Research Article

UV Spectrophotometric Method Development and Validation for Simultaneous Quantification of Clidinium Bromide and Chlordiazepoxide Hydrochloride in Dosage Form

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ABSTRACT

Introduction: Derivative UV spectroscopic method is one of the multicomponent analytical method. The combination of Clidinium bromide (CLB) and Chlordiazepoxide hydrochloride used for to treat stomach or bowel problems including peptic ulcers, irritable bowel syndrome (IBS), and enterocolitis (inflammation of the colon and small intestines.

Aim/Objectives: To develop Derivative UV Spectrophotometric method for simultaneous quantification of Clidinium bromide (CLB) and Chlordiazepoxide hydrochloride (CLDZ) in their combined dosage form.

Method: This method utilizes ratio of solvent, which is Methanol: Distilled water (10:90 v/v) which make method economic. The λmax of Clidinium bromide and Chlordiazepoxide hydrochloride selected for analysis was found to be 245.46 nm (at ZCP of CLDZ) and 280.06 nm (at ZCP of CLB) respectively.

Result: Linearity was observed in the concentration range of 20-120µg/mL for CLB ($r2$= 0.9997) and 3-18µg/mL for CLDZ ($r2$= 0.9997) by first order derivative method. The accuracy was found to be 99.62 to 100.33% and for CLB and 99.16 to 99.84 % for CLDZ. The intraday precision was found to be 0.27 - 0.70 and 0.12 - 0.46 for CLB and CLDZ respectively. The Inter day precision was found to be 0.46 – 0.70 and 0.32 -0.66 for CLB and CLDZ respectively. LOD for CLB and CLDZ was found to be 0.1807µg/ml and 0.0097µg/mL and LOQ doe CLB And CLDZ was found to be 0.5477 µg/mL and 0.0294 µg/mL respectively.

Conclusion: The validation parameters found to be complying with ICH guidelines showing % RSD in the desired range. The use of distilled water as a major solvent makes the method economic as well as ecofriendly. The proposed methods can be successfully applied for the routine analysis of both the drugs in dosage form.

Key Words: Method Development, Validation, Simultaneous, Derivative Spectrophotometric, Clidinium bromide, Chlordiazepoxide hydrochloride, Dosage form

INTRODUCTION[1,2]

Chlordiazepoxide Hydrochloride (CLDZ) (7-chloro-N-methyl-5-phenyl 3H-1, 4-benzodiazepin-2-amina-4-oxide) is a sedative-hypnotic drug widely employed as a tranquilizer and antidepressant and Clidinium bromide (CLB) (3- [(hydroxy-diphenyl acetyl) -oxy]-1- methyl-1- azoniabicyclo-[2.2.2] octane bromide) is effective for anxiety-related conditions including spastic colon.

LIBRAX® and NORMAXIN® [3,4] are pharmaceutical formulations which contain both drugs. Some chromatographic techniques, including liquid chromatography and a reversed-phase high performance liquid-chromatography have been reported for their simultaneous determination. The chemical structures of Clidinium bromide and Chlordiazepoxide hydrochloride are shown in Fig. 1 and 2 respectively.
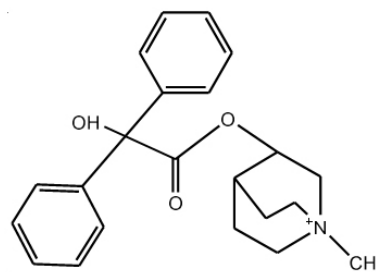

Figure 1: Chemical structures of Clidinium bromide.

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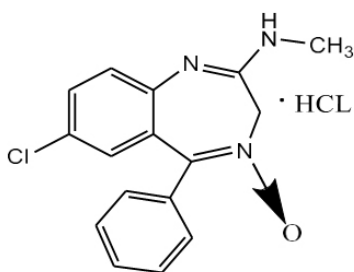
Figure 2: Chemical structures of Chlordiazepoxide hydrochloride.

Literature survey reveals that four HPLC and no UV Spectrophotometric analytical methods [5,6,7] have been reported for the quantitative determination of Clidinium bromide and Chlordiazepoxide hydrochloride separately as well as combination with others drug only one HPTLC method.

So, the approach is made to develop Derivative spectrophotometric method for quantification of Clidinium bromide and Chlordiazepoxide hydrochloride.

Simultaneous determination of both compounds was carried out using methanol: water (10:90 V/V) as solvent for extracting the drugs from the formulations and subsequently the samples were evaluated by first order derivative spectrophotometry.[8,9]

A study of the spectral behavior of these compounds in different solvents[10] the optimization of the spectral variables, the determination of pKa values and the application of the proposed method to the determination of these drugs in tablets are included in this work

MATERIALS AND METHODS

Instruments

A Shimadzu UV-1800 Spectrophotometer with 10-mm cells was used for measurements of the absorbance and derivative absorption spectra. For all solutions, the first order derivative spectra were recorded. The spectra derivatives were obtained digitally by software UV Probe incorporated in the Shimadzu UV-1800 spectrophotometer.

Reagents

Clidinium bromide and Chlordiazepoxide were gift sample provided from TORRENT PHARMACEUTICAL LTD Gujarat, India.

All chemical and solvent were of analytical grade provided by Department of Pharmaceutical Sciences, Sardar Patel University, Vallabh Vidyanagar. Anand.

Preparation of Standard Stock solution of CLB and CLDZ

Accurately weighed quantity 100 mg of CLB and CLDZ were transferred into separate 100 ml volumetric flask, dissolved in 10 ml methanol and diluted up to mark with distilled water (100 ml). This will give a stock solution having strength of 1000 µg/mL of each.

Preparation of solution for calibration curve of CLB

Suitable aliquots of the stock solution were diluted up to the mark with distilled water to get the concentration range of 20, 40, 60, 80, 100, 120µg/mL for CLB.

Preparation of solution for calibration curve of CLDZ:

Suitable aliquots of this solution were diluted up to the mark with distilled water to get the concentration range of 3, 6, 9, 12, 15, 18 µg/ml for CLDZ.

Selection of analytical wavelength: 20-120µg/mL solutions of CLB and 3-18 µg/mL solutions of CLDZ were prepared in methanol: distilled water (10:90 v/v) by appropriate dilution of working standard solution and spectrum was recorded between 200-400 nm and all zero order spectrums (D^0) were converted to first order derivative spectrum (D_1) using delta lambda 2.0 and scaling factor 1. The overlain first derivative spectrums of CLB and CLDZ at different concentration were recorded. The zero-crossing point (ZCP) of CLB was found to be 280.06 nm (Figure 2) and ZCP of CLDZ was found to be 245.46 nm is shown below Figure 3.

VALIDATION PARAMETERS[11,12,13]

Validation of developed method was carried out as per ICH guideline. Parameters such as Linearity and range, Accuracy, Precision, LOD and LOQ were taken up as tests for analytical method validation. [12-14]

Linearity and Range:

Appropriate volume of aliquot from CLB and CLDZ working standard solution was transferred to volumetric flask of 10mL capacity. The volume was adjusted to the mark with distilled water to give a solution containing 20-120 µg/ml (20, 40, 60, 80, 100, 120µg/mL) CLB and 3-18 µg/mL (3, 6, 9, 12, 15, 18 µg/mL) CLDZ. All D^1 Spectrum were recorded using above spectrophotometric condition. D^1 absorbance at 245.46 nm and 280.06 nm were recorded for CLB and CLDZ, respectively (n=5) is shown in overlain spectra of CLB and CLD, figure 4 and 5 respectively. Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs are shown figure 6 and 7. Straight line equations were obtained from the calibration curves.

Precision

Repeatability (*n*=6)

The repeatability was checked by scanning and measurement of the responses of solutions of CLB (80μg/mL) and CLDZ (12μg/mL) without changing the parameters of the proposed methods. The procedure was repeated six times and % RSD was calculated.

Intermediate Precision (*n*=3)

The intraday and interday precisions of the first order derivative method were determined by analyzing corresponding responses in triplicate on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CLB (40, 80 and 120μg/mL) and CLDZ (6, 12 and 18 μg/mL). Similarly, the intraday and interday precisions of ratio first order derivative method were determined by taking mixtures having concentration of CLB (40, 80 and 120 μg/mL) and CLDZ (6, 12 and 18 μg/mL).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

They were calculated as 3.3σ/S and 10σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S, is the mean of the slope of calibration plot.

Accuracy:

Recovery studies were done so as to check the accuracy of the method. Known amount of standard solutions of CLB and CLDZ were added to pre-quantify dosage form solution of CLB and CLDZ and D^1 absorbance were determined at 245.46nm and 280.06nm respectively. Concentration of the drug in the mixture was calculated using the regression equations. The analysis was done in a set of 3 replicates.

Application of Proposed Method to dosage form:

First Order Derivative Spectrophotometry An accurately weighed quantity of the powder equivalent to about 10mg of CLB and taken in 100ml volumetric flask and dissolved with methanol: water (10:90v/v) and further diluted up to the mark with same solvent. The solution was then filtered through the Whatman filter paper No. 41. Necessary dilutions are made with distilled water to give final concentration 6 μg/ml and 40μg/ml of CLB and CLDZ respectively. The solutions are then scanned between 200-400nm and absorbance are measured at respective wavelengths. The concentration of each drug was calculated using equation of straight line.

RESULTS

Zero cross point (ZCP):

ZCP of CLB and CLDZ were detected at 245.46 nm and 280.06 nm, respectively and overlain D^1 spectra were recorded (figure3).

Method Validation:

The linearity range for CLB and CLDZ were found to be 20-120μg/mL and 3-18μg/mL respectively. Recovery studies were carried out by addition of standard drug solution to pre-analyzed dosage form solution at four different concentration levels (0%, 80%, 100% and120%) taking into consideration percentage purity of added bulk drug sample. The results of the recovery studies are found to be satisfactory for CLB and CLDZ and shown in Table 3 and 4 respectively. Table 1 and table 3 shows the data of Inter and Intraday precision. The LOD and LOQ was calculated by formula. The result of assay procedure obtained was shown in Table 5. Summary of other validation parameters including Repeatability, Intraday, Interday, LOD and LOQ were found to be satisfactory and are shown in Table 6.

DISCUSSION

The first order derivative UV spectrophotometric method for simultaneous quantification of Clidinium bromide and Chlordiazepoxide hydrochloride from pharmaceutical dosage form was developed. The main focus of the method development for this combination was to make it economic with the use of distilled water instead of using methanol only as a solvent. The developed method was validated as per ICH guidelines. The results obtained for each validation parameters confirmed linearity, precision, accuracy and sensitivity of the developed analytical method as each parameter were in acceptable range. The marketed pharmaceutical formulation containing Clidinium bromide and Chlordiazepoxide hydrochloride was subjected to quantitative analysis using the developed method, yielded nearly 100% assay result for Clidinium bromide and Chlordiazepoxide hydrochloride.

CONCLUSION

The above developed methods is cost effective because of 90% distilled water as a solvent used for method development, sensitive as the method can analyze the sample in microgram quantity upto 20 and 3 for CLB and CLDZ respectively. Developed method is validated as per ICH guideline, hence can also be applied for routine analysis of CLB and CLDZ in analytical testing laboratories and industries.
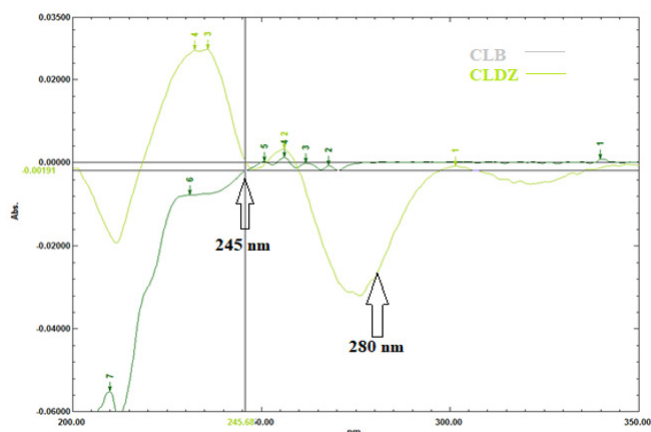
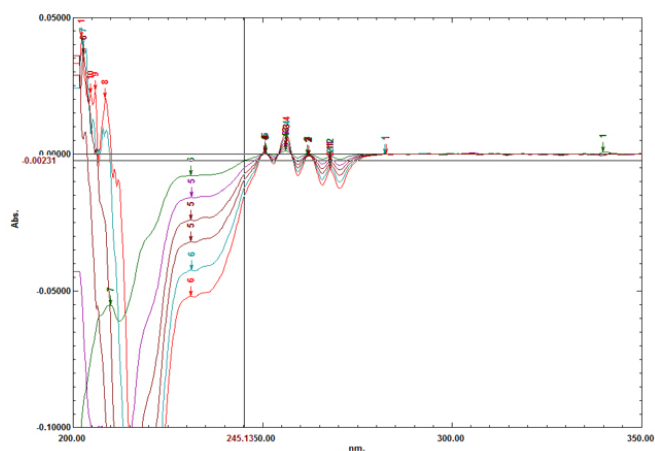**Figure 3:** First order UV derivative spectra of CLB and CLDZ

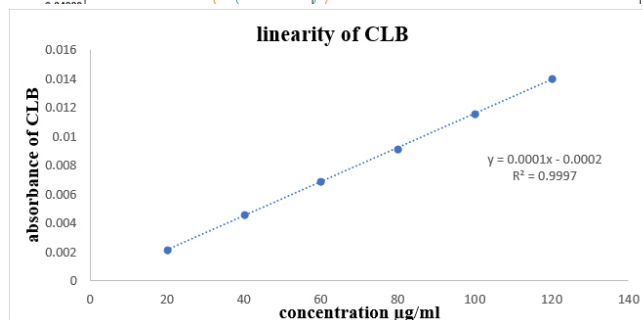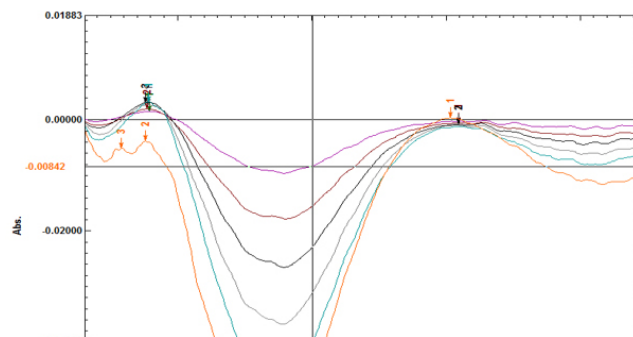**Figure 4:** First order derivative overlay spectra of CLB (20-120µg/ml)

Figure 6: calibration curve of CLB
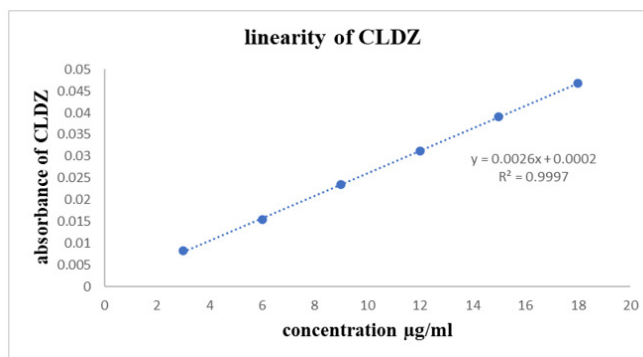
Figure 7: calibration curve of CLDZ

Table 1: Inter day precision data for CLB and CLDZ

Concentration (µg/ml)	CLB		CLDZ	
	Mean absorbance ± SD	%RSD	Mean absorbance ± SD	%RSD
40	0.004537 ± 0.0000321	0.70	0.01536 ± 0.000101	0.66
80	0.009063 ± 0.0000725	0.52	0.03128 ± 0.000153	0.49
120	0.001402 ± 0.0000065	0.46	0.046667 ± 0.000152	0.32

Table 2: Intraday precision data for CLB and CLDZ (n=3)

Concentration (µg/ml)	CLB		CLDZ	
	Mean absorbance ± SD	%RSD	Mean absorbance ± SD	%RSD
40	0.004537 ± 0.00003214	0.70	0.01536 ± 0.0000529	0.34
80	0.009047 ± 0.00002516	0.27	0.031273 ± 0.000144	0.46
120	0.001404 ± 0.000005131	0.36	0.046763 ± 0.00005686	0.12

Table 3: Accuracy data of CLB

Amount of sample (µg/ml)	Amount of std. spike (µg/ml)	Actual amount (µg/ml)	Amount of standard recovered (mean) (µg/ml)	Recovery (%) (mean ± SD)	%RSD
40	0	40	40.133	100.33 ± 0.00006	1.60
40	32	72	72.033	100.04 ±0.00011	1.57
40	40	80	79.7	99.65 ± 0.00006	0.78
40	48	88	87.833	99.81 ± 0.00044	1.68

Table 4: Accuracy data of CLDZ

Amount of sample (µg/ml)	Amount of std. spike (µg/ml)	Actual amount (µg/ml)	Amount of standard recovered (mean) (µg/ml)	Recovery (%) (mean ± SD)	%RSD
6	0	6	5.95	99.16 ± 0.000121	0.77
6	4.8	10.8	10.7371	99.41 ± 0.00034	1.21
6	6	12	11.916	99.30 ± 0.000104	0.33
6	7.2	13.2	13.17	99.84 ± 0.000503	1.46

Table 5: % assay of CLB and CLDZ combination marketed formulation dosage form

Tablet	Label claim (mg/tablet)		Assay ± SD (% of label claim)	
	CLB	CLDZ	CLB	CLDZ
LIBRAX®	2.5	5	99.38 ± 0.87	99.53 ± 1.27
LINOMAX™	2.5	5	98.86 ± 0.83	99.52 ± 1.47

Table 6: Summary of validation parameters

Sr. No.	Summary of validation parameters	CLB	CLDZ
1.	Linearity range (µg/ml)	20-120	3-18
	Slope	0.0001	0.0026
	Intercept	0.0002	0.00002
	Co-Efficient of Determination (r²)	0.9997	0.9997
	Regression Equation	y=0.0001x − 0.0002	y =0.0026x + 0.0002
2.	LOD(µg/ml)	0.1807	0.0097
3.	LOQ(µg/ml)	0.5477	0.0294
4.	Recovery (%)	99.62 – 100.33	99.16 – 99.84
	Precision(%RSD)		
5	Repeatability(%RSD)	0.15	0.18
	Intra-day(%RSD)	0.27 - 0.70	0.12 - 0.46
	Inter-day(%RSD)	0.46 – 0.70	0.32 – 0.66
6.	Specificity	Specific	specific
7.	Selectivity	Selective	selective
8	Assay (%purity)	99.38	99.53

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