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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PREGABALIN, METHYCOBAMIN, AND ALPHA LIPOIC ACID IN MULTICOMPONENT DOSAGE FORM

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Pregabalin,ZCP, ICH guideline.

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ABSTRACT: Objective: To develop and validate a novel and easy first order derivative spectroscopic method for the simultaneous determination of multicomponent dosage form which contains Methylcobalmine, Alpha lipoic acid and Pregabaline. **Method:** Measurement was achieved by selecting different Zero crossing points i.e ZCP for Methylcobalamine and Alpha lipoic acid at 436.24 nm, ZCP for Alpha lipoic acid and Pregabalin at 338.0 nm and ZCP for Pregabalin and Methylcobalmin at 307.03 nm. The method was validated according to ICH guideline. The proposed method was applied for quantification of all three drugs in the marketed formulations. Results: The method was found linear in the range of 100-140µg/ml for Pregabalin,1-1.4µg/ml for Methylcobalmine and 130-170µg/ml for Alpha lipoic acid respectively. The co-efficient co-relation was found to be 99.5 % for Pregabalin, 99.56 for Methylcobalmine and 99.61 for Alpha lipoic acid. The accuracy and precision were within acceptable limits. **Conclusion:** The method is simple, accurate, precise, rapid and cheap. The proposed method can be used for analysis of multicomponent marketed formulation.

INTRODUCTION: Pregabalin (PRG), (S)-3-(aminomethyl)-5-methylhexanoic acid (**Fig.1**) is an antiepileptic used in the treatment of peripheral neuropathy. Its molecular weight is 159.2 g/mol with empirical formula $C_8H_{17}NO_2$ [1, 2]. Methylcobalamin (MCA), (1R,2R,4S,7S)-7-{[(2S)-3-hydroxy-2-phenylpropanol]oxy}-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.02,4]nonane (**Fig.2)** is vitamin supplement used in inadequacy of Vitamin-B_{12} [3].

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Its molecular weight is 1344.38 g/mol with empirical formula $C_{65}H_{91}CoN_{13}O_{14}P$ [4]. Alpha lipoic acid (ALA), (R)-5-(1,2-dithiolan-3-yl)pentanoic acid (**Fig. 3**) [5]. It is an universal reducing agent which prevents oxidative damage of brain cells [6]. Its molecular weight is 1344.38 g/mol with empirical formula $C_{65}H_{91}CoN_{13}O_{14}P$ [5].

Combination of PRG, MCA and ALA treats the problems related to all types of neuropathy and epilepsy [7]. PRG is manifested to be very efficacious and well permitted in the treatment of neurogenic dysaesthesia. MCA increases myelin sheath formation thereby reforms brain cells [6]. ALA is also used as universal antioxidant which disallows oxidative damage of brain cells [7]. The marketed formulation is NERVUP PG which contains pregabalin 75 mg, methylcobalamin 750

mcg and alpha lipoic acid 100 mg. Literature survey revealed that only few HPLC methods for the analysis of simultaneous estimation of Pregabalin, Methylcobalamin and Alpha lipoic acid have been reported.

In the present work we are focused to accomplish the optimum chromatographic conditions for the simultaneous determination of PRG, MCA and ALA in the combined dosage form. The developed method can be implemented successfully as quality control tool. The method was validated as per ICH guideline to access the replicability and extensive practicability of the developed method [8, 9].

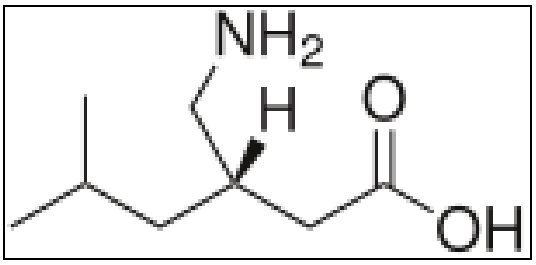

FIG. 1: CHEMICAL STRUCTURE FOR PREGABALIN

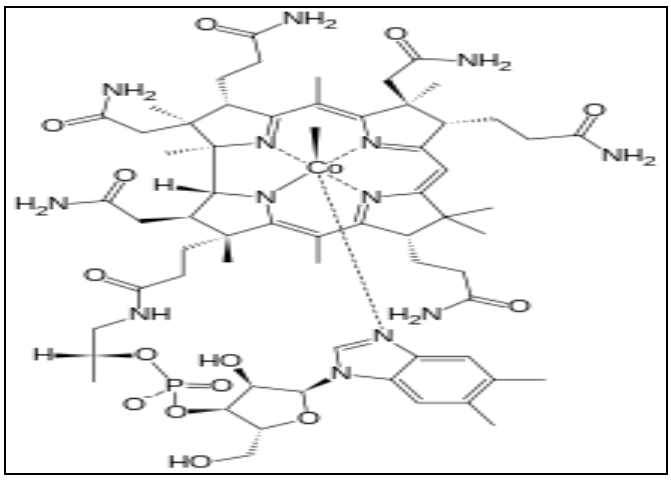

FIG. 2: CHEMICAL STRUCTURE FOR METHYLCOBALAMIN

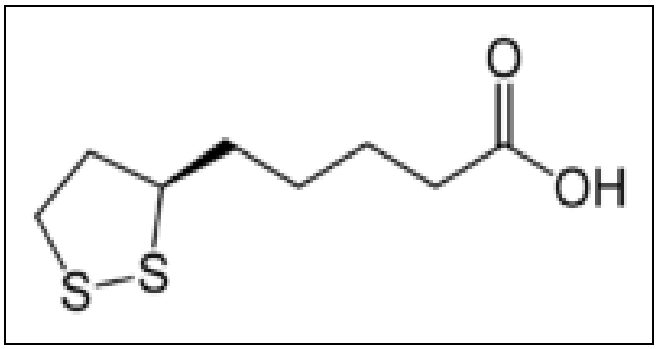

FIG. 3: CHEMICAL STRUCTURE FOR ALPHA LIPOIC ACID

MATERIALS AND METHODS:

Material Procurement:

Pregabalin, Methylcobalamin and Alpha lipoic acid was procured as a gift sample from Alembic Pharmaceuticals, Vadodara, Influx Pharmaceuticals, Gandhinagar, Sunvij drugs ltd., Vadodara. NERVUP PG was purchased from local pharmacy. Analytical Grade solvents were procured from Merck, Mumbai.

Instruments and Equipment:

UV Spectrophotometer:

- Make and Model: Shimadzu UV 1800
- Type: Double Beam Spectrophotometer
- Scanning Speed: Fast, Medium, Slow
- Cuvettes: Matched Quartz cuvettes pair with 1 cm path length

Electronic Weighing Balance:

- Make and Model: Shimadzu, Japan; AUX220
- Capacity: MAX.: 220 gm; Min: 10 mg
- Readability (Deviation): 0.1 mg

First Order Derivative Spectrophotometric Method:

Selection of Suitable Wavelengths for Analysis:

Solutions of PRG (10 µg/ml), MCA (10 µg/ml) and ALA (10 µg/ml) were derivatized using 200 µg/ml Bromocresol green reagent in water. Solutions containing appropriate concentration of PRG, MCA and ALA in water were scanned using UV spectrophotometer in "Spectrum mode" in the range of 800 - 200 nm and their spectra were stored in computer.

Spectra were converted to first order derivative spectra ($\Delta\lambda = 10$ and scaling factor = 15) using UV Probe software (Ver.2.33). First order derivative spectra of drugs were overlaid. From overlaid spectra ZCP of both the drugs were selected as analytical wavelengths for detection.

Preparation of Standard Solutions:

Preparation of Derivatizing agent (BCG solution): 200µg/ml of bromocresol green was prepared by accurately weighing 200 mg and dissolving in 100water.

Preparation of PRG standard stock solution: Accurately weighed 10 mg of PRG was transferred to 10 ml volumetric flask, diluted up to the mark with water to give a stock solution having strength of 1000 µg/ml.

Preparation of MCA standard stock solution: Accurately weighed 10 mg of MCA was transferred to 10 ml volumetric flask, diluted up to mark with water to obtain final concentration of 1000 µg/ml MCA.

Preparation of ALA standard stock solution: Accurately weighed 10 mg of ALA was transferred to 10 ml volumetric flask, diluted up to the mark with water to give a stock solution having strength of 1000 µg/ml.

Preparation of Calibration Curves:

Spectra of prepared tertiary mixture containing concentration 100 + 1 + 120, 110 + 1.1 + 130, 120 + 1.2 + 140, 130 +1.3 + 150 and 140 + 1.4 + 160 µg/ml PEG + MCA + ALA were recorded in the range of 200 to 800 nm using BCG solution prepared in water as blank. Spectra were converted to first order derivative spectra (Δλ = 10 and scalling factor = 15) using UV Probe software (Ver.2.33). Amplitude (dA/dλ) of both the drugs was measured at selected wavelengths. PRG was measured at 436.24 nm (ZCP of ALA and MCA), ALA was measured at 307.03 nm (ZCP of PRG and MCA) and MCA was measured at 338 nm (ZCP of PRG and ALA). Standard calibration curves of dA/dλ against concentration were plotted.

TABLE 1: RESULT OF CALIBRATION CURVE OF PRG, MCA AND ALA FOR FIRST ORDER DERIVATIVE METHOD

Parameter	PRG	MCA	ALA
Regression equation	Y = -0.0006x + 0.0216	Y = -0.0069x – 0.0087	Y = 0.0010x –0.1176
Correlation coefficient (R^2)	0.9950	0.9956	0.9961

Preparation of Sample Solution:

Test solution of 7500 µg/ml of PRG, 750 µg/ml of MCA, 1000 µg/ml of ALA were taken for analysis of PRG, MCA and ALA.

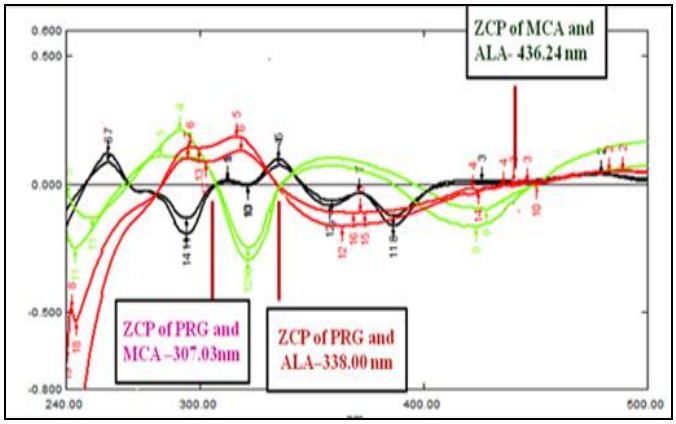

FIG.3: OVERLAID FIRST ORDER DERIVATIVE SPECTRA OF PRG, MCA AND ALA AFTER DERIVATIZATION WITH BCG SOLUTION.

RESULTS AND DISCUSSION:

Validation of Proposed Method:

Validation of developed method was carried out according to ICH guideline for validation of Analytical Procedure Q2(R1):

Linearity and Range:

tertiary mixture solutions having concentration 100, 110, 120, 130 and 140 µg/ml for PRG, 10, 11, 12, 13 and 14 µg/ml for MCA and 120, 130,140,150 and 160 µg/ml for ALA were prepared from stock solution as described in 5.2.1.4 section . Prepared solutions were analyzed as per the proposed method. Six replicate analysis were carried out. The mean amplitude (dA/dλ) with its standard deviation and % relative standard deviation were calculated for the drugs. Mean dA/dλ against concentration were plotted to obtain the calibration curves. Regression equations, co-relation coefficients were computed form calibration curves.

TABLE 2: RESULT OF LINEARITY, RANGE BY FIRST ORDER DERIVATIVE METHOD

Parameter		PRG	MCA	ALA
Range		100-140 µg/.ml	1-1.4 µg/.ml	130-170 µg/.ml
Linearity (n=6)	Equation	Y = -0.0006x + 0.0216	Y = -0.0069x – 0.0087	Y = 0.0010x + 0.1176
	R^2	0.9950	0.9956	0.9961

Limit of detection (LOD) and limit of quantitation (LOQ):

LOD and LOQ were calculated from the data obtained from the linearity studies. For each of the six replicate determinations, slope and y-intercept of the plot was determined. Average of slope (s) and standard deviation of the y intercept (σ) were computed. From these values, the parameter LOD and LOQ were determined using following equations (On the basis of response and slope of the regression equation):

$$LOD = (3.3 \times SD) / Slope$$

$$LOQ = (10 \times SD) / Slope$$

Where; σ = Standard deviation of response

S = Slope of calibration curve

TABLE 3: RESULT OF LOD, LOQ BY FIRST ORDER DERIVATIVE METHOD

Parameter	PRG	MCA	ALA
LOD	5.0915 µg/ml	0.01893 µg/ml	5.4640 µg/ml
LOQ	15.4290 µg/ml	0.05737 µg/ml	16.5576 µg/ml

Accuracy:

Accuracy was calculated by addition of standard drug to preanalyzed sample at 3 different concentration level and computing percentage recoveries. Accurately weighed content equivalent to 750 mg PRG, 0.75 mg MCA and 100 mg ALA were taken according to amount given in **Table 5**. To that amount of standard added according to **Table 5**, 0.15 ml was taken from above solution to have 80%, 100% and 120% level and AUC were determined. Percentage recovery were found and it was obtained between 98 – 102 %. Accuracy was assessed using 9 determinations over 3 concentration level covering the specific range (e.g., 3 concentration and 3 replicates each of the total analytical procedure).

Sample solutions for accuracy were prepared as follows:

TABLE 4: ACCURACY STUDY OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD

Level	PRG		MCA		ALA		PRG	MCA	ALA
	Amount of sample taken in (mg)	Amount of standard added	Amount of sample taken in (mg)	Amount of standard added	Amount of sample taken in (mg)	Amount of standard added			
80%	45	15	0.45	0.15	60	20	60	0.54	80
100%	45	30	0.45	0.30	60	40	75	0.68	100
120%	45	45	0.45	0.45	60	60	90	0.82	120
Standard solution of PRG (1000 µg/ml): Weigh accurately 10mg of PRG transferred in to 10ml volumetric flask, made up to mark with methanol. Standard solution of PCM (1000 µg/ml): Weigh accurately 10mg of MCA transferred in to 10ml volumetric flask, made up to mark with methanol. Standard solution of ALA (1000 µg/ml): Weigh accurately 10mg of ALA transferred in to 10ml volumetric flask, made up to mark with methanol.									

Precision:

Precision of method was computed by two means: Repeatability and Intermediate precision.

Repeatability: System Precision and Method Precision:

System Precision:

Solution containing mixture of 120 µg/ml of PRG, 1.2 µg/ml of MCA and 140 µg/ml of ALA (100% Test concentration) were prepared from their respective stock solution. Prepared solution were analysed six times as per the proposed method. The mean amplitude (dA/dλ) with its standard deviation were computed for both the drugs.

Method Precision:

Six replicate solution containing mixture of 120µg/ml PRG, 1.2 µg/ml MCA and 140 µg/ml ALA were prepared from their respective stock solution. Prepared solution were analysed as per the proposed method. The mean % labelled claim with its standard deviation and % relative standard deviation were computed for both the drugs.

TABLE 5: RESULT OF REPEATABILITY BY FIRST ORDER DERIVATIVE METHOD

Sr. no.	Concentration (µg/ml)			dA/dλ		
	PRG	MCA	ALA	PRG	MCA	ALA
1	120	1.2	150	-0.0521	-0.0914	0.0254
2	120	1.2	150	-0.0529	-0.0936	0.0251
3	120	1.2	150	-0.0514	-0.0926	0.0250
4	120	1.2	150	-0.0526	-0.0921	0.0252
5	120	1.2	150	-0.0531	-0.0918	0.0259
6	120	1.2	150	-0.0517	-0.0949	0.0253

Drug	dA/dλ mean (n= 6)	SD (n= 6)	%RSD
PRG	-0.0521	0.0008	1.6240
MCA	-0.0927	0.0013	1.4071
ALA	0.0259	0.0003	1.2590

- **Intermediate Precision:** Intraday and interday precision was determined in terms of % RSD. Intraday precision was determined by analysing PRG, MCA and ALA in combined solution at three independent concentration range of their respective calibration range for three days.

- **Procedure for Intraday Precision:** Combined solution containing the mixture of PRG, MCA and ALA as 120, 1.2 and 140 µg/ml (100% Test concentration) were prepared as described in section 5.2.1.5 were analysed on 3 times on the same day and %RSD were calculated.

TABLE 6: RESULT OF INTRADAY PRECISION FOR FIRST ORDER DERIVATIVE METHOD

PRG			MCA			ALA		
Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD	Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD	Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD
100	-0.0378 ± 0.0006	1.7388	1	-0.0775 ± 0.0012	1.6473	130	0.0071 ± 0.0001	1.7129
120	-0.0513 ± 0.0006	1.2968	1.2	-0.0925 ± 0.0011	1.1904	150	0.0253 ± 0.0003	1.2594
140	-0.0560 ± 0.0007	1.3410	1.4	-0.1048 ± 0.0014	1.3393	170	0.0205 ± 0.0002	1.4137
Average % RSD		1.3138	Average % RSD		1.3923	Average % RSD		1.4620

Preparation for Interday Precision: Combined solution containing the mixture of PRG, MCA and ALA 120, 1.2 and 140 µg/ml (100% Test concentration) were prepared as described in section 5.2.1.5 were analysed 3 times on 3 different days and %RSD were calculated.

TABLE 7: RESULT OF INTER DAY PRECISION FOR FIRST ORDER DERIVATIVE METHOD

PRG			MCA			ALA		
Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD	Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD	Conc. (µg/ml)	Mean dA/dλ ± S.D (n= 3)	% RSD
100	-0.0378 ± 0.0006	1.7388	10	-0.0771 ± 0.0013	1.7953	120	0.0071± 0.0001	1.4478
120	-0.0523 ± 0.0006	1.2961	12	-0.0927± 0.0013	1.4077	150	0.0253 ±0.0003	1.3965
140	-0.0616 ± 0.0011	1.8856	14	-0.1051 ± 0.0017	1.6447	160	0.0443 ± 0.0006	1.4733
Average % RSD		1.6401	Average % RSD		1.6159	Average % RSD		1.4992

Robustness:

Solution containing mixture of 120 µg/ml PRG, 1.2 µg/ml MCA and 140 µg/ml ALA was prepared from their respective sample solution prepared as per described in section 5.2.1.5. Prepared solution was analyzed as per proposed method with small but

deliberate change in spectroscopic conditions as listed below:

i. Scanning speed: Fast, Medium and Slow

ii. Methanol from different manufacturers.
Methanol GR Grade: Merck ltd., India,
Qualigens Fine Chemicals Pvt. Ltd., India

The mean % labeled claim with its standard deviation and % relative standard deviation was computed at each level.

TABLE 8: RESULT OF ROBUSTNESS FOR FIRST ORDER ORDER DERIVATIVE METHOD

Robustness	PRG % RSD	MCA % RSD	ALA % RSD
Change in Scanning speed	1.5297	1.4156	1.5109
Change in BCG brand	1.4560	1.3867	1.4289

Analysis of Pharmaceutical Dosage Form:

Twenty capsules were weighed accurately and their average weight was determined. The capsule content equivalent to 75 mg PRG, 0.75 mg MCA and 100 mg of ALA were weighed and transferred to 10 ml volumetric flask. To this flask, 5 ml of water was added and the flask was sonicated for 5 min. The volume was adjusted up to the mark with water. The solution was then filtered through Whatman filter paper no. 41µm. Filtrate contained mixture of 120µg/ml PRG, 1.2µg/ml MCA and 140µg/ml ALA. An aliquots of 0.12 ml of the filtrate solution was suitably diluted to 10 ml with water to get a final concentration of 120µg/ml of PRG, 1.2µg/ml of MCA and 130 µg/ml of ALA. Spectrum of prepared solution was recorded in spectroscopic condition.

Spectrum was converted to first order derivative spectrum (Δλ = 10, scaling factor = 15) using UV Probe software (Ver.2.33). Amplitudes (dA/dλ) of PRG, MCA and ALA were measured at 252.58 nm and 369.84 nm respectively. Concentrations of PRG, MCA and ALA 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 dosage form.

TABLE 9: ANALYSIS OF PHARMACEUTICAL FORMULATION

Sr. no.	Labelled Claim (mg)			Amount obtained (mg)			% Labelled claim		
	PRG	MCA	ALA	PRG	MCA	ALA	PRG	MCA	ALA
1	75	0.75	100	73.7475	0.7630	100.6213	98.3321	101.7412	100.6213
2	75	0.75	100	75.6225	0.7414	99.1004	100.8313	98.8578	99.1004
3	75	0.75	100	75.2100	0.7199	97.2104	99.7212	100.2856	97.2104
4	75	0.75	100	73.0575	0.7375	101.2351	98.3387	97.4131	101.2351
5	75	0.75	100	74.0400	0.7413	99.8703	99.7223	98.8526	99.8703
6	75	0.75	100	75.6225	0.7511	98.2301	100.8354	100.2814	98.2301

Drug	Label claim	% Labelled claim	SD (n=6)	% RSD
PRG	75 mg	99.6266	1.0833	1.4531
MCA	0.75 mg	99.5983	1.9357	1.9357
ALA	100 mg	100.2083	1.5053	1.5147

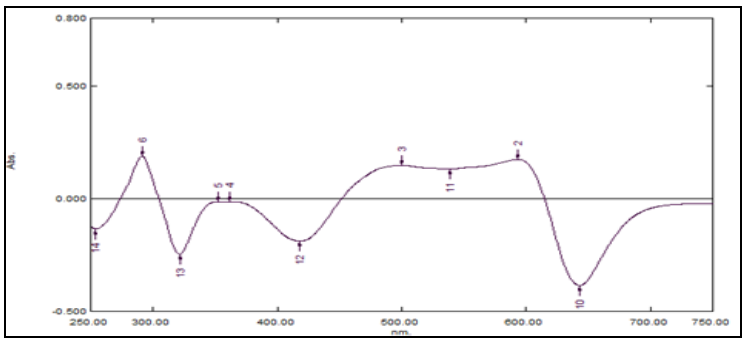

FIG.4: FIRST ORDER SPECTRUM OF CAPSULE FORMULATION BY FIRST ORDER DERIVATIVE METHOD

CONCLUSION: The developed method was established to be precise accurate, linear, robust and specific for determination of PRG, MCA and ALA. The method was developed and validated as per ICH guideline and all parameters tested were found to be within limits.

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REFERENCES:

1. Indian Pharmacopoeia. Volume II, The Indian Pharmacopoeia Commission, Ghaziabad, 2010:1960 – 1961.
2. Ahuja S. and Scypinski S: Handbook of Modern Pharmaceutical Analysis. Edn 1, Vol III - Separation Science and Technology, Academic Press, New York, 2001:1-22.
3. Sweetman SC. Martindale: The Complete Drug Reference. Edn 36, Pharmaceutical Press, London, 2009:1818-1981.
4. The Merck Index. Edition 14, Merck Research Laboratories Division of Merck and Co., Inc., USA, 2006:6045.
5. The United State Pharmacopeia 30. United States Pharmacopoeial Convention, INC., 2009:956-2200.
6. Rang HP. Dale NM. Ritter JM. and Flower RT. Rang and Dale's Pharmacology 6, Elsevier Limited, 2007:352.
7. "Brand name of combination of Pregabalin, Methylcobalamin and alpha lipoic acid", November 3013, http://www.drugsupdate.com/brand/showavailablebrands/421/2
8. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guidelines, 1994, ICH Harmonised Guideline.
9. Code Q2B-Validation of Analytical Procedure Methodology Step-4 Consensus Guidelines, 1994, ICH Harmonised Guideline.
10. Jagani, NM, Prajapati VD, Shah JS and Patel PB: Development and validation of reverse phase high performance liquid chromatography method for simultaneous estimation of cinitapride and omeprazole in combined capsule dosage form. International journal of pharmaceutical sciences review & research 2012;1:67-71
11. Olajire A: Chemical derivatization methodologies for UV-Visible Spectrophotometric determination of Pharmaceuticals. International Journal of. Pharmaceutical Science Review & Research 2012**;** 14:6-24.
12. Sowjanya K, Thejaswini JC, Gurupadayya BM, and Indupriya M: Spectrophotometric determination of Pregabalin using Gibb's and MBTH reagent in Pharmaceutical dosage form.Der Pharma Chemica 2011; 3:112-122.
13. Oommen MK, Eapen SC, Velayudhankutty S and Haribabu Y: UV – Vis spectrophotometric method for estimation of Pregabalin and Methylcobalamin in bulk and capsule dosage form. International Journal of Pharmaceutical Research and Life Sciences 2013; 1:115-124.
14. Shep SG and Lahoti SR: Development and validation of UV spectrophotometric method of pregabalin in bulk and pharmaceutical formulations. International Journal of Chem Tech and Reearch 2013; 5:1264-1270.
15. Kavitha MP and Rajasekhar A: A validated HPLC method for the analysis of pregabalin and methylcobalamin in bulk and pharmaceutical formulation. International Journal of Comprehensive pharmacy 2013; 7:1-4.
16. Shah GR, Ghosh C and Thaker BT: Determination of pregabalin in human plasma by electrospray ionization tandem mass spectroscopy. Journal of Advance Pharma Tech Research 2010; 1:354-357.
17. Shah DA, Patelia EM, and Mori A: Simultaneous estimation of pregabalin and methylcobalamin in pharmaceutical formulation by HPTLC densitometry Method. Journal of Chromatography and separation technique 2013; 4:145-152.

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