AN ECONOMICAL ASSAY METHOD FOR THE SPECTROSCOPIC QUANTIFICATION OF AN ANTIVIRAL DRUG

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Abstract

Objective: UV spectroscopy method provides a new and economical path for precise, linear and accurate estimation of the drug Favipiravir. Because of its broad field of antiviral activities efforts were made for collection of qualitative and quantitative data for development and validation according to ICH guidelines.

Materials & method: Zero order and first order derivative methods were developed using phosphate buffer pH 4.0, 6.4 and borate buffer 9.0 as solvents. Favipiravir showed absorption maxima at 322 nm, 361 nm and 237 nm respectively.

Results: Favipiravir obeyed linearity in the range of 1- 40 µg/mL and 1 – 50 µg/mL (D^0 & D^1; phosphate pH 4.0); 1 – 40 µg/mL (D^0 & D^1; borate pH 9.0) and 1-30 µg/mL (D^0 & D^1; phosphate pH 6.4) respectively. The % RSD obtained in various precision studies was less than 1.9. The % recovery of the analyte was within 98.1 – 101.28 indicating the accuracy. The LOD values were less than 0.151 µg/mL and LOQ values were lower than 0.468 µg/mL making the methods highly sensitive. The assay obtained was within 98.38 – 103.44 % w/w which was within the specified limits without any interference from the excipients.

Conclusion: The UV spectroscopic methods for the analysis of Favipiravir by zero order spectroscopy and first order derivative were found to be simple, sensitive, precise and accurate as per the Q2(R1) guidelines and can be used for assay of bulk drug and pharmaceutical dosage forms.

Keywords: Favipiravir; UV spectroscopy; Zero order; First derivative method; Validation

Introduction

Toyama Chemical in Japan developed favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) (Fig.1), a purine nucleic acid analogue for the treatment of viral diseases, including influenza. This has recently been investigated and proven to be a promising option for COVID-19 management. It operates by preventing the replication of RNA viruses by inhibiting the RNA-dependent RNA polymerase enzyme (Rd-Rp). Rd-Rp is a common enzyme in many RNA viruses. So, Favipiravir can be employed in various types of RNA virus infections such as arenavirus, bunyavirus, and filovirus, also including influenza strains that are resistant to currently available antiviral drugs [1]. During stability investigations and quality examination of Favipiravir, chromatographic techniques like HPLC [2-4], UV spectrophotometric techniques [5,6] and spectrofluorimetry [7] have been employed for quantification of the drug in formulations and human plasma. Derivative spectrophotometry is a powerful analytical tool that is used for collecting qualitative and quantitative data from spectra with several unresolved bands. In this study, an attempt has been made to apply the principles of derivative spectroscopy along with fundamental UV spectroscopy using simple buffers (to improve the stability of the solutions) for quantification of Favipiravir. Favipiravir was estimated by using zero order (D^0) and first order derivative (D^1) methods.

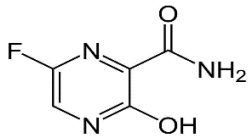

Fig. 1: Chemical structure

Materials and methods

Instruments

The instrument used for the entire analysis was SHIMADZU double beam UV Visible spectrophotometer (Model-UV 1800) with slit width fixed at 1nm, equipped with UV-Probe

system software. A pair of 10 mm matched quartz cells were used to measure the absorbance. The samples were weighed on Shimadzu electronic balance.

Reagents and chemicals

Favipiravir reference standard was gifted by Dr. Reddy's laboratory, Hyderabad. All the chemicals such as methanol, potassium dihydrogen phosphate, disodium hydrogen phosphate, boric acid, sodium hydroxide and glacial acetic acid were obtained from Thermo Fisher Scientific Pvt Ltd. Distilled water was used throughout the study. Commercial Favipiravir tablets (FABIFLU, 400 mg) used for estimation were manufactured by Glenmark pharmaceuticals and procured in the local pharmacy.

Preparation of Phosphate buffer pH 4.0:

3.01 g of potassium dihydrogen phosphate and 5.04 g of disodium hydrogen phosphate was dissolved in 800 mL of water, the pH was adjusted to 4.0 with glacial acetic acid and sufficient water was added to produce 1000 mL [8].

Preparation of Phosphate buffer pH 6.4:

About 1.79g of disodium hydrogen phosphate, 1.36g of potassium dihydrogen phosphate and 7.02g of sodium chloride was dissolved in sufficient water to produce 1000 mL [8].

Preparation of Borate buffer pH 9.0:

6.20 g of boric acid was dissolved in 500 mL of water, the pH was adjusted to 9.0 with 1M sodium hydroxide and sufficient water was added to produce 1000 mL [8].

Preparation of stock solution and working standard

Accurately weighed about 50 mg of Favipiravir was transferred to a 50 ml volumetric flask and dissolved in 50 mL of methanol (1000 µg/mL). From the stock solution 10 mL was diluted in a 100 mL volumetric flask with the respective buffer solution.

Preparation of standard solutions

Aliquots from working standard solution were taken in the range of 0.1 - 50 µg/mL in a series of 10 mL volumetric flasks. The volume was made up to the mark with phosphate buffer pH 4.0, 6.4 and borate buffer pH 9.0 and scanned at 322 nm, 361 nm and 237 nm respectively for zero order and amplitude was measured at the range of 240 nm - 305 nm (phosphate pH 4.0) and 227 nm – 249 nm (phosphate pH 6.4).

Preparation of sample solution

For analysis of commercial tablets (Fabiflu), 20 tablets containing Favipiravir were taken and weighed. The tablets were powdered and the powder equivalent to 50 mg of Favipiravir was taken in a 50 ml volumetric flask, containing 25 mL of methanol and sonicated for 30 minutes. The volume was made up to 50 mL with methanol which was filtered to obtain a clear solution. This was further diluted with phosphate buffer pH 4.0, 6.4 and borate buffer pH 9.0 to get required concentrations.

Method optimization

A 10 µg/mL of Favipiravir was prepared and scanned in the UV region using various solvents like phosphate buffer, sodium acetate buffer, borate buffer and 0.1 N NaOH. Based on the spectral characteristics of Favipiravir obtained with various solvents phosphate pH 4.0, pH 6.4 and borate buffer pH 9.0 were chosen as the suitable solvent for analysis. Two methods namely

zero order and first order derivative spectroscopy were developed for the quantification of Favipiravir.

D^0: Zero order spectroscopic method

The solutions were scanned in the range from 200-400 nm against the respective blanks. Absorption spectra were observed which gave maximum absorbance at 322 nm, 361 nm and 237 nm for phosphate buffer pH 4.0, 6.4 and borate buffer pH 9.0 respectively. The calibration curve was plotted with concentrations vs absorbance and regression coefficient was calculated [9].

D^1: First order derivative spectroscopic method

The zero order spectra were transformed into first order derivative spectra (delta lambda 8, scaling factor 1) using the inbuilt software of the instrument. The first order derivative spectra showed maxima and minima at 305 and 240 nm respectively for phosphate buffer pH 4.0 and 227 nm and 249 nm for phosphate buffer pH 6.4. The amplitudes were calculated by considering the maxima and minima of the curve in the concentration range of 1-50 µg/mL and 1-30 µg/mL for phosphate buffer pH 4.0 and 6.4 respectively. The graph was plotted by using amplitude against concentration and regression equation was calculated [9].

Method validation

The method developed was validated according to International Council of Harmonization (ICH) Q2 (R1) guidelines [10]. The parameters that were determined are linearity, accuracy, precision, quantitation limit and detection limit.

Linearity:

Calibration curves were plotted over a concentration range of 1-40 µg/mL, 1-30 µg/mL and 1-40 µg/mL for phosphate buffer pH 4.0, 6.4 and borate buffer pH 9.0 respectively. Absorbances

were measured at 322 nm, 361 nm and 237 nm respectively for the above solutions. The obtained results are shown in Table 1. For D^0 absorbance versus concentration was plotted and from this curve regression equation was calculated. For D^1 amplitude against concentration was plotted.

Precision:

The precision of the proposed method was ascertained by determining triplicates of three different concentrations 10, 20 and 30 µg/mL (phosphate buffer pH 4.0 and borate buffer pH 9.0) and 5,10 and 15 µg/mL (phosphate buffer pH 6.8) within the linearity range at three different intervals of time on same day (intraday precision) and on different days (interday precision). The % RSD was calculated for both D^0 and D^1 and the obtained results are shown in Table 2a &2b.

Accuracy:

The accuracy of developed method was determined by carrying out recovery studies by standard addition method at three different levels. Pure drug solution at 50 %, 100 % and 150 % were added to pre analyzed formulation solution. Percentage recovery was calculated and the results are shown in Table 3.

Detection Limit and Quantitation Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Detection limit can be determined based on the standard deviation of y- intercepts of regression lines and slope value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. $LOD = 3.3\sigma/S$; $LOQ = 10\sigma/S$. where σ is the standard deviation of the response and S is the slope of the standard curve.

Assay:

Dilutions were prepared within the linearity range from the sample solution as mentioned earlier using phosphate buffer pH 4.0, 6.4 and borate buffer pH 9.0. These solutions were subjected for UV analysis and assay was calculated considering the label claim specified on the tablets. (Table 4)

Results and discussion

The UV visible spectroscopic methods for Favipiravir by zero order derivative (D^0) and first order derivative (D^1) were found to be simple, sensitive, accurate, economical and reproducible. For D^0 the concentration was found to be linear in the range of 1-40 µg/mL (phosphate buffer pH 4.0 and borate buffer pH 9.0) and 1-30 µg/mL (phosphate buffer pH 6.8). The regression coefficient was 0.9997 (phosphate buffer pH 4.0), 0.9981 (phosphate buffer pH 6.4) and 0.9991(borate buffer pH 9.0). For D^1 the concentration was found to be linear in the range of 1-50 µg/mL (phosphate buffer pH 4.0) and 1-30µg/mL (phosphate buffer pH 6.8). The regression coefficient was 0.9996 (phosphate buffer pH 4.0) and 0.0089 (phosphate buffer pH 6.4). This indicates that the developed methods were linear as per the Beer's law. The linearity plots and overlain spectra in both the methods are given in figure 2a-2e and 3a-3e.

Table 1: Linearity study data

Conc. (µg/mL)	Absorbance				
	Phosphate pH 4.0		Phosphate pH 6.4		Borate 9.0
	D^0	D^1	D^0	D^1	D^0
1	0.0555	0.0045	0.079	0.013	0.066
2	0.1114	0.009	-	-	0.144
5	0.2626	0.0205	0.305	0.034	0.355
10	0.5151	0.0396	0.589	0.063	0.625
15	-	-	0.865	0.092	-
20	1.0636	0.0767	1.194	0.121	1.280
30	1.5325	0.1139	1.715	0.179	1.880
40	2.0679	0.1489	-	-	2.424
50	-	0.1835	-	-	-

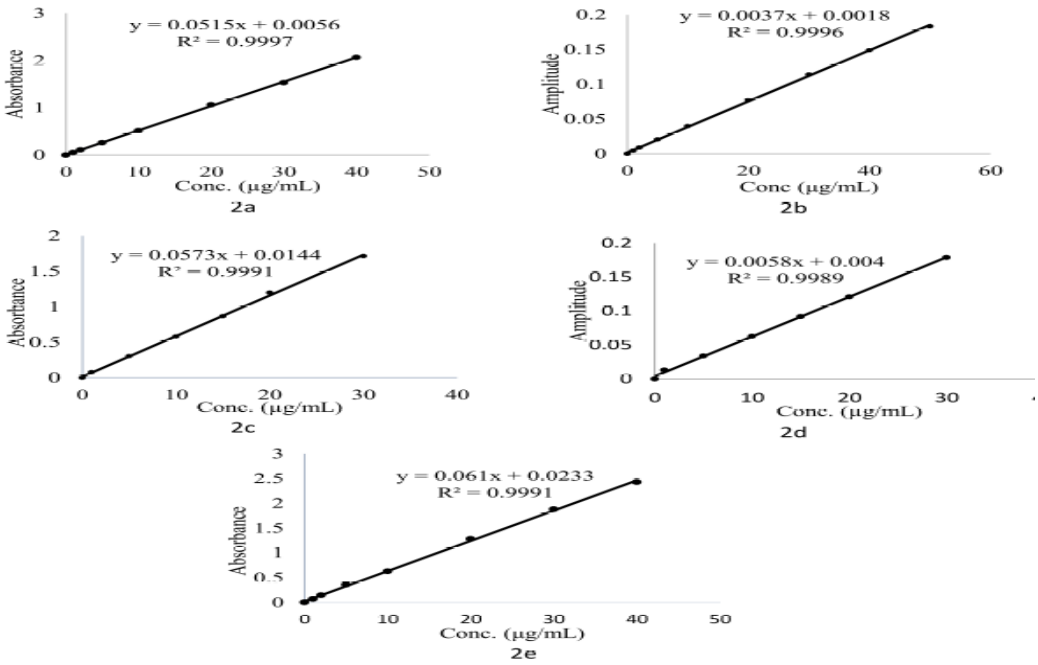

Fig. 2a-2e: Linearity plots for linearity study; 2a&2b: D^0, D^1(phosphate buffer pH 4.0); 2c&2d: D^0, D^1(phosphate buffer pH 6.4); 2e: D^0(borate buffer pH 9.0)

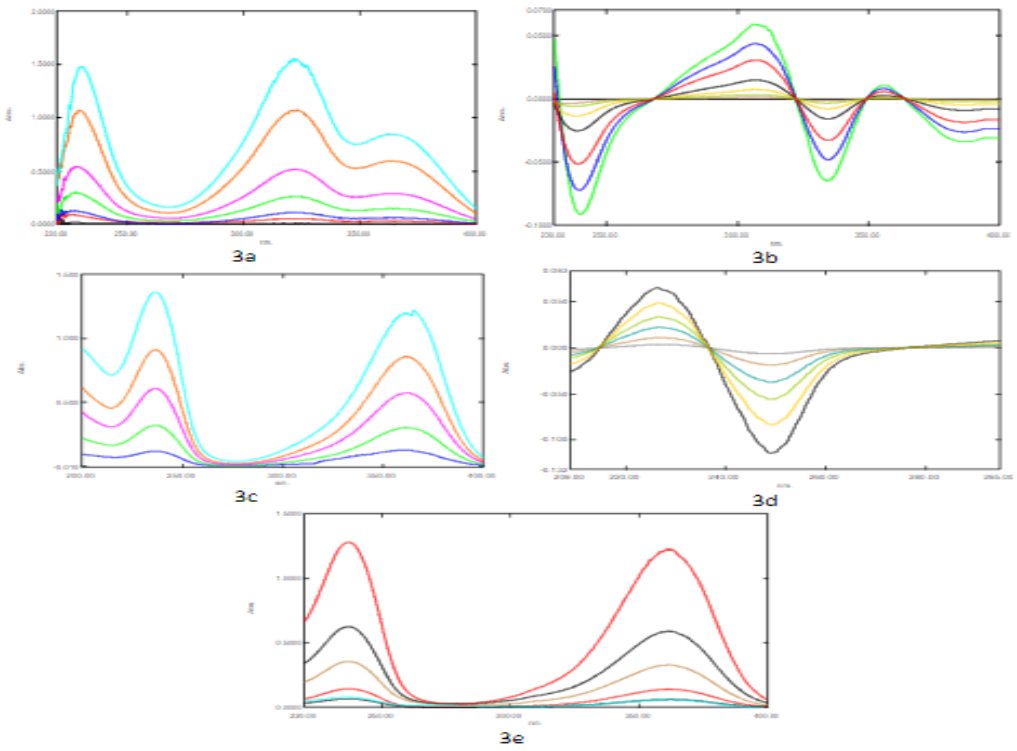

Fig. 3a-3e: Overlain spectrum for linearity study; 3a&3b: D^0, D^1(phosphate buffer pH 4.0); 3c&3d: D^0, D^1(phosphate buffer pH 6.4); 3e: D^0(borate buffer pH 9.0)

In precision study, for intraday the percent relative standard (% RSD) for D^0 was found to be in the range of 0.089 - 1.28 (phosphate buffer pH 4.0); 0.354 - 0.669 (phosphate buffer pH 6.4); 0.680 - 1.120 (borate buffer pH 9.0) and for D^1 it was found to be in the range of 0.187 - 0.681 (phosphate buffer pH 4.0) and 0.398 - 1.007 (phosphate buffer pH 6.4). Interday % RSD value for D^0 was found to be in the range of 0.444 - 1.825 (phosphate buffer pH 4.0); 0.432 - 0.556 (phosphate buffer pH 6.4); 0.90 - 1.43 (borate buffer pH 9.0) and for D^1 was found to be 0.432-0.556 (phosphate buffer pH 4.0) and 0.658-1.438 (phosphate buffer pH 6.4). The % RSD values for precision studies were found to be less than 2.0, which indicate the methods are precise.

Table 2a: Precision study data (phosphate pH 4.0 & borate pH 9.0)

Conc. (µg/mL)	*Assay (% w/w) ± SD, % RSD					
	Phosphate pH 4.0				Borate pH 9.0	
	Intraday		Interday		Intraday	Interday
	D^0	D^1	D^0	D^1	D^0	D^0
10	98.13 ± 1.16, 1.18	99.89 ± 0.68, 0.68	98.70 ± 0.43, 0.44	101.15 ± 0.56, 0.55	101.13 ± 1.13,1.12	98.70 ± 0.43, 0.44
20	99.16 ± 1.27, 1.28	100.58 ± 0.20, 0.20	98.66 ± 0.46, 0.46	100.44 ± 0.43, 0.43	100.59 ± 0.69,0.68	98.66 ± 0.46, 0.46
30	98.28 ± 0.57, 0.08	100.29 ± 0.18, 0.18	98.69 ± 1.80, 1.82	101.44 ± 0.47,0.47	100.41 ± 0.83, 0.83	98.69 ± 1.80, 1.82

* Mean of three determinations

Table 2b: Precision study data (phosphate buffer pH 6.4)

Conc. (µg/mL)	*Assay (% w/w) ± SD, % RSD			
	Phosphate pH 6.4			
	Intraday		Interday	
	D^0	D^1	D^0	D^1
5	99.38 ± 0.35, 0.35	100.15 ± 0.39, 0.39	98.92 ± 0.73, 0.74	100.44 ± 1.44, 1.43
10	99.48 ± 0.66, 0.66	98.81 ± 0.99, 1.01	99.90 ± 0.36, 0.36	99.96 ± 1.24,1.24
15	99.44 ± 0.42, 0.42	99.70 ± 0.78, 0.78	99.32 ± 0.47, 0.47	101.06 ± 0.67, 0.65

* Mean of three determinations

The accuracy of the method was assessed by recovery studies at three different levels i.e., 50%, 100%, 150%. The values of % recovery was close to 100 % for both D^0 and D^1 which indicates the accuracy of the methods.

Table 3: Accuracy data

Level (%)	*Recovery (%) ± SD, % RSD				
	Phosphate pH 4.0		Phosphate pH 6.4		Borate 9.0
	D^0	D^1	D^0	D^1	D^0
50	101.28 ± 0.19, 0.19	100.64 ± 0.55, 0.54	98.15 ± 0.703, 0.71	99.82 ± 1.40, 1.40	100.40 ± 0.65, 0.65
100	98.71 ± 0.58, 0.59	100.08 ± 0.47, 0.47	99.60 ± 0.99, 1.01	99.07 ± 0.81, 0.82	100.84 ± 0.69, 0.68
150	100.95 ± 0.74, 0.73	100.35 ± 0.34, 0.34	98.401 ± 0.33, 0.37	99.68 ± 0.58, 0.58	100.40 ± 0.82, 0.81

* Mean of three determinations

The LOD values for D^0 were 0.151µg/mL (phosphate buffer pH 4.0); 0.029 µg/mL (phosphate buffer pH 6.4); 0.106 µg/mL (borate buffer pH 9.0) and for D^1 the values are 0.145 µg/mL (phosphate buffer pH 4.0) and 0.087µg/mL (phosphate buffer pH 6.4). The LOQ values for D^0 were 0.461 µg/mL (phosphate buffer pH 4.0); 0.087 µg/mL (phosphate buffer pH 6.4); 0.323 µg/mL (borate buffer pH 9.0) and for D^1 the values were 0.468 µg/mL and 0.263 µg/mL for phosphate buffer pH 4.0 and 6.4 respectively. The obtained values indicate the sensitivity of the proposed methods which is comparable and lower than the reported methods The methods were successfully used to determine the amount of Favipiravir present in tablet without any interference from the excipients. The results obtained are in good agreement with the corresponding labeled amount.

Table 4: Assay data

Buffer	Obtained amount (mg)		*Assay (% w/w) ± S.D, % RSD	
	D^0	D^1	D^0	D^1
Phosphate pH 4.0	413.75	404.04	103.44 ± 1.72, 1.66	101.01 ± 0.42, 0.42
Phosphate pH 6.4	396.89	393.52	99.22 ± 0.55, 0.55	98.38 ± 1.60, 1.70
Borate pH 9.0	399.01	-	99.75 ± 0.90, 0.90	-

* Mean of three determinations

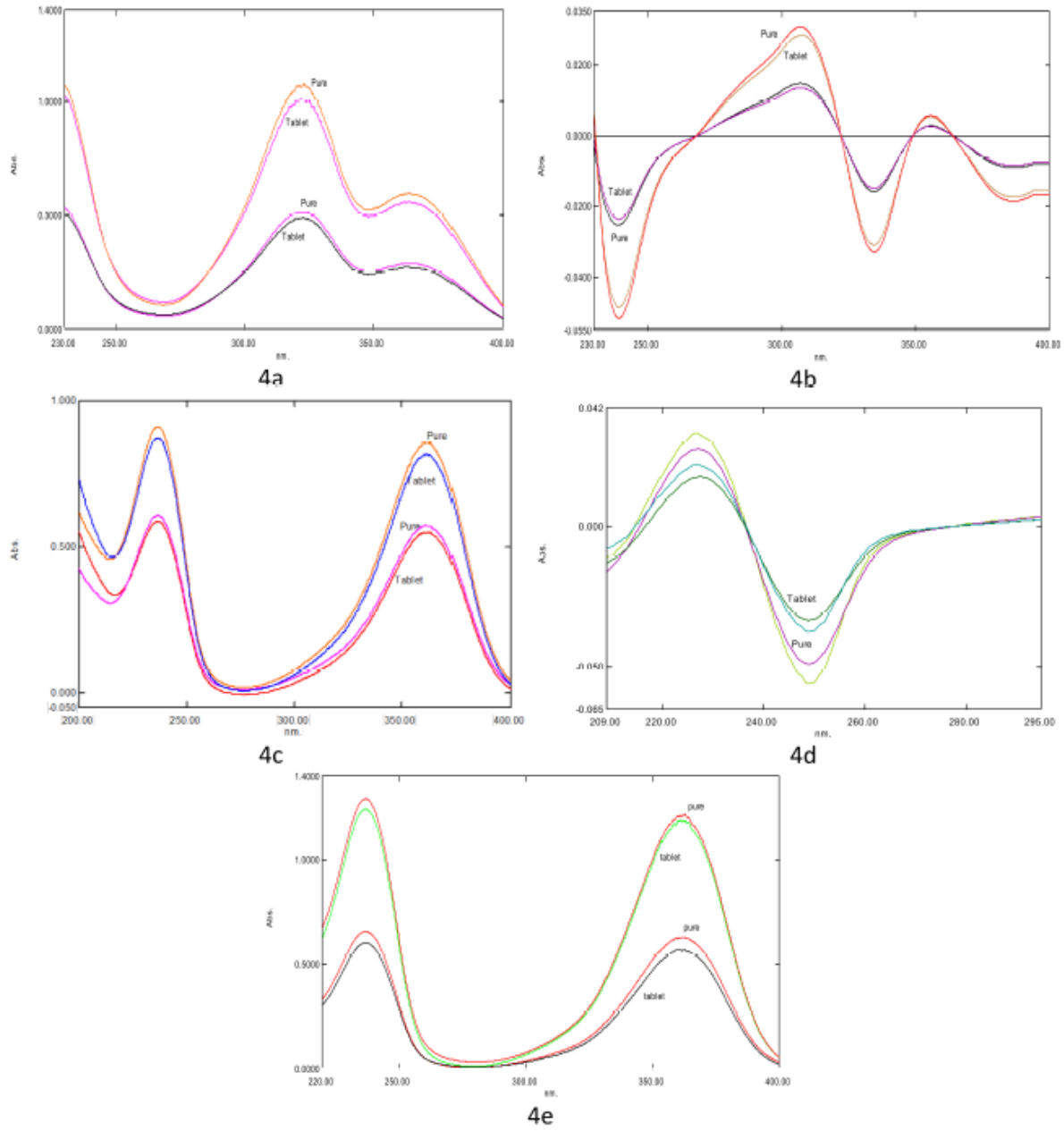

Fig. 4a-4e: Overlain spectrum for assay; 4a&4b: D0, D1(phosphate buffer pH 4.0); 4c&4d: D0, D1(phosphate buffer pH 6.4); 4e: D0(borate buffer pH 9.0)

Conclusion:

The UV spectroscopic methods for the analysis of Favipiravir by Zero order spectroscopy (D^0) and First order derivative (D^1) were found to be simple, economical, precise, accurate and can

be used for the assay of bulk drug and pharmaceutical formulations as a part of regular quality control analysis.

Table 5: Optical characteristics and summary of validation parameters

Parameter		Phosphate pH 4.0		Phosphate pH 6.4		Borate pH 9.0
		D^0	D^1	D^0	D^1	D^0
λ/Amplitude (nm)		322	240 - 305	361	227 – 249	237
Linearity (µg/mL)		1.0 - 40.0	1.0 - 50.0	1.0 - 30.0		1.0 - 40.0
Sandell's sensitivity (µg/cm^2/0.001 abs unit)		0.01941	-	0.0169	-	0.01601
Molar extinction coefficient (L mol^{-1} cm^{-1})		8283.17	-	9754.85	-	10296.3
Precision (% RSD)	Intraday	0.089 – 1.28	0.187 – 0.681	0.354 – 0.669	0.398 – 1.007	0.680 – 1.120
	Interday	0.444 – 1.825	0.432 – 0.556	0.366 – 0.742	0.658 – 1.438	0.90 – 1.43
Accuracy (% Recovery)		98.71 – 100.24	100.08 – 100.6	98.15 – 99.60	99.07 – 99.82	100.40 – 101.58
LOD (µg/mL)		0.151	0.145	0.029	0.087	0.106
LOQ (µg/mL)		0.461	0.468	0.087	0.263	0.323

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References:

1. Umang A, Reyma R, Zarir U. Favipiravir: A new and emerging antiviral option in COVID-19, Med J Armed Forces India. 2020; 1(1):1-7.
2. Ramarao N, Abhinandana P, A validated high performance liquid chromatographic method for the quantification of Favipiravir by PDA detector, Int J Life Sci Pharma Res. 2021; 11(2): 181-187.
3. Sayyed A, Lajporiya M. Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. J Pharm Res Int, 2021; 33(48B): 255-271.

4. Srinivas L, Neelu J. Stability indicative and costeffective analytical method development and validation of Favipiravir and Peramivir in bulk and pharmaceutical dosage form by using RP-HPLC. Int. J. Appl. Pharm, 2021; 13(4): 65-71.
5. Jeevana B, Venkata R. UV method development for estimation of new antiviral repurposing drug Favipiravir. Asian J Pharm Clin Res, 2021; 14 (7): 67-69.
6. Ibrahim B. HPLC-UV method for quantification of Favipiravir in pharmaceutical formulations. Acta Chromatogr, 2020; 33(3): 209-215.
7. Megahed SM, Habib AA, Hammad SF, Kamal AH. The experimental design approach for the development of a spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma. Spectrochim Acta A Mol Biomol Spectrosc. Biomol. 2021; 249(1):1-7.
8. Indian Pharmacopoeia 2018, published by The Indian Pharmacopoeia Commission Ghaziabad, Government of India Ministry of Health and Family Welfare.
9. Beckett AH & Stenlake JB. Textbook of Practical Pharmaceutical Chemistry, 4th edition, Part II, C.B.S., Publishers & Distributors, 281-288.
10. ICH Guidelines Q2(R1) (2005) Validation of analytical procedures: text and methodology, International Council for Harmonisation. Geneva.