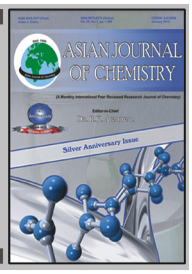
Asian Journal of Chemistry; Vol. 25, No. 1 (2013), 581-582

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13192



NOTE

HPLC Method Development for Estimation of Citicoline and Methylcobalamine in Tablet

SURAJ SAHOO[1,*], PRASANNA KUMAR PANDA[2], SAGAR KUMAR MISHRA[2] and SABUJ SAHOO[2]

[1]School of Pharmaceutical Education & Research, Berhampur University, Berhampur-760 007, India
[2]University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar-751 004, India

*Corresponding author: Email: sahoosuraj@yahoo.co.in

(*Received*: 7 December 2011; *Accepted*: 7 August 2012) AJC-11933

This work describes simple, accurate and precise, RP-HPLC procedure for the estimation of citicoline and methylcobalamine in tablet dosage forms. An isocratic separation was achieved using a Inertsil ODS,C18, 250 × 4.6 mm, 5 µ column with a flow rate of 1 mL/min using UV detection at 270 nm and 550 nm for citicoline and methylcobalamine respectively. The mobile phase for the separation consisted of acetonitrile:buffer (0.5 M KH_2PO_4 + 2.0 mL triethylamine) 25:75 v/v ratio. The retention times of citicoline and methylcobalamine were 2.25 and 6.71 min, respectively. The method was statistically validated for linearity, accuracy and precision. The linearity of citicoline and methylcobalamine shows a correlation coefficient of 0.999 and 0.999. The method was reproducible with intra and inter-day variations. The simplicity and accuracy of the proposed method ensures its use in routine quality control analysis of pharmaceutical formulations. The method established is robust, resisting small deliberate changes in flow rate and the ratio of the organic components in the mobile phase which is useful for the routine determination of citicoline and methylcobalamine in its pharmaceutical dosage form.

Key Words: Citicoline, Methylcobalamine, HPLC, Validation.

Citicoline[1] is 5'-O-[hydroxyl({hydroxyl[2-(trimethylammonio)ethoxy]phosphoryl}oxy)phosphoryl]cytidine and is a polarized molecule with a molecular weight of 488.33. It is a white crystalline, highly hygroscopic powder and is soluble in water.

Methylcobalamine[2] is Co α-[α-(5,6-dimethylbenz-1*H*-imidazolyl)]-Co β-methylcobamide and occurs as dark red crystals or crystalline powder with a molecular weight of 1344.38. It is sparingly soluble in water, slightly soluble in ethanol. It is affected by light. Both the drugs are manufactured in combined pharmaceutical formulation, profound search from data and literature available, it reveals that several methods have been reported including LC, ultraviolet spectrophotometry, high performance liquid chromatography for the analysis of citicoline and methylcobalamine either alone or in combinations with others[3-6].

Comparatively few reports have appeared dealing with the estimation of citicoline and methylcobalamine by HPLC method in combination so far. Taking simplicity, cost-effectiveness, in the sector of chromatographic techniques for pharmaceutical analysis into account, HPLC method was developed. Therefore, this paper proposes a RP-HPLC procedure for the assay of citicoline and methylcobalamine in combination tablet dosage form. The present RP-HPLC method was validated following the ICH guidelines[7].

Citicoline and methylcobalamine were obtained as a memento sample from Cellogen Pharma Ltd., Navi Mumbai. Acetonitrile and water used were of HPLC grade. In this study all other reagents used were of AR grade.The analysis was carried out on HPLC (make Shimadzu) configured with dual mode pump (model 10 AT VP) and detector (model SPD-10AVP), to monitor and integrate output signal.

Chromatographic conditions: Column: Intersil 250 mm × 4.6 mm; Particle size: packing 5 µ; Stationary phase: C18, ODS; Column temperature: 25 ºC; Mobile phase: Buffer (0.5 M KH_2PO_4 + 2.0 mL triethylamine):acetonitrile (75:25); Flow rate: 1.0 mL/min; Detection: 270 and 550 nm for citicoline and methylcobalamine, respectively; Retention time: About 2.25 and 6.71 min for citicoline and methylcobalamine respectively; Injection volume: 10 µL and100 µL for citicoline and methylcobalamine, respectively.

Preparation of mobile phase: To optimize the HPLC parameters several mobile phase compositions were tried. Satisfactory peak symmetry (Tailing factor and theoretical plates Table-1) were obtained with mobile phase consisting of buffer:acetonitrile in ratio of 75:25. The mobile phase was filtered through 0.45 µ filter and degassed by ultrasonication for 20 min.

TABLE-1
SYSTEM SUITABILITY TEST PARAMETERS FOR THE PROPOSED METHOD

Parameters	Citicoline	Methylcobalamin
Retention time (min)	2.25	6.71
Theoretical plates	2554	2010
Tailing factor	0.75	1.08

Assay standard preparation

Citicoline standard solution: An accurately weighed quantity of 25 mg equivalent to citicoline was transferred in to a 25 mL volumetric flask, dissolved in a sufficient quantity of HPLC water. The volume was made up to the mark with water to get a final concentration of 1000 µg/mL.

Methylcobalamine standard solution: An accurately weighed quantity of 25 mg methylcobalamine was transferred in to a 100 mL amber coloured volumetric flask, at heat and light protective place and dissolved in a sufficient quantity of HPLC water. The volume was made up to the mark with water. 1 mL of this solution was diluted with water in a 50 mL amber coloured volumetric flask up to mark to get a final concentration 5 µg/mL.

Assay sample preparation: To get equivalent concentration with respect to standard preparation 20 tablets each containing 500 mg citicoline and 0.5 mg of methylcobalamine were accurately weighed and average weight was calculated. Crushed fine powder equivalent to 500 mg citicoline and 0.5 mg of methylcobalamine were weighed accurately and transferred in to 100 mL Amber coloured volumetric flask, at heat and light protective place and dissolved and made up to the volume with water. From the above sample preparation assay was determined for methylcobalamine and 1 mL of this sample solution were diluted to 5 mL with water for citicoline determination.

Procedure for analysis: Chromatographic conditions set for the analysis was optimized to get a study base line. After the stabilization of base line separate injections with equal volume of placebo, standard and sample preparation were injected in to the HPLC system and chromatographed. Similarly duplicate injections of sample were chromatographed and the average peak area of the drugs was computed from the chromatograms and the amount of the drugs present in the tablet dosage form was calculated. Assay for citicoline and methylcobalamine are 99.8 and 100 % respectively.

Method validation: The developed analytical method was validated with respect to parameters which are specificity, linearity, precision, accuracy, robustness and ruggedness and are executed as per the ICH guidelines. The results obtained are narrated in tables ahead (Tables 2-5).

TABLE-2
REGRESSION ANALYSIS OF THE CALIBRATION CURVE FOR THE PROPOSED METHOD

Parameters	Citicoline	Methylcobalamine
Linearity range (µg/mL)	500-1500	2.5-7.5
Slope	530.8	8365.8
Intercept	1.6	2863.6
Correlation coefficient	0.999	0.999

TABLE-3
RESULTS FOR PRECISION WITH RUGGEDNESS

Drug	Intra-day assay (Analyst 1)		Inter-day assay (Analyst 2)	
	Obtained (%)	RSD (%)	Obtained (%)	RSD (%)
Citicoline	99.8	0.14	100.2	1.06
Methylcobalamine	100.0	0.64	99.2	1.07

TABLE-4
RESULTS FOR ACCURACY

Levels	Citicoline		Methylcobalamine	
	Recovered (%)	RSD (%)	Recovered (%)	RSD (%)
50 %	99.5	0.5	99.4	0.6
100 %	99.6	0.7	100.0	1.6
150 %	99.9	0.7	100.0	1.4

TABLE-5
RESULTS FOR ROBUSTNESS

Factor	Change	Retention time		RSD (%)	
		CIT	MET	CIT	MET
Flow rate (mL/min)	-0.2	2.5	6.6	0.8	0.2
	+0.2	2.1	6.4	0.3	0.5
Acetonitrile (%)	-5.0	2.3	6.5	1.5	0.3
	+5.0	2.2	6.7	0.6	0.1

CIT = Citicoline; MET = Methylcobalamine.

Placebo injection was chromatographed at 270 and 550 nm which apparently depict non interference of excipients at the retention times of citicoline and methylcobalamine respectively.

Results obtained with respect to individual parameter are within the acceptance criteria and as stated earlier are validated as per ICH guidelines.

Conclusion

Data indicates that the procedure for assay of tablet dosage form is validated which is simple as well. Therefore it can be used for the routine quality control analysis for the determination of the citicoline and methylcobalamine in their tablet dosage forms.

REFERENCES

1. J.J. Secades and G. Frontera, *Methods Find Exp. Clin. Pharmacol.*, **17**, 1 (1995).
2. A.H. Beckette and J.B. Stenlake, Practical Pharmaceutical Chemistry, CBS Publishers, New Delhi, India, Vol. 2, edn. 4, p. 275 (1997).
3. B.R. Ganduri, R.J. Peddareddigari, R.N. Dasari and K.R. Saiempu, *Int. J. Pharm. Tech. Res.*, **2**, 427 (2010).
4. S.Q. Gu, *Chin. J. Pharmceut.*, **33**, 397 (2002).
5. R.V. Galande, K.G. Baheti and M.H. Dehghan, *Int. J. Chem. Tech. Res.*, **2**, 695 (2010).
6. J. Saravanan, A. Shajan, N.H. Joshi, R. Varatharajan and K.Valliappan, *Int. J. Chem. Pharm. Sci.*, **1**, 13 (2010).
7. ICH Topic Q 2 (R1), ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures, pp. 1-6 (1995).