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RP-HPLC Method Development and Validation for Estimation of Dalfampridine in Pure and Tablet Dosage Form

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Abstract

A simple, economic, rapid, accurate and stability indicating RP-HPLC method was developed for the estimation of amount of of Dalfampridine in pure and tablet dosage form. The method was performed on Phenomenex C18 (125 X 4.6 mm, 5 µm) using the mobile phase composed of buffer (0.01M sodium acetate pH 4.5): methanol in the ratio of 60:40 v/v. Th flow rate was maintained at 0.8 mL/min. The retention time for Dalfampridine was found to be 1.713 min. The method was found to be linear in the range of 5-25 µg/mL and the regression equation was found to be y=14691x-12844. For intra- and inter-day precision the %RSD for Dalfampridine was found to be 0.218 and 0.622%. Percentage mean recovery was found to be 98.36%. LOD and LOQ values obtained for Dalfampridine were found to be 0.107 µg/mL and 0.323 µg/mL respectively. Acid, alkali, oxidative, thermal and neutral degradation studies were performed. The results are analysed statistically and are found to be satisfactory. Hence this method can be routinely applicable for analysis of Dalfampridine in pure and tablet dosage form.

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Introduction

Dalfampridine (Fig. 1), is a potassium channel blocker prescribed for the treatment of multiple sclerosis. It is chemically pyiridine 4-amine or 4-Amino pyridine. It is also useful as an antagonist or non-depolarising neuro muscular blocking agents such as d-tubocurarine, gallamine, pancuroniun. Dalfampridine (DFP) which acts as at central and peripheral nervous system enhances conduction in demylinated axons and improve walking ability of multiple sclerosis patients [1]. It strengthens brain signals through the nerves that have been damaged by multiple sclerosis [2]. The use of Dalfampridine is to stimulate the demylinated axons that are exposed in multiple sclerosis patients.

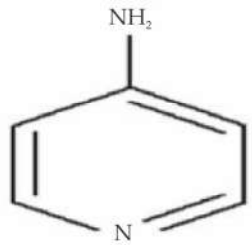

Fig. 1: Structure of Dalfampridine

Literature survey [3-8] reveals that very few HPLC methods were reported for estimation of Dalfampridine in pharmaceutical dosage forms. In the present work an attempt has been made to develop a novel, rapid and economic RP-HPLC method for estimation of Dalfampridine in pure and tablet dosage form.

Materials and Methods

Instrument

Agilent 1260 infinity binary pump HPLC with open lab software was used for chromatographic studies.

Chemicals

The pure sample of Dalfampridine was obtained from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. HPLC grade methanol, analytical grade sodium acetate, ortho phosphoric acid were purchased from E. Merck (India) Ltd., Mumbai. Dalfampridine tablets were purchased from local market. Triple distilled water was used throughout experiment.

Buffer Preparation (0.01M Sodium acetate)

Transfer 0.136 g of sodium acetate into 100 mL volumetric flask, dissolved in some water and diluted upto mark with water. Then pH was adjusted to 4.5 using ortho phosphoric acid.

Preparation of standard stock solution

Accurately weigh about 100 mg of Dalfampridine working standard and transferred into a 100 mL clean volumetric flask, dissolve in 20 mL of mobile phase, sonicated for 5 minutes, and diluted upto mark with mobile phase.

Preparation of sample stock solution

Weigh 5 tablets of Dalfampridine and powdered them in a mortar and pestle and a quantity of tablet powder equivalent to 10 mg of DFP was transferred to 10 mL volumetric flask. Add small quantity of mobile phase to dissolve the sample and the volume was adjusted up to the mark with mobile phase. The prepared solution was filtered through a 0.22 µm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution.

Results

Method development

The present method was developed by performing various trails with different mobile phases in different compositions and with different columns. Finally the mobile phase consisting of 0.01M sodium acetate (pH 4.5): methanol (60:40, v/v) and the phenomenex C18 (125×4.6 mm, 5 µm) column was selected for analysis. The flow rate was maintained at 0.8 mL/min flow rate and the response was detected at 272 nm which gave sharp peak, minimum tailing factor with short run time for DFP. The retention time for DFP was found to be 1.713 minutes. The optimized chromatogram was shown in Figure 2.

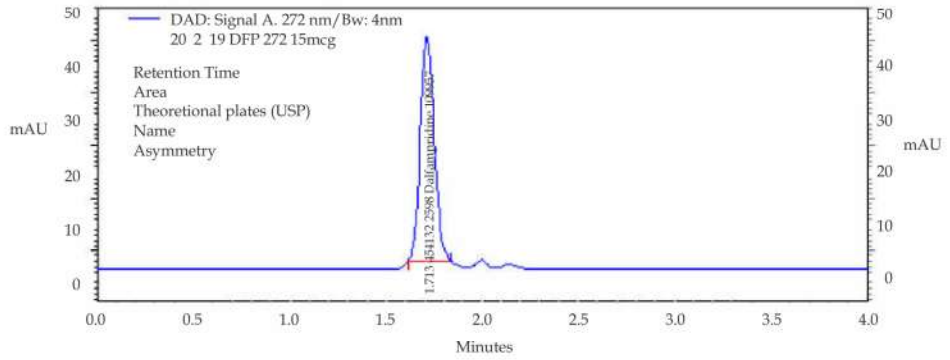

Fig. 2: Optimized Chromatogram of Dalfampridine

Validation

System Suitability

System suitability was known by injecting the solution into system for several times and the parameters like theoretical plates, resolution and asymmetric factor were evaluated and the values are shown in Table 1.

Table 1: System suitability results for Dalfampridine

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.713	386818	2899	1.02
2	1.707	386823	2892	0.99
3	1.713	381565	2889	1.03
4	1.712	371256	2952	1.02
5	1.713	385623	2923	1.05
6	1.711	370246	2944	1.03
7	1.712	380145	2925	1.08
8	1.713	380136	2912	1.02
9	1.713	379985	2825	0.99
10	1.712	380625	2913	1.06
Mean	1.712	380322	-	-
%RSD	0.1082	1.513	-	-

Specificity

The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms there was no interference from blank and standard Chromatogram.

Linearity

Linearity was estimated by preparing five different standard solutions of Dalfampridine in the range of 5-25 µg/mL, injected into system and the response was measured at 272 nm. Calibration curve (Fig. 3) was constructed by taking the response on Y-axis and concentration on X-axis. The linearity results were depicted in Table 2.

Table 2: Linearity results for Dalfampridine

S. No.	Conc.(µg/mL)	Peak area	Statistical Analysis	
1	0	0	Slope	14691
2	5	197562	Intercept	12844
3	10	386691	Regression equation	y = 14691x -12844
4	15	551497		
5	20	755951	Correlation coefficient	R^2 = 0.999
6	25	934197		

Precision

The precision of the method was performed at two levels i.e intra-day and inter-day analysis. For intra-day precision, the selected solution (10 µg/mL) was injected into system for six times in the same day, whereas in inter-day precision the analysis was carried out for six days. The %RSD was calculated and the results of intra-day inter-day precision were tabulated in Table 3.

Table 3: Intra & inter-day results for Dalfampridine

S. No.	Intra-day Time (Hours)	Peak area	Inter-day Days	Peak area
1	0	387562	1	385641
2	3	388835	2	384932
3	6	387423	3	385621
4	9	387256	4	382347
5	12	388265	5	388168
6	15	389246	6	381642
	Mean	388098	Mean	384725
	SD	817.416	SD	2395.78
	%RSD	0.21	%RSD	0.622

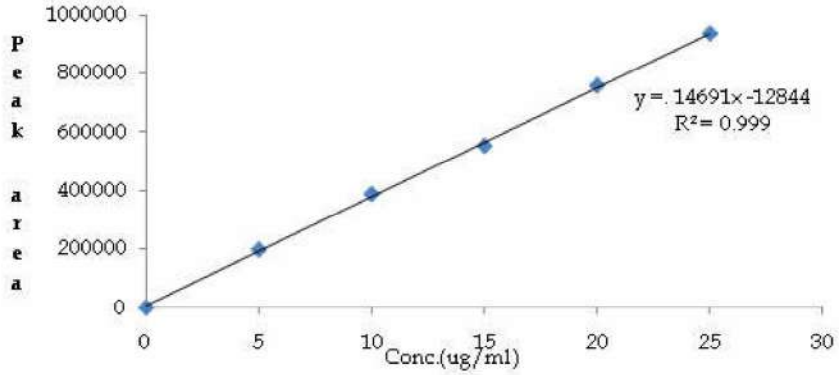

Fig. 3: Linearity curve of Dalfampridine

Accuracy

Accuracy was determined by preparing the standard solutions which are spiked with the 50%, 100% and 150% of sample in triplicate and injected into chromatographic system. Calculate the % recovery and the accuracy results were given in Table 4.

Table 4: Accuracy results for Dalfampridine

Recovery/ Spike level at about [%]	Amount of DFP added (pap)	Peak Area	Conc. Found (µg/mL)	% Recovery	% Mean recovery
50	5	584129	5.041	100.08	100
50	5	583945	5.083	101.6	
50	5	589795	4.97	99.4	
100	10	774832	9.857	98.57	99.86
100	10	771564	9.903	99.03	
100	10	772431	10.02	100.2	
150	15	941562	14.74	98.26	100.32
150	15	939634	15.29	101.93	
150	15	941265	15.08	100.53	

Ruggedness

Ruggedness of the method was confirmed by the analysis of samples was done by different analysts. Samples of Dalfampridine 10 µg/mL concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The robustness results were furnished in Table 5.

Table 5: Robustness results of Dalfampridine

S. No.	Parameter	Optimised	Used	Rt (min)	Peak area	% RSD
1	Flow rate	0.8 mL/min	0.6 mL/min	1.71	379461	0.35
			0.8 mL/min	1.73	385692	0.75
			1.0 mL/min	1.68	383914	0.95
2	Wavelength	230 nm	270 nm	1.88	371564	0.59
			272 nm	1.87	371462	0.75
			274 nm	1.88	371356	0.69
3	Mobile phase	MeOH:Buffer (40:60)	38:62	1.91	382456	0.95
			40:60	1.88	373257	0.84
			42:58	1.85	334267	0.69

Limit of detection and Limit of quantification (LOD & LOQ)

LOD and LOQ values are calculated from calibration curve method and the LOD and LOQ of Dalfampridine is given in Table 6.

Table 6: LOD and LOQ of Dalfampridine

Parameter	Measured value (µg/mL)
Limit of detection	0.107
Limit of quantification	0.323

Estimation of Dalfampridine tablet dosage forms

From the sample stock solution 1 mL was taken and transferred to 100 mL volumetric flask and injected into instrument. The assay results were shown in Table 7 and sample chromatogram was represented in Figure 4.

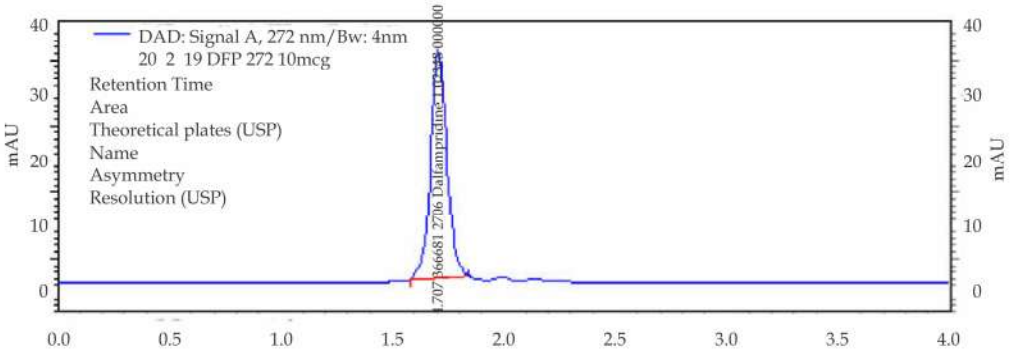

Fig. 4: Sample Chromatogram of Dalfampridine

Table 7: Assay results of Dalfampridine formulation

Formulation		Label claim	Amount found	% Assay
Dalfampridine	Ampyra	10 mg	9.91	99.12

Degradation studies

Acid degradation studies

To 1 ml of stock solution of Dalfampridine, 1 ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 10 µg/ml solution and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies

To 1 ml of stock solution of Dalfampridine, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 10 µg/ml solution and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies

To 1 ml of stock solution of Dalfampridine, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^{0}C. The resultant solution was diluted to obtain 10 µg/ml solution and 10 µl solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation studies

The standard drug solution was placed in oven at 105^{0}C for 6 hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 10 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60^{0}C. For HPLC study, the resultant solution was diluted to 10 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

The degradation results were tabulated in Table 8.

Table 8: Degradation data of Dalfampridine formulation

Type of degradation	Dalfampridine		
	Area	% Recovered	% Degraded
Acid	290174	95.18	4.82
Base	301623	96.41	3.59
Peroxide	365214	95.39	4.61
Thermal	382565	99.25	0.75
Water	387324	99.23	0.77

Table 9: Method Validation Summary

S. No.	Parameter	Observation
1	System suitability	The %RSD for retention time is 0.1082% and for peak area is 1.513%
2	Precision	%RSD for Intra and Inter-day Precision 0.21 & 0.622
3	Accuracy	%Mean recovery is between 99.862-100.32%
4	Linearity	Regression equation: y=14691x-12844; R2= 0.999
5	LOD & LOQ	LOD: 0.107 µg/mL & LOQ:0.323 µg/mL
6	Ruggedness	%RSD value is below 2%
7	Robustness	The % variation change in wavelength and flow rate is within limits

Summary

The present RP-HPLC method was developed for estimation of Dalfampridine by using Phenomenex C18 (125 x 4.6 mm, 5 µm) as stationary phase with mobile phase containing mixture of 0.01M sodium acetate (pH 4.5) and methanol (60:40 v/v). The eluted compound was monitored at 272 nm. Dalfampridine peak was eluted at 1.713 min. The developed method was validated for parameters of specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness as per approved ICH guidelines [9] and validation results are summarized in Table 9. The method was subjected to acid, alkali, thermal, oxidative and neutral degradation studies and degradation data demonstrate that the method was stability indicating method.

Conclusion

A novel, reverse phase stability indicating HPLC method has been developed for quantitative estimation of Dalfampridine in pure and tablet dosage form. A few methods were reported for estimation of Dalfampridine so an attempt has been made to develop a simple, accurate, sensitive and precise RP-HPLC method. Initially various trails were conducted with different mobile phases and columns. Based on peak parameters 0.01M sodium

acetate buffer (pH4.5): methanol in the ratio of 60:40 v/v selected as optimized mobile phase. Phenomenex (125 mm x 4.6 mm, 5µm) column was optimized for the drug analysis. Flow rate was optimized to 0.8 mL/min. The peak was detected with a short retention time, i.e. 1.713 min with good peak area, theoretical plates and low tailing factor. The developed RP-HPLC method was validated as per the ICH guidelines. No interference peaks reveals that the method is specific. %RSD results were proved that the method was precise. Good percentage recoveries demonstrate the accuracy of the method. Degradation data reveal that the methd was stable to acid, alkali, oxidative, thermal and neutral degradation. These results have disclose that the method was stability indicating and can be suitable for analysis of Dalfampridine in pharmaceutical dosage forms in quality-control laboratories.

References

1. Andrew RB, Herbert RH, and Ron C. Development of Dalfampridine, a novel pharmacologic approach for treating walking impairment in multiple sclerosis. Annals of the New York Academy of Sciences. 2014:33-44.
2. Raymond L, Elsen J, Susan C, Jacob and Olga H. Dalfampridine (Ampyra) an aid to walking in patients with multiple sclerosis. Pharmacy and Therapeutics. 2010;35(12):665-69.
3. Dharani NR, Padmini K and Sumakala S. Stability indicating RP-HPLC method development and validation for estimation of Dalfampridine in its bulk and formulation. International Journal of Advanced Research. 2016;4:184-91.
4. Madhumathi CH, Padmalatha M, Sneha S and Divya CB. Development and validation of UV spectrophotometry method for the estimation of Dalfampridine in tablets. International Journal of Innovative Pharmaceutical Sciences and Research. 2014;2:1019-26.
5. Vivekkumar KR, Gazala LS, Sanjay and Surana J. Development and validation of spectroscopic methods for the estimation of Dalfampridine in bulk and in tablet formulation. Analytical Chemistry. 2014;14(1):37-41.
6. Fatatry HM, Hammad SF and Elagamy SH. Validated spectrofluorimetric determination of Dalfampridine in its synthetic mixture and spiked human plasma through derivatization with flurescamine. Journal of Analytical Techniques. 2013;3:23-26.
7. Jain M, Srivastava V, Kumar R, Dangi, Hiriyanna SG and Kumar A. Determination of five potential genotoxic impurities in Dalfampridine using liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 2016;133:273-78.
8. Babu C, Narasimha Rao KL, Devanna N and Suresh KVN. Development and validation of stability indicating RP-HPLC for the determination of substances in Dalfampridine substances and tablet dosage forms. Asian Journal of Pharmaceutical and Clinical Research. 2017;10(10):334-38.
9. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization. 2005.pp.1-13.