DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PROPRANOLOL HYDROCHLORIDE AND FLUNARIZINE DIHYDROCHLORIDE IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

The first order derivative of UV spectrometry method for simultaneous determination of Propranolol hydrochloride (PRO) and Flunarizine dihydrochloride (FLU) in pure bulk drug and combined dosage form was found to be simple, accurate, fast, precise and reproducible. The first derivative values measured at 289nm for PRO and 253nm for FLU. The linearity for zero order derivative method was carried out by using the concentration range 4-28µg/ml for PRO and 3-7µg/ml for FLU. The coefficient correlation of PRO and FLU for zero order was found to be 0.9995 and 0.9991 respectively. At zero crossing point of PRO (289nm) FLU showed a measurable derivative absorbance where as at the zero crossing point of FLU (253nm), PRO showed appreciable derivative absorbance value. The coefficient correlation of PRO and FLU for first order derivative was found to be 0.9991 and 0.9995 respectively. Precision study showed that % RSD was within the range of acceptable limits (<2). The % recovery for PRO and FLU was found to be in the range of 98-102% and 100-101% respectively. The percentage assay was found to be as 99.5 and 100.12% for PRO and FLU. The results of analysis have been validated as per ICH Q2 (R1) guidelines. This method has applied successfully for the determination of PRO and FLU in its combination with a high percentage of recovery good accuracy and precision.

Keywords: Propranolol Hydrochloride, Flunarizine Dihydrochloride, UV Spectrophotometry, First order derivative spectrophotometry.

INTRODUCTION

Propranolol hydrochloride [1-[(1-methyl ethyl) amino]-3-(1-naphthylenyl)-2-propanol hydrochloride]¹ is a non selective beta adrenergic antagonist and used in the management of hypertension, angina pectoris, myocardial infarction and cardiac failure¹. Propranolol hydrochloride is official in IP² and USP³. Flunarizine dihydrochloride [1-[Bis (4-fluorophenyl) methyl] 4-[2E)-3-phenylprop-2-enyl] piperazine dihydrochloride]⁴ is a calcium channel blocker and used in migraine prophylaxis, epilepsy and vascular disease⁵. Flunarizine dihydrochloride is official in BP⁶.

Figure 1: Chemical Structures of Propranolol Hydrochloride and Flunarizine Dihydrochloride

Literature survey reveals that some methods have been developed for their determination by HPLC⁷⁻⁹ or spectrophotometry¹⁰⁻¹² either alone or in combination, but no method was found for the selected combined dosage form by first order derivative UV spectrophotometry. Derivative spectrophotometry¹³⁻¹⁵ is one of the advanced spectrophotometric techniques useful for determination of quantitative and qualitative analysis. It has a great capability to resolve the overlapping spectra in those methods which

lack selectivity. The purpose of this work was to develop a simple, accurate and sensitive UV derivative method for determination of Propranolol hydrochloride and flunarizine dihydrochloride in bulk as well as combined dosage form.

MATERIALS AND METHODS

Apparatus

The instrument used was double beam UV/V is spectrophotometer (Make: Jasco, Model: V- 530, Software: spectra manager 2.32) with a matched pair of 1cm quartz cell and sonication of sample solution was done using ultrasonic cleaner (Spectra lab, Model: UCB-40, Mumbai, India).

Materials

Propranolol hydrochloride (PRO) drug sample was procured from Shreepati Pharmaceuticals Pvt. Ltd. Indore (Madhya Pradesh, India) and Flunarizine dihydrochloride (FLU) drug sample was gifted by FDC India Ltd. Jogeshwari (Mumbai, India). Both drugs were used without any further purification. Capsule (BETACAP PLUS LS, Sun Pharmaceuticals India Ltd, Silvassa, India) was purchased from local market, containing Flunarizine dihydrochloride 5mg and Propranolol hydrochloride 20mg per capsule. All chemicals and reagents used were of analytical grade. They were purchased from Merck Chemicals, Mumbai, India and LOBA Chemie Pvt. Ltd., Mumbai, India.

Methods

Preparation of standard stock solution

The stock solutions having 1mg/ml concentration of PRO and FLU were prepared separately by dissolving accurately weighed quantities of both drugs in methanol. Further dilutions of the standard stock solutions of both drugs were made with methanol to get the working standard solutions of 100µg/ml concentration of PRO and FLU.

Method Development

Selection of scanning range and sampling wavelength

The standard solutions of PRO and FLU were diluted with methanol individually to get the concentration of 20 µg/ml and 5 µg/ml respectively and were scanned in the UV range 400-200nm. The λ_{max} of both the drugs were found to be 289nm and 253nm respectively.

Development of first order derivative spectra

The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2nm for the range of 400-200nm with scanning speed of 400nm/min. It was observed that PRO shows zero crossing at 289nm while FLU shows zero crossing at 253nm. At zero crossing point of PRO (289nm), FLU showed a measurable $dA/d\lambda$ whereas at zero crossing point of FLU (253nm), PRO showed a measurable $dA/d\lambda$. Hence the wavelengths 289nm and 253nm were selected as analytical wavelengths for determination of PRO and FLU first order derivative method respectively.

Method Validation

The above proposed method was validated according to International conference on harmonisation Q2 R1 guidelines¹⁶ for validation of analytical procedures in order to determine the linearity, Accuracy, Precision and assay of market formulation.

Linearity

For linearity studies, aliquots of drug solutions were further diluted with methanol to get the final working standards of concentration range of PRO as 4-28 µg/ml and FLU as 1-7 µg/ml respectively. The linearity was evaluated by the regression analysis. The corresponding regression equation was found to be for

$$\text{Propranolol: } y = 0.0243x - 0.0057, R^2 = 0.9995$$

$$\text{Flunarizine: } y = 0.0463x + 0.0071, R^2 = 0.9991$$

Accuracy

To assess accuracy of the method, recovery studies were carried out by the standard addition method. For this, known quantities of pure PRO and FLU equivalent to 80,100,120% of their label claim in commercial tablets were mixed with corresponding definite amounts of pre-analysed formulations such that final concentrations of the drugs were within the linearity range. The total amount of each drug was then determined and the drug added amount was calculated.

Precision

The precision was determined with standard samples of both drugs prepared in triplicates at three different concentration levels covering the entire linearity range. The precision was calculated by intraday and interday and reported as % RSD.

Assay of marketed formulation

Ten capsules were weighed and powder equivalent to 20mg of PRO and 5mg of FLU was weighed and transferred to 100ml volumetric flask, volume adjusted with methanol. 2.5ml of this standard solution was pipette out and diluted to 10ml with methanol to get the solution containing 20 µg/ml PRO and 5 µg/ml FLU. Absorption spectra of the prepared solution were recorded and concentration of each drug was calculated using calibration curve equation.

Calibration curves of first order derivative

The aliquots of both the drugs used in linearity studies were converted to first derivative spectra and the derivative absorbance at 289nm and 253nm for PRO and FLU were measured respectively. The calibration graphs of both drugs were plotted at 289nm 253nm (Figure 5). The following regression equation for both the drugs were obtained as

$$\text{Propranolol: } y = -0.0003x + 0.0008, R^2 = 0.999$$

$$\text{Flunarizine: } y = 0.0002x + 0.0006, R^2 = 0.9995$$

Table 1: Results showing linearity of PRO and FLU

S. No.	Conc. Of drug (µg/ml) (PRO)	Absorbance by zero order	Conc. Of drug (µg/ml) (FLU)	Absorbance by zero order
1	4	0.25428	1	0.368
2	8	0.28243	2	0.4319
3	12	0.3441	3	0.4897
4	16	0.37933	4	0.5279
5	20	0.39997	5	0.5529
6	24	0.4934	6	0.6848
7	28	0.6274	7	0.7476
Regression Equation	Y=0.243X-0.0057		Y=0.00463X+0.0071	
Correlation Coefficient	R ² = 0.9995		R ² = 0.9991	
Slope	0.0243		0.0463	
Intercept	0.0057		0.0071	

Table 2: Results of Recovery studies

Drug	Conc. of std. drug	Recovery level	Amt. of drug added	Total amount of drug	% recovery (n=3)
PRO	12 µg	80%	9.6	21.6	98.60
		100%	12	24	100.17
		120%	14.4	26.4	102.95
FLU	3 µg	80%	2.4	5.4	100.59
		100%	3	6	100.74
		120%	3.6	6.6	100.31

Table 3: Results showing precision studies

Drug	Conc of drug (µg/ml)	%RSD (n=3)	
		Intraday	Interday
PRO	8	0.1878	0.1883
	12	0.2795	0.2812
	16	0.3841	0.3817
FLU	2	1.136	0.1556
	3	0.997	1.345
	4	1.172	1.031

Table 4: Result of assay of marketed formulation

Drug	Labelled claim (mg)	Amount found (mg)	Assay value	% RSD
PRO	20	19.9	99.5%	1.2
FLU	5	5.0012	100.12%	0.58

Table 5: Results of first order derivative linearity of PRO and FLU

S. No.	Conc. Of drug PRO (µg/ml)	Absorbance by first order derivative	Conc. Of drug FLU (µg/ml)	Absorbance by first order derivative
1	4	-0.000567273	1	0.000786143
2	8	-0.00182645	2	0.00099325
3	12	-0.00331994	3	0.00120711
4	16	-0.00460363	4	0.00139471
5	20	-0.00605984	5	0.00158639
6	24	-0.00743571	6	0.00181279
7	28	-0.00852786	7	0.00199561
Regression equation		$y = -0.0003x + 0.0008$	$y = 0.0002x + 0.0006$	
Correlation coefficient		$R^2 = 0.999$	$R^2 = 0.9995$	
Slope		-0.0003	0.0002	
Intercept		0.0008	0.0006

Figure 2: Overlapped spectra of PRO (20µg/ml) and FLU (5µg/ml)

Figure 3: Linearity graph for zero order of PRO

Figure 4: Linearity graph for zero order of FLU

Figure 5: Linearity graph for first order derivative of PRO

Figure 6: Linearity graph for first order derivative of FLU

RESULTS AND DISCUSSION

From experimental condition described, linearity, Accuracy, Precision and assay of marketed formulation were performed. Results of linearity for zero order and first order derivative are shown in Table 1. Both the drugs obey Beer's law in the concentration range of 4-28 $\mu\text{g/ml}$ for PRO and 1-7 $\mu\text{g/ml}$ for FLU for zero order. Result of recovery studies are shown in Table 2. The recovery study done by standard addition

method has given satisfactory results as PRO 98-103% and FLU 100-101% at three concentration levels. Precision study showed that % RSD was within the range of acceptable limits (<2), as shown in Table 3. The optimized method was applied to simultaneous determination of Propranolol hydrochloride and Flunarizine dihydrochloride in their combined marketed formulation and the satisfactory results were obtained as shown in Table 4. Results of linearity for first order

derivative are shown in Table 5. Both the drugs obey Beer's law in the concentration range of 4-28 µg/ml for PRO and 1-7 µg/ml for FLU for first order derivative method.

CONCLUSION

A fast, simple, accurate and precise method was developed for the estimation of PRO and FLU in combination using first order derivative UV spectrophotometric technique. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength. The developed method can be successfully applied to analysis of marketed formulation.

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REFERENCES

1. Merck and Co. Inc. The Merck Index, 13thed. Whitehouse station New Jersey; 2001. p. 2030, 346
2. Indian Pharmacopoeia, Volume III, The Indian Pharmacopoeia commission, Gaziabad, Government of India Ministry of Health and Family Welfare; 2010.
3. United States Pharmacopoeia, USP 30/N 25, The official compendia of standards, Asain edition, USP Convention, Rockville, MD; 2007.
4. Merck and Co. Inc., The Merck Index, 13thed. White house station New Jersey; 2001. p. 2030, 346
5. British Pharmacopoeia, Vol. I, Her Majesty's Stationary Office London; 2007. p. 890
6. Tulja Rani G, Gowri Shankar D, Kadgapathi P, Satyanarayana B. A Validated RP HPLC method for simultaneous determination of propranolol hydrochloride and alprazolam in bulk and in pharmaceutical formulations. *J Pharm Research* 2011; 4(2): 358-360.
7. Kartinasari WF, Chufianty H, Indrayanto G. HPLC determination of flunarizine dihydrochloride in tablets and its validation. *J Liq Chromatogr R T* 2003; 26(7): 1059-67. <http://dx.doi.org/10.1081/JLC-120020093>
8. Ming-Ren S Fuh, Chi-Jen Hsieh, Yu-Lun Tai. Optimization for detection of flunarizine by high performance liquid chromatography/electrospray/mass spectrometry. *Talanta* 1999; 49: 1069-75. [http://dx.doi.org/10.1016/S0039-9140\(99\)00072-7](http://dx.doi.org/10.1016/S0039-9140(99)00072-7)
9. Jończyk A, Nowakowska Z. Determination of hydrochlorothiazide, triamterene and propranolol hydrochloride by the spectrophotometric method and high-performance liquid chromatography (HPLC). *Acta Pol Pharm* 2001; 58(5): 339-44. PMID:11876440
10. Mohammad MA. Spectrophotometric and spectrofluorimetric determination of cinnarizine and flunarizine dihydrochloride in pure and dosage forms. *Bull Fac. Pharm Cairo University* 2004; 42: 27-40.
11. Patel SA, Patel PU, Patel NJ. Development and validation of spectrophotometric method for simultaneous estimation of diazepam and propranolol hydrochloride in combined dosage form. *Int Research J Pharm* 2011; 2(8): 119-123.
12. Patil AS, Shirkhedkar AA, Surana SJ *et al.* Q-absorbance and multicomponent UV spectrophotometric methods for simultaneous estimation of propranolol hydrochloride and flunarizine dihydrochloride in capsules. *Der Pharma Chemica* 2011; 3(3): 404-8.
13. Doshi AK, Patel BN and Patel CN. Development and validation of spectrophotometric method for simultaneous determination of propranolol hydrochloride and flunarizine dihydrochloride in their combined dosage formulation. *International Journal of Pharmaceutical science and Research* 2012; 3(6): 1741-1744.
14. Bengi Uslu, Sibel A Özkan. Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography-UV methods, *Analytica Chimica Acta* 2002; 466: 175-185. [http://dx.doi.org/10.1016/S0003-2670\(02\)00545-7](http://dx.doi.org/10.1016/S0003-2670(02)00545-7)
15. Patel Deep P, Shah Ragin R, Patel Alisha P and Tank Ponal K. Development and validation of first order derivative UV-spectroscopic method for estimation of ibuprofen and famotidine in synthetic mixture, *Pharma science monitor, an international journal of pharmaceutical sciences*.
16. ICH Harmonised-Tripartite Guidelines, Validation of analytical procedures: Text and Methodology Q2 (R1); 2005.

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