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UV-Visible First Order Derivative Spectrophotometric Method Development and Validation for Simultaneous Estimation of Amitriptyline Hydrochloride and Chlordiazepoxide in Tablet Dosage Form

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A simple, precise, fast, accurate and sensitive UV-visible first order derivative spectrophotometric method was developed and validated for amitriptyline and chlordiazepoxide estimation in pure and tablet dosage form. The method involved determination of amitriptyline and chlordiazepoxide using first derivative spectrophotometric technique at 219 nm and 239 nm over the concentration ranges of 5-17 µg/mL and 1-7 µg/mL. Mean recoveries were found to be about 98.33 ± 0.45 % w/w for amitriptyline and 99.75 ± 1.16 % w/w for chlordiazepoxide. The coefficient (r^2) were 0.9998 for amitriptyline and 0.9997 for chlordiazepoxide, respectively. The limit of detection and limit of quantification were found to be 131 and 398 ng/mL, respectively for amitriptyline and 26 and 79 ng/mL, respectively for chlordiazepoxide. The assay percentage of the marketed formulation calculated were 99.87 ± 0.03 % w/w for amitriptyline and 98.81 ± 1.04 % w/w for chlordiazepoxide, respectively. This study provides a validated UV spectrophotometric method by using a first order derivative method. This validated method was carried out with respect to the parameters such as linearity, specificity, stability, accuracy, precision, limit of quantification and limit of detection in the light of internationally accepted ICH guidelines.

Keywords: Validation, Amitriptyline hydrochloride, Chlordiazepoxide, UV spectrophotometry and Development.

INTRODUCTION

Amitriptyline hydrochloride chemically is 3-(10,11-dihydro-5H-dibenzo[a,d]cyclopenten-5-ylidine)propyldimethylamine hydrochloride (Fig. 1). It is a dibenzocycloheptene derivative tricyclic antidepressant (TCA), structurally similar to phenothiazines. Amitriptyline can elevate mood in depressive illness and also inhibit serotonin transporter located at platelet membrane [1-3]. Chlordiazepoxide, 7-chloro-4-hydroxy-N-methyl-5-phenyl-3H-1,4-benzodiazepin-2-imine (Fig. 1), is a benzodiazepine derivative, used to treat anxiety which was the first benzodiazepine to be used clinically [4-6]. The published research data showed that there are many spectrophotometric methods [7-15] and HPLC [16-18] methods for the estimation of amitriptyline or chlordiazepoxide in bulk and pharmaceutical dosage forms.

First order derivative spectrophotometry is used for extracting both qualitative and quantitative evidence from spectra composed of unresolved bands and for eliminate the effect of baseline tilts and baseline shifts [8,9]. It is also used for spectral resolution of multicomponent mixtures and simultaneous estimation of compounds with overlapped spectra. These methods could be used without any pre-treatment processes and tedious sample preparations [10,11]. To the best of our knowledge, there is no first order derivative spectrophotometric method reported in the literature for simultaneous estimation of amitriptyline and chlordiazepoxide in pharmaceutical formulations. The goal of the present workis to develop a simple, reliable and fast derivative spectrophotometric method for simultaneous estimation of amitriptyline hydrochloride and chlordiazepoxide in tablet dosage form [12-16].

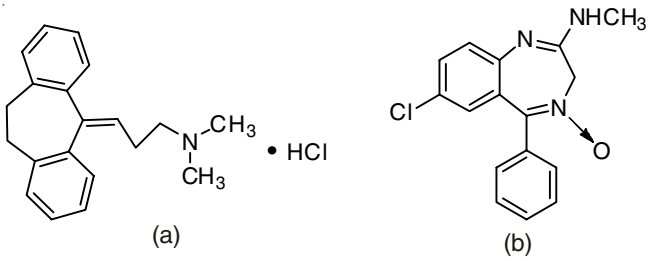

Fig. 1. Chemical structure of amitriptyline hydrochloride (a) and chlordiazepoxide (b)

EXPERIMENTAL

Marketed formulation of Libotryp-10 (Batch Number-JK10448, Wockhardt Ltd., Solan, India) was purchased from the local drug store of Moga, Punjab. Methanol (AR grade) was purchased from Rankem (New Delhi, India). All the other chemicals and solvent of analytical grade were used without any further purification. Milli-Q water was used throughout the whole study. Unless otherwise specified, all solutions were filtered through a 0.22 µm Nylon 6, six membrane filter (Pall Life Sciences, USA) prior to use.

Perkin Elmer Lambda 35 UV/visible spectrophotometer coupled with computer loaded with UV WinLab PC software version 6.0.3 was used for spectrophotometric determinations. The first order derivative spectra were recorded in the range of 200-400 nm.

Preparation of stock and standard solutions: Stock solutions of 1 mg/mL of pure amitriptyline and chlordiazepoxide were separately prepared in methanol and stored at 2-8 °C until used. Combined standard solutions of amitriptyline and chlordiazepoxide was prepared by diluting required quantity of stock solution with same solvent to obtain the linearity range. Marketed formulation was prepared in methanol and diluted.

Preparation of calibration standards and quality control samples: From standard solutions, the final concentration of calibration standards having 5, 8, 11, 14, 17 µg/mL for amitriptyline hydrochloride and 1.0, 2.5, 4.0, 5.5, 7.0 µg/mL for chlordiazepoxide were prepared by appropriate dilution with methanol. Quality control samples at 3 concentrations in the linearity range which were across the range of 80-120 % of the target concentration *i.e.* 8, 11 and 14 µg/mL for amitriptyline and 2.5, 4.0 and 5.5 µg/mL for chlordiazepoxide were prepared from the standard solutions as mentioned above.

Assay of tablets: Twenty tablets of Libotryp were weighed and finely powdered. An amount of the tablet powder equivalent to 12.5 mg of amitriptyline and 5 mg of chlordiazepoxide was transferred to a 100 mL volumetric flask and add 70 mL methanol. After sonication for 15 min, the flask was made up to volume by methanol.

RESULTS AND DISCUSSION

Method development and optimization

First derivative zero crossing spectrophotometry: Zero order spectra of amitriptyline and chlordiazepoxide were recorded between 200-400 nm against blank (AR grade methanol) using a 1.0 cm quartz cell. Zero order spectra of pure amitriptyline and chlordiazepoxide were stored individually within the 5-17 µg/mL and 1-7 µg/mL concentration ranges respectively and was derivatized in first order using delta lambda 5 and scaling factor 10. The first derivative amplitudes of amitriptyline and chlordiazepoxide were recorded at 219 and 239 nm, respectively.

Considering all the derivative order spectra of amitriptyline hydrochloride and chlordiazepoxide from first to fourth order derivative, the first order derivative spectra was found suitable. Zero crossing point on the first derivative spectra of both drug shows substantial absorbance. From the derivatized spectra of prepared mixtures, absorbances were measured at 219 and 239 nm for amitriptyline hydrochloride and chlordiazepoxide respectively and can be employed for the estimation of amitriptyline hydrochloride and chlordiazepoxide without interference from other drug in combined formulations (Fig. 2). These absorbances *vs.* concentration were plotted in quantitative mode to obtain the working curves from which by extrapolating the value of absorbances of the sample solution, the concentration of corresponding drugs were determined.

Method validation

Specificity: The specificity of the method was evaluated by scanning different ratios of amitriptyline and chlordiazepoxide. The excipients used in formulation did not interfere with the amitriptyline and chlordiazepoxide peaks and thus the method is specific.

Linearity: Calibration curve was constructed from the data of five different concentrations of amitriptyline and chlordiazepoxide. Results of the regression analysis and the coefficient of determination (r^2) are listed in Table-1. The high coefficient of determination values *i.e.* 0.9998 for amitriptyline and 0.9997 for chlordiazepoxide, respectively indicated excellent linearity between their peak areas (Y) and standard drug concentrations in the range 5.0-17 µg/mL for amitriptyline and 1-7 µg/mL for chlordiazepoxide, respectively and overlay of peaks are shown in Fig. 2.

TABLE-1
VALIDATION PARAMETERS OF FIRST DERIVATIVE SPECTROPHOTOMETRY METHOD FOR AMITRIPTYLINE AND CHLORDIAZEPOXIDE

Parameters	Amitriptyline	Chlordiazepoxide
Absorption maxima (nm)	219	239
Linearity range (µg/mL)	5- 17	1-7
Coefficient of determination (r^2)	0.9998	0.9997
Correlation coefficient (r)	0.9996	0.9994
Regression equation (Y)	Y = 0.045X + 0.181	Y = 0.028X – 0.00
LOD (ng/mL)	131	26
LOQ (ng/mL)	398	79
Precision (%RSD) Intra-day	0.06	0.91
Inter-day	0.08	1.16

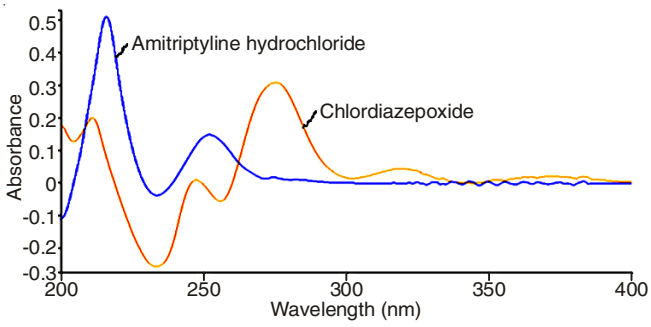

Fig. 2. Overlay spectra of first derivative spectrophotometry method

Precision and accuracy: Intra-day and inter-day precision results listed in Tables 2 and 3 in terms of % RSD were found to be 0.8987 and 0.5515 for amitriptyline and 1.5306 and 1.1578 for chlordiazepoxide (% RSD < 2.0) which indicated that the propose method is highly precise and reproducible. Accuracy results listed in Table-4 were found to be 98.33 ± 0.45 % w/w for amitriptyline and 99.75 ± 1.16 %

TABLE-2
PRECISION DATA OF FIRST DERIVATIVE SPECTROPHOTOMETRY METHOD FOR AMITRIPTYLINE

Concentration (µg/mL)	Intra-day		Inter-day	
	SD	RSD (%)*	SD	RSD (%)*
8	0.0471	1.1913	0.0306	0.7702
11	0.0458	0.9207	0.0265	0.5326
14	0.0345	0.5841	0.0208	0.3517
	Mean	0.89	Mean	0.55

*Mean and standard deviation for 3 determinations.

TABLE-3
PRECISION DATA OF FIRST DERIVATIVE SPECTROPHOTOMETRY METHOD FOR CHLORDIAZEPOXIDE

Concentration (µg/mL)	Intra-day		Inter-day	
	SD	RSD (%)*	SD	RSD (%)*
2.5	0.0234	1.4842	0.0173	1.0914
4.0	0.0306	1.5501	0.0252	1.2729
5.5	0.0367	1.5576	0.0265	1.1091
	Mean	1.53	Mean	1.15

*Mean and standard deviation for 3 determinations.

TABLE-4
ACCURACY DATA OF FIRST DERIVATIVE SPECTROPHOTOMETRY METHOD FOR AMITRIPTYLINE AND CHLORDIAZEPOXIDE

Concentration (µg/mL)		Recovery (%)* (w/w)	
Amitriptyline	Chlordiazepoxide	Amitriptyline	Chlordiazepoxide
8.0	2.5	101.17	98.96
11.0	4.0	98.98	100.59
14.0	5.5	97.52	99.70
	Mean recovery	98.33	99.75
	SD	0.45	1.16

*Mean and standard deviation for 3 determinations.

w/w for chlordiazepoxide, which indicates high recovery of the method.

Limit of detection and low limit of quantification: The limit of detection and limit of quantification were found to be 131 and 398 ng/mL, respectively for amitriptyline and 26 and 79 ng/mL, respectively for chlordiazepoxide. These data show that the method was highly sensitive and specific.

Analysis of marketed sample: The proposed method was applied for the analysis of amitriptyline and chlordiazepoxidein pharmceutical dosage form. Amitriptyline and chlordiazepoxide was found to be 99.87 ± 0.3 % w/w for amitriptyline and 98.81 ± 1.04 % w/w for chlordiazepoxide respectively.

Conclusion

A first order derivative spectrophotometric method has been developed for the simultaneous estimation of amitriptyline and chlordiazepoxide in pharmaceutical dosage forms. The developed method is simple, rapid, accurate, low cost and practical for routine quality control analysis. Furthermore, a simple and rapid sample preparation is needed when applied to the analysis of tablet dosage forms. It is concluded that derivative spectrophotometry is successfully utilized for the estimation of amitriptyline and chlordiazepoxide.

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