IJBPAS, September, 2023, 12(9): 4022-4031

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UV SPECTROPHOTOMETRY AIDED MULTIVARIATE CALIBRATION TECHNIQUE FOR THE QUANTIFICATION OF DALFAMPRIDINE AND ITS APPLICABILITY IN ITS PHARMACEUTICAL DOSAGE FORM

SUBASH M[1], KAVITHA J[1*], SEETHARAMAN R[1] AND KOKILAMBIGAI K.S[1]

[1]Department of Pharmaceutical Analysis, SRM College of Pharmacy,

SRM Institute of Science and Technology, Kattankulathur, Chengalpattu district – 603 203,

Tamil Nadu, India

[*]**Corresponding Author: Dr. J.Kavitha: E Mail: kavitha0208@gmail.com**

Received 20th June 2022; Revised 25th Aug. 2022; Accepted 4th Dec. 2022; Available online 1st Sept. 2023

https://doi.org/10.31032/IJBPAS/2023/12.9.7366

ABSTRACT

The purpose of the study is to develop and validate a simple, sensitive, and accurate UV spectrophotometric method for the quantification of Dalfampridine in bulk drug and its pharmaceutical dosage form employing multivariate linear regression analysis. Multivariate linear regression analysis was based on the relationship between concentration and absorbance, where the absorbance was measured from five distinct wavelengths, and the results were obtained statistically. The developed approach was linear throughout a concentration range of 5-15 µg/mL, with a correlation coefficient value greater than 0.998. The Absorption maximum (λmax) of Dalfampridine was observed at 244 nm. The developed method was found to be simple, rapid, accurate, and precise in accordance with the ICH guidelines Q2(R1). This approach using statistics gives reliable results without any deviations coming out of the instrument or by experimental conditions.

Keywords: Dalfampridine, Multivariate Linear Regression Analysis, Validation, UV Spectrophotometry

INTRODUCTION:

Multiple sclerosis (MS) is a progressive neurologic disease that results in long-term disability and affects an estimated 400,000 individuals in the United States[1]. Individuals with MS confront a variety of symptoms and disabilities, which can

include ambulatory impairment, visual loss, numbness, weakness, imbalance, bowel and bladder urgency, fatigue, and pain **[1]**. Dalfampridine (DFP) is the first drug approved in the United States by USFDA to improve walking in patients with multiple sclerosis and is chemically known as 4-aminopyridine or fampridine. Ampyra® is an extended release tablet formulation of DFP which was previously called Fampridine-SR **[2]**. Understanding the mechanisms by which DFP exerts its therapeutic effects is a complex issue as it blocks a wide variety of K+ channels that are distributed across multiple cell types in the nervous system but also in the immune system, and because of their molecular identities remaining unknown **[3]**. On literature survey, several analytical methods have been identified to be reported for the quantification of DFP employing techniques such as RP-HPLC **[4]**, HPLC **[5-7]** and GLC **[8]**. The proposed method is optimized and validated ac- cording to ICH guidelines **[9]**.

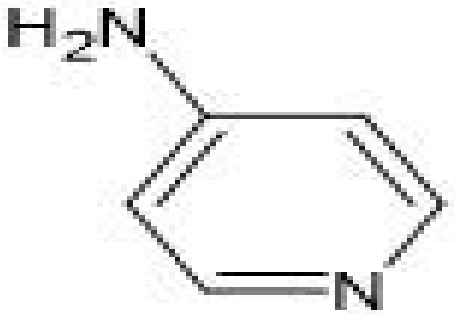

Figure 1: Chemical Structure of DFP

Most laboratories utilize spectrophotometric procedures because of their accuracy, precision, reproducibility, and low cost. The preferred method is based on a direct calculation of DFP that has a high degree of accuracy and precision. The method can be applied to examine DFP and is easy and affordable. The proposed method outlines how to assess DFP in pharmaceutical formulations by means of a UV spectral multilinear regression methodology with fundamental mathematical building blocks.

A single common class determination is converted to "m" dependent variables that can be added to the calibration model at any moment using multilinear regression **[10]**. Under ideal experimental conditions, this statistical technique offers significant sensitivity and resolving ability at a low cost for routine quality control analysis **[11]**. Application of the stated approach is confirmed in accordance with the International Conference on Harmonization (ICH) **[12]**. It was advised that the created technique be verified utilizing the ICH Q2(R1) procedure for analytical method validation in order to confirm its validity.

Amongst some of the recently reported complex analytical techniques, the current study was conceived with the goal of developing a simple, accurate, precise, and sensitive, rapid analytical approach for

the measurement of HC. A simple analytical approach based on UV spectrophotometry facilitated multivariate calibration procedure was suggested to be created based on the preceding assertion **[13, 14]**.

EXPERIMENTAL:

Chemicals and solvents employed:

- Methanol
- The reference standard of DFP (purity – 99.8 %w/w) was obtained as a gift sample from Ideal testing laboratory, Pondicherry.
- AMPYRA® TABLETS – (Label claim – 10 mg of DFP), manufactured by Sun Pharma Laboratories Pvt. Ltd., The marketed tablet formulations were procured from the local market.

Solubility:

- Soluble in water, Methanol, Acetone, Tetrahydrofuran, Isopropanol, Dimethyl formaide, Dimethyl sulphoxide, Ethanol.

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).

METHOD DEVELOPMENT:

Selection of solvent:

Methanol, which was used as the solvent throughout the analysis to solubilize the medication, was reported to be easily soluble in DFP.

Preparation of standard stock solution:

A 100 mL volumetric flask was filled with precisely 10 mg of DFP. The volumetric flask's contents were dissolved using 50mL of the solvent. The final amount was 100 mL and thoroughly mixed with the solvent (1 mg/mL). Pipetting 1 mL of the aforementioned solution into a 100 mL volumetric flask, followed by adding solvent to fill the remaining space and thoroughly mixing the mixture. The resultant solution was subsequently diluted with the solvent to produce concentrations ranging from 5 to 15 g/mL.

Determination of λ_{max}:

The solvent was employed to dilute the standard stock solution of DFP to a concentration of 10 µg/mL. The UV range of 400-200 nm was used to scan this solution. The UV spectra of DFP is shown in figure 2 and the absorbance maxima of DFP was found to be 244 nm. Five wavelengths were selected in and around the absorbance maxima of DFP, such as 240, 242, 244,246 and 248nm for the study.

Preparation of standard solution for linearity:

The solvent was used to further dilute the standard stock solution of DFP to create

concentrations of 7, 8, 9, 10, 11, 12, and 13 g/mL for linearity experiments.

Preparation of sample solution:

The average weights of 10 DFP tablets (DALSTEP TABLETS - Label claim - 10 mg of DFP) were calculated. From the combined content, a weight equal to 10 mg of DFP was measured, dissolved in 50 mL of solvent using sonication for 15 minutes, and then the solvent was added to bring the volume to 100 mL. It was well blended and filtered before use. For further analysis, the filtrate was appropriately diluted.

METHOD VALIDATION:

In accordance with the ICH Q2(R1) procedure, which looked at validation factors like linearity, precision, and accuracy, the created approach was validated.

Linearity:

The absorbance of the prepared linearity concentrations was measured at five different wavelengths around the drug's maximum (244 nm), namely 240, 242, 244, 246 and 248 nm **(Table 1)**, and the overlay UV spectra demonstrating linearity is shown in **Figure 3**. This was done to establish linear correlation and eliminate instrumental fluctuations. The correlation coefficient values for the established linear regression equations were obtained separately for each of the five wavelengths **(Table 2)**. The multivariate calibration linearity built at five different wavelengths and the total absorbance are shown in **Figure 4 (a & b)**.

Precision:

The generated linearity concentrations' absorbance was evaluated at five different wavelengths near the drug's maximum (244 nm), namely 240, 242, 244, 246 and 248 nm **(Table 1)**. **Figure 3** displays the overlay UV spectra proving linearity. To establish linear correlation and get rid of instrumental variations, this was done. For each of the five wavelengths, the correlation coefficient values for the established linear regression equations were determined individually **(Table 2)**. **Figure 4** displays the overall absorbance and the multivariate calibration linearity built at five different wavelengths (a & b).

Accuracy:

Recovery studies were conducted to evaluate the devised technique's accuracy at concentration levels of 50%, 100%, and 150% utilising the conventional addition approach. 0.1 mL of the sample solution was pipetted into three separate 10 mL volumetric flasks from the prepared stock solutions of the standard and sample, and 0.1, 0.5, and 0.7 mL of the standard stock solution were pipetted into the same volumetric flasks, respectively. To raise the final volume to the appropriate level, methanol was utilised. The recovery rates were calculated. The recovery investigations' results are summarised in

Table 7, and the overlay UV spectra showing accuracy are shown in **Figure 7**.

Assay:

At a wavelength of 244 nm, the extracted sample solutions' absorbance was measured. The formulations' drug content was estimated, and the assay findings are summarised in **Table 8**.

RESULTS AND DISCUSSION:

The absorption maxima of DFP were observed at 244 nm using methanol as solvent **(Figure 2)**.

Linearity:

Within the defined concentration range of 5 – 15 µg/mL, the devised technique was found to be linear. All five wavelengths of 240, 242, 244,246 and 248nm were used to construct a linear regression equation. The resulting correlation coefficient values were determined to be more than 0.998 **(Figure 3)**.

Precision:

Intraday and interday precision studies were carried out. The % RSD values for intraday and interday precision were determined to be in the ranges of 0.516 – 0.550 and 0.524 - 0.570, respectively, which were considered within the ICH guidelines' acceptability requirement of 2%. The low estimated % RSD value illustrates the precision of the established approach. **Tables 5 & 6** provide the mean, Standard deviation, and % RSD Relative standard deviation at five selected wavelengths.

Recovery:

The percentage recovery of the drug was calculated and determined to be between 98.2-102 % w/w, which was found to be within the ICH protocol's limit of 97 – 103 percent w/w. As a result, the approach can be considered accurate **(Table 7, Figure 7)**.

Assay:

The absorbance of the sample solution was recorded at 242 nm and the quantity of DFP present in the tablet formulations was calculated. The assay percentage of the drug was found to be 99.58 % w/w and the calculated % RSD was found to be less than 2% **(Table 8)**.

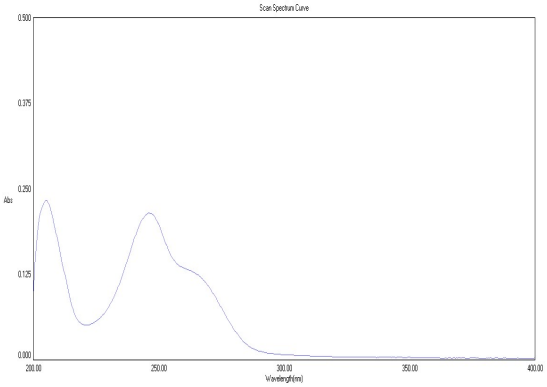

Figure 2: UV spectra of DFP

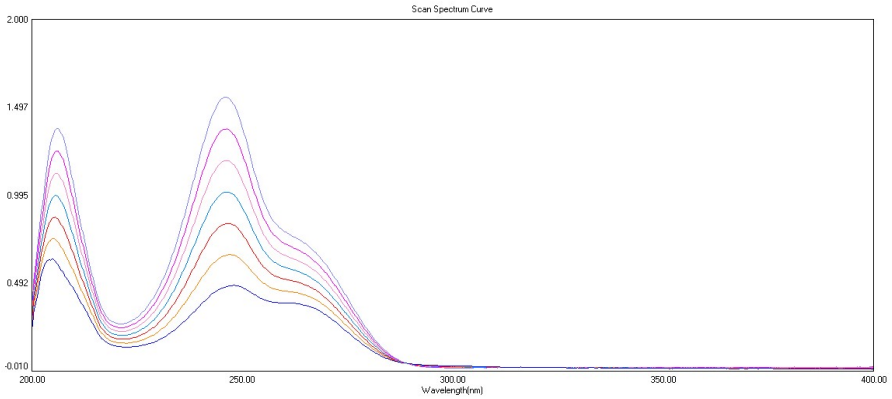

Figure 3: Overlay spectra of DFP showing Linearity

Table 1: Absorbance values at five selected wavelengths

Concentration (µg/mL)	240 nm	242 nm	244 nm	246 nm	248 nm
7	0.361	0.408	0.448	0.469	0.479
8	0.504	0.57	0.652	0.65	0.652
9	0.646	0.733	0.825	0.832	0.825
10	0.789	0.895	0.998	0.789	0.998
11	0.931	1.058	1.153	1.194	1.172
12	1.074	1.220	1.330	1.375	1.345
13	1.216	1.383	1.506	1.557	1.518

Table 2: Linearity data showing statistical parameters at all five wavelengths

Wavelength (nm)	Slope	Intercept	Regression equation	r^2
240	0.032	0.0003162	y = 0.1425x -0.6363	0.9995
242	0.033	0.00031622	y = 0.1625x - 0.7297	0.9991
244	0.0314	0.00036515	y = 0.1763x - 0.7829	0.9996
246	0.0327	0.0003162	y = 0.177x - 0.7657	0.997
248	0.0326	0.00036148	y = 0.1732x - 0.7337	0.998

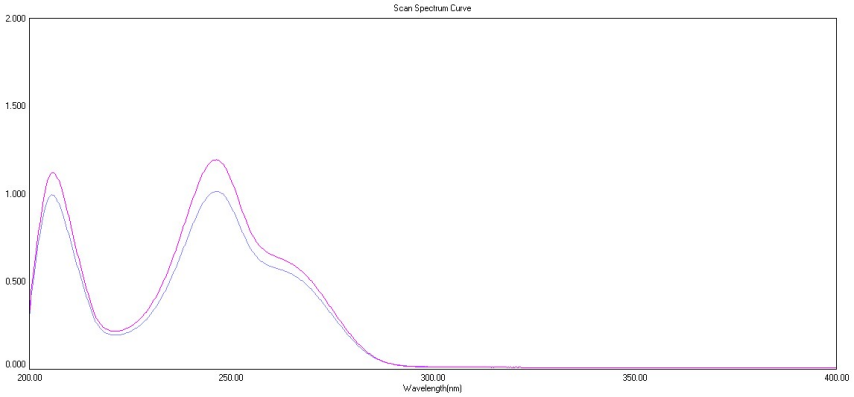

Figure 5: Overlay UV Spectra of DFP showing intraday precision studies

Table 3: Intraday precision at five selected wavelengths

Concentration (µg/mL)	No. of Repetitions	Absorbance (nm)				
		240	242	244	246	248
10	1	0.642	0.667	0.627	0.658	0.651
	2	0.649	0.671	0.629	0.662	0.657
	3	0.646	0.733	0.801	0.832	0.825
	4	0.651	0.671	0.637	0.659	0.649
	5	0.639	0.672	0.639	0.659	0.646
	6	0.637	0.668	0.641	0.661	0.654

Table 4: Intraday Precision of DFP showing Mean, SD, and % RSD

Concentration (µg/mL)	Description	240 nm	242 nm	244 nm	246 nm	248 nm
10	Mean	0.6440	0.6803	0.6623	0.6885	0.6803
	SD	0.0056	0.0259	0.0682	0.0703	0.0710
	% RSD	0.8673	3.8031	10.2910	10.2129	10.4324

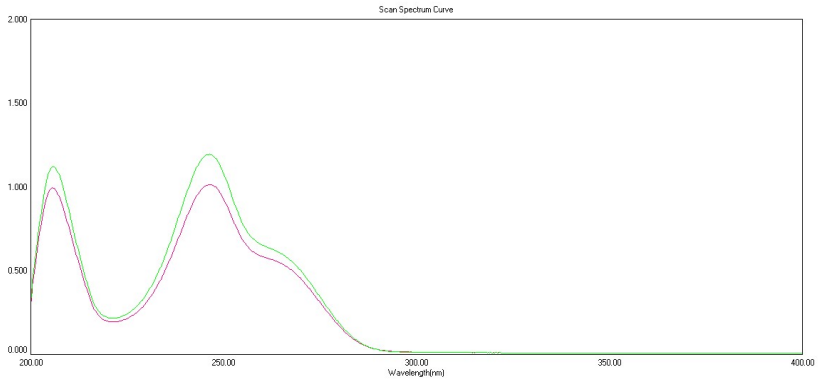

Figure 6: Overlay UV Spectra of DFP showing interday precision studies

Table 5: Interday precision at five selected wavelengths

Concentration (µg/ mL)	No. of Repetitions	Absorbance (nm)				
		240	242	244	246	248
10	1	0.642	0.667	0.631	0.659	0.651
	2	0.649	0.669	0.636	0.662	0.657
	3	0.646	0.733	0.801	0.832	0.825
	4	0.648	0.671	0.637	0.659	0.659
	5	0.649	0.67	0.639	0.659	0.656
	6	0.641	0.668	0.631	0.661	0.654

Concentration (µg/mL)	Description	240 nm	242 nm	244 nm	246 nm	248 nm
10	Mean	0.6458	0.6797	0.6625	0.6887	0.6837
	SD	0.0035	0.0262	0.0679	0.0702	0.0693
	% RSD	0.5489	3.8498	10.2534	10.1980	10.2534

Table 6: Interday Precision of DFP showing Mean, SD, and % RSD

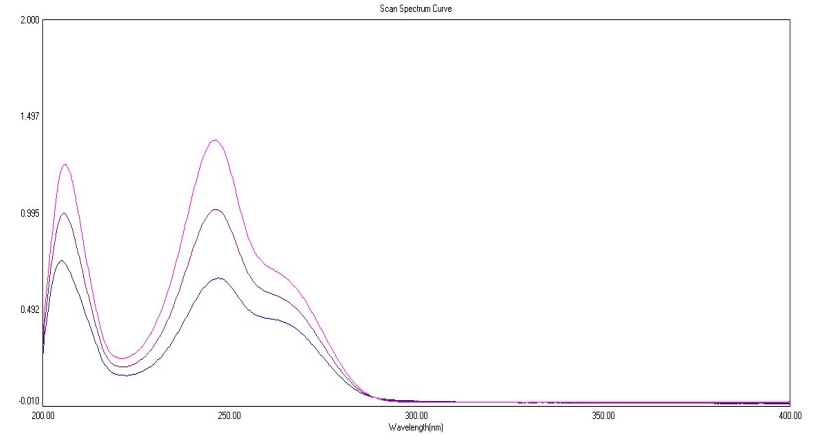

Figure 7: Recovery studies of DFP at five selected wavelengths

Table 7: Recovery studies of DFP at five selected wavelengths

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery
240	2	3	4.9	98.00
		8	9.9	99.00
		13	14.7	98.00
242	2	3	4.79	95.80
		8	9.95	99.50
		13	14.86	99.07
244	2	3	4.81	96.20
		8	9.91	99.10
		13	14.6	97.33
246	2	3	4.9	98.00
		8	9.9	99.00
		13	14.7	98.00
248	2	3	4.81	96.20
		8	9.91	99.10
		13	14.6	97.33

Table 8: Assay of DFP in marketed pharmaceutical formulations

Label claim (mg)	Amount estimated (mg)	%Assay	Average (n = 3)	SD	% RSD
DFP (10mg)	9.9	99.00	98.83	0.7638	0.7728
	9.8	98.00			
	9.95	99.50			

CONCLUSION:

For evaluating DFP in pharmaceutical formulation, the suggested straightforward and speedy UV spectrophotometric-assisted multivariate calibration approach was proven to be linear, sensitive, accurate, and exact. Since the drug's absorbance is measured at five different selected wavelengths, the multivariate calibration methodology has been said to be superior to the previous processes published. This makes the method more accurate. As a result, a quick and easy method based on mathematical elements was created. This method is highly recommended for routine quality control testing of DFP in pharmaceutical formulations since it is more predictable than prior spectrophotometric procedures.

ACKNOWLEDGEMENT:

The authors are thankful to The Management of SRM Institute of Science and Technology and SRM College of Pharmacy, Kattankulathur, for successfully providing various reprographic sources for this research work.

CONFLICT OF INTEREST:

The authors report no conflicts of interest in the study.

REFERENCE:

[1] J. Dunn and A. Blight, "Dalfampridine: A brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis," *Curr. Med. Res. Opin.*, vol. 27, no. 7, pp. 1415–1423, 2011, doi:

10.1185/03007995.2011.583229.

[2] S. Thomas, S. Shandilya, A. Bharti, and A. Agarwal, "A stability indicating simultaneous dual wavelength UV-HPLC method for the determination of potential impurities in fampridine active pharmaceutical ingredient," *J. Pharm. Biomed. Anal.*, vol. 58, no. 1, pp. 136–140, 2012, doi: 10.1016/j.jpba.2011.09.009.

[3] C. Espejo and X. Montalban, "Dalfampridine in multiple sclerosis: From symptomatic treatment to immunomodulation," *Clin. Immunol.*, vol. 142, no. 1, pp. 84–92, 2012, doi: 10.1016/j.clim.2011.06.004.

[4] V. Jain, R. Dehariya, and G. Parkhe, "Method development and validation for estimation of dalfampridine in synthetic mixture by using UV spectrophotometry and RP-HPLC," *EAS J Pharm Pharmcol*, vol. 1, no. 4, pp. 1–4, 2019, doi: 10.36349/easjpp.2019.v01i04.001.

[5] M. Jain *et al.*, "Determination of five potential genotoxic impurities in dalfampridine using liquid chromatography," *J. Pharm. Biomed. Anal.*, vol. 133, pp. 27–31, 2017, doi: 10.1016/j.jpba.2016.10.013.

[6] A. van der Horst, P. N. F. C. de Goede, H. A. M. van Diemen, C. H. Polman, and H. J. M. Martens, "Determination of 4-aminopyridine in serum by solid-phase extraction and high-performance liquid chromatography," *J. Chromatogr. B Biomed. Sci. Appl.*, vol. 574, no. 1, pp. 166–169, 1992, doi: 10.1016/0378-4347(92)80114-6.

[7] S. W. Casteel and B. R. Thomas, "A High-Performance Liquid Chromatography Method for Determination of 4-Aminopyridine in Tissues and Urine," *J. Vet. Diagnostic Investig.*, vol. 2, no. 2, pp. 132–134, 1990, doi: 10.1177/104063879000200210.

[8] J. Evenhuis, S. Agoston, P. J. Salt, A. R. De Lange, W. Wouthuyzen, and W. Erdmann, "Pharmacokinetics of 4-aminopyridine in human volunteers: A preliminary study using a new GLC method for its estimation," *Br. J. Anaesth.*, vol. 53, no. 6, pp. 567–570, 1981, doi: 10.1093/bja/53.6.567.

[9] Koppala Srinivasarao; and Parsharamulu Rayam;, "An Indian Journal Full Paper," *Anal. Chem. an indian J.*, vol. 13, no. 1, pp. 69–76, 2013.

[10] D. Naveena Rani, K. S.

Kokilambigai, and K. S. Lakshmi, "Multivariate calibration technique for the spectrophotometric quantification of rasagiline in bulk drug and pharmaceutical formulations," *Res. J. Pharm. Technol.*, vol. 13, no. 2, pp. 843–849, 2020, doi: 10.5958/0974-360X.2020.00159.6.

[11] Sai Susmitha A, Kokilambigai KS, and Lakshmi KS. 2019. Spectrophotometric Quantification of Telmisartan Employing Multivariate Calibration Technique in Bulk and Pharmaceutical Formulations. Res. J. Pharm. Technol. 12, 1799. doi: 10.5958/0974-360X.2019.00300.7.

[12] Kokilambigai KS, Seetharaman R, Kavitha J, Sowndaravel P, and Lakshmi KS. 2017. Multivariate calibration technique for the spectrophotometric quantification of Zaleplon in bulk drug and pharmaceutical formulations. J. Pharm. Sci. Res. 9, 824–829. doi: 10.22159/ajpcr.2019.v12i2.29416.

[13] Manikanthakumar J, Chandrasekaran N. 2010. UV-Spectrophotometric Determination of Abacavir Sulphate in Bulk and Tablet Formulations. Res. J. Pharm. Technol. 3, 1122–1124.

[14] Varadharajan K, Anandakumarss K. 2010. Estimation of Tadalafil in Bulk and in Formulation by UV-Visible Spectrophotometry. Asian J. Res. Chem. 3, 54–57.