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Development and Validation of Analytical Methods for Simultaneous Estimation of Amitriptyline Hydrochloride and Methylcobalamin in their Tablet Dosage Form by UV Spectrophotometric Method

Chetna V. Karchaliya*, Parula B. Patel
Department of Quality Assurance, S. J. Thakkar Pharmacy College, Rajkot, Gujarat, India
sonichetna158@gmail.com

ABSTRACT

The simple, accurate and precise Absorption Correction Method has been developed for the simultaneous estimation of Amitriptyline hydrochloride and Methylcobalamin in combined tablet dosage form. The method utilizes distilled water as solvent and λmax of Amitriptyline hydrochloride and Methylcobalamin selected for analysis were found to be 239 nm and 351 nm respectively. The method was validated as per International Conference on Harmonization (ICH) guidelines. The Linearity range lies between 20-60 µg/ml (R^2 0.9998) for Amitrityline hydrochloride and 3-9 µg/ml (R^2 0.9990) for methylcobalamin. The accuracy and precision were determined and found to comply with ICH guidelines. The method showed good reproducibility and recovery with %RSD in desired range.The proposed method can be applied for routine analysis of both drugs

Keywords: Drug Utilization Pattern, Effectiveness, Oral hypoglycemic Agents, Diabetes Mellitus

INTRODUCTION

Amitriptyline Hydrochloride (ATH) is chemically, 3-(10, 11- Dihydro- 5H- dibenzo [a, d] cycloheptен- 5-ylidene)- N, N- dimethyl-1- propanamine[1]. It is a tricyclic antidepressant used in case of anxiety and also exerts an anticholinergic activity[1]. AMI is official in IP, BP and USP. The IP[1], BP[2] and USP[3] describe HPLC, non-aqueous titration and titrimetric methods, respectively for estimation of AMI.

Methylcobalamin (MCA) is chemically, (1R,2R,4S,7S)-7-{[(2S)-3-hydroxy-2-phenylpropanol]oxy}-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane[4]. It is a form of Vitamin B12 used in the treatment of trigeminal neuralgia, megaloplastic anaemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome. It is official in Japanese Pharmacopoeia.JP[4] describe HPLC method for estimation of MCA.

The Extensive review of Literature revealed that many analytical methods like UV spectrometry[5-8], RP-HPLC[9-10] and HPTLC[11-12] have been reported for estimation of Amitriptyline hydrochloride and Methylcobalamin in individual and combined (with other drugs) dosage form. But no any spectrophotometric or RP-HPLC method was available for simultaneous estimation of Amitriptyline hydrochloride and Methylcobalamin in their combined dosage form.

So, it was thought of interest to develop simple, rapid, accurate and precise spectrophotometric and RP-HPLC methods for simultaneous estimation of Amitriptyline hydrochloride and Methylcobalamin in bulk and in tablet dosage form. The developed methods were validated for its linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to the ICH guidelines (Q2R1).

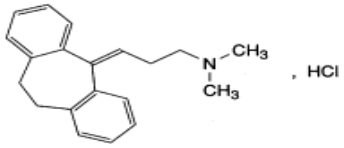

Figure 1: Chemical Structure of Amitriptyline hydrochloride

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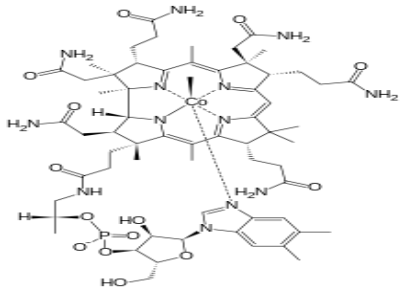

Figure 2: Chemical Structure of Methylcobalamin

MATERIALS AND METHODS

An UV-Visible double beam spectrophotometer (Shimadzu, Model - 1800) having two matched quartz cells with 10 mm light path. All weighing were done on electronic balance (Shimadzu, Model-AUX-220). Distilled water was used as solvent. Amitriptyline hydrochloride and Methylcobalamin reference standard was provided as gift sample by La Pharmaceuticals, Ahemdabad, Gujarat and Athene Chemical Pvt. Ltd., Ahemdabad, Gujarat respectively. The commercial fixed dose combination product (AMNURITE 10) tablet was procured from the local market. Each tablet containing 10 mg ATH and 1500mcg MCA.

Preparation of working standard solution for ATH: Accurately weighed 10 mg of ATH was transferred to 10 ml volumetric flask, dissolved and diluted up to mark with Distilled water to obtain final concentration of 1000 µg/ml ATH. Solution was further diluted with Distilled water to obtained working standard solutions of ATH.

Preparation of working standard solution for MCA: Accurately weighed 10 mg of MCA was transferred to 10 ml volumetric flask, dissolved and diluted up to mark with Distilled water to obtain final concentration of 1000 µg/ml MCA. Solution was further diluted with Distilled water to obtained working standard solutions of MCA.

Selection of suitable wavelengths for analysis: Solutions containing appropriate concentration of ATH (10 µg/ml) and MCA (10 µg/ml) in Distilled water were scanned using UV spectrophotometer in "Spectrum mode" in the range of 400 – 200 nm and their spectra were overlaid. From overlaid spectra of both the drugs analytical wavelengths for detection were selected.

Detection Wavelengths: 239 nm (λ_2) and 351 nm (λ_1).

Preparation of calibration curves: Absorbance of prepared standard solutions having concentration 20, 30, 40, 50 and 60 µg/ml for ATH and 3, 4.5, 6, 7.5 and 9 µg/ml for MCA were measured at 239 nm and 351 nm. Standard calibration curves of absorbance against concentration were plotted. Absorptivity coefficients were determined using calibration curves at both the wavelengths.

RESULT AND DISCUSSION:

Selection of Suitable Wavelength for Analysis:

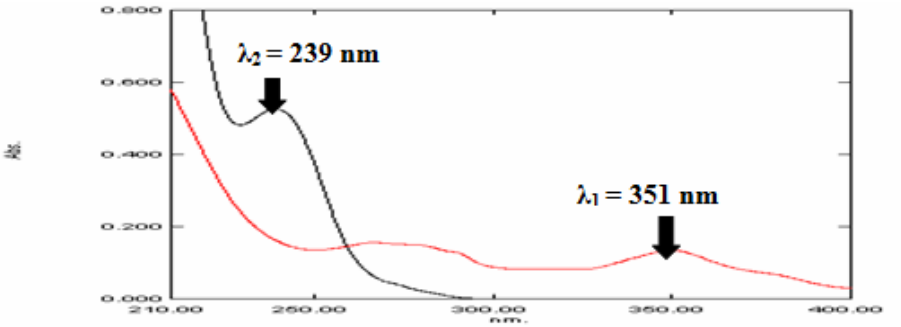

Figure 3: Overlaid spectra of ATH (10 µg/ml) and MCA (10 µg/ml) in Distilled water

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VALIDATION PARAMETERS

Linearity:

Linearity study was carried at different concentration levels and from that according to dose ratio working range was selected to be 20-60 µg/ml and 3-9 µg/ml for ATH and MCA, respectively at both the wavelengths.

Table 1: Result of Calibration Curves of ATH and MCA for Absorption Correction method

Parameters	MCA		ATH
Wavelength	λ_1 = 351 nm	λ_2 = 239 nm	λ_2 = 239 nm
Regression Equation	Y= 0.0277x-0.0478	Y= 0.0325x-0.0611	Y= 0.0271x+0.4130
Correlation Coefficient	0.9990	0.9993	0.9998
Absorptivity	0.0277	0.0325	0.0271
Linearity	3-9 µg/ml		20-60 µg/ml

Precision:

Precision of the method was determined in the terms of Repeatability, Intraday and Interday precision. Repeatability (% RSD) was assessed by analyzing test drug solution within the calibration range, six times on the same day. Intraday variation (% RSD) was determined by analysis of this solution three times on the same day. Interday variation (% RSD) was determined by analysis of this solution on three different day.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

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

Table 2 Result of LOD, LOQ and Precision by Absorption Correction method

Parameters	**MCA**		**ATH**
Wavelength	**λ_1 = 351 nm**	**λ_2 = 239 nm**	**λ_2 = 239 nm**
Precision (%RSD)			
Repeatability (n=6)	0.6739	0.4683	0.1327
Intraday (n=3)	0.5050-1.6806	0.5426-1.3089	0.7477-1.5145
Interday (n=3)	0.8810-1.9021	0.9329-1.2820	0.5722-1.8862
LOD (µg/ml)	0.1596	0.1464	2.4476
LOQ (µg/ml)	0.4837	0.4369	

Accuracy:

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels. % Recovery was calculated from absorbance ratio. % Recovery was found to be between 98.34 – 100.21 for ATH and 98.73 – 102.11 for MCA.

Table 3: Result of accuracy of ATH for absorption correction method

Level of Recovery	Amount of drug taken(mg)	Amount of API added(mg)	Total amount of ATH (mg)	Amount of ATH recovered mg (Mean ± S.D.)*	% Recovery of ATH*
0	40	0	40	-	-
80	40	2	42	42.09 ± 0.0202	100.21
100	40	4	44	43.69 ± 0.5858	99.29
120	40	6	46	45.24 ± 0.2858	98.34

(n=3)

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Table 4: Result of accuracy of MCA for absorption correction method

Level of Recovery	Amount of drug taken(mg)	Amount of API added(mg)	Total amount of MCA (mg)	Amount of MCA Recovered mg (Mean ± S.D.)*	% Recovery of MCA*
0	6	0	6	-	-
80	6	3	9	9.19 ± 0.1858	102.11
100	6	6	12	12.09 ± 0.0665	100.75
120	6	9	15	14.81 ± 0.6658	98.73

(n=3)

Analysis of Pharmaceutical formulation by Content uniformity:

The method was successfully applied to determine the amounts of ATH and MCA present in the pharmaceutical formulation. The results obtained were in good agreement with the corresponding 49 labeled amount.

Table 5: Content uniformity result by Absorption Correction Method:

Sr. No	Labelled claim (µg)		Amount obtained		% Labelled claim		STD Criteria
	ATH	MCA	ATH	MCA	ATH	MCA	
1	40	6	39.67	5.91	99.17	98.50	
2	40	6	39.98	5.93	99.95	99.33	
3	40	6	39.45	6.05	98.62	101.02	
4	40	6	39.05	5.54	97.62	97.33	
5	40	6	39.60	5.82	99.00	97.03	
6	40	6	40.65	5.93	101.50	98.83	85%-115%
7	40	6	40.05	6.02	100.12	100.50	
8	40	6	39.53	5.88	98.82	98.33	
9	40	6	39.83	6.13	99.57	102.16	
10	40	6	39.68	5.90	100.07	98.45	
					97.62%-101.52%	97.03%-102.16%	

CONCLUSION

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The developed method is simple, accurate, precise and economical. Hence the method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Amitriptyline Hydrochloride and Methylcobalamin in their tablet dosage forms.

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