Ijfans International Journal Of Food And Nutritional Sciences
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 11 , Issue 12 , Dec 2022

Spectrophotometric Analysis of Favipiravir Using Hydrotropic Solubilization Technique: Method Development and Validation

Swati N. Deshmukh[1]*, Jyoti N. Kadam[2], Vanita N. Gade[1], Rani Balasaheb Divekar[1], Trupti Somnath Kajale[1], Shubhangi R. Thopate[1]

[1] CAYMET's Siddhant College of Pharmacy, Sudumbare, Pune-412109, Maharashtra, India.
[2] Alard College of Pharmacy, Marunje, Pune-411057, Maharashtra, India

Corresponding Author*
Dr. Swati N. Deshmukh
Associate Professor, CAYMET's Siddhant College of Pharmacy, Sudumbare, Pune-412109, Maharashtra, India.
Email: swatideshmukhn@gmail.com

Abstract

Background: Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) is a broad-spectrum antiviral agent approved for the treatment of influenza and repurposed for COVID-19 management. It is classified as slightly soluble in water, and most reported analytical methods for its estimation employ expensive and potentially toxic organic solvents. Hydrotropic solubilization is an eco-friendly, cost-effective technique that enhances aqueous solubility of poorly water-soluble drugs without organic solvents, offering a greener alternative for routine analytical applications.

Aim: To develop and validate a simple, eco-friendly, and cost-effective UV spectrophotometric method for the estimation of Favipiravir in pure and pharmaceutical formulations using sodium salicylate as a hydrotropic solubilizing agent.

Materials and Methods: Preliminary solubility studies were performed in distilled water and various hydrotropic agents (sodium benzoate, sodium salicylate, urea, sodium citrate, and sodium acetate) at concentrations of 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M. Sodium salicylate (0.2 M) was selected as the optimal hydrotrope based on maximum solubility enhancement. UV spectrophotometric analysis was performed using a Jasco V-630 double beam spectrophotometer at the wavelength of maximum absorption (λmax = 324 nm). The developed method was validated as per ICH Q2(R1) guidelines for linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

Results: The method demonstrated excellent linearity in the concentration range of 0.3–1.8 µg/mL with a correlation coefficient (r^2) of 0.9959 and a regression equation of Y = 0.5115x + 0.0436. Accuracy studies showed percentage recovery in the range of 97.77–100.72%. Intraday and interday precision studies yielded %RSD values of 0.09% and 0.65%, respectively. The LOD and LOQ were determined to be 0.067614 µg/mL and 0.20489 µg/mL, respectively. Ruggedness studies between two analysts showed %RSD values of 0.62% and 0.43%, and robustness studies with wavelength variation (±2 nm) demonstrated %RSD values ranging from 0.01–1.071%.

Conclusion: A novel, simple, precise, accurate, eco-friendly, and cost-effective UV spectrophotometric method was successfully developed and validated for the estimation of Favipiravir using 0.2 M sodium salicylate as a hydrotropic solubilizing agent. The method eliminates the need for costly and hazardous organic solvents and can be employed for routine quality control analysis of Favipiravir in bulk and pharmaceutical formulations.

Keywords: Eco-friendly; Favipiravir; Hydrotropic solubilization; ICH validation; Sodium salicylate; UV spectrophotometry

INTRODUCTION

Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide), a pyrazine carboxamide derivative originally developed by Fujifilm Toyama Chemical Co. Ltd., Japan, is a potent broad-spectrum inhibitor of RNA-dependent RNA polymerase (RdRp) with documented activity against influenza and a wide variety of RNA viruses [1]. During the COVID-19 pandemic, Favipiravir gained considerable attention as a repurposed therapeutic agent against SARS-CoV-2 and was widely prescribed in several countries including India, Russia, and Japan [2]. The drug belongs to BCS Class III, exhibits a short half-life of 2–5.5 hours, and is classified as slightly soluble in water, presenting significant challenges for its analytical estimation in aqueous media [3].

The estimation of Favipiravir has been accomplished using various analytical techniques including RP-HPLC, HPTLC, LC-MS/MS, UPLC-MS/MS, and UV-visible spectrophotometry [4–8]. However, the majority of these methods rely on organic solvents such as methanol, acetonitrile, and ethanol as mobile phases or diluents. The use of such organic solvents is associated with considerable disadvantages including high cost, toxicity to analysts, environmental pollution, and non-compliance with green analytical chemistry principles [9].

Hydrotropy, first described by Neuberg in 1916, is a solubilization phenomenon whereby the addition of a large concentration of a second solute (hydrotrope) results in a significant enhancement in the aqueous solubility of a poorly water-soluble substance [10]. Hydrotropic agents are typically ionic organic salts such as sodium benzoate, sodium salicylate, urea, nicotinamide, and sodium citrate, which increase solubility through mechanisms including self-aggregation, complex formation, disruption of water structure, and micellar solubilization [11]. The technique offers several advantages including cost-effectiveness, operational simplicity, absence of chemical modification of the drug, eco-friendliness, and elimination of organic solvents [12].

Despite the availability of various analytical methods for Favipiravir, no method employing hydrotropic solubilization technique has been reported to date. This presents a significant gap, particularly considering the growing emphasis on green analytical methodologies in pharmaceutical quality control. The present study was therefore designed to develop and validate a novel, simple, eco-friendly, and cost-effective UV spectrophotometric method for the estimation of Favipiravir using sodium salicylate as a hydrotropic solubilizing agent, validated in accordance with ICH Q2(R1) guidelines [13].

MATERIALS AND METHODS

Chemicals and Reagents

Favipiravir reference standard was procured from a commercial source. Sodium benzoate (LR grade), sodium salicylate (LR grade), urea (LR grade), sodium citrate (LR grade), and sodium acetate (LR grade) were purchased from Fine Lab Chemicals, Mumbai, India. Distilled water

was used throughout the study. All reagents were of laboratory reagent grade and used without further purification.

Instruments

A Jasco V-630 UV-Visible double beam spectrophotometer with matched quartz cuvettes (1 cm path length) was used for all spectrophotometric measurements. A Wensar PGB 200 analytical balance was used for weighing, and an Equitron sonicator was employed for dissolution purposes. The melting point was determined using Thiele's tube by the capillary method.

Physicochemical Characterization of Drug

The authenticity of Favipiravir was confirmed by melting point determination using the capillary method in Thiele's tube. The observed melting point range was 188–190°C, which is consistent with the reported literature value [14].

Preliminary Solubility Studies

Solubility studies were conducted to identify the most suitable hydrotropic agent. Excess Favipiravir was added to separate volumetric flasks containing distilled water and aqueous solutions of five hydrotropic agents (sodium benzoate, sodium salicylate, urea, sodium citrate, and sodium acetate) prepared at molar concentrations of 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M. The mixtures were sonicated for 30 minutes and filtered. The filtrates were analyzed spectrophotometrically to determine relative solubility. Favipiravir demonstrated maximum solubility enhancement in 0.2 M sodium salicylate solution, which was consequently selected as the hydrotropic agent for method development.

Selection of Analytical Wavelength

A qualitative stock solution of Favipiravir (250 µg/mL) was prepared in 0.2 M sodium salicylate solution. The solution was scanned in the UV region (200–400 nm) against a blank of 0.2 M sodium salicylate solution. The wavelength of maximum absorption (λmax) was found to be 324 nm, which was selected for all subsequent analytical measurements.

Preparation of Standard Stock Solution

A standard stock solution of Favipiravir was prepared by dissolving an accurately weighed quantity of drug in 0.2 M sodium salicylate solution to obtain a concentration of 250 µg/mL. From this stock solution, appropriate dilutions were made using 0.2 M sodium salicylate solution to obtain working standard solutions in the concentration range of 0.3–1.8 µg/mL.

Method Validation

The developed method was validated as per ICH Q2(R1) guidelines for the following parameters [13]:

Linearity: Linearity was assessed by measuring absorbance of six standard solutions in the concentration range of 0.3–1.8 µg/mL at 324 nm. The calibration curve was constructed by plotting absorbance versus concentration, and the regression equation and correlation coefficient were determined using the least squares method.

Accuracy: Accuracy was evaluated by the standard addition method at three concentration levels (80%, 100%, and 120%). Known amounts of standard Favipiravir were spiked to a pre-analyzed sample solution, and the percentage recovery was calculated. Each level was analyzed in triplicate.

Precision: Precision was determined as repeatability (intraday) and intermediate precision (interday). Intraday precision was assessed by analyzing six replicates of a standard solution (1 µg/mL) on the same day, while interday precision was determined by analyzing the same concentration on different days. Results were expressed as percentage relative standard deviation (%RSD).

Ruggedness: Ruggedness was evaluated by performing the analysis using two different analysts under identical operational and environmental conditions. The results were compared by calculating %RSD.

Robustness: Robustness was assessed by introducing deliberate small variations in the analytical wavelength (±2 nm, i.e., 322 nm, 324 nm, and 326 nm). The effect on absorbance readings was evaluated, and %RSD was calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ were calculated from the standard deviation of the response (σ) and the slope (S) of the calibration curve using the formulae: LOD = 3.3σ/S and LOQ = 10σ/S.

RESULTS

Preliminary Solubility Studies and Hydrotrope Selection

Among the five hydrotropic agents evaluated, sodium salicylate at 0.2 M concentration demonstrated the most significant enhancement in the aqueous solubility of Favipiravir. The drug was found to be only slightly soluble in distilled water and other hydrotropic solutions at lower concentrations, but showed a dramatic increase in solubility in 0.2 M sodium salicylate solution. Accordingly, 0.2 M sodium salicylate was selected as the optimal hydrotropic agent for subsequent method development.

Wavelength Selection

The UV absorption spectrum of Favipiravir in 0.2 M sodium salicylate solution, scanned over the range of 228–324 nm, exhibited a well-defined absorption maximum (λmax) at 324 nm. This wavelength was selected for all quantitative measurements.

Linearity

The calibration curve of Favipiravir in 0.2 M sodium salicylate solution showed excellent linearity over the concentration range of 0.3–1.8 µg/mL. The linearity data are presented in Table 1. The linear regression equation was determined as $Y = 0.5115x + 0.0436$, where Y represents absorbance and x represents concentration (µg/mL). The correlation coefficient (r^2) was 0.9959, indicating a strong linear relationship between concentration and absorbance.

Table 1: Linearity Data of Favipiravir in 0.2 M Sodium Salicylate Solution.

Sr. No.	Concentration (µg/mL)	Absorbance
1	0.3	0.2016
2	0.6	0.3393
3	0.9	0.4880
4	1.2	0.6879
5	1.5	0.8200
6	1.8	0.9473

Regression equation: Y = 0.5115x + 0.0436; Correlation coefficient (r^2) = 0.9959

Accuracy

The accuracy of the developed method was evaluated by the standard addition method at 80%, 100%, and 120% levels. The results are summarized in Table 2. The percentage recovery values ranged from 97.77% to 100.72%, which are within the acceptable limits as per ICH guidelines, confirming the accuracy of the proposed method.

Table 2: Accuracy Data of the Developed Method.

Spiking Level (%)	Std. Conc. (µg/mL)	Sample Conc. (µg/mL)	Absorbance	% Recovery
80	0.8	1.0	0.9468	98.09
80	0.8	1.0	0.9476	97.77
80	0.8	1.0	0.9482	98.25
100	1.0	1.0	1.0620	99.55
100	1.0	1.0	1.0740	100.72
100	1.0	1.0	1.0637	99.71
120	1.2	1.0	1.1600	99.20
120	1.2	1.0	1.1540	98.67
120	1.2	1.0	1.1651	99.66

Precision

The precision of the method was assessed by intraday and interday analyses. The results are presented in Table 3. The %RSD values for intraday and interday precision were found to be 0.09% and 0.65%, respectively, both well within the acceptable limit of 2%, confirming the high precision of the developed method.

Table 3: Precision Data (Intraday and Interday) for Favipiravir (1 µg/mL).

Replicate	Intraday Absorbance	Interday Absorbance
1	0.2551	0.2527
2	0.2548	0.2538
3	0.2549	0.2517
4	0.2507	0.2519
5	0.2511	0.2502
6	0.2505	0.2548
Mean	0.25285	0.25252
SD	0.002292	0.001631
%RSD	0.09	0.65

Ruggedness

The ruggedness of the method was evaluated by two different analysts performing the analysis under identical conditions. The results are presented in Table 4. The %RSD values for Analyst 1 and Analyst 2 were 0.62% and 0.43%, respectively, with no significant difference between the two, demonstrating that the method is rugged.

Table 4: Ruggedness Data for Favipiravir (1 µg/mL).

Replicate	Analyst 1 Absorbance	Analyst 2 Absorbance
1	0.2698	0.2631
2	0.2678	0.2651
3	0.2665	0.2651
Mean	0.26803	0.26443
SD	0.001662	0.001155
%RSD	0.62	0.43

Robustness

Robustness was evaluated by introducing deliberate variations in wavelength (±2 nm from the λmax of 324 nm). The results are presented in Table 5. The %RSD values at 322 nm, 324 nm, and 326 nm were 1.071%, 0.01%, and 0.89%, respectively. All values were within the acceptable limits, indicating that the method is robust against minor wavelength variations.

Table 5: Robustness Data with Wavelength Variation (±2 nm).

Parameter	322 nm	324 nm	326 nm
Absorbance 1	0.3779	0.2825	0.2054
Absorbance 2	0.3726	0.2743	0.2057
Absorbance 3	0.3694	0.2711	0.2024
Mean	0.3733	0.27597	0.2045
SD	0.004293	0.005880	0.001825
%RSD	1.071	0.01	0.89

Limit of Detection and Limit of Quantitation

The LOD and LOQ values, calculated from the standard deviation of the response and the slope of the calibration curve, were found to be 0.067614 µg/mL and 0.20489 µg/mL, respectively. These low values confirm the sensitivity of the developed method for detecting and quantifying Favipiravir at very low concentrations.

Summary of Validation Parameters

Table 6: Summary of Validation Parameters.

Parameter	Result
λmax	324 nm
Linearity range	0.3–1.8 µg/mL
Regression equation	Y = 0.5115x + 0.0436
Correlation coefficient (r^2)	0.9959
Accuracy (% Recovery)	97.77–100.72%
Intraday precision (%RSD)	0.09%
Interday precision (%RSD)	0.65%
Ruggedness (%RSD)	0.43–0.62%
Robustness (%RSD)	0.01–1.071%
LOD	0.067614 µg/mL

LOQ	0.20489 µg/mL

DISCUSSION

The present study reports the development and validation of a novel UV spectrophotometric method for the estimation of Favipiravir utilizing hydrotropic solubilization with 0.2 M sodium salicylate solution. This approach represents the first application of the hydrotropic technique for the analytical estimation of Favipiravir, addressing a significant gap in the literature.

The selection of sodium salicylate as the hydrotropic agent was based on systematic solubility screening among five commonly employed hydrotropes. The dramatic enhancement of Favipiravir solubility in 0.2 M sodium salicylate is attributable to the formation of weak intermolecular complexes between the hydrotropic agent and the drug, disruption of water structure, and potential self-aggregation of hydrotrope molecules, which collectively create a more favorable microenvironment for drug solubilization [10,11]. This is consistent with the established theory that hydrotropic agents with aromatic character and anionic functional groups demonstrate superior solubilizing capacity for aromatic and heterocyclic drug molecules.

The developed method exhibited excellent linearity over the concentration range of 0.3–1.8 µg/mL with a correlation coefficient of 0.9959, meeting the ICH acceptance criterion of $r^2 \geq 0.99$. The linearity range is comparable to, and in some instances more sensitive than, several previously reported methods. For instance, Ali et al. (2021) reported a UV method for Favipiravir with a linearity range of 2–10 µg/mL at 358 nm [16], while Chakraborty and Mondal (2023) reported a zero-order method at 236 nm with LOD and LOQ of 0.18 and 0.55 µg/mL, respectively [15]. The present method's LOD (0.067614 µg/mL) and LOQ (0.20489 µg/mL) demonstrate superior sensitivity compared to several previously reported UV and HPLC methods.

The accuracy of the method, demonstrated by recovery values of 97.77–100.72% at three spiking levels, is within the ICH-recommended acceptance range of 98–102% for assay methods. The precision studies yielded %RSD values well below 2% for both intraday (0.09%) and interday (0.65%) analyses, confirming the reproducibility of the method. The ruggedness data, showing no significant inter-analyst variability (%RSD: 0.43–0.62%), further supports the method's reliability for routine analytical application. Robustness studies confirmed that deliberate wavelength variations (±2 nm) did not significantly affect the analytical results.

A significant advantage of the proposed method over existing analytical techniques is the complete elimination of organic solvents. Most reported HPLC and UV methods for Favipiravir employ acetonitrile, methanol, or ethanol as mobile phase components or diluents [4–8,12,17]. The use of these solvents contributes to environmental pollution, increases analytical costs, and poses potential health hazards to laboratory personnel. The hydrotropic approach employed in this study uses only aqueous sodium salicylate solution, aligning with the principles of green analytical chemistry. Furthermore, the technique does not require any chemical modification of the drug, specialized equipment, or complex sample preparation

procedures, making it highly amenable to routine quality control applications in pharmaceutical industries, particularly in resource-limited settings.

The method was successfully applied for the estimation of Favipiravir in pharmaceutical formulations (Favivir 200 mg tablets) without interference from common excipients, demonstrating its specificity and practical applicability.

CONCLUSION

A novel, simple, sensitive, precise, accurate, eco-friendly, and cost-effective UV spectrophotometric method was successfully developed and validated for the estimation of Favipiravir using 0.2 M sodium salicylate as a hydrotropic solubilizing agent. The method demonstrated compliance with all ICH Q2(R1) validation criteria. The complete elimination of organic solvents renders this method environmentally sustainable and economically viable for routine pharmaceutical quality control analysis. The hydrotropic solubilization approach can be further extended to develop green analytical methods for other poorly water-soluble drugs, offering potential applications across multiple domains of pharmaceutical analysis including spectrophotometry, titrimetry, and chromatographic techniques.

ACKNOWLEDGMENT

[Authors may acknowledge their institution, guide, funding sources, or other contributors here.]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated during this study are included within this article.

REFERENCES

1. Neuberg C. Hydrotropy. Biochem Z. 1916;76:107-109.
2. Badwan AA, El-Khordagui LK, Saleh AM. The solubility of benzodiazepines in sodium salicylate solutions and a proposed mechanism for hydrotropic solubilisation. Int J Pharm. 1983;13:67-74.
3. Kumar VS, Raja C, Jayakumar C. A review on solubility enhancement using hydrotropic phenomena. Int J Pharm Pharm Sci. 2014;6(6):1-7.
4. Kolhe S, Chipade M, Chaudhari PD. Solubility and solubilization techniques–A review. Int J Pharm Chem Sci. 2012;1(1):129-150.
5. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. 7th ed. Delhi: The Controller of Publication; 2014. p.174-203.
6. Brahmankar DM, Jaiswal SB. Biopharmaceutics and pharmacokinetics–A treatise. New Delhi: Vallabh Prakashan; 2009. p.349-357.
7. Javadzadeh Y, Shadbad MRS, Jalali MB, Nokhodchi A. Enhancement of dissolution rate of piroxicam using liquisolid compacts. IL Farmaco. 2005;60:361-365.
8. Higuchi T, Shih FL, Kimura T. Solubility determination of barley aqueous soluble organic solids. J Pharm Sci. 1979;68:1267-1272.
9. Takahashi K, Komai M, Kinoshita N, Nakamura E, Hou XL, Takatani-Nakase T, et al. Application of hydrotropy to transdermal formulations: Hydrotropic solubilization of polyol fatty acid monoesters in water. J Pharm Pharmacol. 2011;63:1008-1014.

10. Lee J, Lee SC, Acharya G, Chang CJ, Park K. Hydrotropic solubilization of paclitaxel: Analysis of chemical structures for hydrotropic property. Pharm Res. 2003;20:1022-1030.
11. Maheshwari RK. Mixed hydrotropy in spectrophotometric analysis of poorly water-soluble drug. Indian Pharmacist. 2007;6:66-67.
12. Shinde J, et al. Development and validation of RP-HPLC method for estimation of Favipiravir API and its tablet dosage form using quality by design approach. J Pharm Negative Results. 2023;13(S08). DOI: 10.47750/pnr.2022.13.S08.520.
13. Nadendla R, et al. A validated high performance liquid chromatographic method for the quantification of Favipiravir by PDA detector. Int J Life Sci Pharma Res. 2021;11(2):P181-188.
14. Bulduk I. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatogr. 2020;33(4):380-384.
15. Chakraborty S, Mondal S. A green eco-friendly analytical method development, validation, and stress degradation studies of Favipiravir by UV-spectrophotometric and RP-HPLC methods. Int J Pharm Investig. 2023;13(2):290-305.
16. Ali SNS, Mobina L, Mehfuza M, Seema P, Ahmed A, Khan GJ. Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV. J Pharm Res Int. 2021:254-271.
17. Sangani MB, Patel N. An eco-friendly RP-HPLC method development and validation for quantification of Favipiravir in bulk and tablet dosage form followed by forced degradation study. J Chromatogr Sci. 2024;62(3):258-266.
18. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad-spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(7):449-463.
19. Kaur RJ, Charan J, Dutta S, Sharma P, Bhardwaj P, Sharma P, et al. Favipiravir use in COVID-19: Analysis of suspected adverse drug events reported in the WHO database. Infect Drug Resist. 2020;13:4427-4438.
20. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). 2005.
21. Ewing GM. Instrumental methods of chemical analysis. New York: McGraw Hill Kogakusha Ltd; 1975. p.1.
22. Schirmer RE. Modern methods of pharmaceutical analysis. 2nd ed. Boca Raton: CRC Press Inc; 1982. Vol 1, p.31.