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Development and validation of spectroscopic methods for the estimation of Dalfampridine in bulk and in tablet formulation

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ABSTRACT

Four simple, precise and cost effective spectrophotometric methods have been developed for the estimation of Dalfampridine in bulk and its tablet formulation. Dalfampridine was estimated at 262 nm in UV-spectroscopy (Method A), 274.5 nm in first order derivative spectroscopy (Method B), scanned at 254.2 - 269.0 nm in area under curve for zero order derivative spectroscopy (Method C) and at 267.2 - 284.2 nm in area under curve for first order derivative spectroscopy (Method D). The drug follows Beer-Lambert's law in the concentration range of 2.0 -7.0 µg/ml in all the methods. All the methods were validated by following the analytical performance parameters suggested by International Conference on Harmonization (ICH) guidelines. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of dalfampridine in bulk and in tablet formulation.

KEYWORDS

Dalfampridine;
UV spectroscopy;
Derivative spectroscopy;
AUC;
Validation.

INTRODUCTION

Dalfampridine (DFP, Figure 1), is an oral potassium channel blocker recently approved by FDA (Food and Drug Administration) for symptomatic treatment of multiple sclerosis. It acts at the central and peripheral nervous systems, enhances conduction in demyelinated axons, and improves walking ability. Chemically it is 4-aminopyridine, *p*-aminopyridine or Fampridine[1]. Literature survey reveals the clinical overview of Dalfampridine[2], pharmacokinetic analysis of extended release tablets[3], development for symptomatic improvement and management in patients with multiple sclerosis[4,5], phase 3 trial of extended release oral drug[6] and its efficacy as a treatment to improve walking in patients with multiple sclerosis[7]. Thus at the moment, available literature only highlights therapeutic and pharmacological profile of drug but no published methods validated for its estimation in pharmaceutical formulations.

This encourages us to undertake this work, so that quantitative estimation of DFP can be done and hence can be used for routine analysis of bulk and formulation

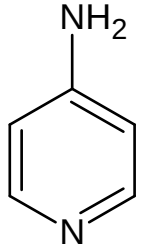


Figure 1 : Chemical Structure of Dalfampridine

as well. The present study describes the development and validation of rapid, simple, specific, sensitive, accurate and precise UV-spectroscopic methods for the determination of DFP in bulk and tablet dosage form. The proposed method is optimized and validated according to ICH guidelines[8].

EXPERIMENTAL

Reagents and chemicals

Dalfampridine was supplied as a gift sample by Apollo Pharmaceuticals Ltd., Mumbai (India). All chemicals and reagents used were of analytical grade (Merck Chem. Ltd., Mumbai). Double R.O. water was selected as the solvent for sample preparation.

Instrument

A double beam UV-Visible spectrophotometer (UV-2450, Shimadzu, Japan; software UV Probe 2.21) with spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock solutions

Accurately weighed 10 mg of DFP transferred to 100 ml volumetric flask. It was dissolved in water and volume was made up to the mark with same solvent to obtain the final strength 100 µg/ml. Then aliquots of standard stock solution were prepared by suitably diluting with same solvent to get the final concentrations of 2, 3, 4, 5, 6 and 7 µg/ml.

Method-A (Zero order derivative spectrophotometry)

From the stock solutions, 0.3 ml of DFP was transferred to 10 ml volumetric flasks and the volume was adjusted to the mark with same solvent to obtain strength 3 µg/ml. The solution was scanned in the UV range 200-400 nm and DFP showed absorbance maximum at 262 nm (Figure 2a).

Method-B (First order derivative spectrophotometry)

The zero-order derivative spectra of concentration 3 µg/ml was derivatized into first order using UV-probe software of the spectrophotometer; amplitude of the trough was recorded at 274.5 nm (Figure 3a).

Method-C (Zero order derivative spectrophotometry using area under curve)

The zero-order spectrum of drug concentration 3 µg/ml was selected for determination of area under curve (AUC). The wavelength range 254.2 – 269.0 nm was selected to record the AUC (Figure 4a).

Method-D (First order derivative spectrophotometry using area under curve)

The wavelength range 267.20 – 284.20 nm was selected to record the AUC for first-order derivative

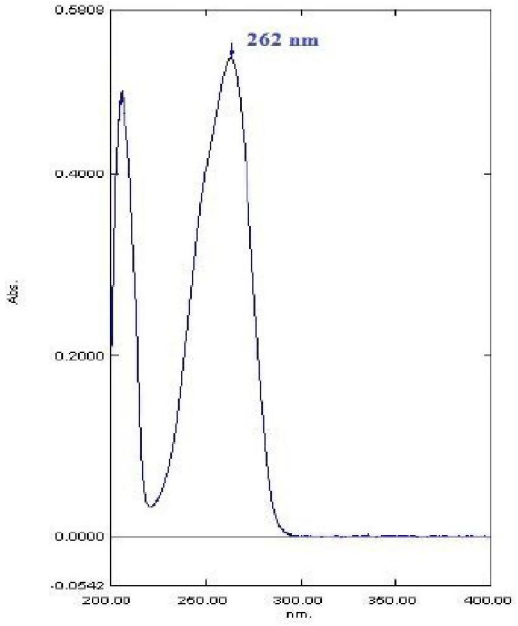
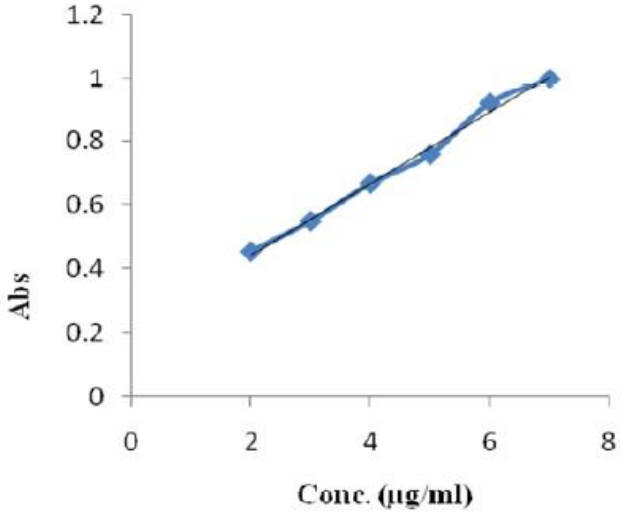


Figure 2a : UV Spectrum of DFP in water



Y = 0.112 x + 0.22 Correlation coefficient = 0.9937

Figure 2b : Calibration Curve of DFP

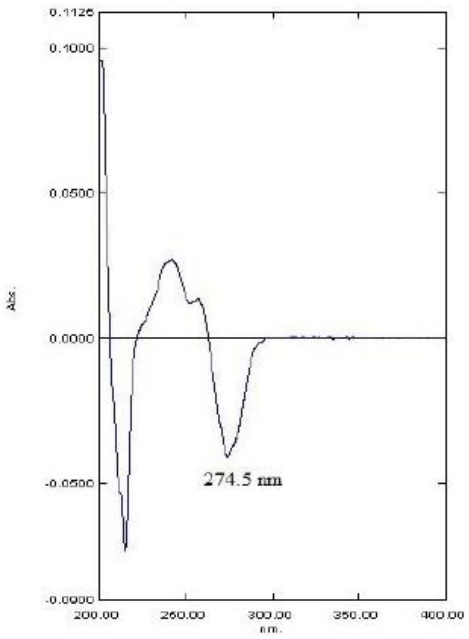
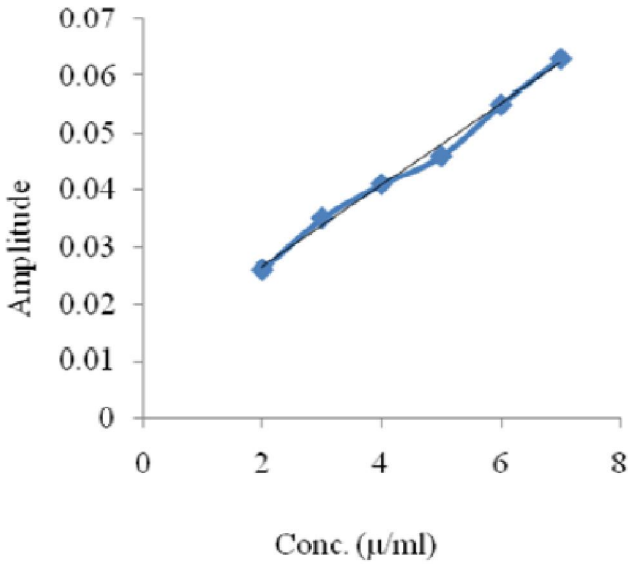


Figure 3a : First order derivative spectrum of DFP



Y = 0.007 x + 0.012 Correlation coefficient = 0.9928

Figure 3b : Calibration curve of DFP

spectrum of concentration 3 µg/ml (Figure 5a).

Analysis of in-house tablets

From tablets prepared by using tablet compression machine twenty tablets were accurately weighed and powdered finely. A quantity equivalent to 10 mg of DFP was accurately weighed, transferred to 10 ml volumetric flask and sonicated for 5 min with sufficient quantity of water. The solution was filtered through Whatman filter paper and the resultant solution was diluted with same solvent to get concentration 10 µg/ml for all methods. The amount of drug present in the sample solution was determined using the calibration curve of standard drug.

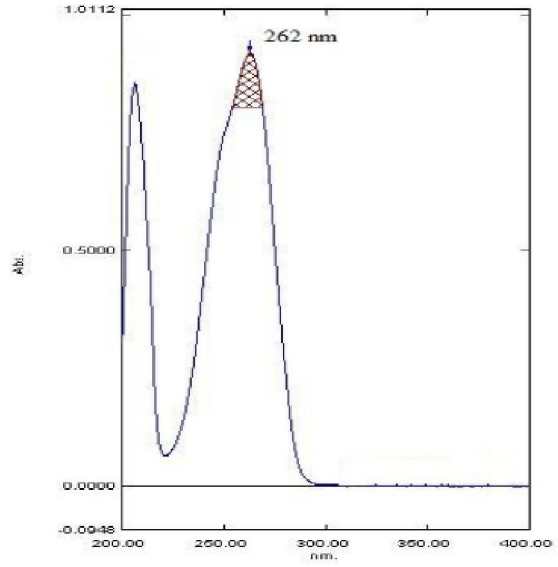
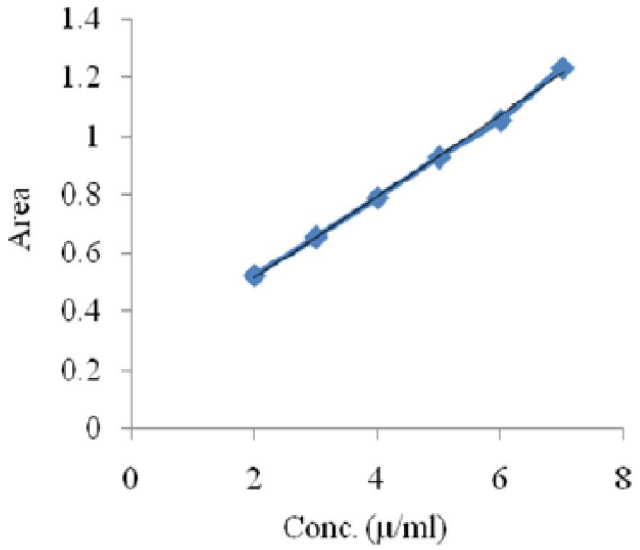


Figure 4a : UV spectrum of DFP showing AUC



Y = 0.140 x + 0.235 Coefficient of correlation = 0.998

Figure 4b : Calibration curve of DFP

Validation of proposed method

Linearity

Aliquots 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml of standard stock solution of DFP was transferred to series of 10 ml volumetric flasks and made up to volume with water. Each solution was analyzed. Linearity of the concentration was taken in range of 2–7 µg/ml for each method.

The Calibration curves were plotted as concentra-

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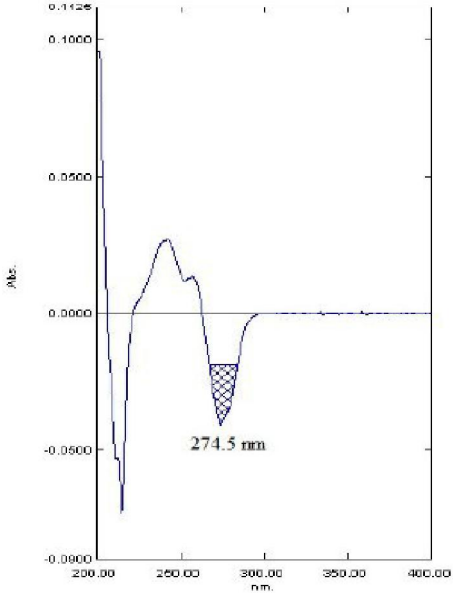
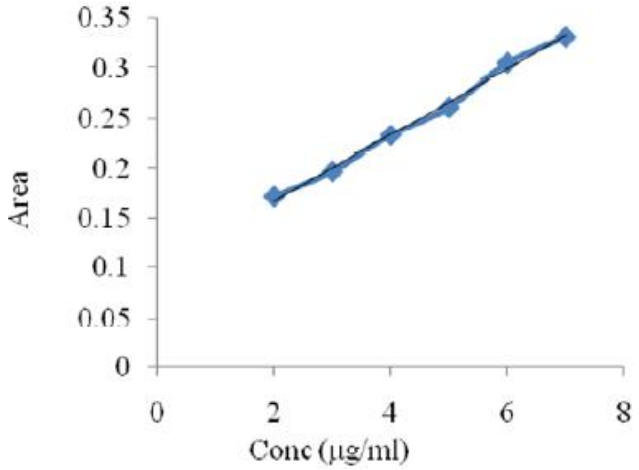


Figure 5a : First order UV spectrum of DFP showing AUC



Y = 0.033 x + 0.102 Coefficient of correlation = 0.994

Figure 5b : Calibration curve of DFP

tion *vs* absorbance at 262 nm (Figure 2b), concentration vs amplitude (Figure 3b), and concentration vs AUC (Figure 4b, Figure 5b).

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 2, 3 and 4 µg/ml of DFP solutions for three times in the same day. Inter-day precision was determined by analyzing daily for three consecutive days over a period of week using same concentrations.

Accuracy

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80, 100 and 120 %. The absorbance of solutions was recorded and spectra were derivatized. AUC was measured for each derivative.

Repeatability

Repeatability was determined by analyzing 3 µg/ml concentration of DFP solution for six times. The absorbance of solutions was recorded and spectra were derivatized. AUC was measured for each derivative.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated as LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), where SD is the residual standard deviation of the peak areas of the drug (n=6) and 'S' is the slope of the line. Sensitivity was performed between 2 – 3 µg/ml for each spectroscopic method.

Ruggedness

Ruggedness of the proposed methods was determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions.

RESULTS AND DISCUSSION

DFP in water showed maximum absorbance at 262 nm. For the estimation of DFP four spectroscopic methods have been developed. In these methods DFP followed linearity in the concentration range of 2 - 7 µg/ml. The amount of DFP estimated by methods was found to be within the acceptance criteria. Results obtained indicate that there is no interference of the excipients commonly used in tablets. These methods were validated for accuracy, precision, repeatability, sensitivity and ruggedness. The results are shown in TABLE 1. Precision study at different time and day interval for each method, showed low standard deviation and % R.S.D less than 2, indicates that the proposed methods are precise for the determination of DFP. High recovery and low standard deviation confirmed that proposed methods are accurate to for determination in pharmaceutical formulation. Also these methods were proved to be rugged as low values of % RSD were obtained.

TABLE 1 : Summary of validation parameters for proposed method

Parameters	Method A	Method B	Method C	Method D
Linearity (µg/mL)	2 – 7	2 – 7	2 – 7	2 – 7
Y = mx + C	Y = 0.112 x + 0.22	Y = 0.007 x + 0.012	Y = 0.140 x + 0.235	Y = 0.033 x + 0.102
Correlation coefficient	0.9937	0.9928	0.998	0.994
LOD (µg/mL)	0.288	0.634	0.175	0.267
LOQ (µg/mL)	0.874	1.921	0.531	0.809
% Recovery*	100.23	100.58	100.25	99.98
%RSD	0.82 – 1.30	0.69 – 1.10	0.71 – 0.81	0.84 – 0.99
Precision (%RSD)				
Intra-Day*	0.79 – 1.02	0.99 – 1.14	0.59 – 1.03	0.60 – 1.11
Inter-Day*	0.40 – 0.82	0.13 – 0.96	0.51 – 1.09	0.35 – 0.82
Repeatability#	0.84	1.03	1.23	0.92
Ruggedness (%RSD)#				
Analyst I	0.40	0.84	1.08	0.99
Analyst II	0.45	1.03	1.15	1.10

n = 3 #n = 6

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REFERENCES

[1] J.O.Maryadele; The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edition, Merck and Co. Inc.; Whitehouse Station, NJ, USA, **(2006)**.

[2] M.D.Egeberg, C.Y.Oh, J.L.Bainbridge; Clin. Therap., **34**, 2185-2194 **(2012)**.

[3] Y.Gao, L.Marinucci, S.L.Way, H.R.Henney III, R.Wada; Curr. Medi. Res. Opi., **11**, 16 **(2012)**.

[4] M.Malhotra, P.Ghai, B.Narasimhan, A.Deep; Arabian Journal of Chemistry (in press).

[5] S.McDonald, J.N.Clements; Am. J.Health Syst. Pharm., **69**, 544 **(2012)**.

[6] A.D.Goodman, T.R.Brown, K.R.Edwards, L.B.Krupp, R.T.Schapiro, R.Cohen, L.N.Marinucci, A.R.Blight; Annals of Neurology, **68**, 494–502 **(2010)**.

[7] J.Dunn, A.Blight; Curr. Med. Res. Opin., **7**, 1415-23 **(2011)**.

[8] ICH Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology, **(2005)**.