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Spectrophotometric Multivariate Calibration Approach: Application in Quantitative Determination of Mebeverine in Bulk Drug and Pharmaceutical Formulations

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Abstract: Multivariate calibration technique using spectrophotometer is underway to assess quantitatively mebeverine in intermediate and fished stage of different dosage forms of pharmaceutical. This approach bases on the use of linear regression analysis equation by using correlation of concentration of drugs and corresponding absorbance taken, at five unlike wavelengths. Statistical evaluation of the results finds the method to be highly accurate, precise and reproducible. Drug molecule was studied in concentration range of 5-80 µg/mL and correlation coefficient(r) of 0.9966 was obtained. Proposed method was found to have considerable resolving power, rapidity, sensitivity and low cost for the quantification of the drugs analysis, in quality control samples and schedule analysis of subject compounds. Common excipients like magnesium stearate, Pyrrolidone K 30, lactose and hydroxypropylmethylcellulose were studied and no interference was countered. This approach was highly useful in eliminating the common error that comes from experimental design or instruments errors.

Key words: Mebeverine · Multivariate · Pharmaceuticlas

INTRODUCTION

Mebeverine hydrochloride (MVH) is 4-[ethyl(4-methoxy-α-methylphenethyl) amino]butylveratrate hydrochloride, its molecular formula is C25H35NO5HCl having molecular weight 466 and melting point 105 -107 °C. It is white or almost white, crystalline powder. It is fairly soluble in both water and ethanol (96%) and insoluble in diethyl ether [1].

It has been known to be musculotropic antispasmodic drug acting on the smooth muscle of the gastrointestinal tract. It relieves spasm and do not affect gut motility because its action is not interceded by the autonomic nervous system [1]. Mebeverine rapidly shows complete absorption and is metabolized completely. Its hydrochloride derivative has antispasmodic action and acts directly on smooth muscles of gastrointestinal tract [1]. It is indicated in irritable bowel syndrome [2-4].

Various analytical methods for determination of MVH are reported in literature. Karemer *et al.,* reported the fluorescence polarization immunoassay (FPIA) and gas chromatographic mass spectrophotometric studies on the toxicological analysis of mebeverine [5]. Another method was reported by Stockis *et al* [6] which identified mebeverine acid as the main circulating metabolite in man, while in another reported method the metabolism of mebeverine in man is described. This method identified

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urinary metabolites using gas chromatography/ mass spectrometry [7], plasma concentration of mebeverine using HPLC was reported by Dickinson *et al* [8].

Multivariate UV Technique: Multivariate statistics is a form of the simultaneous examination and analysis of more than one result variable. The function of multivariate statistics is multivariate analysis. In this current work mathematical algorithm was used as bellow.

The absorbance of an analyte (X) is measured at five wavelength sets (λ = 258, 260, 262, 264 and 266 nm). The corresponding equations can be written as follows,

$$\begin{aligned} A_{\lambda 258} &= a \times C_X + k_1 \\ A_{\lambda 260} &= b \times C_X + k_2 \\ A_{\lambda 262} &= c \times C_X + k_3 \\ A_{\lambda 264} &= d \times C_X + k_4 \\ A_{\lambda 266} &= e \times C_X + k_5 \end{aligned} \quad (1)$$

Here A_λ represent the absorbance of the analyte under test, a, b, c, d, e are the slopes and k_1, k_2, k_3, k_4 and k_5 are the intercepts of linear regression equation for the analyte corresponding to five wavelengths and C_X represents the concentration of analyte. All these equations (1) can be combined as follows

$$A_T = a \times C_X + b \times C_X + c \times C_X + d \times C_X + e \times C_X + K_T \quad (2)$$

The simplified form of this equation is

$$A_T = C_X (a + b + c + d + e) + K_T \quad (3)$$

where A_T and K_T correspond to the sum of absorbance and sum of intercepts obtained of equations. So from the above equation concentration of analyte X, in an unknown solution can be found as fallows,

$$C_X = \frac{AT - KT}{(a + b + c + d + e)} \quad (4)$$

In this case, the multivariate spectrophotometric calibration mood utilizes the linear relation of concentration with absorbance which predicts the amount of unknown concentration of analyte.

Experimental

Apparatus: Shimadzu 1601 UV–visible spectrophotometer coupled with a P-IV computer loaded with Shimadzu UVPC version 3.91 software.

Materials: Mebeverine hydrochloride was gifted by a local Pharmaceutical. Their purity was confirmed by melting point and IR spectra. Commercial pharmaceutical formulations tablets of a fresh lot were procured from local market manufactured by AGP (Private) Limited Karachi. Throughout the study deionized water was used and all other chemical and reagents were of analytical grade.

Preparation of Standard Solutions: Stock standard solutions of MVH (1000 μGmL-1) was prepared by dissolving 100 mg of mebeverine hydrochloride in little amount of deionized water and then diluted with same solvent.

A validation set of seven different concentrations were prepared in working range of 5 to 80 $\mu g ml^{-1}$ for MVH. These solutions were scanned in the UV region and absorbances were taken at 258, 260, 262, 264 and 266 nm wavelength and plotted against concentration, which followed Beer & Lambert's law.

Analysis of Tablets: An average wt of 20 tablets was powdered and homogenized and a bulk mass equivalent to 100 mg of MVH was transferred to a 100 ml calibrated volumetric flask. The solution was stirred on magnetic stirrer for 30 min; the volume was adjusted to the mark level with deionized water. A portion of the solution was filtered through whatman filter paper 42 micron (Germany) and clear filtrate was diluted with deionized water to obtain desired concentrations.

RESULTS AND DISCUSSION

Linear regression functions obtained at five different wavelength sets forms the foundation of multi variation approach and this experimental design is manipulating this technique in devising analytical tool for mebeverine. Multi-linear regression functions derived from different data set is reduced to single linear regression equation and this helps in providing a more sensitive determination than the classical UV method. Random error associated with instruments as well as interference form excipients are eliminated using this approach. Under optimized conditions the applied statistical method is highly advantageous over the classical method with respect to resolution, sensitivity, rapidity and low cost for quantization of mebeverine in quality control laboratories.

In this paper, statistical tools in combination to multivariate spectral technique was used and the resultant data set acquired for the determination of mebeverine in

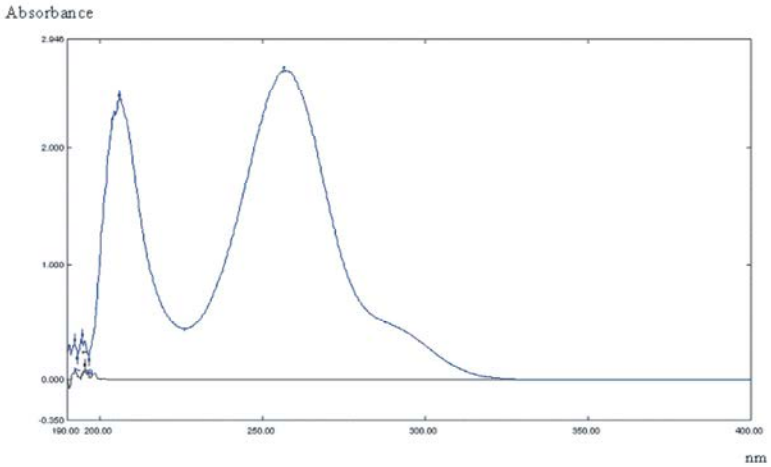

Fig. 1: Representative ultraviolet spectra of mebeverine (Standard)

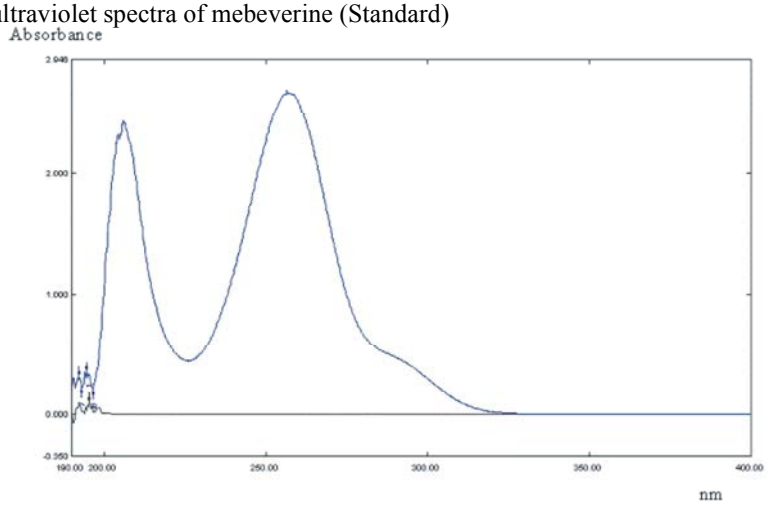

Fig. 2: Representative ultraviolet spectra of mebeverine (Pharmaceutical Formulation)

dosage forms in bulk and finished forms substantiated the high level accuracy and precision. Consistent recovery from the commercial pharmaceutical product further confirmed the applicability of the method. Hence, this method is extremely constructive with very easy mathematical contents, is more consistent than the other spectrophotometric methods and strongly suggests the application in calibration models for a usual analysis.

Specificity: The placebo analysis was used to determine the selectivity of method. The placebos of mebeverine hydrochloride tablet formulation were prepared in which all the normal ingredients except the mebeverine were present. They were given the same treatment as was given to normal samples. The absorbance was recorded to study the excipient meddling (maize starch, sodium starch glycolate, lactose, magnesium stearate and hydroxypropylmethylcellulose). No intrusion was observed from the excipients in the tested range of wavelengths. The% recovery of tablets formulations was the approximate proof that the method is specific.

Emblematic spectra for standard preparation Figure 1, pharmaceutical formulation Figure 2 and placebo Figure 3 evidently showed that there were no interference.

Linearity and Range: The linearity of the applied analytical procedure was assessed across a wide range of concentration. The selected range over which linearity was performed ranged between 5–80 μg ml^{-1}. The equation A = B C + D was used to calculate the regression line where C is the mebeverine concentration (μg ml^{-1}) and A was the absorbance. D is intercept and B is slope of regression line as mentioned in Table 1. Least squire regression procedure was used to obtain the calibration curve. Table 1 also has the

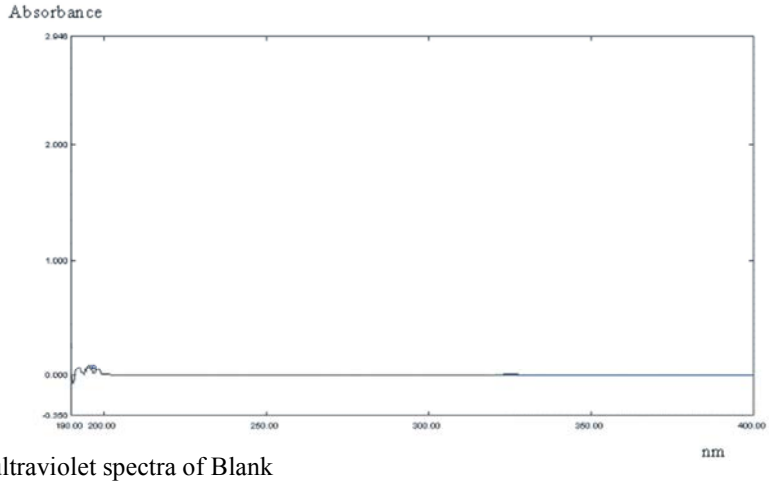

Fig. 3: Representative ultraviolet spectra of Blank

Table 1: Linear regression functions and their statistical parameters at the five-wavelength sets

Drug	λ	Regression equation	r	LOD (ug/ml)	LOQ (ug/ml)	%R.S.D.*
Active	258	A = 0.0226 Cx +0.0125	0.9998	0.0884	0.62	0.944
	260	A = 0.0242 Cx – 0.0212	0.9983	0.039	0.33	0.669
	262	A = 0.0242Cx +0.0098	0.9976	0.0272	0.16	0.317
	264	A = 0.0237 Cx +0.0175	0.9966	0.0567	0.38	1.02
	266	A = 0.0236 Cx +0.004	0.9973	0.0791	0.49	0.559

* =% Relative Standard Deviation

Table 2: Intra and interday precision and accuracy of the method

	Intraday			Interday		
Drug added (mg)	Drug found (mg)	%Recovery (Mean)	%RSD	Drug	%Recovery found (mg)	%RSD (Mean)
32 (80%)	32.16	100.5	0.841	32.33	101.03	0.87
40 (100%)	40.52	101.3	1.27	40.71	101.77	1.34
48 (120%)	48.41	100.85	1.19	48.69	10143	0.72

Table 3:% recovery of at different wavelengths range

	% Recover					
Concentration ug/ml						
260	258	260	262	264	266	Multi.[a]
5	99.22	99.38	101.29	98.63	99.47	100.06
10	100.17	99.58	98.94	100.47	101.29	100.28
20	100.31	100.39	100.08	99.85	98.84	99.97
40	99.74	98.57	100.67	99.69	100.33	100.27
60	101.47	101.35	99.26	101.22	101.03	99.84
80	98.97	100.57	99.88	99.54	98.92	100.19

[a]Multivariate data

representation of linear equation for five different wavelengths. The minimum correlation coefficient (r) value was 0.9966.

Limits of Detection and Quantitation: Using empirical formula of 3.3s/m for limit of detection (LOD) and 10s/m limits of detection (LOQ) the value of LOD and LOQ were established, where s is the standard deviation of the absorbance (for five replicates) and m is the slope of the calibration curve, determined from linearity investigation [10]. The LOD and LOQ have been shown in Table 1.

Accuracy: For accuracy a reference standard solution was analyzed and compared the measured value with the true value by using spiking method also called as the method of standard additions.

The results of accuracy and precision are summed up in Table 2, expressed as percent recovery and relative standard deviation (R.S.D). The results indicated in table are a clear representation of a good recovery. Hence the Table 2 demonstrates good% recovery values of the method and multivariate data. Table 3 represents the% recovery of the method at different wavelengths which is derived using multivariate mathematical calculations.

Precision: Precision of an assay method was evaluated as measurement of repeatability, intermediate precision and reproducibility. Reproducibility is the most commonly used analytical procedure in laboratories. Intra-day and interday precession was established for method by evaluating reference standard solutions of MVH concentration 5, 10, 20, 40, 60 and 80 μg ml^{-1}. This procedure was repeated for 4 days under the same conditions. The results are indicated in table 2. These results show that there was no significant difference between assay results in within-day or between-day analysis. Thus this implies that the precision of the assay method was good (R.S.D = 1.34%).

Stability of the Mebeverine Solutions: Next step was to scrutinize the stability of mebeverine solution with time. For this purpose a 20 μg ml^{-1} of mebeverine was prepared and was scanned on the spectrophotometer at 0, 2, 6, 9 and 12 h after its preparation. The data obtained after 12 hours indicates that the absorbance of sample was not significantly changed. Hence it was concluded that mebeverine stays stable after its solution preparation in water for up to 12 hours.

Application of the Method to Pharmaceutical Analysis: Two different brands were studied for the successful application of the new established assay method. Both brands are available in local market. Pharmaceutical formulation was presented as Spasler neo 135 (% recovery 98.6±1.76). Colofac™ 135 (% recovery 97.86±1.09) mg tablet.

CONCLUSIONS

Multi variation calibration technique in line with statistical approach has been found to be an efficient way of reducing errors and inaccuracies associated with instruments when subjected to analysis. Excellent analytical response was obtained using this analytical procedure and the results suggest that this method is a powerful tool with very simple mathematical content, is more reliable than other spectrophotometric methods. This method is highly simple, economical and at the same time accurate and precise, gives an acceptable recovery of the mebeverine in pharmaceutical tablet formulations.

REFERENCES

1. Arayne, M.S., N. Sultana and F.A. Siddiqui, 2005. A new RP-HPLC method for analysis of mebeverine hydrochloride in raw materials and tablets, Pakistan Journal of Pharmaceutical Sciences, 18(2): 11-14.
2. British Pharmacopoeia 2003. Published by The Stationary office London, pp: 1194-95.
3. The Merck Index.. 2001. An Encyclopedia of Chemical, Drugs and Biologicals, 13th Ed., Merck Research Laboratories. Division of Merck & Co Inc. Whitehouse Station, NJ, pp: 1030.
4. Martindale, The extra pharmacopoeia. 1996. Thirty-first Edition. Published by Royal Pharmaceutical society London, pp: 1227.
5. Kraemer, T., R. Wennig and H.H. Maurer, 2001. The antispasmodic drug mebeverine leads to positive amphetamine results by fluorescence polarization immunoassay (FPIA)—studies on the toxicological analysis of urine by FPIA and GC-MS. J. Anal. Toxicol., 25(5): 333-338.
6. Stockis, A., P.J. Guelen and D. deVos, 2002. Identification of mebeverine acid as the main circulating metabolite of mebeverine in man. J. Pharm. Biomed. Anal., 29(1-2): 335-340.
7. Kristinsson, J., A. Snorrad and M. Hannsson, 1994. The metabolism of mebeverine in man: Identification of urinary metabolites by gas chromatography/mass spectrometry. Pharmacol. Toxicol., 74(3): 174-180.
8. Dickinson, R.G., P.V. Baker, M.E. Frankin and W.D. Hooper, 1991. Facile hydrolysis of mebeverine *in vitro* and *in vivo*: negligible circulating concentration of the drug after oral administration. J. Pharmacol. Sci., 80(10): 952-957.
9. Arayne, M.S., N. Sultana and F.A. Siddiqui, 2007. Optimization of levofloxacin analysis by RP-HPLC using multivariate calibration Technique, Pak. J. Pharm. Sci., 20(2): 100-106.
10. ICH guideline Q2B: Validation of Analytical Procedures: Methodology (2003).