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## Research Article

### UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ALPRAZOLAM AND MEBEVERINE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATION

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#### ABSTRACT

A simple, accurate, precise, sensitive, rapid and economical spectrophotometric method was developed and validated for simultaneous estimation of Alprazolam (ALP) and Mebeverine HCl (MBH) in bulk drug and pharmaceutical formulation. The estimation of these drugs was carried out by using 0.1M HCl as a solvent. The wavelength maxima for Alprazolam and Mebeverine HCl were found to be 262.3 nm and 222.5 nm. The linearity range was observed in the concentration range of 3-15 µg/ml for both drugs and regression equation was found to be for ALP  $0.0565x+0.0138$  and for MBH  $0.049x-0.0126$ . Percentage recoveries for Alprazolam and Mebeverine HCl were found to be 99.84% and 99.47% respectively. % RSD values for Intra-day precision were found to be for ALP 1.18% and for MBH 0.59%. Inter-day precision %RSD values were found to be for ALP 0.94% and for MBH 0.69%. LOD was found to be for ALP 1.42 (µg/ml) and for MBH 2.1542 (µg/ml). LOQ was found to be for ALP 4.3242 (µg/ml) and for MBH 6.5442 (µg/ml). The %Assay of Alprazolam and Mebeverine HCl were found to be 99.20% and 100.02% respectively. Statistical analysis proved that the developed method can be successfully used for simultaneous analysis of Alprazolam and Mebeverine HCl in pure and tablet dosage forms.

#### INTRODUCTION

Alprazolam [Fig. 1] is chemically 8-chloro-1-methyl-6-phenyl-4*H*-s-triazolo[4,3- $\alpha$ ][1,4] benzodiazepine<sup>[1]</sup>. It is a short-acting anxiolytic of the benzodiazepine class of psychoactive drugs<sup>[2,3]</sup>. Alprazolam possesses anxiolytic, sedative, hypnotic, skeletal muscle relaxant, anti-convulsant and amnesic properties. The exact mechanism of action of Alprazolam is unknown. Benzodiazepines bind nonspecifically to benzodiazepine receptors BNZ1, which mediates sleep, and BNZ2, which affects muscle relaxation, anticonvulsant activity, motor coordination, and memory. As benzodiazepine receptors are thought to be coupled to gamma-aminobutyric acid-A (GABA<sub>A</sub>) receptors, this enhances the effects of GABA by increasing GABA affinity for the GABA receptor. Binding of the inhibitory neurotransmitter GABA to the site opens the chloride channel, resulting in a hyperpolarized

cell membrane that prevents further excitation of the cell<sup>[4]</sup>.

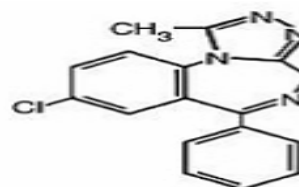
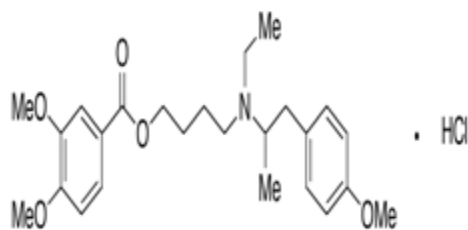


Fig. 1: Structure of Alprazolam

Mebeverine Hydrochloride [Fig. 2] is chemically 4-[ethyl-1-(4-methoxyphenyl)propan-2yl]amino] buty l-3,4- dimethoxybenzoate hydrochloride<sup>[5]</sup>. It is an anti-muscarinic and belongs to a group of compounds called musculotropic antispasmodics. These compounds act directly on the gut muscles at the cellular level to relax them. This relieves painful muscle spasms of the gut, without affecting its normal motility<sup>[6]</sup>. Mebeverine is used to relieve symptoms of irritable bowel syndrome and related

intestinal disorders that are the result of spasms in the intestinal muscles. Mebeverine is also an inhibitor of calcium-depot replenishment. Therefore, it has dual mode of action which normalizes the small bowel motility<sup>[7,8]</sup>.



**Fig. 2: Structure of Mebeverine HCl**

The literature survey demonstrated that few analytical methods like TLC<sup>[9]</sup>, HPTLC<sup>[10]</sup> and HPLC methods were described for estimation of Alprazolam and Mebeverine HCl in single<sup>[11-13]</sup> and combined<sup>[14]</sup> dosage form. However only one UV Spectrophotometric<sup>[15]</sup> method was available for determination of these drugs in combined dosage form. Hence the objective of the present work is to develop and validate a new, simple, sensitive, precise and accurate UV Spectrophotometric method for the simultaneous estimation of Alprazolam and Mebeverine Hydrochloride in bulk drug and in pharmaceutical formulations.

## MATERIALS AND METHODS

### Instrument

Shimadzu UV1800 Double Beam UV-Visible Spectro-photometer was used for analysis.

### Chemical and Reagents

The reference samples of Alprazolam (API) and Mebeverine Hydrochloride (API) were provided as gift samples from Sumages Pharma Pvt. Ltd., Bhimavaram, India. The commercial formulations (tablets) (MEBASAP-AL tablets containing 0.25 mg of Alprazolam and 135 mg of Mebeverine Hydrochloride) were procured from the local market. Hydrochloric acid (AR grade) was purchased from E.Merck (India) Ltd., Mumbai, India and was used as solvent. Fresh purified distilled water was used throughout the experiment.

### Selection of solvent

Based on the solubility of the compounds finally 0.1M Hydrochloric acid was selected as common solvent for both the drugs due to its positive results.

### Detection of wave length maxima

The spectra of diluted solutions contains 10 µg/ml of ALP and MBH were recorded separately on UV spectrophotometer and the solutions were scanned between 200-400 nm by using 0.1M Hydrochloric acid as blank. The peaks of maximum absorbance wavelengths were observed at 262.3 nm for ALP and 222.5 nm for MBH. The overlain spectra give the isobestic point at 263.9 nm.

### Standard solution of Alprazolam and Mebeverine HCl

About 5 mg of Alprazolam and 5 mg of Mebeverine Hydrochloride was accurately weighed and transferred into a 100 ml clean dry volumetric flask containing 70 ml of 0.1M Hydrochloric acid. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the Hydrochloric acid to get a stock concentration of Alprazolam and Mebeverine Hydrochloride. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the 0.1M Hydrochloric acid.

### Sample solution of Alprazolam and Mebeverine HCl

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 5 mg of Alprazolam and 5 mg of Mebeverine Hydrochloride was transferred into a 100 ml clean dry volumetric flask containing 70 ml of 0.1M Hydrochloric acid. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the Hydrochloric acid to get a stock concentration of Alprazolam and Mebeverine Hydrochloride. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the 0.1M Hydrochloric acid. Absorbance of the sample solution was measured at 262.3 nm and 222.5 nm. The data was presented in Table 1.

**Table 1: Assay results of Alprazolam and Mebeverine Hydrochloride formulations**

Formulation		Label claim	Amount found	%Assay
MEBASAP-AL	Alprazolam	5 mg	4.96 mg	99.20%
	Mebeverine HCl	5 mg	5.01 mg	100.02%

## METHOD VALIDATION

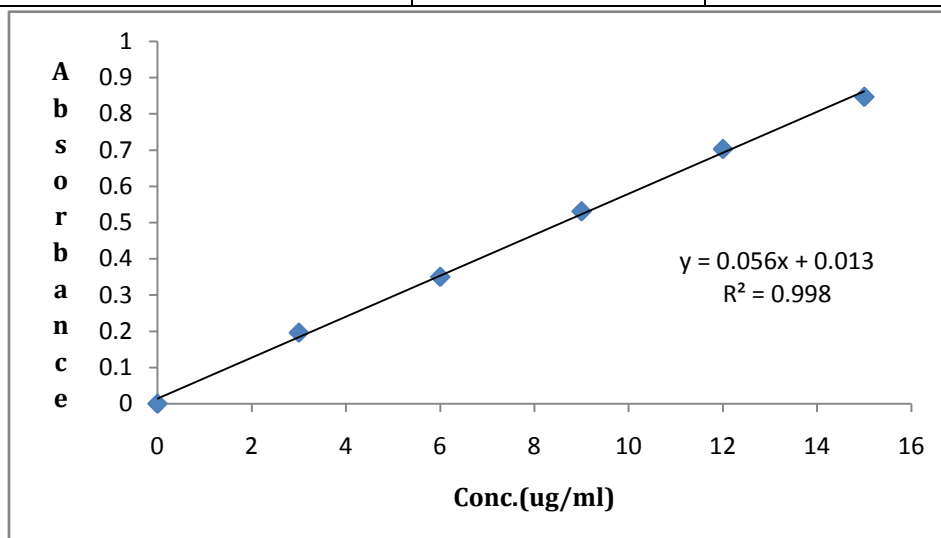
### Linearity

Linearity was performed by preparing standard solutions in between the range of 3-15 µg/ml for both Alprazolam and Mebeverine Hydrochloride. The absorbance were measured at 262.3 nm for ALP and 222.5

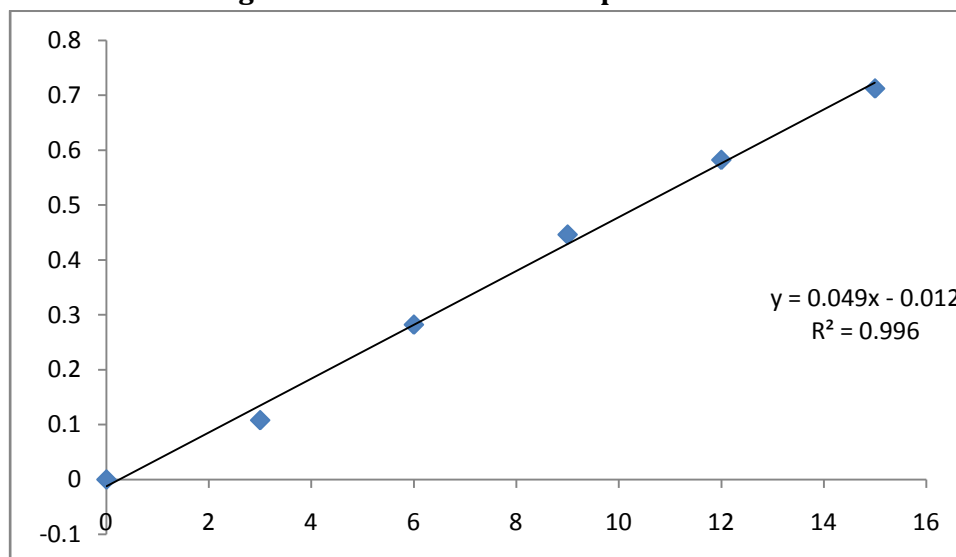
nm for MBH. The linearity results were furnished in Table 2 and calibration curves were depicted in Fig. 3 and Fig. 4.

**Table 2: Linearity results of Alprazolam and Mebeverine HCl**

S. No.	Concentration (µg/ml)	Absorbance (ALP)	Absorbance (MBH)
1	0	0	0
2	3	0.196	0.108
3	6	0.35	0.282
4	9	0.531	0.446
5	12	0.703	0.582
	15	0.847	0.712
Slope		0.055	0.5517
Intercept		0.0277	0.0289
Regression Equation(y)		0.0565x+0.0138	0.049x-0.0126
Correlation Coefficient		0.9985	0.9965



**Fig. 3: Calibration curve of Alprazolam**



**Fig. 4: Calibration curve of Mebeverine Hydrochloride**

**Precision**

Precision was evaluated by conducting intra-day and inter-day precision studies. The results were furnished in Table 3 and Table 4.

**Table 3: Results for Intra-day Precision**

S. No.	Time (Hours)	Absorbance (ALP)	Absorbance (MBH)
1	0	0.472	0.364
2	1	0.469	0.361
3	2	0.465	0.363
4	3	0.463	0.365
5	4	0.462	0.36
6	5	0.457	0.362
7	6	0.458	0.359
Mean		0.46	0.36
SD		0.0055	0.0022
%RSD		1.18	0.597

**Table 4: Results for Inter-day Precision**

S. No.	Time (Days)	Absorbance (ALP)	Absorbance (MBH)
1	0	0.479	0.391
2	1	0.477	0.388
3	2	0.466	0.392
4	3	0.469	0.389
5	4	0.473	0.388
6	5	0.472	0.385
7	6	0.472	0.385
Mean		0.473	0.388
SD		0.004	0.003
%RSD		0.94	0.69

**Accuracy**

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the absorbance before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 50%, 100% and 150%. The results were tabulated in Table 5 and Table 6.

**Table 5: Recovery studies for Alprazolam**

Level	Standard conc. ( $\mu\text{g/ml}$ )	Conc. added ( $\mu\text{g/ml}$ )	Conc. found ( $\mu\text{g/ml}$ )	% Recovery	% Mean recovery
50%	10	5	5.01	100.20	99.84
100%	10	10	9.98	99.80	
150%	10	15	14.93	99.53	

**Table 6: Recovery studies for Mebeverine Hydrochloride**

Level	Standard conc. (µg/ml)	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	% Mean recovery
50%	10	5	4.97	99.40	99.47
100%	10	10	9.93	99.30	
150%	10	15	14.96	99.73	

**Limit of detection and Limit of quantification**

For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Alprazolam and Mebeverine Hydrochloride are given in Table 7.

**Table 7: LOD and LOQ of Alprazolam and Mebeverine HCl**

Parameter	Measured value (µg/ml) ALP	Measured value (µg/ml) MBH
Limit of detection	1.42	2.15
Limit of quantification	4.32	6.54

**RESULTS AND DISCUSSION**

The proposed work describes a method used for the simultaneous estimation of Alprazolam and Mebeverine HCl by using 0.1M HCl as a solvent. The selected analytical wavelengths for were found to be 262.3 nm and 222.5 nm for ALP and MBH respectively. The calibration curve was constructed over a linear range of 3-15 µg/ml for both drugs ALP and MBH. The correlation coefficient was found to be 0.9985 for ALP and 0.9965 for MBH respectively. %RSD values of intra-day and inter-day precision were found to be for ALP 1.18% and 0.94% and for MBH 0.59% and 0.69%. For ALP the mean recovery was found to be 99.84% and for MBH 99.47%. LOD and LOQ values of developed method were found to be for ALP 1.42 (µg/ml) and 4.3242 (µg/ml) and for MBH 2.1542 (µg/ml) and 6.5442 (µg/ml). The %Assay of Alprazolam and Mebeverine HCl were found to be 99.20% and 100.02% respectively.

**CONCLUSION**

The developed spectrophotometric method was simple, reproducible, accurate, rapid and cost-effective method for simultaneous determination of ALP and MBH in bulk and pharmaceutical formulations. The method was validated for parameters like linearity, accuracy and precision as per ICH guidelines. All statistical results and assay results were found to be satisfactory. Hence the proposed method can be successfully employed for quantitative analysis of Alprazolam and Mebeverine HCl in combined dosage form.

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