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EVALUATION OF QUALITY CONTROL OF FAVIPIRAVIR FOR ITS TEN RELATED SUBSTANCES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: DEVELOPMENT OF PROCEDURE AND VALIDATION

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ABSTRACT

This study deals with designing and validating an HPLC procedure for the determination of favipiravir and ten favipiravir-related impurities simultaneously in favipiravir bulk forms. The ten favipiravir related substances investigated herein included 6-Bromo-3-hydroxy-amide; 6-Fluoro-3-hydroxy-nitrile; 6-Chloro-3-hydroxy-amide; Favipiravir acid; 3-Hydroxy amide; 3,6-Difluoro amide; 3,6-Dichloro amide; 6-Chloro-3-hydroxy-nitrile; 3,6-Difluoro nitrile; and 3,6-Dichloro nitrile. Column Inert Sustain C18 is used to analyze favipiravir and favipiravir-related impurities. Mobile phases employed included 20 mM KH_2PO_4 (system A) and 20 mM KH_2PO_4: Acetonitrile combined in 700:300 (vol/vol) parts (system B,) and given to column through gradient elution. The system was examined in accordance with the ICH prerequisite and was capable of providing accurate (96.4-114.8%) and precise (0.1-0.4%) quantitative results. The peak purities measures for favipiravir and its ten impurities revealed specificity. Linearity was evidenced from the LOQ amount level to 120% amount of the specification level (0.0015 mg/mL) with R^2 measures > 0.999 for favipiravir and its ten related impurities. This method concluded it was beneficial for the evaluation expected.

Keywords: Favipiravir, Related Substances, Impurities, Quality Control, HPLC, Evaluation.

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INTRODUCTION

It would need considerable time and plus effort to find a breakthrough antiviral drug that really is uniquely effective towards SARS-CoV-2. Consequently, by default, pharmaceuticals that were previously being administered to treat various viral illnesses have been enacted. One relevant medication is favipiravir (FVPR), which was initially promoted as an antiflu medicine in Japan. The "Drug Controller General of India" has granted emergency authorization for FVPR medication.[1-4] The pharmaceutically potent component must always be monitored in quality-controlling labs at all processes of manufacture pertaining to pharmaceutically acceptable manufacturing standards.[5,6] The screenings of the pharmaceutically potent component as a raw component, as an intermediate while in the controlling process, after packing, and for stability assessment all through the product's shelf life are among these processes. In order to deliver high throughput and reduce analysis costs, such quality-controlling studies demand the availability of quick, environmentally friendly, and economical analytical techniques. Regarding the manufacturing of FVPR molecules, no technique for the quantitative analysis of FVPR-relevant impurities in FVPR bulk molecules has really been disclosed. It is necessitating us to design a methodology for quality control, specifically for the evaluation of FVPR-related substances in FVPR bulk medicine. The ten FVPR-related substances (Table-1) investigated herein this investigation included: 6-Bromo-3-hydroxy-amide (6-B3-HA impurity), 6-Fluoro-3-hydroxy-nitrile (6-F3HN impurity), 6-Chloro-3-hydroxy-amide (6-C3HA impurity), Favipiravir acid (FA impurity), 3-Hydroxy amide (3-HA impurity), 3,6-Difluoro amide (3,6-DFA impurity), 3,6-Dichloro amide (3,6-DCA impurity), 6-Chloro-3-hydroxy-nitrile (6-C3-HN impurity), 3,6-Difluoro nitrile (3,6-DFN impurity), 3,6-Dichloro nitrile (3,6-DCN impurity). The aim of the present research was to establish and validate fully the HPLC method for simultaneously estimating 6-B3-HA

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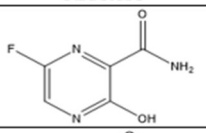
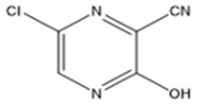
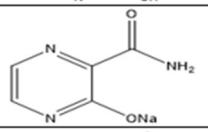
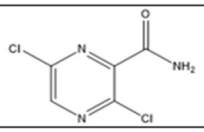
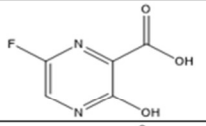
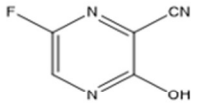
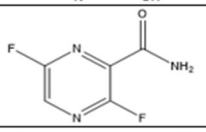
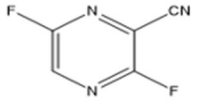
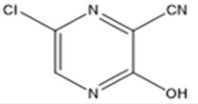
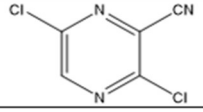
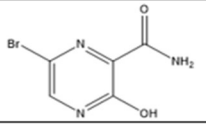
impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity in FVPR bulk medicine.

Table-1: Name and Structures of FVPR and TenFVPR-Related Substances

Impurity/API	Structure	Impurity/API	Structure
Favipiravir		6-chloro -3-hydroxy-amide	
3-Hydroxy amide sodium salt		3,6-Dichloro amide	
Favipiravir acid		6-Fluoro-3-hydroxy nitrile	
3,6-Difluoro amide		3,6-Difluoro nitrile	
6-Chloro-3-hydroxy-Nitrile		3,6-Dichloro nitrile	
6-bromo-3-hydroxy-amide			

EXPERIMENTAL

Equipment

Waters Alliance, 2695 model System chromatography; Photodiode array, 2998 model, Waters alliance; UV detector, 2489 model, Waters alliance; InertSustain C18 (Length = 250 mm, id - 4.6 mm, particle measurement - 3 µm) column; Atlus Sunset. XLST photostability chamber; Julabo SW23 water bath was employed for simultaneously estimating 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity in FVPR bulk medicine.

Chemicals

"Rankem laboratory reagent, India" provided potassium dihydrogen phosphate and phosphoric acid, "Rankem Chemicals, India" provided acetonitrile, and "Milli Q" provided water was employed.

Impurities and FVPR Reference

"Mylan laboratories limited, India" provided 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity, and FVPR bulk reference.

Conditions for Evaluation

The experiment's ideal chromatographic settings were as follows: flow rate, 0.6 mL/min; injection volume, 20 µL; column temperature, 35 °C; sample port temperature, 5 °C. Chromatographic impurity detection was accomplished at 300 nm for 6-F3-HN impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 3,6-DFN impurity, 3,6-DCN impurity & FVPR and 365 nm for 6-C3-HA impurity, 6-B3-HA impurity & 6-C3-HN impurity. Mobile phases included 20 mM KH_2PO_4 (system A) and 20 mM KH_2PO_4: Acetonitrile combined in 300:700 (vol/vol) parts (system B) and given to the column through gradient elution. Phosphoric acid was applied to bring the pH of 20 mM KH_2PO_4 to 2.5 units. The gradient elution line-up included: 0 min (89% system A and 11% system B), 6 min (89% system A and 11% system

B), 14 min (72% system A and 28% system B), 24 min (26% system A and 74% system B), 35 min (26% system A and 74% system B), 38 min (3% system A and 97% system B), 47 min (3% system A and 97% system B), 48 min (89% system A and 11% system B) and 55 min (89% system A and 11% system B). Milli Q water: Acetonitrile combined in 900:100 (vol/vol) parts was exercised as diluent 1 for all FVPR-related impurities while Milli Q water: Acetonitrile combined in 900:100 (vol/vol) parts was exercised as diluent 2, particularly for 3,6-DCN impurity.

Solutions

Stock 1 solution encompassing concentration 0.075 mg/mL each of 6-F3-HN impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 3,6-DFN impurity, 3,6-DCN impurity, 6-C3-HA impurity, 6-B3-HA impurity & 6-C3-HN impurity was prepared using diluent 1. Stock 2 solution encompassing concentration 0.075 mg/mL of 3,6-DCN impurity was prepared using diluent 2. Working FVPR-related impurities solution encompassing concentration 0.0015 mg/mL (0.15 % w.r.t sample amount) each of 3,6-DCN impurity, 6-F3-HN impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 3,6-DFN impurity, 3,6-DCN impurity, 6-C3-HA impurity, 6-B3-HA impurity & 6-C3-HN impurity was prepared through suitable dilution of stock 1 and 2 solutions using diluent 1. Utilizing diluent 1, a stock FVPR solution encompassing a concentration of 1.00 mg/mL was produced. Stock FVPR solution was accurately diluted by diluent 1 to arrange a working FVPR solution encompassing a concentration of 0.0015 mg/mL FVPR. FVPR test solution encompassing a concentration of 1.00 mg/mL was produced with diluent 1.

General Procedure

Injected diluent 1 (blank), working FVPR solution, and FVPR test solution into the chromatograph InertSustain C18 column, analyzed with proposed HPLC procedure, and recorded the peak areas. Contempt the peaks due to diluent 1 (blank). Contempt the peaks due to 6-C3-HA impurity, 6-B3-HA impurity, and 6-C3-HN impurity at 300 nm. Integrate the peaks due to FVPR, 6-C3-HA impurity, 6-B3-HA impurity, and 6-C3-HN impurity only at 365 nm. Calculated the amounts of a 6-F3-HN impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 3,6-DFN impurity, and 3,6-DCN impurity using the expressions a and b below.

$$\text{a) For impurities at 300 nm (\% w/w)} = \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{F}$$

b) Total impurities = Sum of impurities at 300 nm and 365 nm

Where AT infers the area response of impurity in the FVPR test solution; AS infers the Median area response for FVPR from working FVPR solution; DT infers FPVR concentration (mg/mL) in FVPR test solution; DS infers FVPR concentration (mg/mL) in working FVPR solution; P infers FVPR Potency; F infers Measure of the respective impurity's relative response factor in connection to the FVPR (F=1.0). The contents of 6-C3-HA impurity, 6-B3-HA impurity, and 6-C3-HN impurity in the FVPR sample at 365 nm were similarly quantified.

RESULTS AND DISCUSSION

Conditions for Evaluation-Optimization

Throughout this investigation, the HPLC procedure by means of a gradient manner elution was used with 20 mM KH_2PO_4, pH 2.3 (system A), and 20 mM KH_2PO_4 pH 2. 3: Acetonitrile combined in 300: 700 (vol/vol) parts (system B) as mobile phase. The 6-F3-HN impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 3,6-DFN impurity, and 3,6-DCN impurity exhibited intense absorption at 300 nm wavelength, therefore, they were analyzed at 300 nm. Conversely, 6-C3-HA impurity, 6-B3-HA impurity, and 6-C3-HN impurity exhibited intense absorption at 365 nm wavelength. Accordingly, we analyzed 6-C3-HA impurity, 6-B3-HA impurity, and 6-C3-HN impurity at 365 nm. The best column, flow rate, pH, and temperature optimized for simultaneously estimating 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity in FVPR bulk medicine were InertSustain C18 column, 0.6

mL/min, 2.5 units and 35 °C, respectively. Optimum parting was attained at Rt of 19.096 min for 6-B3-HA impurity, 22.680 min for 6-F3-HN impurity, 17.380 min for 6-C3-HA impurity, 7.777 min for FA impurity, 5.529 min for 3-HA impurity, 11.958 min for 3,6-DFA impurity, 21.217 min for 3,6-DCA impurity, 24.246 min for 6-C3-HN impurity, 28.982 min for 3,6-DFN impurity, 34.552 min for 3,6-DCN impurity, and 14.759 min for FVPR.

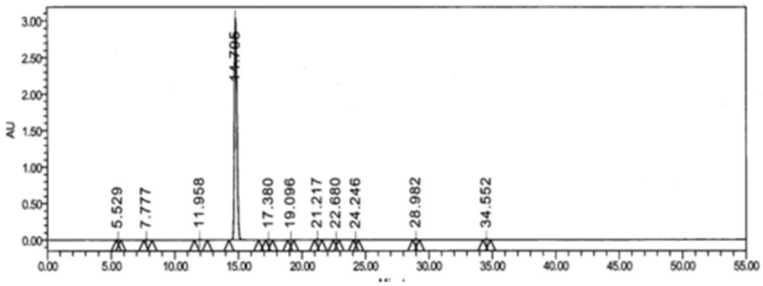


Fig.-1: Chromatogram Got Applying Optimized Set Conditions

Method Validation

Pursuant to "ICH" norms, this novel methodology was validated.[7-10]

Specificity

Spiked 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity at specification level (0.0015 mg/mL) into FVPR test solution (1.00 mg/mL). Utilizing a PDA detection method, the peak purity of FVPR and its mentioned impurities was determined. Table-2 reviews the retention time, purity threshold, relative retention time, purity angle, and resolution estimates obtained for FVPR and its mentioned impurities. The peaks of the FVPR and the aforementioned impurities were suitably separated from one another. The peak purity of the FVPR and its listed impurities was passed, meaning that the purity angle is below the threshold.

Table-2: Summary of Specificity

Impurity/drug	Retention Period(min)	Retention period Ratio	Purity angle	Purity threshold	Resolution
3-HA impurity	5.510	0.37	0.281	1.055	Not available
FA impurity	7.744	0.52	0.152	1.165	8.0
3,6-DFA impurity	11.921	0.81	0.145	1.298	11.7
FVPR (300 nm)	14.784	1.00	0.192	1.004	8.2
FVPR (365 nm)	14.785	1.00	0.192	1.004	Not available
6-C3-HA impurity	17.335	1.17	0.237	1.085	8.1
6-B3-HA impurity	19.049	1.29	0.238	1.080	6.3
3,6-DCA impurity	21.164	1.43	0.182	1.247	8.1
6-F3-HN impurity	22.627	1.53	0.267	1.069	5.8
6-C3-HN impurity	24.196	1.64	0.330	1.053	7.1
3,6- DFN impurity	28.931	1.96	0.099	1.157	21.9
3,6-DCN impurity	34.485	2.34	0.172	1.226	19.7

System Suitability

System compatibility testing is conducted prior to the consideration of each validation criterion. Working FVPR-related impurities solution and working FVPR solution were formulated and analyzed via the proposed HPLC procedure. Measurements for system appropriateness are displayed in Table-3. The system appropriateness measurements met the authorization requirements.

Table-3: Summary of System Suitability

Parameter	1*	2*	3*	4*	5*	6*
LOD and LOQ	7.7	349.4	1.1	27928	0.1	0.4
System precision, Method precision, Accuracy, Solution stability Initial, 22 hr and 44 hr	7.4	43.7	1.4	25526	0.5	0.5
Specificity	8.2	47.2	1.1	31530	0.8	0.5
Linearity	7.8	165.1	1.1	24920	0.4	0.8
Ruggedness	8.1	369.5	1.1	35155	0.5	0.3
Minimum value	7.4	43.7	1.1	24920	0.1	0.3
Maximum value	8.2	369.5	1.4	35155	0.8	0.8

In Table-3, 1* refers to the resolution between FVPR and 6-C3-HA impurity from the working FVPR-related impurities solution at 300 nm; 2* refers to signal to noise value ratio of FVPR from the working FVPR solution at 300 nm; 3* refers to FVPR peak tailing factor from the working FVPR solution at 300 nm; 4* refers to plate count for FVPR peak from the working FVPR solution at 300 nm; 5* refers to % value of RSD for FVPR peak area counts from six duplicate injections of working FVPR solution at 300 nm; 6* refers to % value of RSD for FVPR peak area counts from six duplicate injections of working FVPR solution at 365 nm.

LOD and LOQ

The LOD measure and LOQ measure are calculated centered on the signal response to noise value ratios gained from the working FVPR-related impurities solution. For the FVPR and the aforementioned impurities, LOD & LOQ solutions were formulated in accordance with the desired signal response to noise value ratios. The signal response to noise value ratios observed for FVPR and its mentioned impurities should be at least 3:1 (for LOD) and 10:1 (for LOQ). The LOD measures and LOQ measures obtained for FVPR and its mentioned impurities were itemized in Table-4.

Table-4: Summary of LOD Measures and LOQ Measures

Impurity/drug	LOD measure		LOQ measure	
	Value (mg/mL)	Signal/ noise ratio	Value amount (mg/mL)	Signal/ noise ratio
3-HA impurity	0.00004224059	9.2	0.0001280018	29.5
FA impurity	0.00003393150	7.3	0.0001028227	24.8
3,6-DFA impurity	0.00003300104	6.9	0.0001000031	23.2
FVPR	0.00004337078	8.8	0.0001314266	28.1
6-C3-HA impurity	0.00002069296	6.5	0.0000627060	20.9
6-B3-HA impurity	0.00003421704	8.9	0.0001036880	28.1
3,6-DCA impurity	0.00001987172	6.8	0.0000602173	21.9
6-F3-HN impurity	0.00004444632	7.2	0.0001346858	23.5
6-C3-HN impurity	0.00001978291	9.1	0.0000599482	29.5
3,6- DFN impurity	0.00002011875	8.9	0.0000609659	29.1
3,6-DCN impurity	0.00001960230	6.1	0.0000594009	19.9

Linearity

By examining the solutions spanning from LOQ amount to 120 percent of the specification level, the HPLC procedure's linearity parameter was shown for FVPR and the aforementioned impurities. Linearities were achieved in the range of 0.0001039273 to 0.0062356370 mg/mL (6-B3-HA impurity), 0.0001315908 to 0.0060734210 mg/mL (6-F3-HN impurity), 0.00006206352 to 0.00620635200 mg/mL (6-C3-HA impurity), 0.0001023422 to 0.0061405340 mg/mL (FA impurity), 0.0001275351 to 0.0058862370 mg/mL (3-HA impurity), 0.0001000816 to 0.0060048970 mg/mL (3,6-DFA impurity), 0.00006080976 to 0.00608097600 mg/mL (3,6-DCA impurity), 0.00006093756 to 0.00609375600 mg/mL (6-C3-HN impurity), 0.00006284106 to 0.00628410600 mg/mL (3,6-DFN impurity), 0.00006069960 to

0.00606996000 mg/mL (3,6-DCN impurity) and 0.0001313488 to 0.0060622510 mg/mL (FVPR at 300 nm and 365 nm). All of the impurities originally mentioned, including FVPR, have high linearity ($R^2 \geq 0.999$).

Table-5: Summary of Regression Parameters

Impurity/drug	Slope	Intercept	Correlation Coefficient (R)	R^2
3-HA impurity	26683217.8815	524.7184	1.0000	0.9999
FA impurity	43722791.5200	687.0231	1.0000	0.9999
3,6-DFA impurity	48673373.7050	746.9089	1.0000	0.9999
FVPR at 300 nm	45742017.1021	1898.6639	0.9998	0.9996
FVPR at 365 nm	34511901.4753	1359.0607	0.9998	0.9996
6-C3-HA impurity	49268032.2187	689.2327	1.0000	0.9999
6-B3-HA impurity	42304845.9300	687.1337	1.0000	0.9999
3,6-DCA impurity	60520000.8500	959.5661	1.0000	0.9999
6-F3-HN impurity	28077979.0437	549.9449	1.0000	0.9999
6-C3-HN impurity	55926004.7506	729.1835	1.0000	0.9999
3,6- DFN impurity	78906219.0837	641.6718	0.9999	0.9999
3,6- DCN impurity	67388797.7855	465.5567	1.0000	1.0000

Accuracy

By assessing the solutions comprising the FVPR sample spiked with aforementioned impurities at 10%, 30%, 50%, 100%, and 120% of the specification limit, the accuracy of the procedure was evaluated. Table-6 give the % recovery findings for all aforementioned impurities. The recovery percentages values found for 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, and 3,6-DCN impurity fell between 96.4 and 114.8, which met the standards for acceptability (80.0%- 120.0%).

Table-6: Summary of Recoveries

Level→	Recoveries* of impurities at the level of				
Impurity↓	10%	30%	50%	100%	120%
3-HA	101.77	100.47	98.13	100.57	97.00
FA	102.17	101.13	99.00	99.33	98.03
3,6-DFA	100.57	99.83	99.83	100.03	100.47
6-C3-HA	99.23	99.37	99.40	99.83	98.67
6-B3-HA	113.10	104.47	103.83	101.53	100.60
3,6-DCA	102.57	100.97	100.10	100.57	99.30
6-F3-HN	101.47	100.73	99.00	100.13	98.33
6-C3-HN	101.80	101.03	99.90	100.30	98.93
3,6- DFN	101.10	100.00	97.90	98.87	96.97
3,6- DCN	100.13	101.23	98.83	99.87	98.00

* Mentioned recoveries are the average for three measurements.

Precision

For system precision evaluation, working FVPR-related impurities solution and working FVPR solution were formulated and analyzed via the proposed HPLC procedure. Table-7 presents the data of the peak areas of the FVPR and each of its impurities (6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, and 3,6-DCN impurity). Spiked 6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity at specification level (0.0015 mg/mL) into FVPR test solution (1.00 mg/mL). Utilizing the proposed HPLC procedure, the recovery percentage of FVPR and its mentioned impurities was determined. Table-7 reviews the recovery percentage estimates obtained for FVPR and its mentioned impurities. Both the percentage RSD for peak areas and the percentage RSD for recoveries for each impurity fell within the agreeable ranges (0.1–0.4 for peak areas and 0.4–1.2 for recoveries, respectively).

Table-7: Summary of Precision

Precision →	System		Method	
Impurity↓	Peak area*	RSD %	Recovery* %	RSD %
3-HA impurity	133183.000	0.2	100.367	0.4
FA impurity	214142.167	0.1	99.117	0.5
3,6-DFA impurity	245654.500	0.2	99.850	0.6
6-C3-HA impurity	265558.167	0.2	99.550	0.6
6-B3-HA impurity	211164.833	0.2	101.217	1.2
3,6-DCA impurity	301632.833	0.2	100.283	0.4
6-F3-HN impurity	146520.667	0.1	99.967	0.5
6-C3-HN impurity	268640.833	0.1	100.100	0.4
3,6- DFN impurity	398727.333	0.1	98.583	0.4
3,6- DCN impurity	339603.333	0.2	99.550	0.5
FVPR at 300 nm	224633.667	0.4	Not available	Not available
FVPR at 365 nm	168188.667	0.3	Not available	Not available

* Mentioned recoveries and peak areas are the averages for six measurements.

Ruggedness

The method's robustness has been measured by looking at how comparable the results were from the analysis of six distinctive preparations of an FVPR sample that had been spiked with its mentioned impurities (6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, and 3,6-DCN impurity) at the specification amount limit (0.0015 mg/mL) by various analysts, different devices, and distinct columns on various days. Statistics were applied to compare the outcomes. Ruggedness was made known by the fact that the overall percent RSD measures (0. 3% to 1.3%) of recoveries for every mentioned impurity was beneath 10.0%.

Solution Stability

In order to ascertain how stable, the working solution and the impurities-spiked FVPR solution will be in the diluent as well as sample, the sustainability of the solutions was tested by retaining them in the refrigerator (2–8 °C). The standard working solution and the FVPR solution added with all mentioned impurities were held at the recommended temperature levels and were analyzed at 0 hr, 20 hr, and 44 hr. The percentage basis was gauged (0.2% to 5.4%). The bias from the beginning value must not exceed ±20% for a stable solution. The aforementioned findings show that the working solution and the FVPR solution with added impurities were sustainable for near to 44 hr at 2–8°C.

Robustness

By making subtle adjustments to the method HPLC specifications, such as altering the temperature of the column thermostat, increasing/decreasing pH and flow rate in the mobile phase, and changing the wavelength of the detector, it was probable to test the robustness of this quantitative method. During the course of robustness analysis, a sample of FVPR spiked with all mentioned impurities at a specified limit amount was used. The robustness assessment results are represented in Table-8. Based on percentage recoveries of mentioned impurities (6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, 3,6-DCN impurity), the method proved robust for the temperature of column thermostat alteration, for increasing/decreasing pH and flow rate in the mobile phase and changing the wavelength of the detector.
In Table-8, A* refers to actual conditions (flow: 0.6 mL/min, column oven temperature 35°C, Wavelength 300 nm and 365 nm and pH 2.5); B* refers to low flow: 0.54 mL/min; C* refers too high flow: 0.66 mL/min; D* refers to low column oven temperature 33°C; E* refers to high column oven temperature 37°C; F* refers to low wavelength 298 nm; G* refers to high wavelength 302 nm; H* refers to low wavelength 363 nm; I* refers to high wavelength 367 nm; J* refers to low buffer pH 2.3; K* refers to high buffer pH 2.6.

Table-8: Summary of Data for Robustness

Conditions ↓	Percentage recoveries of impurities									
	3-HA	FA	3,6-DFA	6-C3-HA	6-B3-HA	3,6-DCA	6-F3-HN	6-C3-HN	3,6-DFN	3,6-DCN
A	101.2	100.0	100.9	100.6	102.9	101.1	100.8	100.8	99.3	100.5
B	101.0	100.3	100.4	100.5	100.1	100.4	100.4	100.5	100.3	100.9
C	100.7	100.9	100.9	101.0	101.5	100.8	100.6	100.8	100.6	101.3
D	101.4	100.6	100.7	100.4	100.8	100.9	100.6	100.9	100.5	101.0
E	100.3	100.5	100.3	100.2	100.4	100.5	100.7	100.5	99.9	100.3
F	100.0	100.9	100.8	NO	NO	100.7	100.7	NO	100.6	100.6
G	99.8	101.0	101.2	NO	NO	100.8	100.8	NO	100.8	100.5
H	NO	NO	NO	100.5	100.7	NO	NO	100.5	NO	NO
I	NO	NO	NO	100.5	101.3	NO	NO	100.8	NO	NO
J	100.4	99.9	100.4	100.5	100.2	100.3	100.1	100.3	99.8	100.2
K	100.1	99.7	100.2	100.5	100.0	100.2	100.2	100.3	99.8	100.2

NO – not obtainable

CONCLUSION

In conclusion, a validated HPLC process for FVPR and FVPR-related substances (6-B3-HA impurity, 6-F3-HN impurity, 6-C3-HA impurity, FA impurity, 3-HA impurity, 3,6-DFA impurity, 3,6-DCA impurity, 6-C3-HN impurity, 3,6-DFN impurity, and 3,6-DCN impurity) analysis was optimized and furthermore validated fully. The method formulated was noticed to be robust, easy, accurate, precise, reproducible, and selective as clearly evidenced by statistical data analysis. This procedure may be used for FVPR and its mentioned impurities assessment in quality regulatory laboratories.

CONFLICT OF INTERESTS

A conflict of interest is not present, according to the authors.

AUTHOR CONTRIBUTIONS

All of the authors made predominant contributions to this work, took part in its review and editing, and gave their final approval for publishing. The authors' ORCID identifies, which are shown below, can be used to confirm their research profiles:

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