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DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION OF ALPRAZOLAM AND MEBEVERINE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Simple, sensitive, rapid and cost effective spectrophotometric methods were developed and validated for simultaneous estimation of Alprazolam (ALP) and Mebeverine hydrochloride (MEB) in Bulk Drug and Pharmaceutical Dosage Form. Developed methods were applied to perform analysis of marketed formulation (MEBASPA-AL) used in anxiety and irritable bowel syndrome. The drugs obeyed the Beer's law in the concentration range of 2-10µg/ml for ALP and 10-50µg/ml for MEB. In UV-Spectrophotometric methods, estimation of ALP and MEB was carried out at amplitude 220.45 nm and 242.55 nm for First Order Derivative Spectrophotometric Method (method-A); at difference in absorbance between 217 nm and 227 nm and difference in absorbance between 257 nm and 267nm for Dual Wavelength Method (method-B) and 222 nm and 262.40 nm for Simultaneous Equation Method (method-C) using methanol as solvent. The values of limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.165 µg/ml and 0.498 µg/ml for ALP and 2.26 µg/ml and 6.87 µg/ml for MEB by method-A, 0.241 µg/ml and 0.736 µg/ml for ALP and 0.211 µg/ml and 0.641 µg/ml for MEB by method-B, 0.351 µg/ml and 0.106 µg/ml for ALP and 1.115 µg/ml and 3.37 µg/ml for MEB by method-C respectively. The % assay was found to be 99.40% for APL and 101.36 % for MEB by method-A, and by method-B it was found to be 101.40% for APL and 99.52% for MEB and by method-C it was found to be 99.40% for APL and 98.12% for MEB which within range (99-102%). The precision values were less than 2% R.S.D for all methods. So, all developed methods were validated in terms of linearity, limit of detection, limit of quantification, accuracy, precision, and robustness according to ICH guideline which proved suitability of the developed method for the routine estimation of ALP and MEB in pharmaceutical dosage form.

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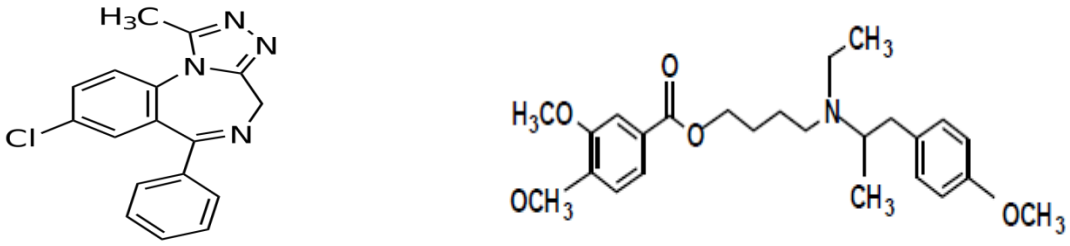
INTRODUCTION

Alprazolam (ALP) is chemically 8-chloro-1-methyl-6-phenyl-4H-[1,2,4] triazolo [4,3,-α]-[1,4] benzodiazepine derived from 1,4-benzodiazepines of new generation.[1] It is a benzodiazepine mainly used as anxiolytic in humans, and may be effective in the treatment of depression and panic disorder.[2] Mebeverine hydrochloride (MEB) is 3,4-Dimethoxybenzoic acid 4- [ethyl[2-(4-methoxyphenyl)-1-methylethyl] amino]-butylester is a potent direct antispasmodic acting mainly on the smooth muscles of the gastrointestinal tract and particularly effective against the colonic spasm.[1-4]MEB belongs to a group of compounds called Musculotropic antispasmodics. These compounds act directly on the gut muscles at the Cellular level to relax them.
The chemical structures of ALP and MEB are shown in Figure 1 (A), (B).



Alprazolam [A] Mebeverine hydrochloride [B]

Figure 1: Chemical structure of [A] alprazolam and [B] Mebeverine hydrochloride

Alprazolam is official in IP 2010, BP 2010, and USP 30 – NF 25. Indian Pharmacopoeia describes liquid chromatography (LC) method for its estimation. Mebeverine hydrochloride is official in BP 2010, IP 2010. Indian Pharmacopoeia describes Non aqueous titrimetric and liquid chromatography (LC) method for its estimation.[1-7]

The review of literature revealed that Few analytical methods like UV - Spectroscopic methods, High Performance Liquid Chromatographic (HPLC) methods, High Performance Thin Layer Chromatographic (HPTLC) methods for Alprazolam in single dosage form and in combination with other drugs have been reported.[8-10] While several methods for mebeverine hydrochloride like UV - Spectroscopic method, Potentiometric method, High Performance Liquid Chromatography (HPLC) and methods for estimation from biological fluid in single dosage form and in combination with other drug been reported[11-14]. But there is no single methods were existed for simultaneous estimation of ALP and MEB in combine dosage form.so The purpose of described method is to develop and validate simple, precise, accurate and reproducible spectrophotometric method for the simultaneous estimation of ALP and MEB in pharmaceutical dosage form.

MATERIALS AND METHODS

Pure Alprazolam was procured as a gift sample from Rhombus pharmaceutical Pvt. Ltd., Ahmedabad and Mebeverine hydrochloride was procured as a gift sample from Taj Pharmaceuticals Pvt. Ltd. Kalgam. Double beam UV/Visible spectrophotometer Shimadzu model 1800 was used to measure absorbance of resulting solutions. Methanol was used as solvent of AR grade. All apparatus and instruments were calibrated and validated as per calibration and validation protocol specified before starting the experiment.

Preparation of Standard Solutions of ALP and MEB:

The standard stock solutions of ALP and MEB were prepared by dissolving 25 mg each of ALP and MEB in methanol separately and final volume was adjusted with same solvent in 25 ml of volumetric flask to get strength of 1000µg/ml of each.

Preparation of Working Standard Solutions of ALP and MEB:

Series A: Solutions of ALP ranging from 2-10µg/ml were prepared by pipetting out 10 ml of the standard stock solution of ALP (1000 µg/ml) diluted to 100 ml. From the above stock solution, 0.2, 0.4, 0.6, 0.8,1 ml was pipette out into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

Series B: Solutions of MEB ranging from 10-50 µg/ml were prepared by pipetting out 10 ml of the standard stock solution of MEB (1000 µg/ml) diluted to 100ml.From the above stock solution, 1,2,3,4,5 ml was pipette out into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol.

Preparation of Sample Solution and Formulation Analysis: (By Standard Addition Method)

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg MEB and 1.85 mg of ALP was transferred to 100 ml volumetric flask, 80ml of methanol was added to the same flask, sonicated for 5 min and diluted to 100 ml with methanol and filtered through Whatman filter paper No. 41. To bring out the concentrations to the ratio 5:1, 18.15mg ALP was spiked. From this prepared formulation stock solution six dilution containing 25µg/ml of MEB and 5µg/ml of ALP were prepared. The sample solution was scanned in the wavelength range of 400–200 nm and measured.

EXPERIMENTAL METHODS

Method A. First order derivative spectroscopic method:

The working standard solutions of ALP and MEB were prepared separately in 10 ml volumetric flask using methanol as a solvent. They were scanned in UV range of 200-400 nm. From the overlain spectra (Fig: 2), two wavelengths were selected for quantitation of both the drugs by proposed first derivative Spectrophotometric method. In first order derivative spectrum ALP showed zero crossing points at 220.45nm. The wavelength selected for estimation of MEB was 220.45nm because it showed adequate absorbance at this wavelength in mixture. Similarly, first order derivative spectrum for MEB was taken and it showed zero crossing point at 242.55nm.The wavelength selected for estimation of ALP was 242.55nm because it showed adequate absorbance at this wavelength in mixture.

Determination of the Zero Crossing Points (ZCP):

Solutions of ALP (5 µg/ml) and MEB (25 µg/ml), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The Zero order spectra of both the drugs were derivatised to first order. First order derivative spectra were selected for analysis of both the drugs. From the overlain spectra of both drugs, wavelengths selected for quantitation were 220.45 nm for MEB (ZCP for ALP) and 242.55 nm for ALP (ZCP for MEB).

Method B. Dual wavelength spectroscopic method:

This method is applicable to calculate the concentration of component of interest found in a mixture containing it along with some unwanted interfering component. The absorbance difference between two points of the mixture spectra is directly proportional to the concentration of the analytic irrespective of the interference.

The working standard solutions of ALP and MEB were prepared separately in 10 ml volumetric flask using methanol as a solvent. They were scanned in UV range of 200-400 nm. From the overlain spectra (Fig: 3), four wavelengths were selected for quantitation of both the drugs by proposed Dual Wavelength Spectrophotometric method. The difference in absorbance between 257 nm and 267 nm (difference is zero for MEB) were plotted against the concentration of ALP. Similarly difference in absorbance between 217 nm and 227 nm (difference is zero for ALP) were plotted against the concentration of MEB and Calibration curve were plotted.

Method C. Simultaneous equation method:

Overlain spectra for both the drugs are shown in Fig.3.Two wavelengths selected for the use of simultaneous equation were 222 nm and 262.40 nm λ_{max} of ALP and MEB. The absorbance was recorded at the selected wavelengths and the absorptivity values were determined for ALP and MEB Statistical parameters like slope, intercept, coefficient of correlation and SD were determined (Fig.4).

Validation Parameters of the Developed Methods:
Validation of the developed method was carried out as per ICH guideline (Q2R1)[15]. Parameter such as linearity, accuracy, precision, LODs and LOQs were taken up as tests for method validation.

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Validation of the developed method was carried out as per ICH guideline (Q2R1). Parameter such as linearity, accuracy, precision, LODs and LOQs were taken up as tests for method validation.

Linearity:
For quantitative analysis of ALP and MEB the linearity curve was plotted. Linearity range of ALP and MEB was established in concentration range of 2-10µg/ml and 10-50µg/ml is shown in Table 1 and 2. The slope and intercept along with its correlation coefficient is given in Figure 5-10 for all developed methods.

Recovery Studies:
Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalyzed sample. Results of the recovery study were found to be within the acceptance criteria 100 ±2 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. In this method the known concentration standard drug was added to the assay sample. The amount present was calculated and the assay amount was reduced from it, which gives the amount recovered. The average per cent recoveries for APL and MEB were obtained are shown in Table 3.

Precision:
The intra-day and inter-day variation for determination of APL and MEB were carried out three times in the same day and three consecutive days and % RSD were calculated. The method was found to be precise due to Low values of the % RSD are shown in Table 4.

Limit of Detection and Limit of quantitation (LOD and LOQ)
The LOD and LOQ of developed method were studied as per ICH guidelines. Several approaches for determining the LOD & LOQ are possible, depending on the procedure i.e, Non-instrumental or instrumental. Among them here employed method was,
LOD= 3.3 σ/S and
LOQ= 10 σ/S
Where σ = the standard deviation of response,
S = the slope of calibration curve. The results obtained are shown in Table 5.

RESULTS AND DISCUSSION
Beer's law is obeyed in the concentration range of 2-50µg/ml for both the drugs APL and MEB. The correlation coefficient was found to be 0.9992 and 0.9991 for APL and MEB respectively by method A and for method B it was 0.9992 and 0.9991 for APL and MEB respectively and for method C it was found to be 0.9991 and 0.9995.The proposed method was also evaluated by the assay of commercially available tablets containing APL and MEB (n = 5). The % assay was found to be 99.40% for APL and 101.36 % for MEB by method-A, and by method-B it was found to be 101.40% for APL and 99.52% for MEB and by method-C it was found to be 99.40% for APL and 98.12% for MEB (Table 6). For APL, the mean recovery was found to be 99.80% with %RSD value 0.198% by method-A, and by method-B it was 90.80% with %RSD value 0.2368 % and by method-C it was 99.71% with %RSD value 0.2772 % and For MEB, the recovery found to be 100.06% with % RSD value 0.1951% by method-A and by method-B it was 99.85% with % RSD value 0.2361% and by method-C it was 99.84 % with %RSD value 0.12185% .The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is <2.

CONCLUSION

The proposed methods provide simple, accurate, fast and reproducible quantitative analysis for simultaneous determination of APL and MEB in tablets. It can be successfully applied for simultaneous estimation of APL and MEB in tablet dosage forms without prior separation and any interference in quality control. Further future research plan is to develop and validate Reversed Phase High Performance Liquid Chromatography methods for simultaneous estimation of Alprazolam and Mebeverine hydrochloride in combined dosage form and to validate the proposed methods as per ICH guideline.

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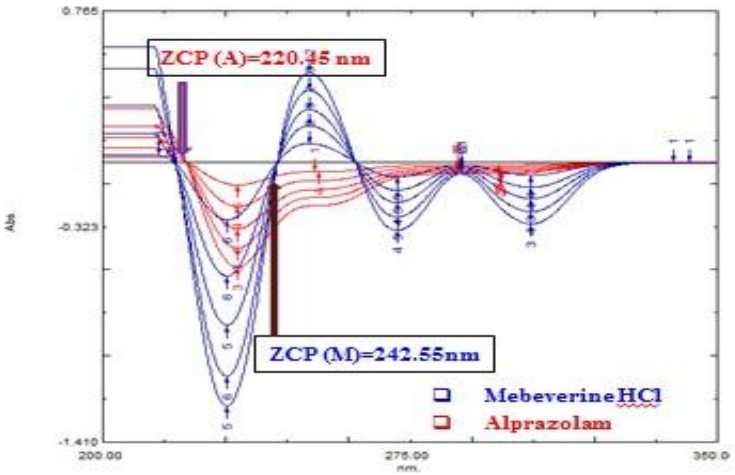

Figure 2: First order Overlaid Spectra of ALP and MEB showing selection of wavelength for detection

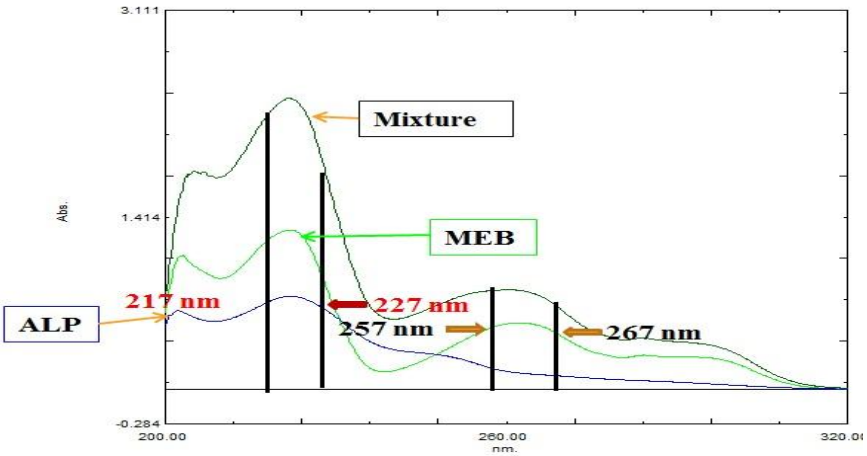

Figure 3: Overlay Spectra of ALP (2 µg/ml), MEB (10 µg/ml) and their Mixture in Methanol

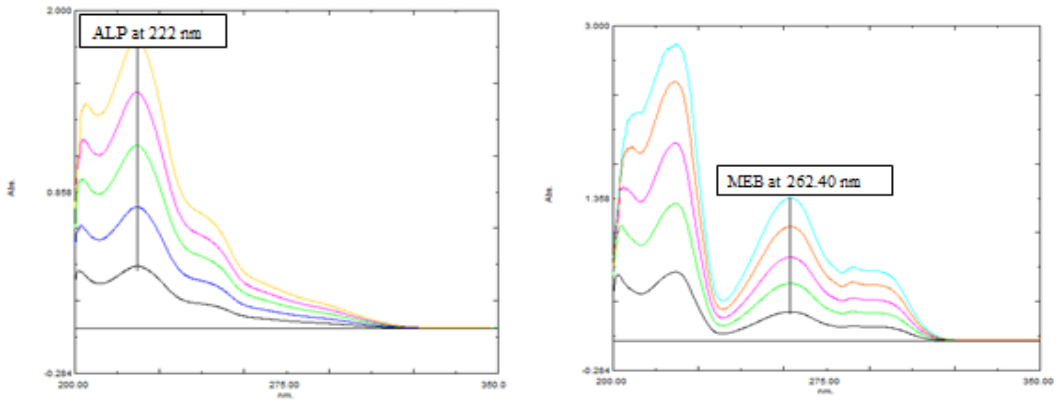

Figure 4: Overlaid Spectra of ALP and MEB showing selection of wavelength for detection

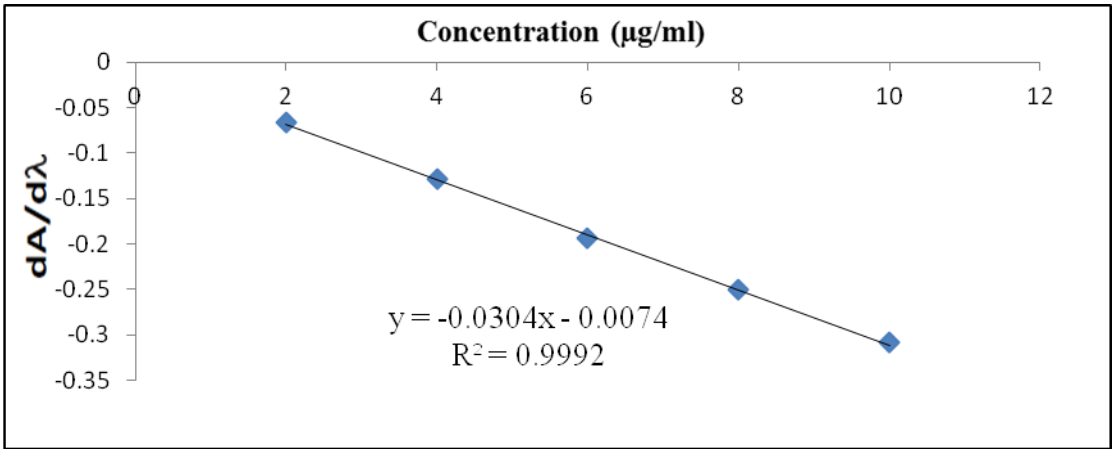

Figure 5: Calibration curve of ALP at 242.55 nm in methanol

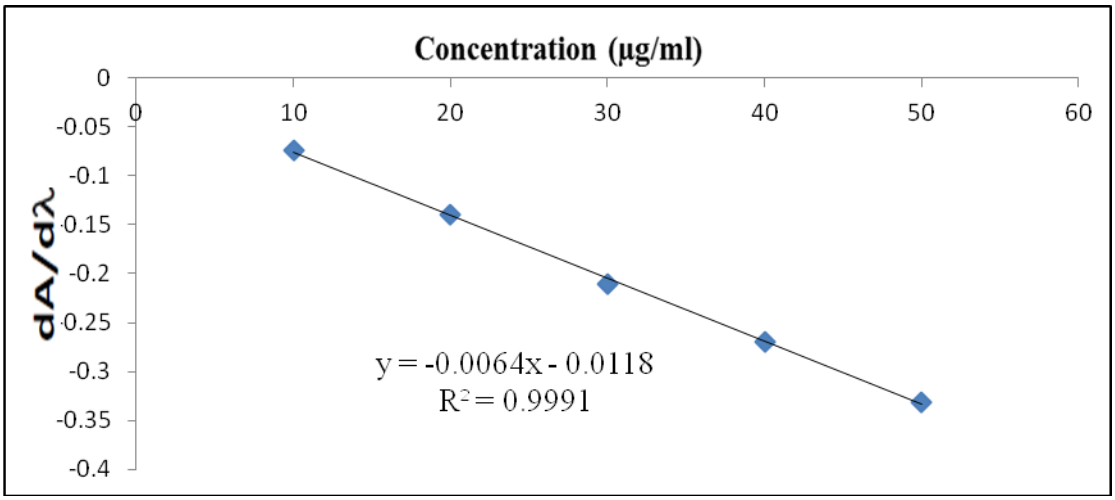

Figure 6: Calibration curve of MEB at 220.45 nm in methanol

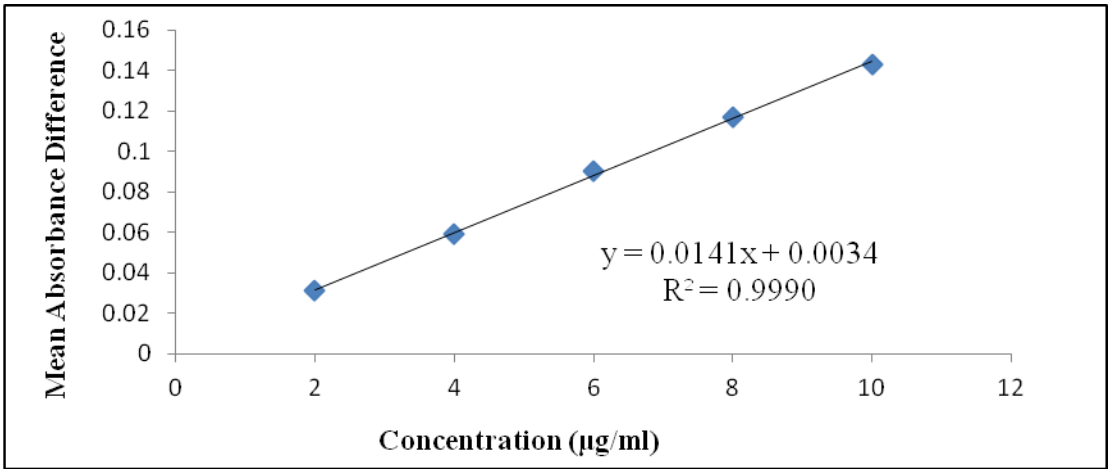

Figure 7: Calibration curve for ALP at difference of absorbance at 257nm and 267nm

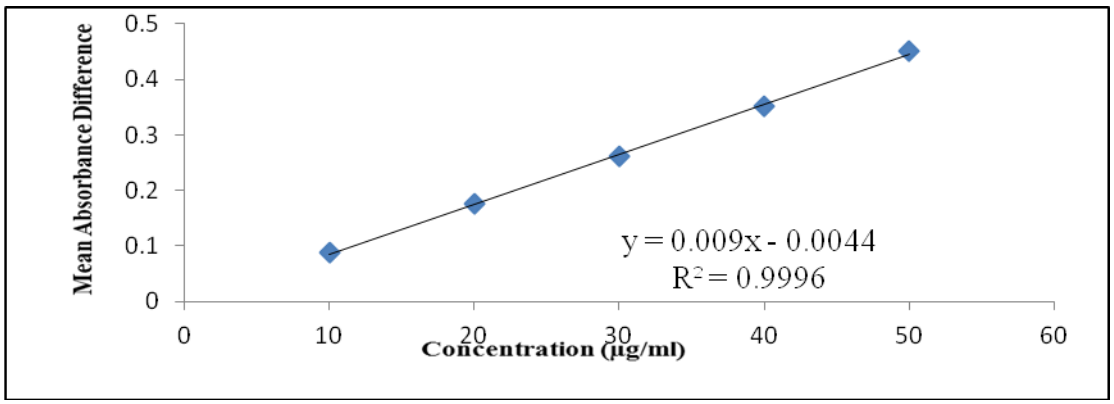

Figure 8: Calibration curve for MEB at difference of absorbance at 217 nm and 227 nm

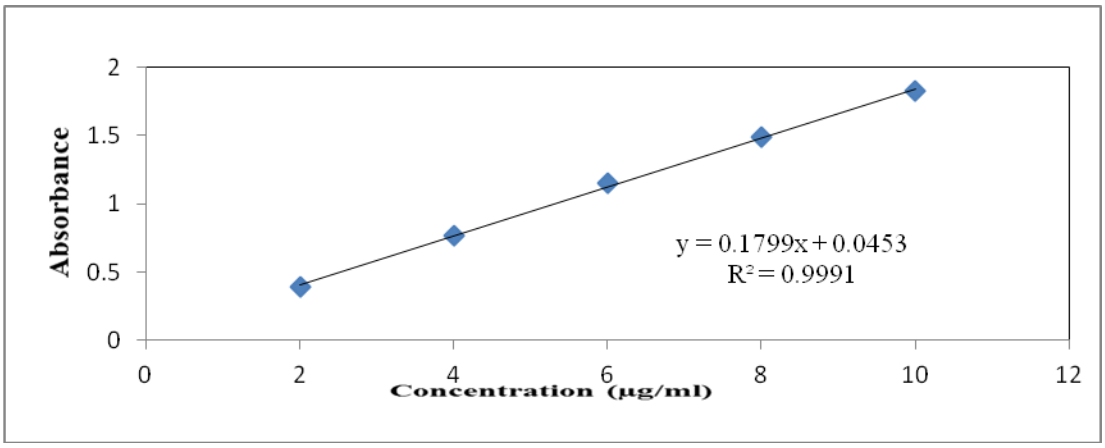

Figure 9: Calibration curve of ALP at 222 nm in methanol

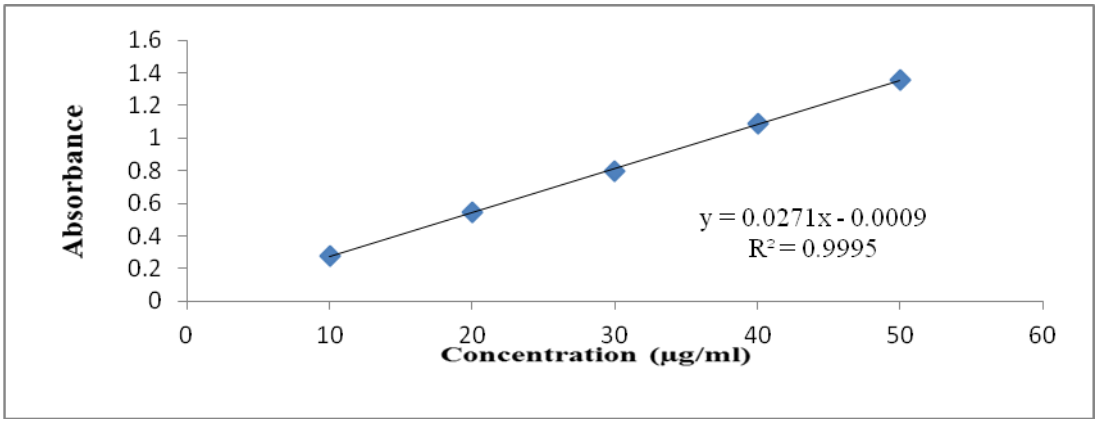

Figure 10: Calibration curve of MEB at 262.40nm in methanol

Table 1: Linearity Data of Alprazolam for First Order, Dual Wavelength and Simultaneous equation Spectroscopic Methods

Sr. No	Conc. (µg/ml)	First Order UV Spectroscopic Method 242.45nm Absorb.* ± %RSD	Dual Wavelength Spectroscopic Method 257-267 Abs. Diff.* ± %RSD	Simultaneous equation Spectroscopic Method Abs. Diff.* ± %RSD	
				(222 nm)	(262.4)
1	2	0.0660±1.1515	0.03033±3.8067	0.391667±0.29487	0.06966±1.6574
2	4	0.1296±0.4452	0.059±3.3898	0.763333±0.151271	0.13033±1.93094
3	6	0.1916±1.0860	0.089±1.1236	1.150333±0.180962	0.19966±0.765038
4	8	0.2501±0.6928	0.117±0.8547	1.488333±0.077583	0.262333±0.440165
5	10	0.3063±0.8215	0.1433±1.0657	1.831667±0.191732	0.32433±0.641829

*Mean value of five determinations

Table 2: Linearity Data of Mebeverine Hydrochloride for First Order, Dual Wavelength and Simultaneous equation Spectroscopic Methods

Sr. No	Conc. (µg/ml)	First Order UV Spectroscopic Method 220.45 nm Absorb.* ± %RSD	Dual Wavelength Spectroscopic Method 217-227 Abs. Diff.* ± %RSD	Simultaneous equation Spectroscopic Method Abs. Diff.* ± %RSD	
				(222 nm)	(262.4)
1	10	-0.07433±2.0549	0.088±1.13634	0.688±1.3801	0.272±0.9229
2	20	-0.144±3.67465	0.1766±0.32680	1.286±0.1552	0.549±0.5464
3	30	-0.2166±2.66469	0.261±0.38312	1.855±0.67379	0.7956±0.4413
4	40	-0.2866±5.32858	0.3516±0.1641	2.390±0.04184	1.08433±0.2320
5	50	0.36033±7..18523	0.449±0.339702	2.929±1.7585	1.3603±0.1530

*Mean value of five determinations

Table 3: Accuracy Study Data for First Order Spectroscopic Method, Dual Wavelength Spectroscopic Method, Simultaneous equation Spectroscopic Method

Level Added %	Zero Order UV Spectroscopic Method		Dual Wavelength Spectroscopic Method		Simultaneous equation Spectroscopic Method	
	ALP %Recovery ± SD	MEB %Recovery ± SD	ALP %Recovery ± SD	MEB %Recovery ± SD	ALP %Recovery ± SD	MEB %Recovery ± SD
80%	98.68±0.1980	99.94±0.1101	100.07±0.6671	99.26±0.2361	99.62±0.2772	99.93±0.09024
100%	99.75±1.0029	99.96±0.1951	99.62±0.4497	99.94±0.0901	99.51±0.5140	100.05±0.1218
120%	100.54±1.195	99.92±0.2596	99.82±0.2601	99.98±0.1493	100.06±0.425	100.34±0.4792

Table 4: Intraday & Interday Precision Data of Developed Spectroscopic Methods

Mixture conc. (µg/m)	First Order UV Spectroscopic Method Absorb.* ± %RSD		Dual Wavelength Spectroscopic Method Abs. Diff.* ± %RSD		Simultaneous equation method Spectroscopic Method Abs. Diff.* ± %RSD	
	ALP	MEB	ALP (257-267nm)	MEB (217-227nm)	ALP (at 222nm)	MEB (at262.42nm)
Intraday precision						
4:20	0.1267±1.2059	0.142±1.2197	0.0576±1.6527	0.181±0.6356	0.762±0.200	1.306±0.15
6:30	0.1960±0.8836	0.216±0.9607	0.092±1.0834	0.259±0.3861	1.154±0.173	1.885±0.05
8:40	0.253±0.7905	0.291±0.3436	0.117±1.2968	0.355±0.2816	1.486±0.067	2.473±0.12
Interday precision						
4:20	0.1280±0.7812	0.143±0.6998	0.057±1.5071	0.184±0.5434	0.765±0.2719	1.309±0.158
6:30	0.1973±0.7740	0.217±0.7017	0.096±1.0463	0.254±0.4534	1.154±0.1323	1.886±0.053
8:40	0.2513±0.6077	0.293±0.6825	0.121±0.1653	0.352±0.4335	1.489±0.0671	2.470±0.123

*Mean value of three determinations

Table 5: Limit of Detection (LOD) and Limit of Quantification (LOQ) for All Developed Methods

Parameters	First Order UV Spectroscopic Method		Dual Wavelength Spectroscopic Method		Simultaneous equation Spectroscopic Method			
					222nm	262.40nm	222nm	262.40nm
	ALP	MEB	ALP	MEB	ALP		MEB	
LOD(µg/ml)	0.165	2.26	0.241	0.211	0.351	0.211	1.115	0.121
LOQ(µg/ml)	0.498	6.87	0.736	0.641	0.106	0.641	3.37	0.369

Table 6: Result of recovery studies of ALP and MEB by using Formulation.

Formulation	Amount present(µg/ml)		Amount Recovered (%)					
	ALP (mg)	MEB (mg)	Zero Order UV Spectroscopic Method*		Dual Wavelength Spectroscopic Method*		Simultaneous equation Spectroscopic Method	
			ALP	MEB	ALP	MEB	ALP	MEB
"MEBASPA-AL (2.5mg ALP & 135 mg MEB)	2.5	135	99.40	101.36	100.42	99.52	99.04	98.12

(* n=6 determination)

Table 7: Summary of Validation Parameters of Alprazolam for First Order Derivative, Dual Wavelength and Simultaneous equation Spectroscopic Method

Sr.no	Parameters	First Order UV Spectroscopic Method	Dual Wavelength Spectroscopic Method	Simultaneous equation Spectroscopic Method
1.	Linearity (Conc. Range) (µg/ml)	2-10	2-10	2-10
2.	Regression Equation*	y = -0.0304x - 0.0074	y = 0.0141x + 0.0034	0.1799x + 0.0453
3.	Correlation Coefficient (r^2)	R^2 = 0.9992	R^2 = 0.9990	R^2 = 0.9998
4.	Precision			
	Intraday (% RSD) (n=3)	0.1267	0.0576	0.762
	Interday (% RSD) (n=3)	0.1280	0.143	0.765
5.	% Recovery	99.65	99.83	99.71
6.	Limit of detection (µg/ml)	0.165	0.241	0.351
7.	Limit of quantification (µg/ml)	0.498	0.736	0.641

Table 8: Summary of Validation Parameters of Mebeverine hydrochloride for First Order Derivative, Dual Wavelength and Simultaneous equation Spectroscopic Method

Sr.no	Parameters	First Order UV Spectroscopic Method	Dual Wavelength Spectroscopic Method	Simultaneous equation Spectroscopic Method
1.	Linearity (Conc. Range) (µg/ml)	10-50	10-50	10-50
2.	Regression Equation*	y = -0.0064x - 0.0118	y = 0.009x - 0.0044	y = 0.0567x + 0.1362
3.	Correlation Coefficient (r^2)	0.9991	0.9993	0.9995
4.	Precision			
	Intraday (% RSD) (n=3)	0.142	0.181	1.306
	Interday(% RSD) (n=3)	0.143	0.184	1.309
5.	% Recovery	100.06	99.99	100.11
6.	Limit of detection (µg/ml)	2.26	0.211	1.11
7.	Limit of quantification (µg/ml)	6.87	0.641	3.37

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