

Research Article

Method Development and Validation for Estimation of Dalfampridine in Synthetic Mixture by using UV Spectrophotometry and RP-HPLC

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Abstract: An accurate, precise and reproducible UV-spectrophotometric and liquid chromatographic assay method were developed and validated for the determination of dalfampridine in synthetic mixture form. Spectrophotometric estimation was done by calibration curve method using 0.1 N NaOH as solvent. In this method λ_{max} for dalfampridine was selected at 244 nm. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Thermo C₁₈ (250 × 4.6 mm, 5 μ m) column with mobile phase consisting of methanol: acetonitrile (50:50v/v) at flow rate of 1.0 ml/min. The retention time for dalfampridine was 4.186±0.3 min. The linearity range for dalfampridine was 5-25 μ g/ml for both HPLC and UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good ($r^2 > 0.999$) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. Moreover, the accuracy and precision obtained with HPLC co-related well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence successfully applied for determining the assay and in vitro dissolution of a marketed formulation.

Keywords: HPLC, UV Spectrophotometry, Dalfampridine, Synthetic mixture, Method validation, Quantitative analysis.

INTRODUCTION

Dalfampridine is the first drug approved in the United States by FDA to improve walking in patients with multiple sclerosis. It is chemically known as 4-aminopyridine or fampridine. Ampyra® is an extended release tablet formulation of dalfampridine which was previously called Fampridine-SR. Fampridine is a potassium channel-blocker that enhances conduction in focally demyelinated axons, improves synaptic transmission and potentiates muscle contraction. It has shown efficacy in patients with all five major types of multiple sclerosis namely relapsing, remitting, secondary progressive, progressive relapsing and primary progressive (Reynolds, J.E.F. 2008; Hayes, K.C. *et al.*, 2004; Vollmer, T. 2009; Vollmer, T., & Blight, A.R., 2009). The structure of dalfampridine is given Fig.1. Dalfampridine is a white crystalline powder with molecular weight 94.11g/mol. At ambient conditions dalfampridine is soluble in methanol,

acetone, acetonitrile, water, tetrahydrofuran, isopropanol, N, N- dimethylformamide, dimethyl sulfoxide and ethanol (Beckett, A.H., & Stenlake, J.B. 1998). Literature survey reveals that few analytical methods were reported for the determination of dalfampridine in bulk and formulation by UV (El-Fataty, H.M. *et al.*, 2013; Madhumathi, C.H. *et al.*, 2014; Vivekkumar, K.R. *et al.*, 2014), HPLC method (Donnelly, R.F. 2004; Jain, M. *et al.*, 2017) and gas chromatographic method (Evenhuis, J. *et al.*, 1981). However there is no combine method available for the determination of dalfampridine drugs. Therefore, an attempt was made to develop a new, rapid and sensitive method for the determination of dalfampridine in bulk drug and synthetic mixture. To assess the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines (International Conference on Harmonisation 2005).

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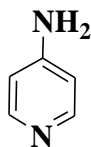


Fig. 1 Chemical structure of dalfampridine

EXPERIMENTAL

Reagents and chemicals

Pure sample of dalfampridine was received as a gift sample from Aurobindo pharma limited, Hyderabad, A.P, India. Acetonitrile (HPLC Grade), methanol (HPLC Grade), supplied by Merck Ltd, New Delhi, India. Triple distilled water was generated in house. The 0.45µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigadh, India. All excepients used were of pharmaceutical grade.

Instrument

In UV-spectrophotometric method, Labindia model- 3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells. Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

UV spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) of dalfampridine

Wavelength of maximum absorption was determined by scanning 10µg/ml solution of dalfampridine using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 244.0 nm (Fig. 2).

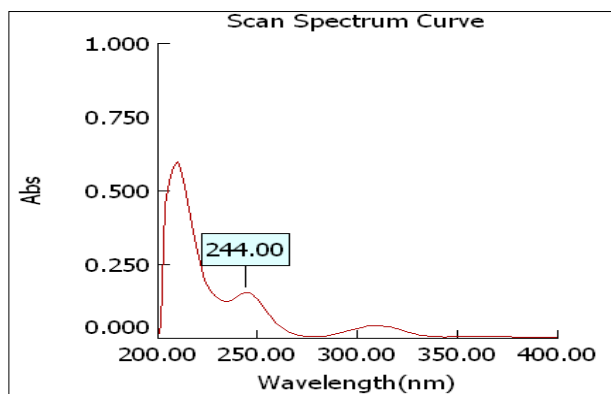


Fig. 2 Selection of λ_{max} of dalfampridine

Preparation of standard stock solution

10 mg of dalfampridine was weighed accurately and transferred to a 10ml volumetric flask and the volume was adjusted to the mark with the diluents (0.1 N NaOH), to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of dalfampridine, 1 ml was taken and diluted up to 10 ml, from this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with 0.1 N NaOH gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25µg/ ml at 244 nm for dalfampridine. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n=5 determinations was plotted as the standard curve (Fig.3).

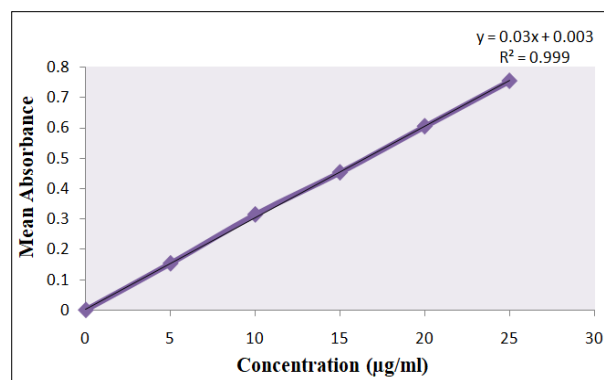


Fig. 3 Calibration curves of the dalfampridine

RP-HPLC METHOD

Chromatographic condition

The isocratic mobile phase consisted of methanol: acetonitrile in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 244 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

10mg of dalfampridine was weighed accurately and transferred to separate 10 ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000 µg/ml.

Working standard solution

From stock solutions of dalfampridine 1 ml was taken and diluted up to 10 ml with methanol. From this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25 µg/ml for dalfampridine solutions manually in triplicate to the HPLC system at detection wavelength of 244 nm. Mean of n =5 determinations was plotted as the standard curve (Fig.4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements. The retention times (Rt) of dalfampridine was 4.186±0.3min. The chromatograms have been shown in Fig. 5.

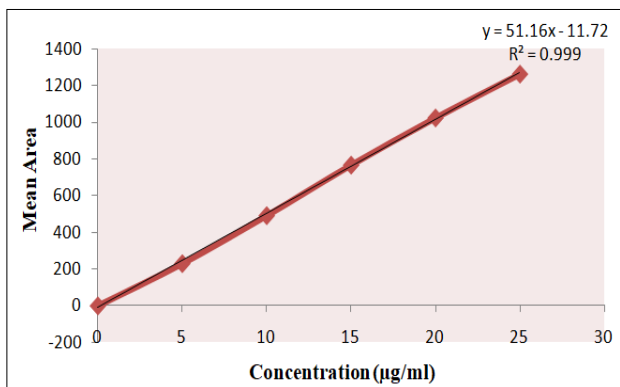


Fig. 4 Calibration graph of dalfampridine

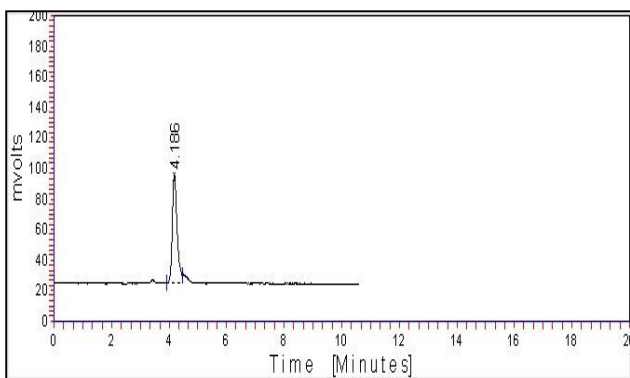


Fig. 5 Chromatogram of Dalfampridine

Validation Parameters

Linearity

Linearity was studied by analyzing five standard solutions (n=5) in the range of 5-25 µg/ml of dalfampridine in both UV spectrophotometric and HPLC method. Calibration curves with concentration verses absorbance or peak area was plotted for each method and the obtained data were subjected to regression analysis using the least squares method. Linearity of dalfampridine was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance or peak area with respective concentration (Table 1).

Table 1 Response ratios of dalfampridine

Concentration (µg/ml)	HPLC Method		UV Method	
	AUC	RR	ABS	RR
5	229.257	45.85	0.15	0.03
10	490.501	49.05	0.31	0.03
15	765.502	51.03	0.45	0.03
20	1022.02	51.10	0.60	0.03
25	1260.08	50.40	0.75	0.03

Accuracy

The validity and reliability of the proposed methods was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies were carried out by adding known amount of standard solution of dalfampridine to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. In UV Spectrophotometric method, the value of mean recoveries was found to be in the range of 99.42 % to 100.17% for dalfampridine. The value of SD and %RSD less than 2 indicate the accuracy of the method. In RP-HPLC method, the value of mean recoveries was found in the range of 100.00% to 100.66 % for dalfampridine. Total amount of drug found and percentage recovery was calculated. Results of recovery studies are reported in Table 2.

Table 2 Results of recovery study

Recovery Level%	% Mean±SD*	
	U.V Method	RP-HPLC Method
80%	99.92±0.156	100.00±0.816
100%	100.17±0.287	100.66±1.247
120%	99.42±0.136	100.26±1.144

* Value of three replicate and three concentrations

Precision

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in Table 3.

Robustness

For the robustness of the analytical method we changed the ratio of mobile phase. As a replacement for the methanol: acetonitrile in a ratio of 50:50v/v, methanol: acetonitrile in a ratio of 55:45 v/v were used as solvent (Results are shown in Table 3).

Table 3 Results of precision

Parameters	UV Method		RP-HPLC Method	
	Dalfampridine (Mean±SD)*	% RSD	Dalfampridine	% RSD
Repeatability	98.50±0.225	0.263	99.98±0.245	0.365
Day to Day	98.56±0.416	0.422	98.057±0.060	0.061
Analyst to Analyst	99.80±0.215	0.312	99.60±0.223	0.232
Robustness*	-	-	99.24 ±0.170	0.171

*Average of 5 replicate and 5 concentration.

LOD and LOQ

LOD and LOQ of described method were observed as 0.125µg/ml and 0.369µg/ml for dalfampridine in UV spectrophotometric method and 0.540 µg/ml and 0.510 µg/ml for dalfampridine in RP-HPLC method, based on the SD of response and slope, which meet the requirement of new method.

Analysis of Synthetic mixture Formulation

Synthetic equivalent to 10 mg dalfampridine were weighed and ground to a fine powder. An equivalent amount to 10 mg of dalfampridine was taken in 10 ml volumetric flask. This was dissolve in 5 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method and RP-HPLC method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The statistical evaluation of tablet analysis by both methods is reported in Table 4.

Table 4 Results and statistical parameters for tablet analysis

S. No	Drug	Label claim	Amount found	% Purity
UV Method	Dalfampridine	10 mg	9.98 mg	99.60
RP-HPLC	Dalfampridine	10 mg	9.95 mg	99.70

*Average of five determination

CONCLUSION

The advantage of UV method over HPLC method is that the proposed UV method does not require the elaborate treatment and procedures usually associated with chromatographic method. It is less time consuming and economical. A statistical comparison of the quantitative determination of dalfampridine shows that HPLC method as more accurate and precise than UV method. The results indicate HPLC and UV spectrotometry methods are adequate methods to quantify dalfampridine in pure form and synthetic form. There was no interference by excipients in the tablets and the mobile phase is easy to prepare. Since these methods are simple, specific, rapid and accurate, they may be successfully and conveniently adopted for routine quality control analysis of dalfampridine in bulk and pharmaceutical dosage form.

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