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Original Article

Development and validation of ratio-derivative spectrophotometric method for simultaneous estimation of Gabapentin, Methylcobalamin and alpha lipoic acid in tablet formulation

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ABSTRACT

Aim: The purpose of this study is to develop simple, economic, specific, rapid, reliable, and reproducible method for simultaneous estimation of the Gabapentin, Methylcobalamin and Alpha lipoic acid.

Method: In this method the overlapping spectra of Gabapentin, Methylcobalamin and Alpha lipoic acid were well resolved by making use of the first-derivative of the ratios of their direct absorption spectra. The derivative ratio absorbance of Gabapentin, Methylcobalamin and Alpha lipoic acid were measured at 731.10 nm, 768.53 nm and 242.21 nm for their quantification. The method was validated for accuracy, precision, linearity, robustness and sensitivity.

Result & discussion: Gabapentin, Methylcobalamin and Alpha lipoic acid were shown linearity in the concentration range of 100–500 μg/ml, 0.5–2.5 μg/ml and 100–500 μg/ml respectively. The LOD & LOQ were found to be 3.09 μg/ml and 9.37 μg/ml; 0.03 μg/ml and 0.10 μg/ml; and 4.79 μg/ml and 14.52 μg/ml respectively. The % labelled claim for Gabapentin, Methylcobalamin and Alpha lipoic acid were found to be 98.71, 98.94 and 98.44 respectively.

Conclusion: Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs in combination.

1. Introduction

Gabapentin (GBP), 1-(aminomethyl) cyclo-hexaneacetic acid, is chemically unique cyclohexane derivative of gabba amino butyric acid (GABA) that was synthesized to cross blood brain barrier, and mimic the inhibitory effects of this neurotransmitter on the CNS. Gabapentin is effective as adjunctive therapy for patients with partial and secondarily generalized tonic-clonic seizures.[1,2] It is official in United State Pharmacopoeia 30.[3] Methylcobalamin (MCB), (1R, 2R, 4S, 7S)-7-{[(2S)-3-

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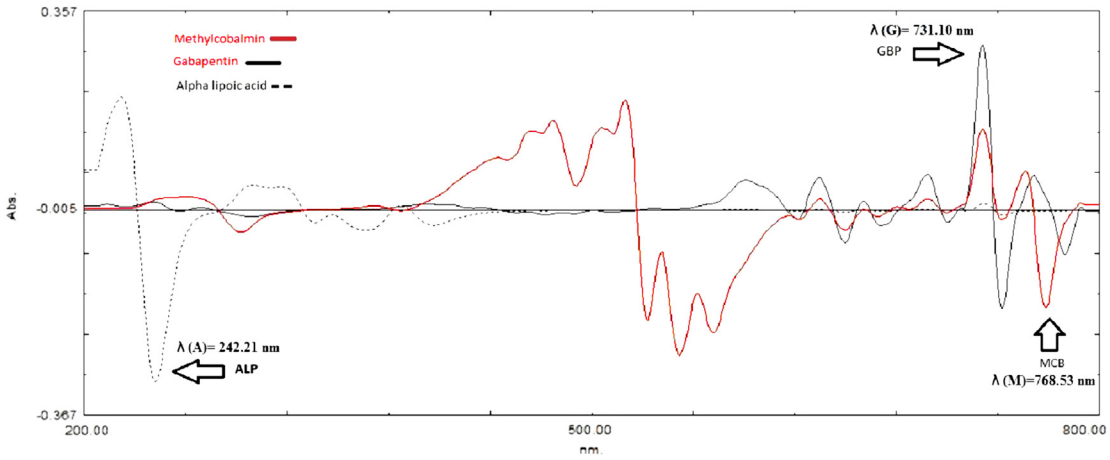

Fig. 1 – Overlaid ratio derivative spectra of Gabapentin, Methylcobalamin and Alpha lipoic acid in methanol.

hydroxy-2-phenylpropanol]oxy}-9,9-dimethyl-3-oxa-9-azonia tricycle [3.3.1.02,4] nonane, is a supplement for vitamin, used in treatment of Vitamin B_{12} deficiency of dietary origin.[1,4] It is official in Japanese pharmacopoeia.[5] Alpha lipoic acid (ALP), (R)-5-(1, 2-dithiolan-3-yl) pentanoic acid, is antioxidant, and used in treatment of diabetes and HIV. It also has been used for cancer, liver ailments, and various other conditions.[1,4] It is official in United State Pharmacopoeia 30.[3] Combination of Gabapentin, Methylcobalamin and Alpha lipoic acid treats both the problems associated with all types of neuropathy i.e., neuralgia (neuronal pain) and neuron degeneration. Gabapentin is proved to be very effective and well tolerated in the treatment of neuropathic pain. Alpha lipoic acid is an universal antioxidant which prevents oxidative damage of neurons. Methylcobalamin increases myelin sheath formation thereby regenerates neuron.

Literature survey reveals many reported methods for the analysis of GBP by ultra-violet (UV),[6,7] high-performance liquid chromatography (HPLC)[8–11] and high-performance thin-layer chromatography (HPTLC).[12] Various methods have been reported for determination of MCB by UV,[13–17] HPLC[5,17,18] and HPTLC.[12] Estimation of ALP by UV,[19,20] HPLC[3,21,22] and GC,[21] either individually or in combination with other drugs are reported. To the best of our knowledge, there is no analytical method reported for simultaneous determination of ternary mixture containing GBP, MCB and ALP. Therefore, an attempt has been made to develop a simple, accurate, rapid and reproducible ratio spectra derivative spectroscopic method for simultaneous determination of GBP, MCB and ALP in tablet dosage form and validate it, in accordance with ICH guidelines.[23]

2. Materials and methods

Pharmaceutical grade of GBP (Zydus Research Center, Ahmedabad, Gujarat, India), ALP (Centurion Laboratories, Vadodara, Gujarat, India) and MCB (Centurion Laboratories, Vadodara, Gujarat, India) were kindly supplied as gift samples, certified to contain >99% (w/w) on dried basis. Commercially available trigabantin 100 (Sun Pharma, Sikkim) tablets claimed to contain 100 mg Gabapentin, 0.5 mg Methylcobalamin and 100 mg Alpha lipoic acid have been utilized in the present work. Methanol of Analytical grade was purchased from Merck Chemicals, India and Rankem Chemicals, India. Sartolon Polyamide, 0.20 μm pore size membrane filter, Sartorius AG. 37070 Goettingen, Germany, and 0.45 μm pore size, 47 mm Ø, Sartolon Polyamide, Sartorious AG, Germany.

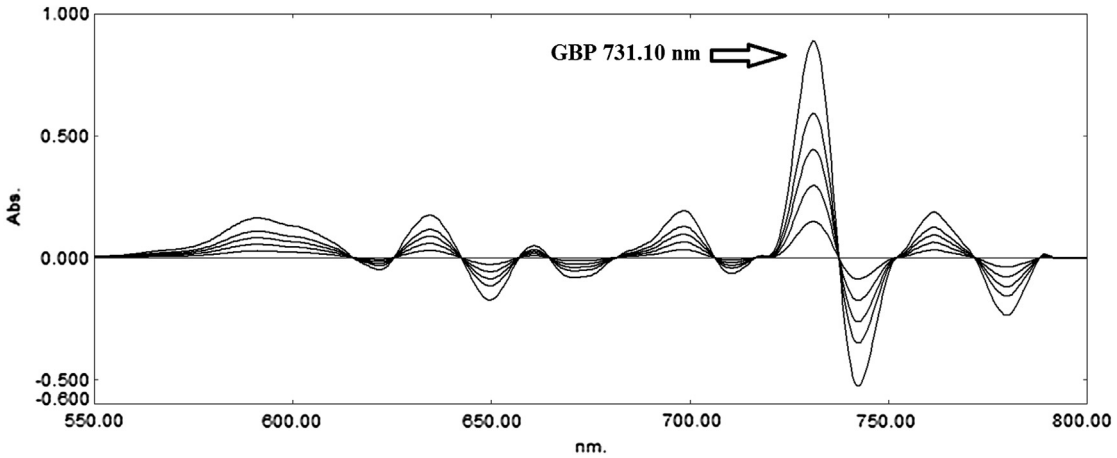

Fig. 2 – Linearity of ratio derivative spectra of Gabapentin (100–500 μg/ml) at 731.10 nm.

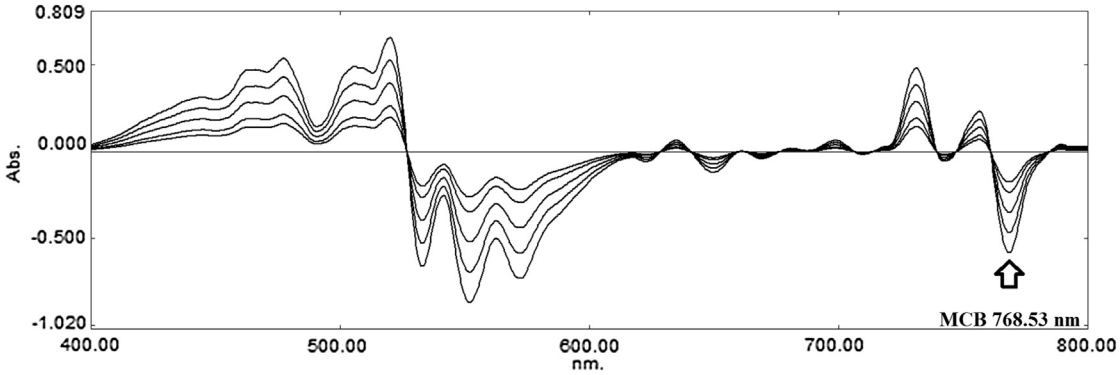

Fig. 3 – Linearity of ratio derivative spectra of Methylcobalamin (0.5–2.5 μg/ml) at 768.53 nm.

3. Selection of suitable wavelengths for analysis

Solutions containing appropriate concentration of GBP, MCB, ALP and mixture of GBP + MCB + ALP in methanol (glassware's protected with aluminium foil & keep all glassware below 25 °C) were scanned using UV–visible spectrophotometer in "Spectrum mode" in the range of 800–200 nm and their spectra were stored in computer. Using UV Probe software spectrum of mixture was divided by spectrum of GBP (100 μg/ml) and MCB (0.5 μg/ml); GBP (100 μg/ml) and ALP (100 μg/ml); MCB (0.5 μg/ml) and ALP (100 μg/ml) to get ratio spectrum of ALP, MCB and GBP respectively. Ratio spectra of the drugs were smoothed ($\Delta\lambda = 10$) and converted to first order derivative spectra ($\Delta\lambda = 10$) using UV Probe software. First order ratio derivative spectra of the drugs were overlaid. From the overlain ratio derivative spectra of GBP, MCB and ALP, 731.10 nm, 768.53 nm and 242.21 nm were selected as suitable analytical wavelengths for analysis of GBP, MCB and ALP respectively (Fig. 1).

4. Preparation of calibration curves

Spectra of prepared standard binary mixture containing 100 + 0.5 + 100, 200 + 1.0 + 200, 300 + 1.5 + 300, 400 + 2.0 + 400, 500 + 2.5 + 500 μg/ml of GBP + MCB + ALP recorded in spectroscopic condition. For ratio spectra of GBP, standard spectra of the drugs mixture were divided by spectra of 0.5 μg/ml MCB and 100 μg/ml ALP. Ratio spectra of GBP were smoothed ($\Delta\lambda = 10$) and converted to first order derivative spectra ($\Delta\lambda = 10$, SF = 10). For ratio spectra of MCB standard spectra of the drugs mixture were divided by spectra of 100 μg/ml GBP and 100 μg/ml ALP. Ratio spectra of MCB were smoothed ($\Delta\lambda = 10$) and converted to first order derivative spectra ($\Delta\lambda = 10$, SF = 10). For ratio spectra of ALP, standard spectra of the drugs mixture were divided by spectra of 0.5 μg/ml MCB and 100 μg/ml GBP. Ratio spectra of ALP were smoothed ($\Delta\lambda = 10$) and converted to first order derivative spectra ($\Delta\lambda = 10$, SF = 1). Amplitudes ($dA/d\lambda$) of obtained ratio derivative spectra of the drugs were measured at selected wavelengths. Standard calibration curves of $dA/d\lambda$ against Concentration were plotted.

5. Validation of method

Validation of developed method was carried out according to ICH Guideline for Validation of Analytical Procedures Q2 (R1) by linearity, limit of detection (LOD) and limit of quantitation (LOQ), accuracy, Precision, robustness and specificity.

5.1. *Robustness*

Solution containing mixture of 300 μg/ml of GBP, 1.5 μg/ml of MCB and 300 μg/ml ALP was prepared and analyzed as per proposed method with small but deliberate change in

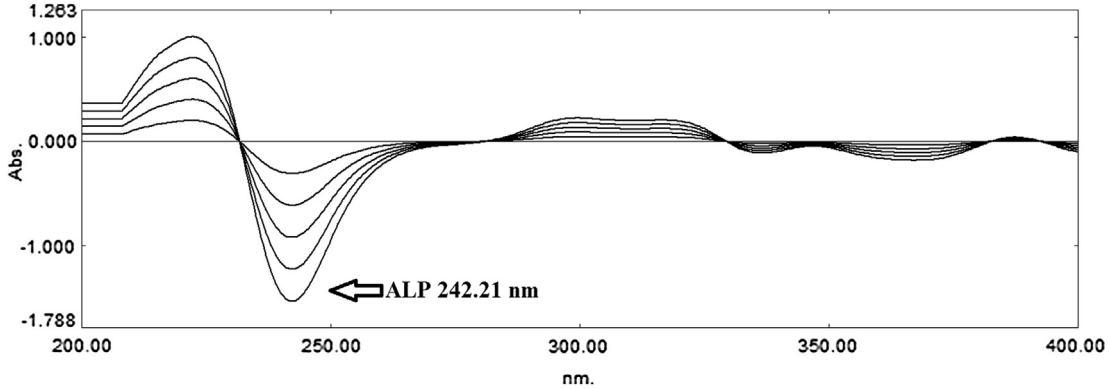

Fig. 4 – Linearity of ratio derivative spectra of Alpha lipoic acid (100–500 μg/ml) at 242.21 nm.

Table 1 – Linearity, range, LOD and LOQ parameter for the simultaneous estimation of GBP, MCB and ALP (N = 6).

Parameter		GBP	MCB	ALP
Range		100–500 μg/ml	0.5–2.5 μg/ml	100–500 μg/ml
Linearity	Equation	y = 0.0015x + 0.1433	y = −0.2306x − 0.1196	y = −0.0031x − 0.0043
	R^2	0.9996	0.9998	0.9999
	% RSD	0.6827 – 1.0350	0.3573 – 1.4616	0.1509 – 1.2399
LOD		3.09	0.03	4.79
LOQ		9.37	0.10	14.52

Table 2 – Results of accuracy studies of GBP, MCB and ALP.

Level		Total concentration (μg/ml)			dA/dλ			Concentration recovered (μg/ml)			% recovery			SD			%RSD		
		GBP	MCB	ALP	GBP	MCB	ALP	GBP	MCB	ALP	GBP	MCB	ALP	GBP	MCB	ALP	GBP	MCB	ALP
L1	1	540	2.7	540	0.957	−0.729	−1.693	542.47	2.64	544.74	100.46	97.88	100.88	0.5567	0.4818	0.3731	0.5571	0.4899	0.3683
	2	540	2.7	540	0.948	−0.732	−1.702	536.47	2.66	547.65	99.35	98.36	101.42						
	3	540	2.7	540	0.953	−0.735	−1.705	539.80	2.67	548.61	99.96	98.84	101.59						
	Mean % recovery										99.92	98.36	101.30						
L2	1	600	3	600	1.043	−0.812	−1.854	599.80	3.00	596.68	99.97	100.09	99.45	0.5592	0.4416	0.7099	0.5590	0.4391	0.7149
	2	600	3	600	1.039	−0.816	−1.863	597.13	3.02	599.58	99.52	100.66	99.93						
	3	600	3	600	1.049	−0.818	−1.837	603.80	3.03	591.19	100.63	100.95	98.53						
	Mean % recovery										100.04	100.57	99.30						
L3	1	660	3.3	660	1.125	−0.895	−2.045	654.47	3.36	658.29	99.16	101.89	99.74	0.2020	0.5256	0.3205	0.2033	0.5185	0.3202
	2	660	3.3	660	1.129	−0.891	−2.053	657.13	3.35	660.87	99.57	101.37	100.13						
	3	660	3.3	660	1.127	−0.887	−2.058	655.80	3.33	662.48	99.36	100.84	100.38						
	Mean % recovery										99.36	101.37	100.08						

Table 3 – Results of results of intra-day, inter-day precision and robustness study for simultaneous determination of GBP, MCB and ALP standards.

Compound	Intra-day precision (n = 6) % RSD	Inter-day precision (n = 6) % RSD	Robustness (n = 6) %RSD		
			Change in scanning speed	Change in methanol manufacturer	Filter variability
GBP	0.6673	0.4854	0.5181	0.7240	0.2845
MCB	1.2385	1.7705	1.4517	1.0498	0.6739
ALP	0.5321	0.3375	1.1022	0.1561	0.4194

spectroscopic condition such as scanning speed, filter variability (0.25 μm and 0.45 μm) and methanol from different manufacturers. The mean amplitude (dA/dλ) with its standard deviation and % relative standard deviation was computed at each level.

5.2. *Specificity*

Specificity of an analytical method was assessed by, defining its ability to measure accurately and specifically the analyte of interest without interferences from blank: Solution containing 300 μg/ml GBP, 1.5 μg/ml MCB, 300 μg/ml ALP, mixture of 300 μg/ml GBP, 1.5 μg/ml MCB and 300 μg/ml ALP were prepared and analyzed as per the proposed method.

5.3. *Stability*

Solution containing mixture of 300 μg/ml of GBP, 1.5 μg/ml of MCB and 300 μg/ml ALP was prepared. Prepared solution is analyzed after 24 h for stability of drugs in 0.1 N HCl, 0.1 N NaOH, light, thermal and hydrogen peroxide.

6. Analysis of pharmaceutical formulation

Twenty tablets were weighed accurately and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to

Table 4 – Results of assay in commercial sample (trigabantin 100).

Drug	% Labelled claim (n = 6)	SD (n = 6)	% RSD
GBP	98.71	1.4657	1.4848
MCB	98.94	1.2594	1.2725
ALP	98.44	0.5827	0.5919

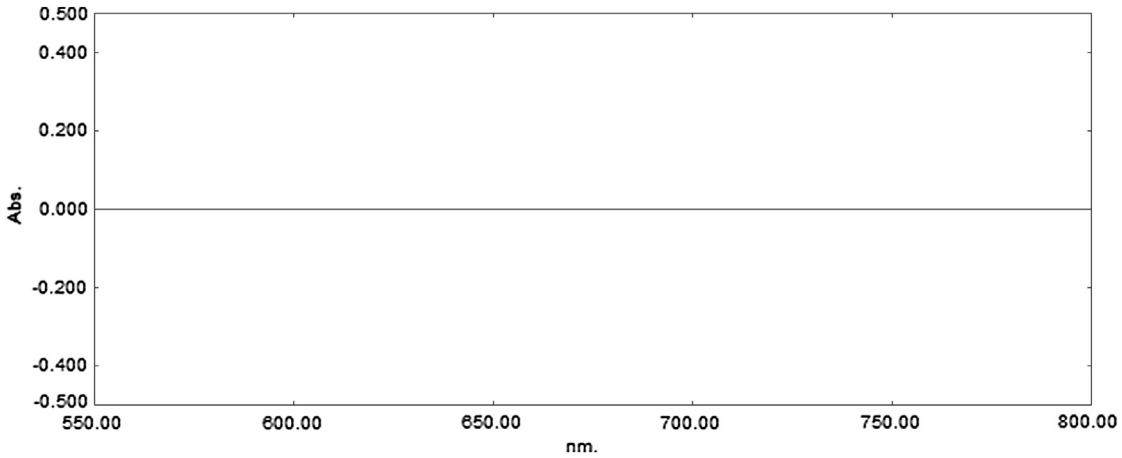

Fig. 5 – Spectrum of blank (placebo).

25 mg of GBP, 0.125 mg MCB and 25 mg of ALP were weighed and transferred to 25 ml volumetric flask. To this flask, 15 ml methanol was added and the flask was sonicated for 5 min. The volume was adjusted up to the mark with methanol. The solution was then filtered through membrane filter paper (0.25 μm). Filtrate contained mixture of 1000 μg/ml GBP, 5 μg/ml MCB and 1000 μg/ml ALP. The filtrate solution was suitably diluted with methanol to get a final concentration of 300 μg/ml of GBP, 1.5 μg/ml of MCB and 300 μg/ml of ALP. For Ratio spectrum of GBP, MCB and ALP, spectrum of the mixture was divided by standard spectrum of MCB (0.5 μg/ml) and ALP (100 μg/ml); GBP (100 μg/ml) and ALP (100 μg/ml); and MCB (0.5 μg/ml) and GBP (100 μg/ml) respectively. Obtained ratio spectra were smoothed ($\Delta\lambda = 10$) and converted to first order derivative spectrum ($\Delta\lambda = 10$, SF = 10 for GBP and MCB; $\Delta\lambda = 10$, SF = 1 for ALP). Amplitude ($dA/d\lambda$) of GBP, MCB and ALP were measured at 731.10 nm, 768.53 nm and 242.21 nm respectively. Concentrations of GBP, MCB and ALP were computed by putting value of their amplitudes in respective standard regression equation obtained from calibration curve. The analysis procedure was repeated six times with tablet formulation.

7. Result & discussion

Excellent linearity was obtained for all the three drugs in the range of 100–500 μg/ml for GBP and ALP; and 0.5–2.5 μg/ml MCB. Linearity of GBP, MCB and ALP were shown in Figs. 2–4 respectively. The correlation coefficients (r^2) were found to be greater than 0.998 ($n = 6$) in all instances. LOD and LOQ were found to be 3.09 μg/ml and 9.37 μg/ml for GBP; 0.03 μg/ml and 0.10 μg/ml for MCB; and 4.79 μg/ml and 14.52 μg/ml for ALP (Table 1). The proposed method afforded high recoveries for GBP, MCB and ALP tablets. Results obtained from recovery studies shown in Table 2 indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in tablets. Precision of the analytical method was found to be reliable based on % RSD (<2%) corresponding to the peak areas. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the three drugs. In all deliberately varied conditions for robustness study, the % RSD of GBP, MCB and ALP were found to be well within the acceptable limit (<1.5%) for robustness study (Table 3). The validated method was used in the analysis of marketed conventional tablet trigabantin 100 with a label claim: 100 mg GBP, 500 μg MCB and 100 mg ALP per tablet. The results for the drugs assay shown in Table 4 indicate a good agreement with the label claims. The spectrum of blank does not show any interference at the detection of GBP, MCB and ALP as it can be seen from the respective spectra (Fig. 5). The results of stability study of drugs shown in Table 5.

Table 5 – Results of stability of drugs in analytical solutions.

Drug	Stability of drugs (after 24 h)				
	0.1 N HCL	0.1 N NaOH	Light	Thermal (80 °C)	Hydrogen peroxide
GBP	Stable	Stable	Stable	Unstable	Unstable
MCB	Stable	Stable	Unstable	Stable	Unstable
ALP	Stable	Stable	Unstable	Unstable	Unstable

8. Conclusion

The developed Ratio spectra derivative spectroscopic method is simple, accurate and precise for the simultaneous determination of GBP, MCB and ALP from tablets. It was successfully validated in terms of linearity, range, accuracy, precision, LOD, LOQ and robustness in accordance with ICH Guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs in combination.

Conflicts of interest

All authors have none to declare.

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