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Development and validation of stability-indicating RP-HPLC method for estimation of dalfampridine in bulk drug and tablet dosage form

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Abstract

Background: In the current study, a simple, improved, precise, rapid, and accurate reverse phase liquid chromatographic method was produced for the estimation of dalfampridine in bulk and tablet dosage form which is a potassium channel blocker used for the treatment of multiple sclerosis (MS). The separation of dalfampridine was achieved isocratically on a C_{18} column (250 × 4.6 mm, 5 μm) using (0.1% v/v) buffer pH 3.0 ± 0.05 adjusted with diluted orthophosphoric acid (OPA) and acetonitrile (ACN) in the ratio of 60:40% (v/v) as a mobile phase, at a flow rate of 0.5 mL/min, and column temperature of 40 °C. HPLC grade methanol as diluents was used. Five microliters of the standard solution of the drug was injected, and the eluted analytes were detected at 262 nm.

Results: Dalfampridine was eluted at 4.5 min with a run time of 10 min. Linearity in the method was measured in the concentration range of 25–75 ppm with a correlation coefficient of 0.999. Limit of detection and limit of quantitation were found to be 0.711 μg/mL and 2.154 μg/mL, respectively. Dalfampridine was subjected for forced degradation stability study in conditions of thermal, acid, alkali, and oxidation and photo-degradation condition. The degradants were well resolved from the dalfampridine main peak. Validation of the developed method is carried as per USFDA and ICH guidelines.

Conclusion: The results of the analysis prove that the method is simple, improved, precise, accurate, and rapid for estimating the content of dalfampridine in bulk drug and tablet dosage form and can be applied for routine analysis.

Keywords: Stability-indicating RP-HPLC, Dalfampridine, Multiple sclerosis, Forced degradation

Background

The dalfampridine, known as 4-aminopyridin and chemically is 1,4-dihydropyridin-4-imine (Fig. 1), is used mostly for the treatment of multiple sclerosis. Dalfampridine is considered a broad- spectrum in action. Pharmacologically, the drug is a potassium channel blocker and mostly lipophilic in nature, which binds favorably to the open state potassium channel in the central nervous system (CNS) [1, 2]. Dalfampridine is rapidly and completely absorbed orally to attain relative bioavailability up to 96%. The excretion takes in unchanged form, mostly from urine (96%) [3]. The reported solubility of dalfampridine was found in water, methanol, acetonitrile (ACN), acetone, ethyl ether, and very soluble in ethanol. It was found slightly soluble in ligroin [4, 5]. The stability of a drug in formulation refers to the ability of a particular formulation to maintain its specifications related to its identity, strength, quality, and purity [6]. Degradation studies over the drug can be calculated by exposing the drug in extremes pH conditions (acidic or basic), oxidative

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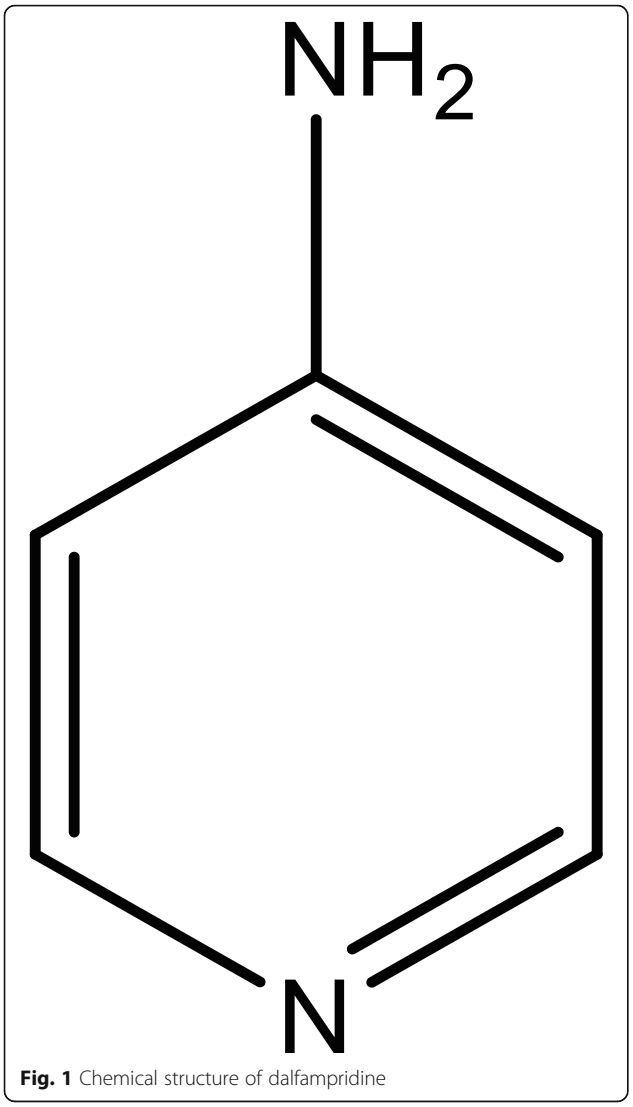

Fig. 1 Chemical structure of dalfampridine

reactions, ecstatic temperature, UV, and dry heat to an extent of 5–20% [7, 8]. For analysis of a drug and its substances, sensitive methods such as LC/MS and GC/MS are preferred but are expensive. The HPLC is found to be the most reliable and cost-effective [9, 10]. The methods reported by the use of reverse phase high-performance liquid chromatography (RP-HPLC) mostly involve the gradient mode of analysis, which makes analysis complex [11]. Hence, the current work aims to develop an accurate, specific, stability-indicating, isocratic method for the estimation of dalfampridine in bulk and tablet form.

Methods

Instrumentation

Waters HPLC (Separation module 2695) chromatographic system equipped with PDA-detector 2487 Xterra C_{18} (250 × 4.6 mm, 5 μL) thermostatic column compartment connected with Empower-3 software, consisting of pump, autosampler, and auto-injector. Shimadzu-(ATX 224)-digital weighing balance, BT ultra sonicator 48, digital systronic pH meter (802), and Millipore vacuum filter pump (XI 5522050) were used for the method development. A 0.22-μm Nylon filter of Merck Millipore was used for filtration.

Materials and reagents

The pharmaceutical grade working standards of dalfampridine were obtained as a gift from Enaltec Pharma. Research Pvt. Ltd (Ambernath, Mumbai, India). Dalfampridine tablet formulation (10 mg) was used of brand AMPYRA, marketed by Accorda Therapeutics Inc. The HPLC grade triethylamine, OPA, methanol, and ACN were procured from SD Fine Chem., Mumbai, India, for the present study. The Milli-Q water procured from Mumbai, India, was used for the analysis.

Chromatographic conditions

The buffer pH 3.0 and ACN in the ratio 60:40% v/v was used as a mobile phase. The flow rate was maintained at 0.5 mL/min. The column temperature was kept at 40 °C, and the detection was carried out at 262 nm with an injection volume of 5 μL.

Preparation of the mobile phase

One milliliter of triethylamine was added to 1000 mL Milli-Q water. The pH was adjusted to 3.0 ± 0.05 with OPA. The solution was filtered through a 0.45-μ membrane filter and was sonicated for 15 min to degas it. The buffer (0.1%) pH 3.0 and ACN in the ratio of 60: 40% v/v was used as a mobile phase. Dalfampridine was separated and eluted in an isocratic program.

Preparation of standard solution (50 ppm)

Accurately weighed 50 mg of dalfampridine as working standard was transferred to a volumetric flask of 50 mL followed by 30 mL of methanol and sonicated. The cooled solution was adjusted up to the mark with methanol. Further 5 mL solution was diluted to 100 mL with diluents.

Preparation of sample solution (50 ppm)

Ten intact tablets of dalfampridine were transferred into a 100-mL volumetric flask. About 70 mL of methanol was added, and the resulting solution was sonicated for 25 min with intermittent shaking, then cooled, and diluted up to the mark with methanol. The resulting solution was then allowed to settle for 15 min and then the solution was centrifuged at 5000 RPM. Further, 5 mL of the supernatant solution was diluted to 100 mL with diluents and filtered through a 0.45-μ Nylon membrane syringe filter or equivalent.

Forced degradation study

Forced degradation studies were carried out on dalfampridine under several conditions as per ICH guidelines Q1A(R2) and Q1 B.

Photolytic degradation

Photolytic degradation was performed by amusing samples under UV and white light for 1.2 million lux hours in a Petri plate for 7 days. The previously exposed sample (10 tablets) was weighed and diluted with methanol to 100 mL after sonication. Supernatant 5 mL solution was diluted to 100 mL with diluent.

Thermal degradation

The thermal stability of the drugs was calculated by keeping the sample in an oven at about 70 °C for 24 h. About 5 mL of this stressed solution was diluted with diluent.

Acid degradation

Degradation under acidic condition was calculated by treating the stock solution with 5 mL of 5N HCl and refluxed at 60 °C for 2 h. The diluent is used for the dilution of the resulting solution.

Alkali degradation

Under alkaline conditions, degradation was studied by refluxing standard solution with 5 mL of 5N NaOH and refluxed at 60 °C for 2 h. The diluent is used for the dilution of the resulting solution.

Peroxide degradation

The standard solution was subjected to oxidative degradation by refluxing with 5 mL of 30% v/v hydrogen peroxide (H_2O_2) solution at 60 °C for 2 h and then treated with diluent.

The chromatogram was studied according to the area of peak of drug and appearance of another/secondary peaks. Any change in area and appearance of secondary peaks will be considered as degradation.

Method validation

The developed method was validated as per ICH Q2 (R1) and USFDA guidelines. The method was validated for the parameters such as linearity and range, specificity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ). The specificity was determined by injecting samples of blank, placebo (except dalfampridine), standard solution, and sample solution from the formulation.

Stability-indicating tests

According to the force degradation experiments and chromatographic analysis, it was concluded that dalfampridine resulted into minimal degradation in conditions such as acid (SF-2), base (SF-3), and peroxide (SF-4) and thermal and photolytic degrading conditions in the range of 1–8%. The higher degradation was found in base degrading condition up to 8.6%, and only one degradation product was found in peroxide degrading condition, which was eluted at a retention time (R_T) of 4.9 min. The control sample (SF-1), i.e., the sample of unstressed condition, was also employed for analysis, which was used for comparison with results of forced degradation. The results of force degradation are tabulated in Table 6.

Results

Method development and optimization of chromatographic conditions

To achieve satisfactory separation of dalfampridine, different buffer at various pH conditions and solvents of different proportions were tested as binary and tertiary eluents. However, the buffer was adjusted at pH 3.0 with dilute OPA and ACN to achieve good satisfactory results at a flow rate of 0.5 mL/min measured at a detection of 262 nm. The optimized chromatogram and optimized conditions are mentioned in Fig. 2 and Table 1, respectively.

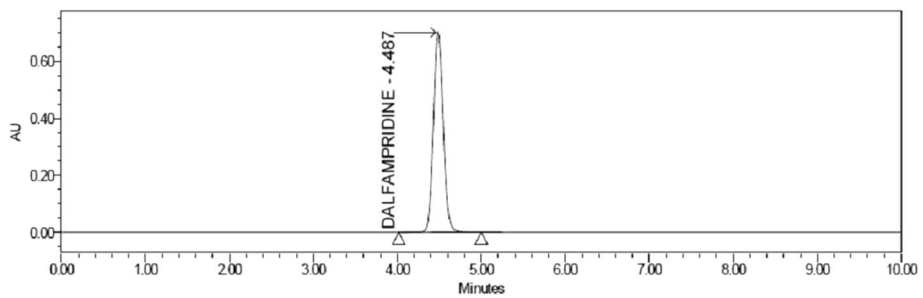

Fig. 2 Typical chromatogram of dalfampridine

Table 1 Optimized chromatographic conditions

Column	Waters XTerra® RP 18 5 μm 4.6 × 250 mm
Flow rate	0.5 mL/min
Injection volume	5 μL
Wavelength	262 nm
Column temp	40 °C
Autosampler temp	25 °C
Run time	10.0 min
Retention time	4.4 min
Mobile phase	Buffer pH 3.0: acetonitrile (60:40)
Theoretical plates	6510
Tailing factor	1.1

System suitability results

The system suitability parameters such as retention time, tailing factor, and theoretical plates for optimized standard mixture chromatogram are tabulated in Table 2.

Method validation

Linearity

The linearity sample preparation was carried out 4 to 15 ppm (ST-1). The calibration curve (Fig. 3) was plotted with a concentration of standard solutions against mean peak areas.

Range

It is derived from linearity. The range was evaluated by measuring different concentrations of standard solutions to dalfampridine, i.e., 25 to 75 ppm (50 to150%) as shown in ST-2.

Specificity

The standard and sample solution peaks were found at R_T 4.5 min within system suitability test acceptance criteria as presented in SF-5. As per degradation studies, the interference with degradants was nil, and the purity angle for the sample solution is less than the purity threshold which indicates the peak is pure. Hence, the results of specificity of the developed method were found to be in acceptance criteria. The results are presented in ST-3.

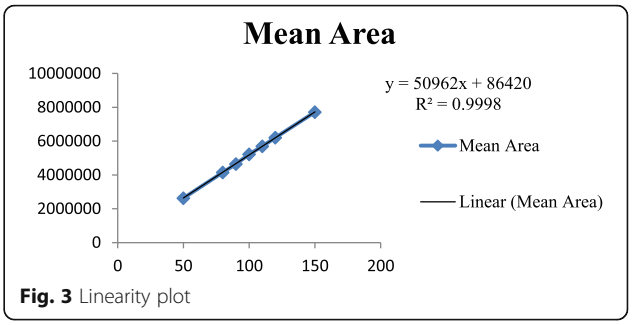

Fig. 3 Linearity plot

Accuracy (% Recovery)

The recovery studies show the accuracy in the proposed method; the known amount of pure drug concentrations was spiked in placebo at three different levels, i.e., 50%, 100%, and 150%, and the study was carried out in triplicates for each level. Accuracy was calculated in terms of the percentage of recovery. The results are tabulated in Table 3.

Precision

System precision, method precision, and intermediate precision are the three levels for evaluation. Each level was investigated by six replicate injections of concentration 50 ppm (100%) of dalfampridine. The results of precision were expressed in terms of % assay and RSD and are tabulated in ST-4.

Robustness

The robustness was believed to be unaffected when small, deliberate changes like flow change, buffer pH change, wavelength change, and column temperature were performed at 100% test (50 ppm) concentration.

Table 2 Results for system suitability

Sr. no.	Parameters	Results	Acceptance criteria
1	Peak area (6 replicate injections)	**Area**	
	1	5325966	
	2	5351414	
	3	5329388	
	4	5320723	
	5	5303219	
	6	5336522	
	Mean peak area	5327872	
	RSD, relative standard deviation	0.3%	RSD must be less than 2
2	Retention time	4.5 min	Must be more than 2 min

Table 3 Result of accuracy

Level (%)	Theoretical concentration (μg/mL)	% Recovery	Mean recovery (%)
50	25.006	98.5	98.4
	25.006	98.2	
	25.006	98.6	
100	49.615	98.9	99.1
	49.615	99.4	
	49.615	99.1	
150	74.720	98.1	98.6
	74.720	99.2	
	74.720	98.5	
Mean recovery			98.7
RSD			0.37 %

The R_T for the control sample was found at 4.9 min. Then, R_T was shifted when the flow rate was changed and found at 6.2 min and 4.1 min at a flow rate of 0.4 and 0.6 mL/min, respectively. The results are summarized in Table 4.

Ruggedness

The ruggedness was carried out by injecting 6 replicate injections of dalfampridine sample solution of conc. 50 ppm, i.e., 100% strength. The analysis was carried out by using two different analysts; hence, the analysis was carried out twice on a system. The results after the test were found within limits, and %RSD was found to be less than 2. The results of ruggedness are tabulated in Table 5.

Limit of detection and limit of quantitation

It was calculated by applying the formula given for LOD and LOQ.

$$\text{Formula}: \quad \text{LOD} = 3.3 \times \text{SD/slope}$$
$$\text{LOQ} = 10 \times \text{SD/slope}$$

The values for LOD and LOQ after calculation were found to be 0.711 μg/mL and 2.154 μg/mL, respectively.

Discussion

Few methods like UV [12–14] spectroscopy and RP-HPLC [15–19] were reported for the estimation of dalfampridine. In the present method, dalfampridine was eluted at 4.5 min with a run time of 10 min. The present method was developed using 0.1% buffer at pH 3.0 ± 0.05 adjusted with diluted OPA and ACN in the ratio of 60:40% v/v. The method was developed with the minimum or reduced amount of organic solvents as the mobile phase which results in a more sensitive and cost-effective method. The analytical methods like stability-indicating RP-HPLC are responsible for idiosyncrasy between active pharmaceutical ingredients from any degradation products

Table 4 Result of robustness

Parameters	Values	Retention time	Tailing factor	Theoretical plates	RSD of standard area (%)	% Assay	Absolute difference
Control	As per method	4.990	1.20	6760	0.16	100.5	–
Flow rate (± 0.1 mL/min)	0.4 mL/min	6.201	1.18	7371	0.22	100.5	0.0
	0.6 mL/min	4.153	1.20	5922	0.26	100.2	0.3
Change in wavelength (± 5 nm)	257 nm	4.967	1.21	6608	0.34	100.5	0.0
	267 nm	4.967	1.21	6656	0.14	98.9	1.6
Buffer pH (± 0.2 unit)	pH – 2.98	4.912	1.20	6230	0.25	98.7	1.8
	pH – 3.02	4.922	1.0	5998	0.68	98.6	1.9
Column temperature (± 5 °C)	35 °C	4.962	1.20	6522	0.57	99.6	0.9
	45 °C	3.781	1.20	6759	0.39	99.7	0.8

Table 5 Result of ruggedness

Sr. no.	% Assay results from analyst I	% Assay results from analyst II
1	99.8	100.1
2	98.1	98.7
3	99.6	99.9
4	98.1	98.4
5	99.0	99.5
6	98.0	98.9
Mean	98.9	99.2
RSD of all determinations	0.65%	0.55%

formed under given conditions. These are the methods where the effect of stressors like pH, temperature, and other conditions helps in understanding the stability of the drug during storage conditions and analysis; the results are summarized in Table 6.

Analysis of marketed sample

The method is applicable for the analysis of dalfampridine in marketed tablet dosage forms of 10 mg. The percentage assay results were found to be 98.3% and the amount found was up to 9.83 mg. The results are summarized in Table 7.

Conclusion

An accurate, precise, specific, stability-indicating, isocratic RP-HPLC method was developed for the estimation of dalfampridine in bulk and tablet dosage form. The compound was evaluated by forced degradation pertaining to several stress conditions, where the developed method separates the compound and degradants successfully and estimated the active contents. The method was successfully validated according to USFDA guidelines for all the parameters which were found within acceptance criteria. The developed method may be useful for routine analysis of dalfampridine tablets or for assay of dalfampridine tablets from stability batches.

Table 6 Forced degradation results

Conditions	% Assay	Degradation achieved	Purity angle	Purity threshold
Control	97.1	–	0.198	0.262
Acid degradation (5N HCl)	91.0	6.1	0.185	0.259
Base degradation (5N NaOH)	88.5	8.6	0.201	0.262
Peroxide degradation (30% H_2O_2)	90.9	6.2	0.690	0.243
Thermal degradation	96.0	1.1	0.201	0.264
Control	102.8	–	0.584	0.254
Photolytic (open Perti plate)	95.5	7.3	0.607	0.252
Photolytic (close Petri plate)	97.7	5.1	0.620	0.256
Photolytic (close Petri plate with aluminum foil)	100.3	2.5	0.610	0.253

Table 7 Result of the assay by the proposed method

Parameters	Results
Standard area	5325966
	5351414
	5329388
	5320723
	5303219
	5336522
Mean area	5327872
Sample area	5184762
Amount found	9.83 mg
% Assay	98.3%

Abbreviations

RP-HPLC: Reverse phase high-performance liquid chromatography; ACN: Acetonitrile; CNS: Central nervous system; OPA: Orthophosphoric acid; Conc.: Concentration; R_T: Retention time; RSD: Relative standard deviation; SST: System suitability test; FD: Forced degradation; LOD: Limit of detection; LOQ: Limit of quantitation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-021-00232-4.

Additional file1: Supplementary data of Control Sample, acid degradation, Base degradation, peroxide degradation and sample preparation under specificity are mentioned as figures indicating **SF-1 to 5.** Supplementary data of results of linearity, results of specificity and results of Precision are mentioned as Table indicating **ST-1 to 3.**

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Authors' contributions

DB, AJ, and AC contributed equally for preceding this research. Concept and guidance were from AS, and the final manuscript was prepared and checked by AN and SK. We declare that all authors have read and approved the manuscript before submission.

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Availability of data and materials

The data for verification is provided with a Supplementary file and the rest of the data, if required, will be available upon request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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