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Simultaneous Estimation of Eprosartan and Hydrochlorthiazide in Pharmaceutical Dosage Forms by RP-HPLC

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An isocratic reverse phase high performance liquid chromatographic method was developed and validated for the simultaneous determination of eprosartan and hydrochlorothiazide in their binary mixtures of pharmaceutical dosage form preparation. The RP-HPLC method was performed by using a Agilent Polaris C_{18} column (150 × 4.6 mm id, ODS 2, 5 µm particle size) where the mobile phase was composed with 0.1 % orthophosphoric acid-methanol [(50:50 v/v) pH 3.10-0.04 adjusted with acetic acid] at a flow rate of 0.8 mL/min. The column temperature was maintained at 27 ± 5 °C and the detection was carried out by means of a UV detector at wavelength of 232 nm. The retention times of eprosartan and hydrochlorothiazide are 4.18 ± 0.10 and 2.53 ± 0.09 min, respectively. The method was found to be linear ($R^2 > 0.99$) precise (RSD < 2.0 %), accurate, specific, simple, sensitive, rapid and robust. The validated method can be used in routine quality control analysis of fixed dose tablet formulations having eprosartan and hydrochlorothiazide without any interference of excipients.

Keywords: Eprosartan, Hydrochlorothiazide, RP-HPLC, Tablet formulation.

INTRODUCTION

The cardiovascular risks are mainly due to age, race and high blood pressure or hypertension. Most of the patients suffering with hypertension require more than one agent in order to achieve satisfactory control on blood pressure [1,2]. Eprosartan mesylate (EPM) is a new antihypertensive agent as an angiotension II receptor antagonist that is highly selective to elicit a higher reduction in systolic blood pressure than other antihypertensive drugs [3,4]. The drug acts on the rennin angiotension system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotension II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic nor epinephrine production, further reducing blood pressure [3-5]. The rennin-angiotensin system plays a vital role in the normal homeostatic regulation of cardiovascular and renal function. Chemically eprosartan is 4-({2-butyl-5[2-carboxy-2-(thiophen-2yl methyl)eth-1-en-l-yl]-1*H*-imidazole-l-yl}methyl)benzoic acid (Fig. 1) has anti-hypertensive activity [6]. Hydrochlorothiazide [HCT] is chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzo-thiadiazine-7-sulfonamide-1,1-dioxide (Fig. 2) is a thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte re absorption, directly increasing excretion of sodium chloride in approximately equivalent amount. It is official in IP, BP, USP [6-9]. Eprosartan and hydrochlorothiazide are available in tablet dosage form in the ratio of 1:24 [10]. Hydrochlorothiazide is official in IP and USP, while eprosartan is not official in any pharmacopoeias [10].

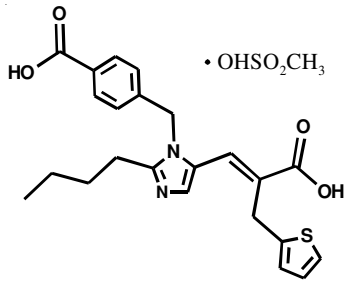

Fig. 1. Molecular structures of eprosartan

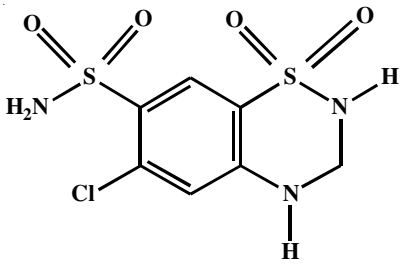

Fig. 2. Molecular structures of hydrochlorothiazide

From the review of the literature, it was observed that few methods have been reported for the estimation of eprosartan and hydrochlorothiazide separately or in combination with other drugs in tablet forms and/or in biological fluids [11-24], such as UV [25], HPLC [26-30] methods for analysis of eprosartan as single dosage forms and hydrochlorothiazide [31] as single component systems. The present method is relatively simple, rapid and highly sensitive for the analysis of eprosartan and hydrochlorothiazide in bulk or in any other formulations. The present method developed is also validated as per ICH guidelines in the analysis of the multi components of interest and it can be used for routine quality control analysis in laboratories [32].

EXPERIMENTAL

The separation was performed on an LC-10 ATVP HPLC system. The module equipped with LC-10 ATVP Binary pump, SIL-10ADvp auto sampler, CTO-10Avp column temperature oven, SPD-10Avp UV-Visible detector. All the system components are controlled using SCL-10Avp system controller. Data acquisition was carried out using LC solutions version 1.23 SP 1 software. This method carried out by using Agilent Polaris column (150 × 4.6 mm, 5 µm), at room temperature (25 °C).

Chromatographic conditions: HPLC separation was tested by using Agilent Polaris C-18 column with 150 × 4.6 mm internal diameter and 2.5 µm of particle size. The mobile phase consists the mixture of 50:50 % (v/v) methanol and 0.1 % orthophosphoric acid (pH adjusted to 3.0 with acetic acid) operated on isocratic mode. It was filtered through 0.45 µ membrane. The flow rate is 0.8 mL/min with detection wavelength of 232 nm at 25 °C. The injection volume is 20 µL.

Preparation of standard solution: Standard solutions of respective drugs can be prepared by taking 21.5 mg of eprosartan and 22.8 mg of hydrochlorothiazide in to a separate 100 mL volumetric flask, dissolved with 50:50 % v/v of mobile phase and milli Q water and solicited until the solutions were completely dissolves. Calibration curve is linear, containing 6 non-zero standards were prepared by using diluents in the concentration range of 5.03-50.31 µg/mL and 5.01-50.16 for eprosartan and hydrochlorothiazide respectively. The mixture was sonicated for 5 min.

Preparation of sample solution: Twenty tablets were accurately weighed and its mean weight was determined. The tablets were made in to a fine homogenized powder by using pestle and mortar. These drug samples were transferred into a separate 100 mL volumetric flask and made into homogeneous solution by using diluents. From this stock, quality control samples were prepared at 3 concentration levels: LQC (12.54 and 12.57 µg/mL for hydrochlorothiazide and eprosartan), MQC (25.08 and 25.15 µg/mL), HQC (37.62 and 37.73 µg/mL) to obtain low, median and high concentration quality control samples. Linearity curve has been calibrated and evaluated by using quality control samples.

Method development and validation: The HPLC procedure was optimized with a view to develop a stability indicating assay method. We had evaluated the chromatographic response by changing the pH values ranging from pH 3.0 to pH 6.5. We tried with different columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-packpro; Spheris orb C18, Phenomenex C18 with different buffer salts such as ammonium formate and orthophosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. Out of these we found that, Agilent Polaris ODS column C18 150 × 4.6 cm 5 µm column with mobile phase composition consisted of (80:20 v/v) of methanol and 0.1 % orthophosphoric acid showed symmetric peaks, less tailing factor and high theoretical plates. The flow rate was maintained at 0.8 mL/min with detection wavelength of 232 nm. Calibration standards were prepared by using 50:50 % v/v of methanol and Milli-Q water. At the proposed flow rate, symmetric peak was obtained, whereas by changing the flow rate results an unacceptable tailing factor and poor peak shape. The chromatogram showing the separation of the drugs is given in Fig. 3.

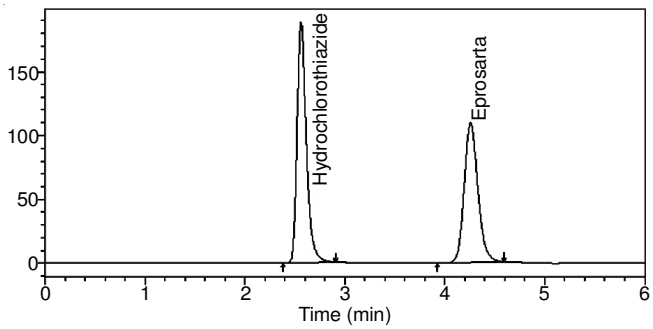

Fig. 3. Observed chromatogram which shows the separation of eprosartan and hydrochlorothiazide

RESULTS AND DISCUSSION

System suitability: System suitability is a pharmacopoeia requirement test which is used to ensure that the proposed method could generate acceptable accuracy and precision results. The study was carried out by injecting six replicate standard solutions, followed by evaluating % RSD of the peak observed. The data was given in the Table-1 within the acceptable limits.

TABLE-1
DATA FOR SYSTEM SUITABILITY

Sample ID	Hydrochlorothiazide		Eprosartan	
	Peak R_T	Peak area	Peak R_T	Peak area
1	2.53	1187607	4.18	1023391
2	2.56	1223586	4.25	1044164
3	2.57	1226642	4.35	1002354
4	2.57	1232197	4.35	1006607
5	2.57	1197494	4.36	977339
6	2.57	1234386	4.36	1004001
Mean	2.562	1216985.3	4.308	1009642.7
Std. EV	0.0160	19565.26	0.0757	22442.97
CV (%)	0.63	1.61	0.82	1.33

Specificity: Specificity can be estimated by comparing the chromatograms obtained between blank and drug solutions. Blank solution means diluent without drug with excipients and prepared the drug solutions. These mixtures were filtered through 0.45 µ membrane filter before injection. Now inject these samples to HPLC system and observed the peaks obtained. There was no interference of any peak for the excipients were

observed with drug peaks (Fig. 4). This indicates that the method is specific.

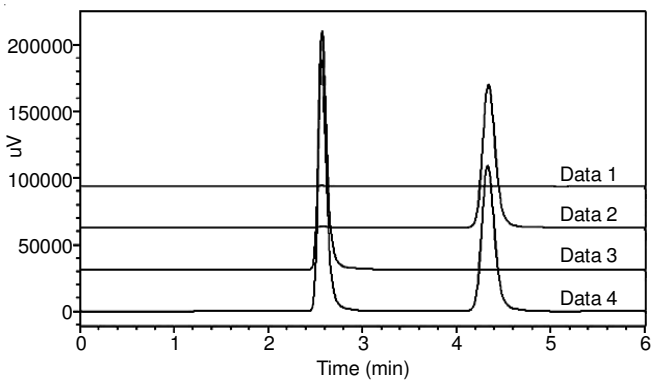

Fig. 4. Overlay chromatogram of specificity

Linearity: Linearity of an analytical method is to estimate test results that are directly proportional to the concentration of the analyte in a sample within the given range. The flow rate of the mobile phase was adjusted to 0.8 mL/min. The column temperature is maintained at 23 ± 1 °C throughout the study. Linearity was performed by preparing mixed standard solutions of eprosartan and hydrochlorothiazide at different concentration levels including working concentration in the range of 25.16 µg/mL of eprosartan and 25.08 µg/mL of hydrochlorothiazide. The solutions were injected in triplicates for each concentration by using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed and the plots of average content *vs.* respective concentration of eprosartan and hydrochlorothiazide were found to be linear in the range of 25.16 and 25.08 µg/mL with coefficient of correlation (r^2) 0.998 and 0.997 for eprosartan and hydrochlorothiazide respectively.

LOQ and LOD values were found to be 4.39 and 2.57 µg/mL, 4.41 and 2.58 µg/mL for eprosartan and hydrochlorothiazide, respectively.

Precision: It is a measure of the degree of reproducibility or repeatability of the analytical method under normal operating conditions. It is expressed in terms of standard deviation or relative standard deviation. Six replicate injections were given to HPLC system and observed the peaks obtained, % RSD of the contents of eprosartan and hydrochlorothiazide were calculated. This act was repeatability performed for the inter-day and intra-day variation studies. The results were compared and given in Tables 2 and 3.

Accuracy: The accuracy of the method should be demonstrated by performing a recovery study. It is the closeness of agreement between true value and measured value. Accuracy of the proposed method can be measured by preparing the samples solutions of known amounts of the pure drugs at different concentration levels and have been added to the pre analyzed standard samples of the drugs from 50 up to 100 %. The sample solutions were subjected to the HPLC system in triplicate and the percent of individual recovery and % RSD for recovery of the respective sample are calculated. A recovery ranged from 98 to 102 % has been observed by the method which indicates its accuracy.

TABLE-2
RESULTS OF INTER AND INTRA-DAY ACCURACY & PRECISION (CV, %) FOR EPROSARTAN BY HPLC

Sample ID	LQC	MQC	HQC
Nominal concentration (µg/mL)	12.57	25.16	37.73
Day 1			
Mean concentration (µg/mL)	12.83	25.02	38.12
SD	0.35	0.23	0.20
CV (%)	2.71	0.94	0.53
Day 2			
Mean concentration (µg/mL)	12.71	25.18	37.25
SD	0.10	0.18	0.26
CV (%)	0.80	0.71	0.70
Day 3			
Mean concentration (µg/mL)	12.55	25.34	38.81
SD	0.18	0.26	0.31
CV (%)	1.43	1.03	0.80

TABLE-3
RESULTS OF INTER AND INTRA-DAY ACCURACY & PRECISION (CV, %) FOR HYDROCHLOROTHIAZIDE BY HPLC

Sample ID	LQC	MQC	HQC
Nominal concentration (µg/mL)	12.72	25.44	37.50
Day 1			
Mean concentration (µg/mL)	12.62	25.12	37.37
SD	0.22	0.23	0.19
CV (%)	1.72	0.92	0.51
Day 2			
Mean concentration (µg/mL)	12.58	25.32	37.25
SD	0.21	0.25	0.49
CV (%)	1.67	0.99	1.32
Day 3			
Mean concentration (µg/mL)	13.12	24.89	37.56
SD	0.25	0.35	0.56
CV (%)	1.91	1.41	1.49

Conclusion

The proposed RP-HPLC method was developed for the simultaneous estimation of eprosartan and hydrochlorothiazide in combined tablets dosage forms which is new, simple, precise, linear, robust, rugged highly accurate, reproducible and less time consumption. The percentage of average recovery was found to be in the range of 98 to 100. The absence of excessive peaks in the drug chromatograms indicates the non-interference of excipients in the drug formulation. The results obtained were satisfactory as per the ICH guidelines. Hence, this RP-HPLC method is suitable for quality control of raw materials and formulations and also for dissolution studies.

REFERENCES

1. J.P. Yaxley and S.V. Thambar, *J. Family Med. Prim. Care*, **4**, 193 (2015); https://doi.org/10.4103/2249-4863.154630.
2. S.N. Covey, *Trends Pharmacol. Sci.*, **5**, 405 (2000); https://doi.org/10.1016/S1360-1385(00)01745-3.
3. A.K. Suresh, R. Sharma, B.K. Bansal, C.J. Singh, *Int. J. Res. Develop. Pharmacy Life Sci.*, **4**, 1559 (2015).
4. P.J. Blankestijn and H. Rupp, *Cardiovasc. Hematol. Agents Med. Chem.*, **6**, 253 (2008); https://doi.org/10.2174/187152508785909500.
5. W.H. Frishman, A. Cheng-Lai and J. Nawarskas, Current Cardiovascular Drugs, Current Medicine Group, p. 53 (2007).
6. G.S. Devika, M. Sudhakar and J.V. Rao, *Pharm. Anal. Acta,* **2**, 3. (2011).

7. Indian Pharmacopoeia Vol, II Published by the Government of India, Ministry of Health and Family Welfare, the Indian Pharmacopoeia Commission, New Delhi, pp. 1194-1196 (2007).
8. British Pharmacopoeia Vol. II, Department of Health and Social Services for Northern Ireland, Stationary Publication, London, p. 330 (2007).
9. United States Pharmacopoeia National Formulary USP23, Asian Edition, United States Pharmacopoeia Convention, Rockville, MD, USA, pp. 2566-2568 (2009).
10. T. Anusha, P. Vishnu Priya, N. Anjali Devi, Shyamala, J.V.C. Sharma, *Indo Am. J. Pharm. Res.*, **3**, 11 (2013).
11. D. Sridharan and A. Thenmozhi, *Asian J. Pharm. Clin. Res.*, **3**, 93 (2010).
12. L.R.P. de Abreu, S.A.C. de Castro and J. Pedrazzoli, *AAPS PharmSci*, **5**, 1 (2003); https://doi.org/10.1208/ps050221.
13. R.K. Barman, M.A. Islam, M. Ahmed, M.I.I. Wahed, R. Islam, A. Khan, M.B. Hossain and B.M. Rahman, *Pak. J. Pharm. Sci.*, **20**, 274 (2007).
14. A.P. Argekar and S.G. Powar, *J. Pharm. Biomed. Anal.*, **21**, 1137 (2000); https://doi.org/10.1016/S0731-7085(99)00210-1.
15. J. Kavitha and S. Muralidharan, *Int. J. ChemTech. Res.*, **2**, 880 (2010).
16. S.R. Sathe and S.B. Bari, *Acta Chromatogr.*, **19**, 270 (2007).
17. T. Sivakumar, P. Venkatesan, R. Manavalan and K. Valliappan, *Indian J. Pharm. Sci.*, **69**, 154 (2007); https://doi.org/10.4103/0250-474X.32137.
18. P. Gupta, C. Issa and A. Bansal, *Indian J. Pharm. Sci.*, **67**, 672 (2005);
19. V. Marinkovic, D. Agbaba, S. Vladimirov and S. Stankovic, *J. Pharm. Biomed. Anal.*, **24**, 993 (2001); https://doi.org/10.1016/S0731-7085(00)00531-8.
20. S.Y. Oh, K.Y. Kim, Y.G. Kim and H. Kim, *J. Pharm. Biomed. Anal.*, **32**, 387 (2003); https://doi.org/10.1016/S0731-7085(03)00129-8.
21. J. Pastera, L. Mejstríková, J. Zoulová, K. Macek and J. Kvìtina, *J. Pharm. Biomed. Anal.*, **44**, 674 (2007); https://doi.org/10.1016/j.jpba.2006.06.045.
22. A.B. Baranda, N. Etxebarria, R.M. Jimenez and R. Alonso, *Talanta*, **67**, 933 (2005); https://doi.org/10.1016/j.talanta.2005.04.028.
23. A.B. Baranda, R.M. Jimenez and R.M. Alonso, *J. Chromatogr. A*, **1031**, 275 (2004); https://doi.org/10.1016/j.chroma.2003.11.019.
24. A.P. Argekar and J.G. Sawant, *J. Liq. Chromatogr. Relat. Technol.*, **22**, 1571 (1999); https://doi.org/10.1081/JLC-100101752.
25. M.T. Raju, S. Gurrala, J.V. Rao and K.R. Sambasiva, *Int. J. Pharm. Pharmaceut. Sci.*, **3**, (2011).
26. A.C. Müller and I. Kanfer, *J. Pharm. Biomed. Anal.*, **53**, 113 (2010); https://doi.org/10.1016/j.jpba.2010.03.019.
27. E. Cateau, N. Tournier, A. Dupuis, G. Le Moal and N. Venisse, *J. Pharm. Biomed. Anal.*, **39**, 791 (2005); https://doi.org/10.1016/j.jpba.2005.04.025.
28. A. Loregian, S. Pagni, E. Ballarin, E. Sinigalia, S.G. Parisi and G. Palù, *J. Pharm. Biomed. Anal.*, **42**, 500 (2006); https://doi.org/10.1016/j.jpba.2006.04.031.
29. R.M.W. Hoetelmans, M. van Essenberg, M. Profijt, P.L. Meenhorst, J.W. Mulder and J.H. Beijnen, *J. Chromatogr. B: Biomed. Sci. Appl.*, **705**, 119 (1998); https://doi.org/10.1016/S0378-4347(97)00500-8.
30. K.C. Marsh, E. Eiden and E. McDonald, *J. Chromatogr. B: Biomed. Sci. Appl.*, **704**, 307 (1997); https://doi.org/10.1016/S0378-4347(97)00454-4.
31. N. Sirisha, A. Haripriya, N.S. Bhavani, R. Bhagirath and M. Satyanarayana, *Scholars Res. Lib.*, **5**, 78 (2013).
32. J. Kavitha, J.S.K. Nagarajan, S. Muralidharan and B. Suresh, *Int. J. Pharm. Pharmaceut. Sci.*, **3**, 113 (2011).