AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Spectrophotometric Method for Simultaneous Estimation of Eperisone Hydrochloride and Aceclofenac Sodium in Synthetic Mixture

Paresh U Patel[1], Bhumi H. Barot [1*]
1. Department of Pharmaceutical Analysis, Center for Health Science Studies, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India.

ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Eperisone hydrochloride and Aceclofenac sodium in mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Eperisone hydrochloride has absorbance maxima at 255 nm and Aceclofenac sodium has absorbance maxima at 275 nm in methanol. The linearity was obtained in the concentration range of 4-20 µg/ml and 4-20 µg/ml for Eperisone hydrochloride and Aceclofenac sodium, respectively. The concentrations of both drugs in synthetic mixture were determined by using simultaneous equations. The mean recovery was 100.34 ± 0.27 and 100.64 ± 0.71 for Eperisone hydrochloride and Aceclofenac sodium, respectively. The method was applied for the determination of these drugs in mixture. The suitability of this method for the quantitative determination of Eperisone hydrochloride and Aceclofenac sodium was proved by evaluating different validation parameters. The proposed method was found to be simple and sensitive for the routine estimation of these drugs in combination. The results of analysis have been validated statistically and by recovery studies.

Keywords: Eperisone hydrochloride, Aceclofenac sodium, Recovery, Simultaneous equations method, Validation.

*Corresponding Author Email: rsp241@yahoo.com
Received 24December 2012, Accepted 03 January 2013

Please cite this article in press as Barot BH *et al.*, Spectrophotometric Method for Simultaneous Estimation of Eperisone Hydrochloride and Aceclofenac Sodium in Synthetic Mixture. American Journal of PharmTech Research 2013.

INTRODUCTION

Eperisone hydrochloride (EPE) is 4-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride[1] (Figure 1) is a well known Skeletal muscle relaxant [1]. It is official in Japanese Pharmacopoeia (JP). JP[2] describe RP-HPLC method for its estimation. Literature survey reveals RP-HPLC[3], LC-ESI/MS[4] and HPLC/MS, GC/MS, NMR[5] methods for estimation of EPE in single dosage form. Aceclofenac sodium (ACE) is chemically 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid[6,7] (Figure 2). Aceclofenac sodium (ACE) is official in IP and BP. IP[7] describes potentiometric method for its estimation. IP[7] and BP[8] describe liquid chromatography. Literature survey reveals UV[8] methods for determination of ACE in single dosage form. Literature survey also reveals UV spectrophotometry [9,10] and HPLC [11-14] method for the determination of ACE with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of EPE and ACE in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of EPE and ACE in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined mixture.

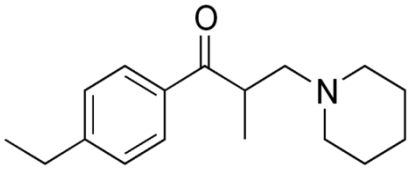

Figure 1: Eperisone hydrochloride (EPE)

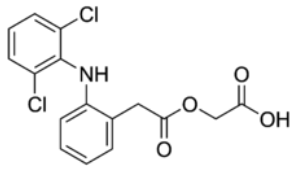

Figure 2:Aceclofenac sodium (ACE)

MATERIALS AND METHODS

Apparatus

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

EPE bulk powder was kindly gifted by Sun Pharmaceutical Industries Ltd, Halol, Baroda, Gujarat, India and ACE bulk powder was kindly gifted by Acme Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India)

and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of standard EPE (10 mg) and ACE (10 mg) powder were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml.

Preparation of synthetic mixture

500 mg of synthetic mixture was prepared by using EPE (50mg) and ACE (50mg) and excipients like MCC (Microcrystaline cellulose), Starch, Magnesium stearate and Talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol.

Preparation of sample solution

Synthetic mixture (0.2 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of EPE (10 µg/ml) and ACE (10 µg/ml).

Methodology

The working standard solutions of EPE and ACE were prepared separately in methanol having concentration of 10 µg/ml. These solution were scanned in the wavelength range of 200-400 nm against methanol as a blank. Maximum absorbance was obtained at 255 nm and 275 nm for EPE and ACE, respectively. These two wavelengths were used for the estimation of these two drugs in mixture.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [15]

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 4-20 µg/ml for EPE and 4-20 µg/ml for ACE. Accurately measured standard solutions of EPE (0.4, 0.6, 0.8, 1.2, 1.4, 1.6, 2.0 ml) and ACE (0.4, 0.6, 0.8, 1.2, 1.4, 1.6, 2.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of these solutions were measured at 255 and 275 nm against methanol as blank. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (*n* = 6) for EPE and ACE (14 µg/ml for both drugs) without changing the parameter of the proposed method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of EPE and ACE (6, 12, 18 µg/ml for EPE and 6, 12, 18 µg/ml for ACE). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of EPE and ACE by the standard addition method. Known amounts of standard solutions of EPE and ACE were added at 50, 100 and 150 % level to prequantified sample solutions of EPE and ACE). The concentrations of these two drugs were determined by solving simultaneous equations. The experiment was repeated for three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for the proposed method were determined by using following equations.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of EPE and ACE in mixture

0.2 ml sample solution was transferred in 10 ml volumetric flask and dilute up to mark with methanol to get a final concentration of EPE(10 µg/ml) and ACE (10 µg/ml). The responses of the sample solution were measured at 255 nm and 275nm for estimation of these drugs in mixture. The amounts of the EPE and ACE present in the sample solution were calculated by solving below mentioned simultaneous equations.

Cx = (A2 aY1 – A1 aY2) / (aY1 aX2 - aY2 aX1)

Cy = (A1 aX2 - A2 aX1) / (aY1 aX2 - aY2 aX1)

Where,

A_1 and A_2 are absorbance of mixture at 255 nm and 275 nm respectively;

aX_1 and aY_1 are absorptivities of EPE and ACE respectively at 255 nm;

aX_2 and aY_2 are absorptivities of EPE and ACE respectively at 275 nm.

RESULTS AND DISCUSSION

The standard solutions of EPE and ACE were scanned separately in the UV range and zero-order spectra for EPE and ACE were recorded. Maximum absorbance was obtained at 255 nm and 275 nm for EPE and ACE, respectively. These two wavelengths were used for the determination of EPE and ACE in mixture. Overlain zero-order absorption spectrum of EPE and ACE in methanol is shown in (Figure 3).

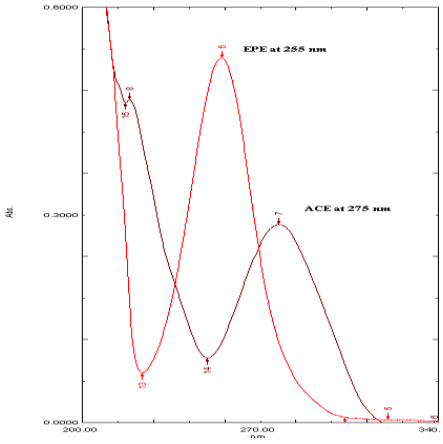

Figure 3: Overlain absorption spectra of EPE (8 µg/ml) and ACE (8 µg/ml) in methanol

Linear correlation was obtained between absorbances and concentrations of EPE and ACE in the concentration ranges of 4-20 µg/ml and 4-20 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The % RSD values of EPE were found to be 0.27 and 0.71 at 255 and 275 nm, respectively. The % RSD value of ACE was found to be 1.16 and 0.42 at 255 and 275 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low % RSD values of interday (0.22-0.59 and 1.06-1.66 for EPE at 255 and 275 nm, respectively and 0.30-0.53 and 0.08-1.28 for ACE at 255 and 275 nm, respectively) and intraday (0.53-0.82 and 0.92-1.82for EPE at 255 and 275 nm, respectively and 0.17-0.66 and 0.24-0.50 for ACE at 255and 275 nm, respectively) reveal that the proposed method is precise. LOD and LOQ values for EPE were found to be 0.082 and 0.21 µg/ml and 0.24 and 0.66 µg/ml at 255 and 275nm, respectively. LOD and LOQ values for ACE were found to be 0.033 and 0.16 µg/ml and 1.01 and 0.48 µg/ml at 255 and 275 nm, respectively. These data show that method is sensitive for the determination of EPE and ACE. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.34±0.27and 100.64 ± 0.71for EPE and ACE, respectively (Table 2). The results of

recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine EPE and ACE in their mixture. The results obtained for EPE and ACE were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method can be is applied for the routine estimation of EPE and ACE in mixture.

Table 1: Regression analysis data and summary of validation parameters for EPE and ACE

Parameters	**EPE**		**ACE**	
Wavelength (nm)	255	275	275	255
Beer's law limit (µg /ml)	4-20	4-20	4-20	4-20
Regression equation (y = a + bc)	y = 0.065x - 0.001	y = 0.019x - 0.012	y = 0.033x + 0.038	y = 0.016x - 0.003
Slope (b)	0.065	0.019	0.033	0.016
Intercept (a)	-0.001	0.0.012	+0.038	-0.003
Correlation coefficient (R^2)	0.9960	0.9930	0.9940	0.9920
LOD[a] (µg/ml)	0.082	0.21	0.16	0.33
LOQ[b] (µg /ml)	0.24	0.66	0.48	1.01
Repeatability (% RSD[c] , n = 6)	0.45	1.60	0.32	0.63
Precision (% RSD, n = 3)				
Interday	0.22-0.59%	1.06-1.66%	0.08-1.28%	0.30-0.53%
Intraday	0.53-0.82%	0.92-1.82%	0.24-0.50%	0.17-0.66%
Accuracy ± S. D[d]. (% Recovery, n = 3)	100.34±0.27		100.64 ± 0.71	

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification [d]S. D. is standard deviation

Table 2: Recovery data of EPE and ACE

Drug	**Amount taken (µg/ml)**	**Amount added (%)**	**% Recovery ± S. D. (n = 3)**
EPE	5	50	98.96 ± 0.047
	5	100	99.42 ± 0.11
	5	150	102.64 ± 0.66
ACE	5	50	100.3 ± 1.89
	5	100	100.4 ± 0.126
	5	150	101.22 ± 0.14

S. D. = Standard deviation., n = Number of determinations.

Table 3: Analysis of EPE and ACE in Synthetic mixture

Synthetic mixture	**Label claim (mg)**		**Amount found (mg)**		**% Label claim ± S. D. (n = 6)**	
	EPE	**ACE**	**EPE**	**ACE**	**EPE**	**ACE**
I	50	50	49.5	49.9	99.01	99.97

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of EPE and ACE in mixture. The method utilizes easily available and cheap solvent for analysis of EPE and ACE hence the method was also economic for estimation of EPE and ACE from mixture. The common excipients and other additives are usually present in the mixture do not interfere in the analysis of EPE and ACE in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in synthetic mixture.

ACKNOWLEDGEMENT

The authors are thankful to Sun Pharmaceutical Industries Ltd, Halol, Baroda, Gujarat, India and Acme Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of EPE and ACE, respectively for carry out the research work.

REFERENCES

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 12th edition, New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station; 2006; 12(3):36-38.
2. Japanese Pharmacopoeia. Society of Japanese Pharmacopeia. 15th ed. Shibuya Tokyo Japan 2006; 618.
3. Mastunga M, Uemura Y, Yonemoto Y, Kanai K, Etoh H, Tankla S., Atsuta Y Nishizawa Y, Yamanishi Y, Long Lasting muscle relaxant activity of eperisone hydrochloride after precutaneous administration in rats, Jpn.J Pharmacol 1997:215-220.
4. Jeoung M, Jeoung E, Kim N, Chung Y, Lee Y, Hwang E, Yhwa L, Hong T. Determination of Eperisone in Humann Plasma by Liquid chromatography-ESI-Tandem Mass Spectroscopy, Arch Pharm Res 2007;30(9):1174-1178.
5. Ding L, Wang X, Yang Z, Chen Y. The use of HPLC/MS, GC/MS, NMR, UV and IR to Identify Degradation product of Eperisone hydrochloride in the tablets. J Pharm and Biomed Analysis. 2008; 6(5):282-287.
6. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 12th edition, New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station. 2006; 12(3):5.
7. Indian Pharmacopoeia. 6th edition, Govt. of India, New Delhi: The Controller of Publication. 2010; 2(4):770-771.

8. British Pharmacopoeia. London: Stationary office Medicines and Healthcare product regulatory agency. 2007; 1(5):41-43.
9. Siva Kumar R, Nallasivan P. Spectrophotometric method for Simultaneous estimation of Aceclofenac and Tizanidine. Ind J Pharm Sci 2008; 2(1):945-949.
10. Garg G, Saraf S, Saraf S. Spectrophotometric method of Aceclofenac, Paracetamol and Chlorzoxazone. Ind J Pharm Sci 2007; 69(5):692-694.
11. Shaikh KA. Simultaneous determination of Diacerein and Aceclofenac in tablet dosage form. Int J Ind Chem 2010; 3(3):2-7.
12. Chandra P, Rathore A. Estimation of Aceclofenac, Tramadol and paracetamol by HPLC. Sci Pharma 2012; 80(2):337-351.
13. Jain J, Shah D, Shah S, Chauhan R. RP-HPLC method for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in their combined tablet dosage form. Derpharma chemica. Schol Res Lib 2011; 3(4):245 -252.
14. Satyanarayana P. Validated HPLC method for Simultaneous Estimation of Zinc Carnosine and Aceclofenac sodium .Int J Pharm Ind Res.2011; 1(2):109-111.
15. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure, Text and Methodology, 2005.