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A Novel UV Spectrophotometric Method Development and Validation of Dalfampridine in Bulk and Pharmaceutical Dosage Form

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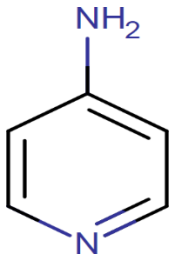
ABSTRACT:
Simple, precise and accurate zero order derivative spectroscopic method has been developed and validated for the estimation of dalfampridine in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ max) at 262nm in 0.1M HCL and obeys Beer's law in the concentration range of 0.5-3.0µg/ml. The linearity study was carried out and regression coefficient was found to be 0.9976 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 98.66% -101.56%. The LOD and LOQ were found to be 0.0360 and 0.109µg/ml. The % relative standard deviation were found to be less than 2. According to ICH guidelines the technique has been validated for linearity, precision, accuracy, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantification of Dalfampridine in bulk and pharmaceutical dosage form.

Keywords: Dalfampridine, zero order derivative spectroscopy, accuracy, 0.1 M HCL.

INTRODUCTION:

Dalfampridine is a potassium channel blocker that enhances conduction in focally demyelinated axons, improves synaptic transmission, and potentiates muscle contraction. Clinically, dalfampridine has been found to improve walking in patients with multiple sclerosis. This is the first drug that was specifically approved to help with mobility in sclerosis patients. It works by strengthening the signals sent by the brain through nerves that have been damaged by multiple sclerosis. Multiple sclerosis is the most common demyelinating disease, in which the insulating covers of nerve cells in the brain and spinal cord are damaged. This damage disrupts the ability of parts of the nervous system to transmit signals. It is an immune-mediated disorder affecting the central nervous system[1-3].

STRUCTURE:



Dalfampridine (4-aminopyridine)

Dalfampridine is chemically known as 4-aminopyridine, with a molecular formula of $C_5H_6N_2$ and a molecular weight of 94.1146 g/mol. Dalfampridine is a white crystalline powder, and it is soluble in polar solvents such as water, methanol, ethanol, and acetonitrile. And consisting of a dose range of 10mg.

MATERIALS AND METHODS:

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Dalfampridine pure drug was obtained as a gift sample from Spectrochem, Mumbai and its pharmaceutical dosage Dalfampridine 20 tablets (Dalstep) labelled claim 10mg from local pharmacy manufactured by sun pharma laboratories ltd.

Solvent: 0.1M HCL is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions of Dalfampridine were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. The absorption spectra obtained and show maximum absorbance at 262nm, as the wavelength for detection.

Preparation of standard stock solution: 100mg of Dalfampridine was weighed accurately and transferred into 100ml volumetric flask and diluted by using 0.1M HCL up to the mark (stock solution 1) From this, stock solution pipetted out 10ml and transferred into 100ml volumetric flask gives 100µg/ml concentration (Stock solution 2). further, prepare the stock solution 3 having the concentration 10µg/ml. From the solution having 10µg/ml concentration pipetted out 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0ml and then transferred into 10ml individual volumetric flask, diluted and made up to the mark by using 0.1M HCL that gives 0.5, 1.0, 1.5, 2.0, 2.5, and 3µg/ml concentration respectively.

Preparation of sample solution: 20 tablets of Dalfampridine marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Dalfampridine was transferred into a 100ml of volumetric flask then it was diluted with 0.1M HCL and made up to the mark.

METHOD AND VALIDATION:

The method was validated according to the ICH guidelines[16-18].

RESULTS AND DISCUSSION:

Method: Zero order derivative spectroscopy

Linearity: The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 0.5-3.0µg/ml was measured at 262nm and absorbance values are shown in table-1. The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in Fig-3. Statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was established by intra-day and inter-day studies. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. shown in table-3.

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table-4.

Ruggedness: The ruggedness is defined as the reliability of results when the method is performed under the variation in conditions. This includes distinct analyst, laboratories, instruments, temperature etc. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (table-5).

LOD and LOQ: The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is a discrete analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated by using following formula.

LOD = 3.3(SD)/S and LOQ = 10(LOD)

LOD and LOQ value of Dalfampridine were found be 0.0360 and 0.109µg/ml.

TABLES:

Table 1: Results of calibration curve at 262nm by zero order spectroscopy

Sl. No	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	0.5	0.127±0.0017
3	1.0	0.222±0.0016
4	1.5	0.307±0.0011
5	2.0	0.403±0.0019
6	2.5	0.503±0.0013
7	3.0	0.611± 0.0013

**Average of six determinations.*

Table 2: Regression parameter of Dalfampridine by zero order spectroscopy

Regression parameter	Results
Range(µg/ml)	0.5-3.0
λmax (nm)	262nm
Regression Equation	Y= 0.0198X+0.0141
Slope(b)	0.0198
Intercept(a)	0.0141
Correlation coefficient(r^2)	0.9978
Sandell's equation	0.0048
Limit of detection(µg/ml)	0.0360
Limit of quantitation(µg/ml)	0.109

Table 3: Determination of precision results for Dalfampridine at 262nm by zero order spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
0.5	0.113±0.0012	1.09	0.127±0.0017	1.04
1.0	0.208±0.0012	0.599	0.222±0.0016	0.734
1.5	0.331±0.0017	0.302	0.307±0.0011	0.306
2.0	0.432±0.001	0.231	0.403±0.0019	0.407
2.5	0.545±0.0014	0.273	0.503±0.0013	0.458
3.0	0.627± 0.0014	0.237	0.611± 0.0013	0.212

**Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Dalfampridine at 262nm by Zero order spectroscopy.

Spiked Levels	Amount of Sample(µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ± Standard deviation*	%RSD**
50	2.0	1	3.02	100.87±0.470	0.468
100	2.0	2	3.09	98.66±0.341	0.345
150	2.0	3	5.02	101.56±0.225	0.221

***Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Dalfampridine at 262nm by Zero order spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.432	0.431
±Standard deviation*	0.0012	0.0011
%RSD	0.277	0.278

***Average of six determinations, **percentage relative standard deviation.*

FIGURES:

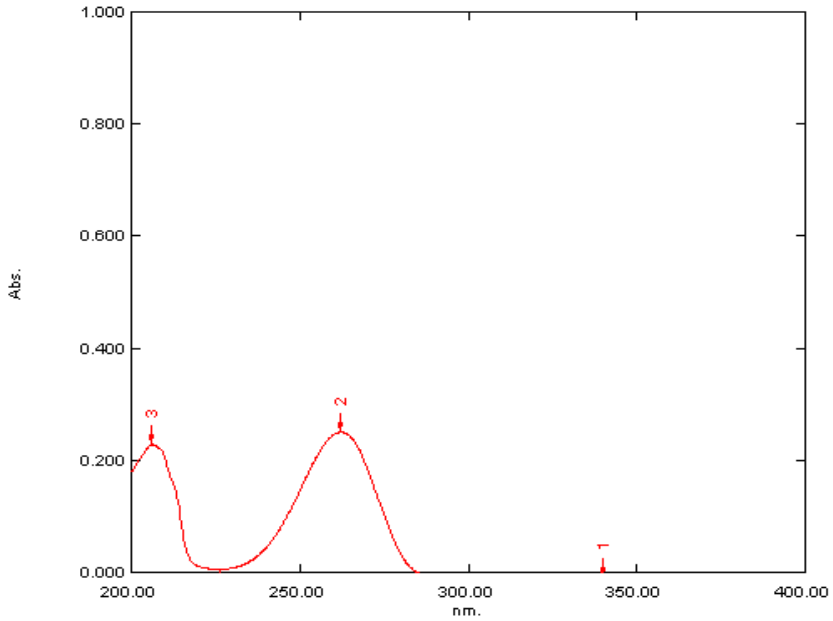


Fig.2: Zero order spectrum of Dalfampridine at 262nm

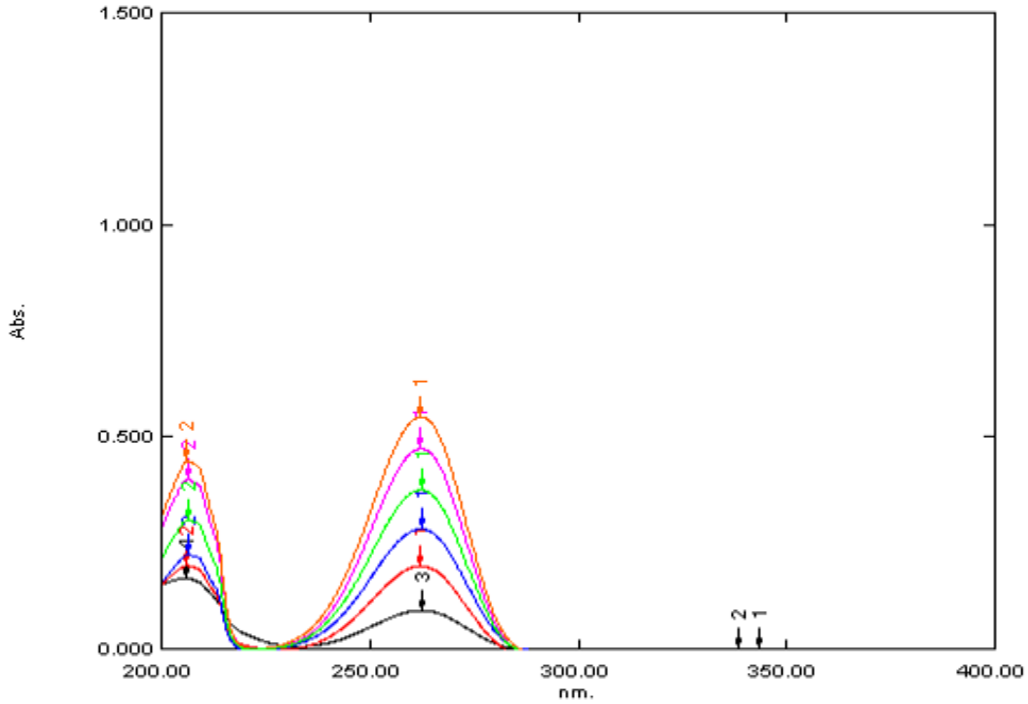


Fig.3: Zero order overlain spectra of Dalfampridine showing absorbance at 262nm.

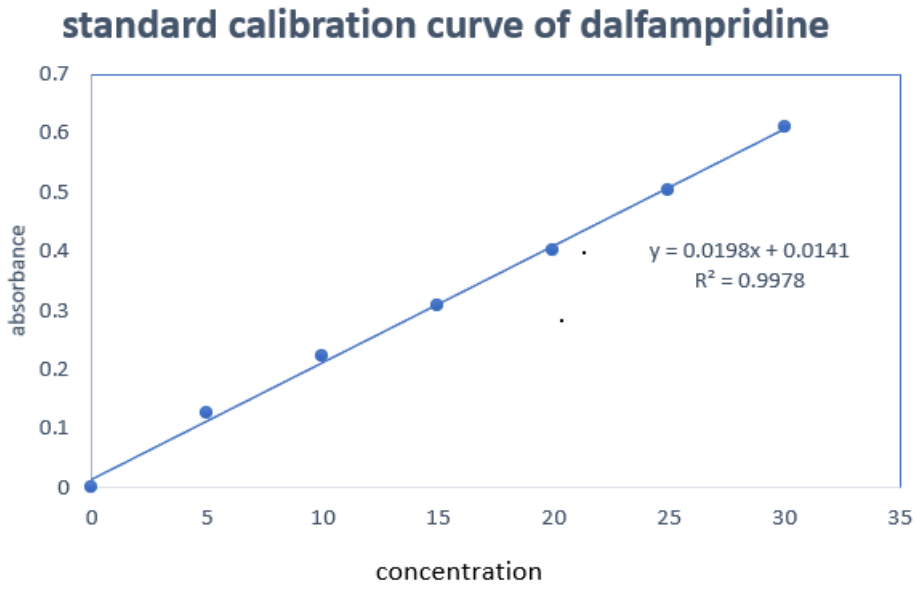


Fig.4: Calibration curve of Dalfampridine by zero order spectroscopy

CONCLUSION:

As per ICH guidelines, the present analytical work was carried out and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Dalfampridine in bulk drug and in pharmaceutical dosage forms.

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