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STABILITY INDICATING RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DALFAMPRIDINE IN ITS BULK AND FORMULATION

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Abstract:

A simple and selective LC method is described for the determination of DALFAMPRIDINE tablet dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of MixedPhosphate buffer (KH2PO4+K2HPO4) pH:3.5 Acetonitrile (30:70v/v/v), with detection of 244 nm. Linearity was observed in the range 35-105 µg /ml for DALFAMPRIDINE (r2 =0.998) for drugs estimated by the proposed methods was in good agreement with the label claim. Several analytical procedures have been proposed for the quantitative estimation of DALFAMPRIDINE separately and in combination with other drugs. To my knowledge simple, rapid analytical method for determination of DALFAMPRIDINE has not been reported so far. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of DALFAMPRIDINE in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

Keywords: *Dalfampridine, RP-HPLC, Method development, Validation*

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INTRODUCTION:

Dalfampridine is a neurofunctional modifier that helps improve walking speed in patients with multiple sclerosis. In MS, axons are progressively demyelinated which exposes potassium channels. As a result, there is a leak of potassium ions which results in the repolarization of cells and a decrease in neuronal excitability. [1] The overall impact is the impairment of neuromuscular transmission as it is harder to trigger an action potential. Dalfampridine inhibits voltage-gated potassium channels in the CNS to maintain the transmembrane potential and prolong action potential. [2] In other words, Dalfampridine works to make sure that the current available is high enough to stimulate conduction in demyelinated axons that are exposed in MS patients. Furthermore, it facilitates neuromuscular and synaptic transmission by relieving conduction blocks in demyelinated axons. [3] IUPAC name is 1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol. Molecular formula $C_5H_5N_2$. Molecular Weight is 94.1.

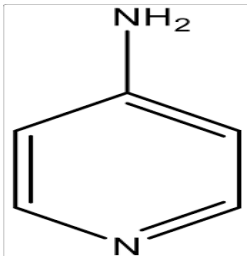

Figure 1: Structure of Dalfampridine

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 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.

MATERIALS AND METHODS:

Chemicals and Reagents: Dalfampridine Gift samples obtained from Madras pharmaceuticals, Chennai. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 244 nm with column INERTSIL column, C18(150x4.6 ID) 5µm, dimensions at Ambient temperature. The optimized mobile phase consists of Methanol: Acetonitrile: water (30:50:20 v/v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Diluent Preparation:
Mobile phase is used as Diluent.

Standard sample
weigh accurately 10 mg of DALFAMPRIDINE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of DALFAMPRIDINE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample:
5 TABLETS(each Tab contains 70 mg of DALFAMPRIDINE were weighed and taken into a mortar uniformly mixed. Test stock solutions of DALFAMPRIDINE (100µg/ml) and was prepared by dissolving weight equivalent to 100 mg of

DALFAMPRIDINE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of DALFAMPRIDINE was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:
To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. The overlay spectrum of Dalfampridine was obtained and the Dalfampridine showed absorbance's maxima at 267 nm. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into INERTSIL column, C18(150x4.6 ID) 5µm column, the mobile phase of composition Methanol: Acetonitrile: water (30:50:20 v/v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation:
The proposed validated method was successfully applied to determine Dalfampridine in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:
Linearity: The linearity study was performed for the concentration of 35 µg/ml to 105 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies:
The accuracy was determined by help of recovery study. The recovery method carried out at three level 80%, 100%,120%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Dalfampridine and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies:
precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness:
To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Forced degradation studies:
The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is "studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline "Stability is defined as the capacity of a drug substance or drug product to remain within

established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods". The results are shown in table 9.

THERMAL DEGRADATION:
Stress testing is likely to be carried out on single batch of the drug substance (API). Thermolytic degradation may lead to hydrolysis / dehydration / isomerization / epimerization / decarboxylation / rearrangements and some kinds of polymerization reactions. ICH guidelines suggest that thermolytic degradation study should be carried out at temperatures (in 10 increments e.g. 50ºC, 60ºC, etc.) above that for accelerated testing and withdraw the sample at different time intervals during reaction condition. If reasonable degradation (i.e. 5-20%) has seen, testing can be stopped at this point.

PHOTOLYTIC DEGRADATION:
The photochemical stability of the drug was studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 24 hours. For HPLC study, the resultant solution was injected into the system and the chromatogram were recorded to assess the stability of sample.

ACIDIC DEGRADATION:
Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N HCl then kept in oven at 60^0c for 1 hour then cool and add 1 ml of 0.1N NaOH it then make up the volume up to 50ml with mobile phase, then place the sample in the vial and measure the chromatogram.

BASE DEGRADATION:
Sample solution (100µg/ml) prepared and transferred into a 50ml volumetric flask and dissolve in mobile phase up to 75% then sonicate it for 10 minutes then add 1 ml of 0.1N NaOH then kept in oven at 60°C for 1 hour then cool it and add 1 ml of 0.1N HCl then make up the volume up to 50ml with mobile. phase, then place the sample in the vial and measure the chromatogram

PEROXIDE DEGRADATION:
Sample solution of Ribociclib (100µg/ml) and 1 ml of 20% hydrogen peroxide (H2O2) was mixed. For HPLC study, 100µg/ml were injected into the system and the chromatogram was recorded to assess the stability of sample.

RESULTS AND DISCUSSION:

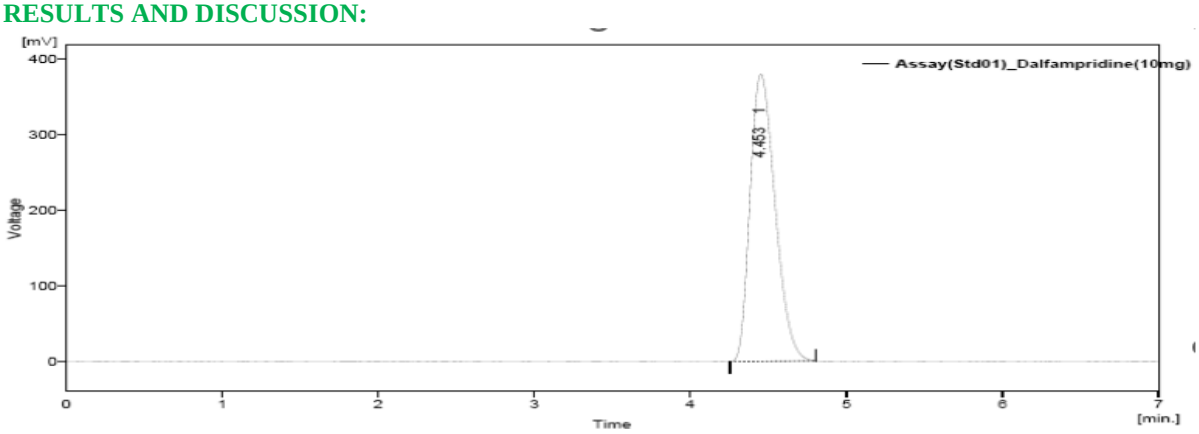

Figure 2: Standard chromatogram

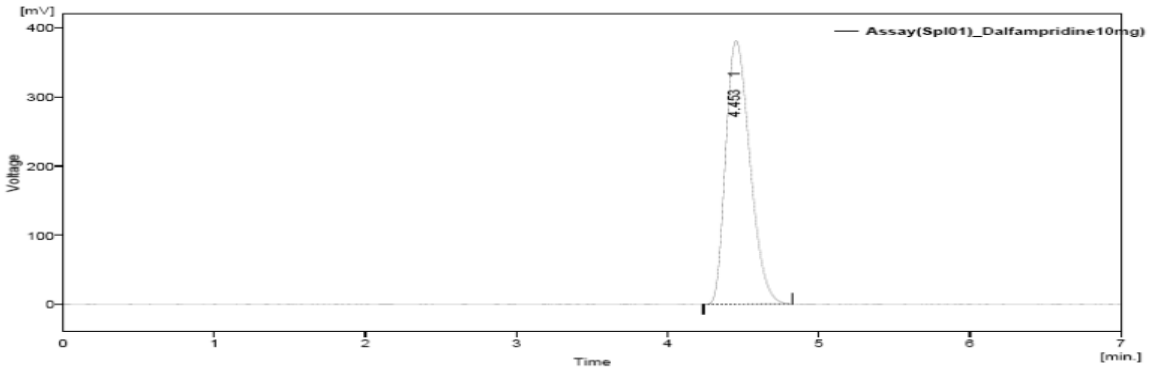

Figure 3: Sample chromatogram

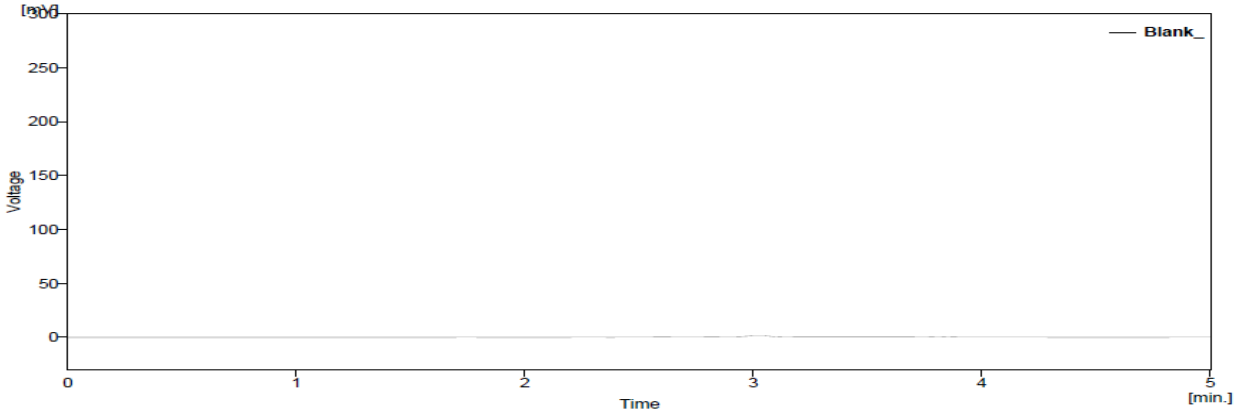

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Injection	**Retention time (min)**	**Peak area**
1	4.389	4256.908
2	4.387	4254.980
3	4.387	4250.876
4	4.387	4256.980
5	4.433	4256.083
6	4.438	4250.980
Mean	4.4035	4254.468
SD	0.0248	2.836
%RSD	**0.14**	**0.27**

Table 2: Assay results for Dalfampridine

DALFAMPRIDINE		
	Standard Area	**Sample Area**
Injection-1	4236.32	4260.493
Injection-2	4256.047	4236.32
Injection-3	4250.49	4256.047
Injection-4	4236.32	4262.647
Injection-5	4260.403	4252.55
Average Area	4247.916	4253.611
Tablet average weight	81	
Standard weight	20	
Sample weight	700	
Label amount	70	
std. purity	99.87	
Amount found in mg	**2.03**	
Assay(%purity)	**101.25**	

Table 3: Linearity results of Dalfampridine

S. No.	**Conc.(µg/ml)**	**Area**
1	35	2490.485
2	52.5	3351.245
3	70	4207.305
4	87.5	5059.046
5	105	5933.427

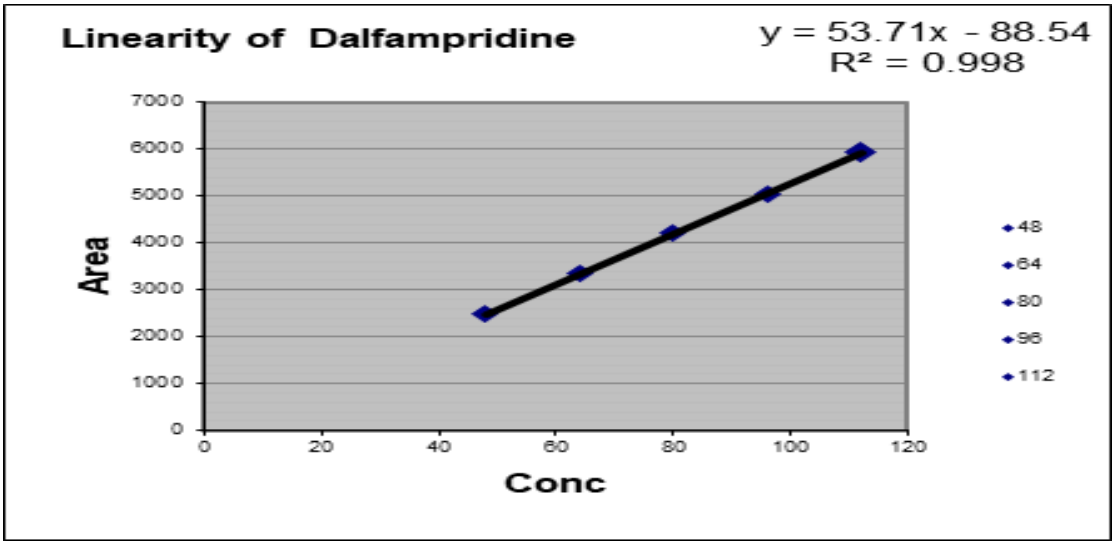

Figure 5: Linearity graph for Dalfampridine

Table 4: Showing accuracy results for Dalfampridine

Recovery level	Accuracy					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	70	**4266.47**	**4255.722**	**71.27**	**101.59**	
	70	**4240.819**				
	70	**4259.877**				
100%	87.5	**5166.633**	**5091.955**	**87.82**	**100.86**	100.45%
	87.5	**5054.273**				
	87.5	**5354.958**				
120%	105	**5833.427**	**5838.054**	**105.78**	**98.91**	
	105	**5822.224**				
	105	**5858.512**				

Table 5: Precision results for Dalfampridine

DALFAMPRIDINE		
S.No.	Rt	Area
1	4.38	4255.073
2	4.367	4184.798
3	4.437	4259.877
4	4.397	4262.854
5	4.437	4286.183
6	4.437	4259.877
avg	4.4092	4251.444
St dev	0.0319	34.450
%RSD	**0.72**	**0.81**

Table 6. Ruggedness results of Dalfampridine

DALFAMPRIDINE	**%Assay**
Analyst 01	99.97
Analyst 02	100.11
%RSD	0.13

Table 7: Robustness results for Dalfampridine

Parameter	DALFAMPRIDINE	
	Retention time(min)	Tailing factor
Flow **0.8ml/min** **1.2ml/min**	 5.383 3.847 2.480	 1.600 1.471
Wavelength **331nm** **335nm**	 4.463 4.450	 1.487 1.526

Table 8: LOD, LOQ of Dalfampridine

Drug	LOD	LOQ
Dalfampridine	61.74	4.73

Table 9: Forced degradation study of Dalfampridine

Name of the Degradation	Condition	Peak Purity	Peak Purity Value	%Assay
Thermal Degradation	60°C/7Days	PASS	+	99.07
Photolytic Degradation	1.2mill/LUX Hours	PASS	+	99.01
Acid Degradation	5mL of 3N HCl/4Hrs at 80°C	PASS	+	98.94
Base Degradation	5mL of 3N NaOH Solution/4Hrs at 80°C	PASS	+	98.97
Peroxide Degradation	5mL of 10% H_2O_2/4Hrs at Bench top	PASS	+	99.00

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Dalfampridine in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Dalfampridine in pure and its pharmaceutical dosage forms.

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