rate of 1 μL/s was used and the space between two bands was 5 mm. The monochromator bandwidth was set at 20 nm, each track was scanned thrice and the baseline correction was used. Ascending development of the plates was performed with Chlroform: Acetonitrile: Glacial Acetic Acid (7:3:1, v/v/v) as mobile phase in a Camag 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland); previously saturated for 30 min with mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was 8 cm. The average development time was 20 min. After development, plates were dried. Densitometric scanning was performed on Camag TLC Scanner 3 in the reflectance absorbance mode at 254 nm for all measurements and operated by Wincats software version 3.15 supplied by Anchrom technologists, (Mumbai).

2.3. Preparation of Standard Stock Solutions

Standard stock solutions with a concentration of 10μg/mL were prepared in methanol for EPS and HYT respectively. The stock solutions were further diluted to enable application of 1 ng/band for both drugs.

2.4. Selection of Analytical Wavelength

Overlay spectra of both drugs was acquired to select appropriate wavelength of analysis.

2.5. Optimization of Mobile Phase and Chromatographic Conditions

Chromatographic separation studies were carried out on the stock solution of EPS and HYT. Various solvent mixtures were tried to achieve optimum resolution between both drugs as shown in Table 1 **.**

2.6. Preparation of Mobile Phase

The mobile phase consisting of Chloroform: Acetonitrile: Glacial Acetic Acid (7:3:1, v/v/v) was prepared. Then the mobile phase prepared was transferred to twin trough chamber in which one side of trough a filter paper wetted with mobile phase was kept to cause saturation of chamber faster.

2.7. Stability of sample solution

The stability of both drugs in the mobile phase was studied for 12 Hours.

2.8. Validation of HPTLC method

2.8.1. Linearity

From stock solution, EPS and HYT were separately spotted on the TLC plate to obtain final concentration 200-700 ng/band respectively. The plate was developed using previously described mobile phase.

2.8.2. Limit of detection (LOD

LOD was calculated from the formula LOD = 3.3 σ/S, where σ = Standard deviation response of the calibration curve, S = Slope of the calibration curve.

2.8.3. Limit of quantification (LOQ

LOQ was calculated from the formula LOQ = 10 σ/S, where σ = Standard deviation response of the calibration curve, S = Slope of the calibration curve.

Table 1: Optimizationof mobile phase

Sr. No	Mobile phase	Remarks
1	Chloroform: Acetonitrile: Glacial Acetic Acid (8:3:1, v/v/v)	Rf values was closer to 1
2	Ethyl acetate: Methanol (9:1, v/v)	No proper resolution
3	Chloroform: Toluene: Methanol (7:2:1, v/v/v)	Tailing observed
4	Chloroform: Acetonitrile: Glacial Acetic Acid (7:3:1, v/v/v)	Well resolved spots

2.8.4. Specificity

The specificity of the method was ascertained by comparing densitogram of tablet formulation with that of diluent, mobile phase, standard drug sample of EPS and HYT.

2.8.5. Recovery

Accuracy of the method was carried out by applying the method to drug sample to which known amount of EPS and HYT standard powder corresponding to 80, 100 and 120% w/w of label claim were added. The mixtures were analysed by running chromatogram in optimized mobile phase.

2.8.6. Precision

Sample solution of concentration 200-700 ng/band for EPS and HYT were used for studying degree of precision of the developed method. The repeatability of sample application and measurement of peak area of the drug was calculated by repeating the assay six times for each concentration on same day. Intra-day and Inter-day precision was determined by repeating assay three times in same day at different time intervals for intraday precision and on different days for inter day precision studies.

2.8.7. Robustness

Robustness was demonstrated by change in chamber saturation time to 10 min and 20 min and change in wavelength to 252 and 256 nm of detection.

2.9. Analysis of tablet formulation

A powder equivalent to 600 mg of EPS and 25 mg of HYT from the trichurated tablet formulation was accurately weighed and dissolved in methanol, sonicated and volume was made to 100 ml with methanol. The solution was filtered through Whatman filter paper no. 41 and volume was adjusted to 100 ml with the same solvent. After filtration, the stock solution was diluted with methanol to give 0.6 μg/ml of EPS and 0.025 μg/ml of HYT. The amount of EPS and HYT present in mixture was calculated.

3. Result and Discussion

EPS and HYT in methanol showed absorption maxima where the isoabsorptive point of these two graphs was observed at 254 nm as shown in Figure 2. Therefore, 254 nm was selected as detection wavelength for both the drugs.

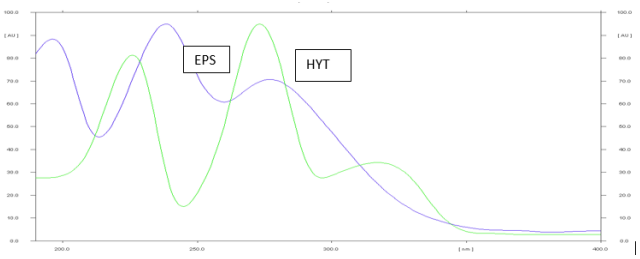

Fig. 2: Typical overlay spectra EPS and HYT

3.1. Optimization of mobile phase

After several trials, mixture of Chloroform: Acetonitrile: Glacial Acetic Acid (7:3:1, v/v/v) was chosen as a mobile phase for analysis. Other chromatographic conditions like run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength were optimized to give reproducible R_f values, better resolution and symmetrical peak shape for the two drugs as shown in Figures 3 and 4The Rf value of EPS and HYT was found to be 0.26 ± 0.02 and 0.42 ± 0.02 respectively. The drug solution diluted with mobile phase was found to be stable for 12 hours.

3.2. Validation of Analytical method

3.2.1. Linearity

From stock solution, EPS and HYT were separately spotted on the TLC plate to obtain final concentration of 200-700 ng/band. The plate was developed using previously described mobile phase. The results obtained are shown in Table 2. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs (Figures 5 and 6).

3.2.2. Limit of Detection and Limit of Quantitation

The LOD and LOQ for EPS and HYT are given in Table 3.

3.2.3. Specificity

The chromatogram of formulation showed only two peaks for EPS and HYT indicating that there is no any interference of any excipients from the formulation.

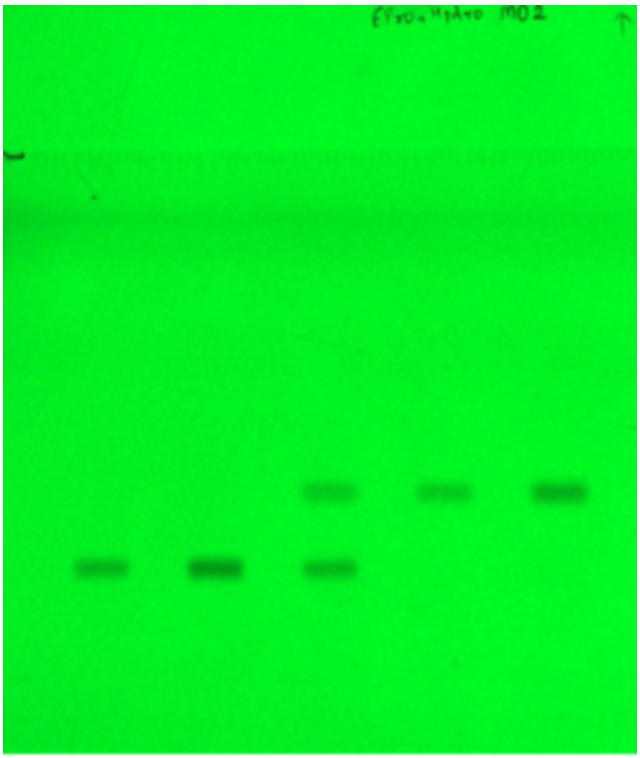

Fig. 3: Densitometric scan of EPS and HYT acquired using Chloroform: Acetonitrile: Acetic Acid (7:3:1, v/v/v) as a mobile phase

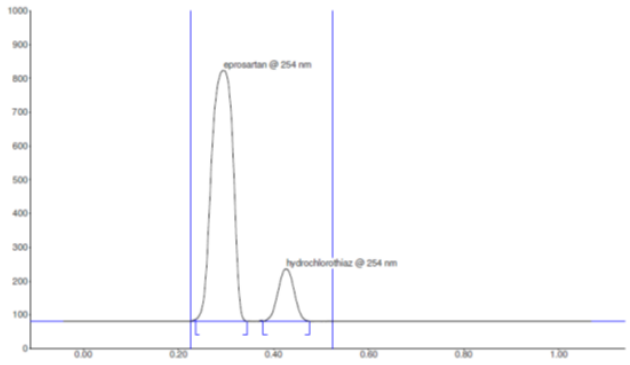

Fig. 4: Chromatogram of EPS and HYT

Table 2: Standard Calibration Curve data for EPSand HYT

Sr No	Concentration (ng/band)	EPS Area*	HYT Area*
1	200	2525.20	2235.43
2	300	3166.73	3042.09
3	400	4048.65	3944.72
4	500	4887.54	4777.36
5	600	5644.36	5460.43
6	700	6276.51	6054.16

*Mean of three estimations.

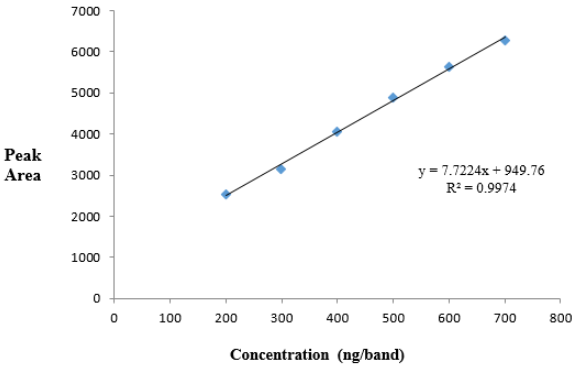

Fig. 5: Standard calibration curve of EPS

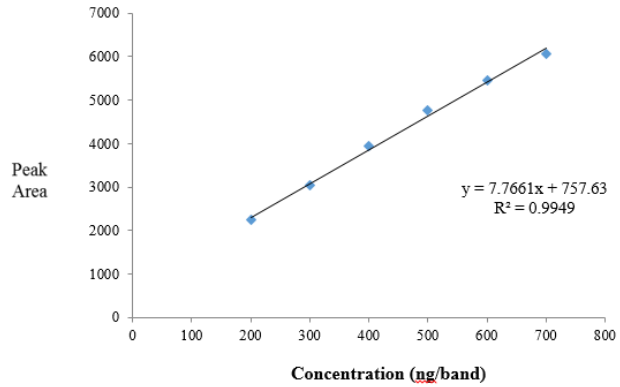

Fig. 6: Standard calibration curveof HYT

Table 3: LOD and LOQ values

Parameter	EPS	HYT
LOD ng/band	0.811	0.332
LOQ ng/band	2.45	1.007

3.2.4. Accuracy

The % recovery was found to be within the limits of the acceptance criteria and the results are given in Table 4. The % recovery value was found to be in the range of 98-102% w/w.

Table 4: Recovery analysis of EPS and HYT

Drug (ng/band)	Amount added (%)	% Recovery*	% RSD
EPS	80	99.4 ± 0.238	0.3401
	100	99.46± 0.208	0.2973
	120	99.48±0.275	0.3941
HYT	80	99.26±0.296	0.084
	100	99.70±0.159	0.045
	120	99.53±0.223	0.063

*Mean of three readings

Table 5: Repeatability, Intraday and Interday precision

Drug	Conc (ng/band)	Intra-day %mean ± RSD	Inter-day %mean± RSD	Repeatability %mean ± RSD
EPS	600	99.67 ± 0.8956	99.41 ± 0.7685	99.67 ± 0.8956
HYT 25		99.87 ± 0.5678	99.30 ± 0.4534	99.87 ± 0.5678

Table 6: Robustness of method to change in Saturation time and Change in detection wavelength

Drug	Conc (ng/band)	Change saturation time(10min)	Change saturation time(20min)	Change in Wavelength At 252nm	Change in Wavelength At 256nm
EPS	600	99.60± 0.8916	99.70± 0.8925	99.80± 0.8020	99.75 ± 0.2010
HYT	25	99.50± 0.50	99.80± 0.800	99.50± 0.8020	99.80± 0.300

Table 7: It shows Assay results for EPS and HYT

Drug	Label Claim(ng/band)	% mean ± S.D.*	% RSD
EPS	600	96.9 ± 0.335	0.345
HYT	25	95.4 ±0.077	0.081

3.2.5. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2%.

Precision of the method was assessed in terms of intra-day and inter-day precision as per the recommendations stated in the ICH guidelines. The % RSD for Intra-day precision was thus found to be 0.8956 and 0.5678 EPS and HYT respectively. The % RSD for Inter-day precision was found to be 0.7685 and 0.4534 for EPS and HYT respectively. The results of the same are given in Table 5.

3.2.6. Robustness

The Robustness of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2%. The results are shown in Table 6.

3.3. Analysis of developed tablet formulation

The amount of EPS and HYT present in tablet formulation was calculated by comparing peak area of sample with that of standard. Densitogram was recorded. The results of analysis of formulation are given in Table 7.

Two peaks were observed in the chromatogram of the formulation, indicating that there is no interference of any extraneous material. The mean % drug content with %RSD of formulation is given in Table 7.

4. Conclusion

A precise, specific, and accurate HPTLC method was developed for simultaneous determination of EPS and HYT from Tablet Formulation without interference from the exicipients. The resolution was achieved using Chloroform: Acetonitrile: Glacial Acetic Acid (7:3:1, v/v/v) as a mobile phase using Aluminum sheet with silica gel G 60 F 254 as a stationary phase. The method was linear in the concentration range of 200-700 ng/band. The method is also robust with respect to chamber saturation time and detection wavelength.

5. Source of Funding

None.

6. Conflict of Interest

None.

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