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Spectrophotometric Methods for Simultaneous estimation of Amitriptyline Hydrochloride and Perphenazine in their Synthetic Mixture

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ABSTRACT:

Two simple, accurate, and precise spectrophotometric method have been developed for Amitriptyline hydrochloride (AMI) and Perphenazine (PER) in their mixture. Amitriptyline hydrochloride has absorbance maxima at 239 nm and Perphenazine absorbance maxima at 257 nm. Method-A area under curve (AUC) method, which involves the calculation of integrated value of the Absorbance with respect to wavelength between 230-242nm for Amitriptyline hydrochloride and 249-259nm for Perphenazine. Method-B First order derivative spectrophotometric method involves the derivatisation of the Zero absorption spectra for the First absorption spectra. The Zero crossing point of Amitriptyline hydrochloride and Perphenazine at 254.9nm and 236.6nm was obtained respectively. Beer's law is obeyed in the concentration range of 5-25 µg/ml and 2-10 µg/ml for Amitriptyline hydrochloride and Perphenazine for two methods. The accuracy and precision of the methods were determined and validated statistically. All the methods sowed good reproducibility and recovery with % RSD less than 1. All method was found to be rapid, precise and accurate and can be successfully applied for the routine analysis of Amitriptyline hydrochloride and Perphenazine in their mixture.

KEY WORDS: Amitriptyline hydrochloride, Perphenazine, Area Under curve method, First order derivative spectroscopic method.

INTRODUCTION

Chemically, Amitriptyline hydrochloride is 3-(5, 6-dihydrobenzo [2, 1-b: 2', 1'-f][7]annulen-11-ylidene)-N, N-dimethylpropan-1-amine;hydrochloride. It is a Tricyclic Antidepressant drug. It is used for Antidepressant. It has anticholinergic and sedative properties (1, 2). Amitriptyline hydrochloride alone or in combination with other drugs is reported to be estimated by spectroscopic method (3-11), RP-HPLC method (12-16), RP-UPLC method, HPTLC and chromatographic methods.

Chemically, Perphenazine is 2-[4-[3-(2-chlorophenothaizine-10-yl) propyl] piperazine-1-yl] ethanol. It is a Typical Antipsychotic drug. It is used for treatment of Schizophrenia (17-18). Perphenazine alone or in combination is reported to be estimated by spectroscopic method and RP-HPLC methods (12-16). In this combination spectrophotometric method was reported. In present work, a successful attempt has been made to estimate both these drugs simultaneously by two simple UV-spectrophotometric methods (Area under curve method, First order derivative spectrophotometric method).

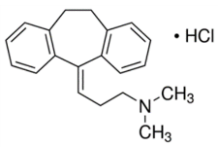

Figure 1 Amitriptyline hydrochloride

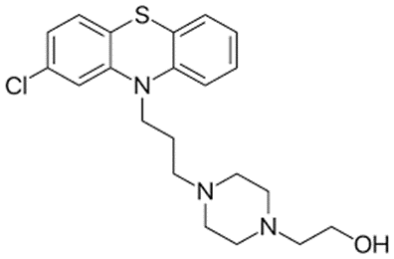

Figure 2 Perphenazine

MATERIAL AND METHOD:

Instrument

Spectrophotometric analysis was carried out by using UV-Visible spectrophotometer having two matched quartz cell with 1cm light path. (Shimadzu 1800)

Reagents and chemicals

Reference standard of Amitriptyline hydrochloride was generous gift sample from Intas Biopharmaceutical Ltd. and Perphenazine was generous gift sample from Shreeji Pharma International. All chemicals used were of analytical grade.

- Methanol (AR Grade-Thomas Baker)
- Distilled water

METHODS AND PROCEDURES

Area Under Curve (Method A)

Area under Curve Method (AUC) method involves measurement of the area between two wavelengths at the point of maximum absorbance. The integrated value of absorbance with respect to the wavelength was selected of AMI were measured between the wavelength range 230-242 nm and for PER were measured between the wavelength ranges 249-259 nm. The calibration curve was plotted at both wavelength and two equations were formed using specific absorbance. The concentration of AMI and PER was calculated from the equations:

$A_1 = a_{x1}\, C(x) + a_{y1}\, C(y)$ (1-2) nm

$A_2 = a_{x2}\, C(x) + a_{y2}\, C(y)$ (3-4) nm

Where,

a_{x1} and a_{x2} are absorptivities of x at (1-2) and (3-4) respectively

a_{y1} and a_{y2} are absorptivities of y at (1-2) and (3-4) respectively

A1 and A2 are AUC of mixed standard at (1-2) and (3-4) respectively.

C(x) and C(y) are the concentration of x and y, respectively.

$$C_x = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}a_{y1} - a_{x1}a_{y2}} \qquad C_y = \frac{A1ax2 - A2ax1}{a_{x2}a_{y1} - a_{x1}a_{y2}}$$

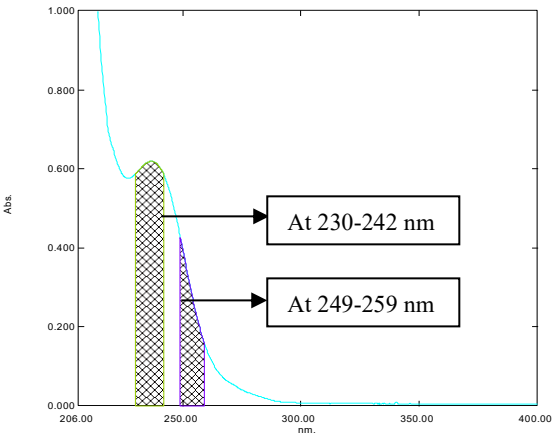

Figure 3 AUC Spectra of Amitriptyline hydrochloride 10 µg/ml in wavelength range 230-242 nm and 249-259 nm.

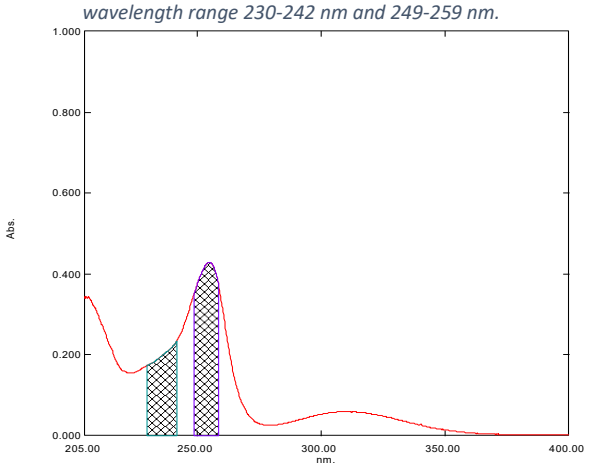

Figure 4 AUC Spectra of Perphenazine 4 µg/ml in wavelength range 230-242 nm and 249-259 nm.

First Order Derivative Spectrophotometric (Method B)

First-order derivative spectrophotometric method Calibration curves were constructed by analysis of working standard solution of AMI and PER. UV absorption spectra and first-order derivative of AMI and PER were measured. The first derivative spectrum of AMI shows absorbance at 254.9 nm (zero-crossing point of PER) and PER shows absorbance at 236.6 nm (zero-crossing point of AMI). The calibration curve was plotted at Abs. vs. Conc. and regression equation obtained.

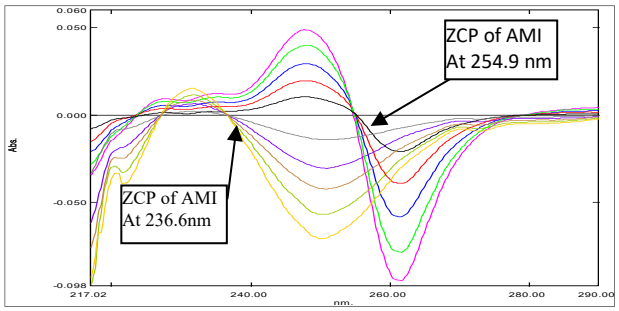

Figure 5 ***Overlain first order spectra of AMI and PER linearity***

Preparation of solution from synthetic mixture

10 mg equivalent of AMI from synthetic mixture was taken into 10 mL of volumetric flask. 5-7 ml Methanol was added and sonicated for 2-3 min and volume was made up with methanol. Solution was filtered through filter paper no. 42. Thus, resulting solution gave 1000 µg/ml of AMI and 400 µg/ml of PER. From the above solution, 0.1 ml was pipette out and transferred ton10 ml of volumetric flask and volume was made up to the mark with methanol in order to get AMI (10 µg/ml) + PER (4 µg/ml) solution.

VALIDATION OF DEVELOPED METHOD

The method was validated according to ICH guideline for validation of analytical procedures in order to determine the linearity, precision, accuracy, LOD and LOQ for the analyte (ICH Q2B).

Preparation of standard stock solution and calibration curve

Standard stock solution of Amitriptyline hydrochloride and perphenazine were prepared using methanol. 10mg of Amitriptyline hydrochloride and 10 mg of Perphenazine was weighed individually and transfer into separate 10ml volumetric flask, dissolve in methanol and dilute to the mark with the same solvent (stock solution 1000 µg/ml). From the above solution pipette out 2.5ml of AMI and 1 ml of PER solution transfer into 10ml volumetric flask and volume was made up to the mark with methanol. (Working stock solution 100 µg/ml).

Linearity

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5 - 25 µg/ml and 2 – 10 µg/ml for Amitriptyline Hydrochloride and Perphenazine respectively. The calibration curve of Area vs. respective concentration was plotted and coefficient and regression line equation for Amitriptyline hydrochloride and Perphenazine were calculated.

Precision

1. Repeatability

1.5 ml of working stock solution of Amitriptyline hydrochloride (100µg/ml) was transferred to 10 ml volumetric flask. 0.6 ml of working stock solution of Perphenazine (100 µg/ml) were respectively transferred to 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 15 µg/ml solution of Amitriptyline hydrochloride and 6 µg/ml solution of Perphenazine. The absorbance of solution was measured spectrophotometry six times and % RSD was calculated.

2. Intraday and Interday Precision

Aliquots of 1.0, 1.5 and 2.0 ml from working stock solution of Amitriptyline hydrochloride (100µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 0.6 and 0.8 ml from working stock solution of Perphenazine (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10, 15 and 20 µg/ml solution of Amitriptyline hydrochloride and 4, 6 and 8 µg/ml solution of Perphenazine. For intraday, the analysis was carried out at different interval of the same day and for interday, the analysis was carried on different day and % RSD was determined.

3. Accuracy

The accuracy of the method was determined by calculating recovery of AMI and PER by the standard addition method. Reference standard solution of each drug was added to samples at three different concentration levels (80%, 100% and 120%). At each level sample were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated.

4. LOD and LOQ

Limit of detection (LOD)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as,

LOD = 3.3*SD/Slope

Limit of quantitation (LOQ)

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity. The LOQ may be calculated as,

LOQ = 10*SD/Slope

RESULTS AND DISCUSSION:

The spectrophotometric method have the advantages of being the most simple, fast and applicable in all laboratories, as most of the active compound show the absorbance in the UV region. The linearity range for AMI 5-25 µg/ml and for PER 2-10 µg/ml. The proposed methods were validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80, 100 and 120 % level. Both drugs showed good regression value at their respective wavelength.

Method A-Area under Curve methods

Table1. Linearity data for AMI at 230-242 nm (n=5)

Sr. No.	Conc. (µg/ml)	AUC Mean ± S.D.	% R.S.D
1	5	3.8122 ± 0.0040	0.1055
2	10	7.3246 ± 0.0484	0.6619
3	15	10.292 ± 0.0268	0.2611
4	20	13.878 ± 0.0428	0.3089
5	25	17.158 ± 0.0239	0.1392

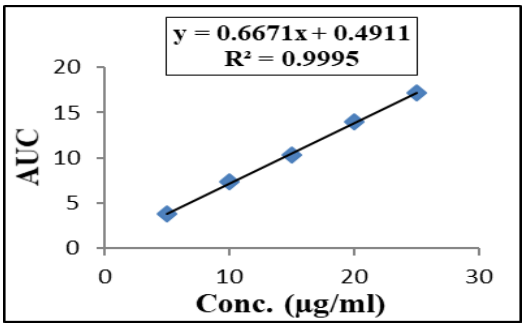

Figure 6 AMI at 230-242 nm

Table 2.Linearity data for PER at 249-259 nm (n=5)

Sr.No.	Conc. (µg/ml)	AUC Mean ±S.D.	% R.S.D
1	2	2.2042 ± 0.0103	0.4689
2	4	4.0854 ± 0.0388	0.8289
3	6	5.9374 ± 0.0477	0.8049
4	8	8.0622 ± 0.0421	0.5228
5	10	9.8050 ± 0.0107	0.11

Table 3.Linearity data for PER at 230-232 nm (n=5)

Sr.No.	Conc. (µg/ml)	AUC Mean ±S.D.	% R.S.D
1	2	1.3952 ± 0.0092	0.6596
2	4	2.4176 ± 0.0160	0.6634
3	6	3.5214 ± 0.0275	0.7814
4	8	4.7222 ± 0.0158	0.3349
5	10	5.8092 ± 0.0435	0.7495

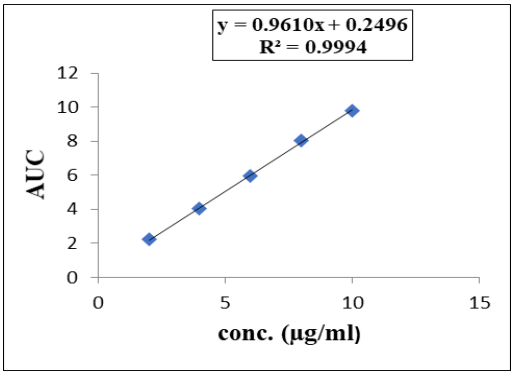

Figure 7 PER at 249-259nm

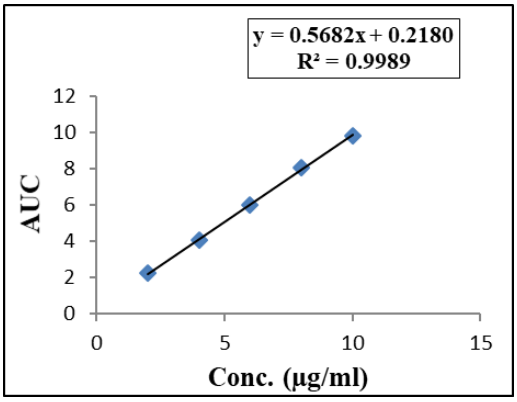

Figure 8 PER at 230-232nm

Table 4 Linearity data for AMI at 249-259 nm (n=5)

Sr.No.	Conc. (µg/ml)	AUC Mean ±S.D.	% R.S.D
1	5	1.6878 ± 0.0178	1.0577
2	10	2.5252 ± 0.0301	1.0669
3	15	4.1134 ± 0.0622	1.5130
4	20	5.3434 ± 0.0614	1.1503
5	25	6.5674 ± 0.0474	0.7229

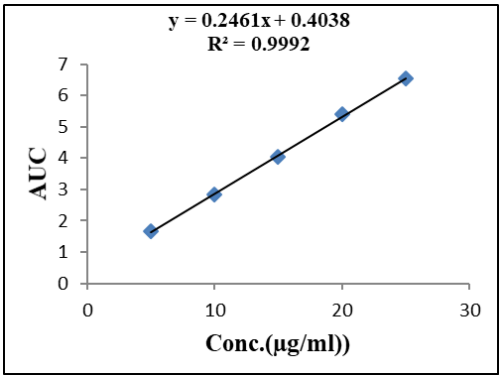

Figure 9 AMI at 249-259 nm

Table 5. Determination of Accuracy of AMI and PER

Drugs	Level	Amt of sample (µg/ml)	Amt of drug spiked (µg/ml)	Total conc. found (µg/ml)	Mean % Recovery
AMI	0%	10	0	10	99.60 %
	80%	10	8	18	101.38 %
	100%	10	10	20	101.50 %
	120%	10	12	22	100.40 %
PER	0%	4	0	4	99.09 %
	80%	4	3.2	7.2	100.97 %
	100%	4	4	8	101.12 %
	120%	4	4.8	8.8	99.43 %

Table 6. Repeatability data of AMI and PER

Sr. No.	Drugs	Conc. (µg/ml)	Mean AUC± S.D. (n=6)	% R.S.D.
1	AMI	15	10.328 ± 0.0416	0.4030
2	PER	6	5.8888±0.0442	0.7512

Table 7 Intraday and Interday Precision data of AMI at 230-242nm and 249-259 nm

Sr.No.	Conc. (µg/ml)	Intraday Precision (n=3)		Interday Precision (n=3)	
		AUC Mean ± SD	% RSD	AUC Mean ± SD	% RSD
1	10	7.3096 ± 0.0289	0.3965	7.3623 ± 0.0401	1.1217
2	15	10.331 ± 0.0490	0.4715	10.385 ± 0.0744	0.7165
3	20	13.866 ± 0.0537	0.3877	13.917 ± 0.0950	0.6827

Table 8 Intraday and Interday Precision data of PER at 230-242nm and 249-259 nm

Sr.No.	Conc. (µg/ml)	Intraday Precision		Interday Precision	
		AUC Mean ± SD	% RSD	AUC Mean ± SD	% RSD
1	4	4.0976 ± 0.0581	1.4192	4.086 ± 0.0401	0.9821
2	6	5.9220 ± 0.0575	0.9710	5.964 ± 0.0468	0.7848
3	8	7.8713 ± 0.1069	1.3588	8.024 ± 0.0565	0.7043

Table 7 Determination of Assay of AMI and PER

Synthetic mixture	Amt. taken (µg/ml)		Amount obtained (µg/ml)		% AMI ± S.D. (n=3)	% PER ± S.D. (n=3)
	AMI	PER	AMI	PER	99.00 ± 0.0005	100.5 ± 0.0003
	10	4	9.9 ± 0.0004	4.02 ± 0.0005		

Method B- First order derivative spectrophotometric method

Table 8 Linearity data for AMI at ZCP of PER at 254.9 nm

Sr.No.	Conc. (µg/ml)	Mean Abs. ± S.D. (n=5)
1	5	0.0137 ± 0.00019
2	10	0.0257 ± 0.00027
3	15	0.0364 ± 0.00057
4	20	0.0475 ± 0.00050
5	25	0.0598 ± 0.00074

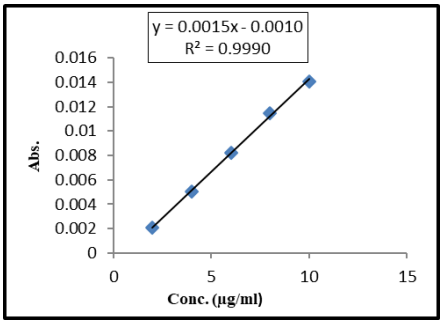

Figure 10 AMI at ZCP of PER at 254.9 nm

Table 9 Determination of Accuracy of AMI and PER

Drugs	Level	Amt of sample (µg/ml)	Amt of drug spiked (µg/ml)	Total conc. found (µg/ml)	Mean % Recovery
AMI	0%	10	0	10	99.50 %
	80%	10	8	18	99.03 %
	100%	10	10	20	102.00%
	120%	10	12	22	98.91 %
PER	0%	4	0	4	98.33 %
	80%	4	3.2	7.2	100.92 %
	100%	4	4	8	100.83 %
	120%	4	4.8	8.8	99.40%

Table 10 Intraday precision and Interday Precision data of AMI at 254.9 nm

Sr.No.	Conc. (µg/ml)	Intraday Precision (n=3)		Interday Precision (n=3)	
		Mean Abs. ± SD	% RSD	Mean Abs. ± SD	% RSD
1	10	0.0257 ± 0.00025	0.9766	0.0258 ± 0.00028	1.1174
2	15	0.0338 ± 0.00028	0.7837	0.0365 ± 0.00050	1.3698
3	20	0.0468 ± 0.00032	0.6858	0.0475 ± 0.00050	1.0526

Table 11 Intraday precision and Interday Precision data of PER at 236.6 nm

Sr.No.	Conc. (µg/ml)	Intraday Precision (n=3)		Interday Precision (n=3)	
		Mean Abs. ± SD	% RSD	Mean Abs. ± SD	% RSD
1	4	0.0050 ± 0.000005	1.1007	0.0049 ± 0.000005	1.3021
2	6	0.0082 ± 0.000007	0.9333	0.0082 ± 0.000120	1.5314
3	8	0.0113 ± 0.000100	0.8849	0.0114 ± 0.000200	1.7543

Table 12 Repeatability data of AMI and PER

Sr. No.	Drugs	Conc. (µg/ml)	Mean Abs. ± S.D. (n=6)	% R.S.D.
1	AMI	15	0.0362 ± 0.000213	0.5889
2	PER	6	0.0081 ± 0.000005	0.6315

Table 12 Determination of Assay of AMI and PER

Synthetic mixture	Amount taken (µg/ml)		Amount obtained (µg/ml)		% AMI ± S.D. (n=3)	% PER ± S.D. (n=3)
	AMI	PER	AMI	PER	100.05 ± 0.0005	99.75 ± 0.0003
	10	4	10.05 ± 0.0004	3.99 ± 0.0005		

CONCLUSION

The proposed methods were found to be simple, accurate and rapid for the routine determination of Amitriptyline hydrochloride and Perphenazine. To study the validity and reproducibility of proposed method, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, specificity and reproducibility. The two methods can be successfully used for simultaneous estimation of Amitriptyline hydrochloride and Perphenazine in combination.

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