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UV-SPECTROPHOTOMETRIC DETERMINATION OF FAVIPIRAVIR IN BULK AND TABLET DOSAGE FORM

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Abstract

Favipiravir is an RNA polymerase inhibitor that moved from a niche influenza drug to a widely stocked antiviral during the COVID-19 pandemic, and tablet quality control had to keep pace. This narrative review draws on published pharmacology, analytical and validation literature to explain how ultraviolet spectrophotometry can be used to determine favipiravir in bulk drug and tablets. It covers the chemistry that makes the molecule suitable for direct UV measurement, the choices made over solvent, wavelength and calibration, the validation parameters set by ICH guidance, and the sample handling needed for 200 mg tablets. Chromatographic and fluorimetric methods are compared. No new experimental data are reported. The main gap is the small number of independent, stability-indicating UV studies.

1. INTRODUCTION

Favipiravir (T-705) is a pyrazinecarboxamide derivative first described as an anti-influenza compound in the early 2000s [1]. For years it was a drug most pharmacists outside Japan had never handled. That changed in 2020, when its broad activity against RNA viruses brought it into clinical trials and national treatment protocols for COVID-19 [2, 3, 4]. Production and dispensing of tablets rose quickly in several countries, and with that rise came a practical question for the quality control laboratory: how can a batch of tablets be released quickly, cheaply and reliably? Early reports from Japan and later from clinical centres elsewhere described it as well

tolerated in short courses, which encouraged wider supply [5, 6]. The shift in demand was abrupt, and it exposed how little published analytical guidance existed for a compound that few laboratories had needed to test before. The tablets are small, the dose is large, and nobody wants a weak batch in the middle of an outbreak. Hospital pharmacies in particular had to qualify new suppliers at short notice.

Liquid chromatography is the usual answer for assay work, and good HPLC methods for favipiravir were published within months of the pandemic starting [7, 8]. Yet many laboratories, especially smaller manufacturers and teaching or regional testing centres, do not run a chromatograph for every batch. Ultraviolet spectrophotometry remains the cheapest quantitative tool on the bench. It needs little solvent, no column and almost no method development time, provided the analyte absorbs well and the excipients do not interfere [9, 10]. Where a method is simple enough to be transferred between sites with minimal training, results become easier to compare, and that comparability is valuable when several manufacturers supply the same market. The trade-off, as always, is specificity. A UV reading reports everything that absorbs, not only the drug [9, 11]. Reagents are inexpensive and results arrive within minutes, so the technique also suits in-process checks during blending and compression [9, 10]. For a manufacturer releasing many lots a week, that saving in analyst time is considerable.

This article reviews what the published literature says about the UV-spectrophotometric determination of favipiravir in bulk drug and in tablet dosage form. It is a narrative review. It reports no new experiments, measurements or tablet analyses. The aim is to gather the pharmacological background, the physical principles, the analytical choices and the validation expectations in one place, so that a reader planning such an assay knows what has been reported by others, where the evidence is firm, and where it is thin. The main findings are brought together in table 1 and grouped in fig. 1. Throughout, the emphasis is on what the evidence allows and does not allow, rather than on a single recommended procedure. Readers looking for a ready-made protocol will not find one here, because the evidence does not yet justify a single standard.

2. PHARMACOLOGY AND DOSAGE FORM

Favipiravir is a prodrug. After uptake into cells it is converted by host enzymes to the ribofuranosyl phosphate and then the active ribofuranosyl triphosphate, which is recognised by viral RNA-dependent RNA polymerase [12, 13]. The triphosphate can be incorporated into nascent RNA or can bind the enzyme and slow chain elongation, and its ambiguous base pairing is thought to drive lethal mutagenesis in some viruses [14, 15]. Because the polymerase active site is conserved across many RNA viruses, activity extends well beyond influenza, and this breadth explains the later interest in Ebola and coronaviruses [16, 17]. In Ebola models, it improved survival in animals even when started after infection was established, which is part of what drew attention to it [17, 18]. Whether the same mechanism explains clinical effect in coronavirus infection was still being debated in 2020 [4, 19]. The prodrug nature also means that the tablet itself contains an inactive precursor, a point that matters little to the UV analyst but a great deal to the clinician.

Table 1: Reported approaches relevant to the determination of favipiravir

Approach	Reported feature or finding	Ref.
Reversed-phase HPLC with UV detection	Isocratic C18 method developed for pharmaceutical formulations; specific and suited to release testing	[7]
Green micellar HPLC	Avoids organic solvent; uses native fluorescence for detection of favipiravir	[8]
Experimental design spectrofluorimetry	Conditions optimised statistically; applied to tablets and human plasma	[20]
Direct zero-order UV Near 320 nm	Simple and fast but specificity depends on the excipients and absence of degradants	[9, 10]

Approach	Reported feature or finding	Ref.
Derivative or difference UV	Can remove background absorbance; needs separate validation of selectivity	[21, 22]
Forced degradation testing	Stress studies show whether a method is stability indicating	[23, 24]
ICH Q2(R1) parameters	Specificity, linearity, accuracy, precision, range and robustness evaluated for assays	[11, 25]
Robustness testing	Deliberate small changes show sensitivity to wavelength, pH and extraction	[26]
Pharmacopoeial tablet assay practice	Powdering twenty tablets, extraction, filtration and dilution to working range	[27, 28, 29]
Pharmacology of tablet dose	200 mg tablets used in large loading regimens, so accuracy near label claim matters	[2, 3]

The clinical record before 2020 was modest. Favipiravir was licensed in Japan for novel or re-emerging influenza that did not respond to other antivirals, a restricted role reflecting concern about fetal toxicity [5, 16]. In Guinea, a single-arm trial during the Ebola outbreak suggested possible benefit in patients with lower viral loads, but the design could not prove it [18]. Pharmacokinetic work showed non-linear behaviour with time-dependent changes in exposure, which complicates dosing and is one reason regulators asked for careful analytical control of the product [30]. Because exposure falls with repeated dosing in some reports, a loading dose is used, and this unusual schedule is directly relevant to how many tablets a patient takes in the first day [2, 3, 30]. Limits on its use in pregnancy followed from animal findings of embryo toxicity, and this shaped how widely clinicians were prepared to prescribe it [5, 6]. Occasional reports of its activity against arenaviruses and noroviruses in laboratory systems widened this interest further [16, 17].

COVID-19 produced a flurry of small, mostly open-label studies. One early comparison reported faster viral clearance than lopinavir and ritonavir [2], and later reviews summarised the accumulating data with cautious optimism while noting heterogeneous designs [3, 6, 19]. Reported adverse effects include raised serum uric acid, mild liver enzyme changes and gastrointestinal upset, while teratogenicity in animals keeps pregnancy a firm contraindication [5, 6]. For the analyst, the practical point is simple. A drug used at high loading doses in many patients needs reliable tablet assay and content uniformity data, and these should be quick to obtain. None of these studies was designed to settle efficacy, and later larger trials were still awaited when this review was prepared [3, 19]. Clinical pharmacists were left to weigh a plausible mechanism against thin trial data, and that uncertainty is another reason why product quality should not be a second variable in the equation [3, 6]. Dose and duration varied among trials, which makes pooled comparison difficult.

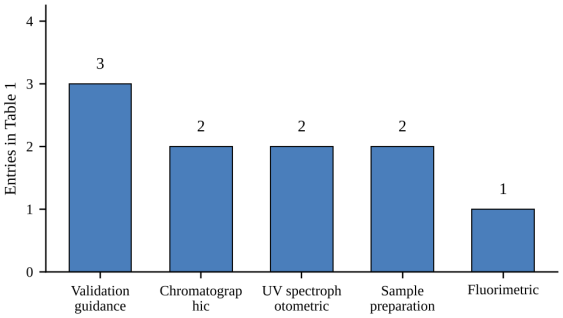


Fig. 1: Number of entries in table 1 by analytical approach group

3. PRINCIPLES OF UV DETERMINATION

Direct UV assay depends on the Beer–Lambert relationship, in which absorbance at a chosen wavelength is proportional to concentration within a limited range [9, 10]. Favipiravir carries a conjugated pyrazine ring bearing hydroxyl, fluorine and carboxamide substituents, an arrangement that absorbs strongly in the near-UV. Published chromatographic and fluorimetric studies place the useful absorption maximum at roughly 320 nm, though the exact value moves a few nanometres with solvent and pH [7, 8, 20]. A band that far from 250 nm is helpful, because many tablet excipients and solvent impurities absorb little above 300 nm. The molar absorptivity of a compound with such a chromophore is high enough that dilute

solutions in the microgram per millilitre range give readable absorbance, and this is why UV assays rarely need preconcentration [7, 20]. Stray light and cell contamination can distort readings at high absorbance, so a reading above about 1.0 should be treated with suspicion [9, 31]. Pyrazines of this type typically show two absorption regions, one deeper in the UV and one at longer wavelength, and the longer band is the useful one for assay.

Choice of solvent matters more than it might seem. Favipiravir dissolves sparingly in water but readily in methanol, dimethyl sulfoxide and alkaline aqueous media, and its acidic hydroxyl group means that the spectrum can shift with pH [8, 20]. Analysts working with pharmacopoeial practice usually favour a solvent that is transparent at the working wavelength, inexpensive and miscible with the dissolution medium [27, 28]. A scan of a dilute solution against a matching blank, repeated in the chosen solvent, is the standard way to fix the wavelength before any calibration is attempted [29, 31]. Methanol is a common choice because it also dissolves excipient-bound drug efficiently under sonication, while still being transparent in the near-UV [8, 29]. Reporting the solvent and pH alongside the wavelength is therefore not pedantry, since a method described without them cannot be reproduced [8, 31]. Water-methanol mixtures are a practical compromise when the aqueous solubility of the powder alone is poor.

Calibration is typically built from a stock solution diluted into a series covering a modest concentration window, with absorbance kept well inside the range where the instrument is linear, often between about 0.2 and 0.8 [9, 31]. Least-squares regression of absorbance on concentration yields a slope, intercept and correlation coefficient, but a high correlation coefficient alone does not prove linearity, and residual plots are the better check [21, 31]. Where excipients contribute background, derivative spectra or the absorbance-difference approach can subtract it, although these tricks need their own validation [21, 22]. Standard additions can be used when the matrix is difficult to match, though for tablets a placebo-based approach is more common and easier to defend [22, 31]. Whatever the approach, replicate readings and a bracketing standard at intervals guard against drift during a long run of tablet samples [21, 22]. A calibration should bracket the expected sample concentration, with the working solution sitting near the middle of the line rather than at an end.

Instrument factors are easy to overlook. Slit width, scan speed, cuvette matching and lamp warm-up all influence repeatability, and quartz cells are essential below about 350 nm because plastic and ordinary glass absorb in this region. Wavelength accuracy and photometric accuracy should be checked against certified filters or holmium oxide standards before a validation exercise begins, as pharmacopoeial general chapters describe [27, 28]. Neglecting these checks is a common source of unexplained day-to-day drift in routine UV work, and they take little time. Small practical measures like these keep a simple method reliable over months of routine use.

4. REPORTED ANALYTICAL METHODS

Most of the quantitative methods published for favipiravir around 2020 and 2021 are chromatographic. A reversed-phase HPLC method with UV detection was reported for pharmaceutical formulations, using a C18 column and an isocratic aqueous-organic mobile phase [7]. A green micellar method avoided organic solvent altogether and used the same compound's native fluorescence as a second detection mode [8]. These methods show that the molecule is easy to detect optically, which is the first condition for a simple spectrophotometric assay [7, 8]. Detection wavelengths in these reports cluster in the near-UV, which is reassuring for anyone planning a plain absorbance method [7, 20]. Run times of a few minutes made these methods practical for routine batch release, and the reported linear ranges comfortably covered tablet strength after dilution [7, 8]. The choice of buffer and organic modifier in these separations indicates how sensitive retention is to pH, consistent with the ionisable hydroxyl group [7, 8].

Spectrofluorimetry has also been developed, with an experimental design approach used to optimise conditions and the method applied to tablets and to human plasma [20]. Fluorimetric detection is more sensitive than absorbance and is useful for biological fluids, but it asks for a spectrofluorometer, which many quality control laboratories do not own. For tablets, where the analyte is present at milligram levels per unit and sensitivity is not limiting, direct absorbance is usually adequate [9, 20]. Reported methods in plasma are a reminder that UV absorbance alone would not be enough for bioanalysis, where selectivity demands separation [8, 20]. The fluorescence route has a further disadvantage, which is that quenching by excipients or by dissolved oxygen must be excluded [8, 20]. Sample volume is small, which gives these methods an advantage when only a little material is available.

Stability-indicating capability is the sharper issue. A chromatographic method can separate degradation products from the parent drug, whereas a plain zero-order UV measurement may add their absorbance to the result [24]. Forced degradation under acid, base, oxidative, thermal and photolytic stress is the usual way to test this, as reviewed for stability-indicating methods [23, 24]. For favipiravir the published stress data are limited, so a UV method intended for release testing of fresh batches should be presented as such, and it should not be assumed suitable for stability samples [7, 23, 24]. Without such studies the analyst cannot know if a rising absorbance on storage reflects loss of drug or accumulation of products that absorb at the same wavelength [7, 24]. Peak purity assessment with a diode array detector is a partial answer in chromatography, though it is not a substitute for proper stress testing [11, 24]. A plain UV method can still be used, but its report should say so openly.

Table 1 collects the main reported approaches and the points a reader should take from each, with the grouping used in fig. 1. The comparison favours UV for speed and cost, chromatography for specificity, and fluorimetry for sensitivity. None of the three is better in every respect, and a laboratory's choice depends on what question the assay must answer [7, 11, 20]. The numbers of entries in each group in the figure reflect how often each approach appears in the sources reviewed, not how well it performs. That plain statement may disappoint those who hoped for a single best method, yet it is what the evidence supports.

Green analytical chemistry has pushed method developers toward solvents that are safer and cheaper, and water-methanol or aqueous alkaline media suit UV work well. A method that uses a small volume of methanol and avoids acetonitrile also removes waste handling costs, which matters in laboratories running hundreds of samples [8, 9]. Disposal of organic solvent in particular is a cost that is often forgotten when comparing methods, even though it can exceed reagent purchase. Such savings are modest per sample but real over a year of routine testing.

5. VALIDATION AND TABLET ASSAY

ICH Q2(R1) sets out the characteristics normally evaluated for an assay: specificity, linearity, range, accuracy, precision, detection and quantitation limits, and robustness [25]. For a tablet assay, the guideline expects the range to cover at least 80 to 120 per cent of the test concentration, accuracy to be shown by recovery of known amounts added to placebo, and precision to be reported at repeatability and intermediate levels. Reviews comparing the FDA, USP and ICH positions note that the principles agree closely, even where the vocabulary differs [11]. Limits of detection and quantitation, by contrast, matter less for a tablet assay at label strength, although they remain part of a complete validation report [11, 25]. Specificity is generally demonstrated by comparing the response of a placebo, the drug and the finished product at the chosen wavelength [11, 25]. Acceptance criteria should be fixed before the work begins, not adjusted afterwards to fit the results.

Robustness is often treated as an afterthought, but small deliberate changes in solvent composition, pH, wavelength setting and sonication time reveal how forgiving a method really is [26]. For UV work, a shift of a few nanometres near the maximum should leave absorbance almost unchanged, because

the peak is broad. That behaviour makes the technique attractive for routine use, but it also means that specificity, rather than robustness, is the property most likely to fail [11, 26]. Method transfer between laboratories adds another layer, since differences in instruments and operators can reveal weaknesses that a single-site validation missed [11, 26]. Replicate preparations by a second analyst on another day give a sense of intermediate precision at very little extra cost [25, 26]. Together these checks are inexpensive and give a realistic picture of day-to-day performance.

Sample preparation for tablets follows a familiar pattern. Twenty tablets are weighed and powdered, a portion equivalent to a stated amount of drug is dispersed in solvent with sonication, and the extract is filtered and diluted to the working range [27, 28, 29]. Insoluble excipients such as microcrystalline cellulose, magnesium stearate and talc can scatter light, so filtration through a membrane of suitable pore size is essential. Coloured film coat residues may also absorb and should be checked with a placebo blank [21, 29]. Dissolving the powder in a volumetric flask, with a proper allowance for the volume taken up by insoluble matter at the largest scale, avoids a small but real positive bias in the final result [27, 28]. Reporting the recovery as a mean with its spread, instead of a single figure, tells the reader whether the extraction is consistent from one preparation to the next [29, 31]. Sonication for too long can warm the solution and, in principle, degrade a labile analyte, so time and temperature should be controlled [21, 27].

Comparing UV results against a validated chromatographic method on the same batch is a sensible way to build confidence, and statistical tests such as the paired t-test and the variance ratio test are standard tools for that comparison [31]. Agreement between methods does not remove the need for specificity data, but it does show that excipients are not driving a systematic bias [7, 11, 31]. Where the two methods disagree, the reason should be investigated before either result is trusted [11, 31]. The tablet matrix itself therefore matters as much as the instrument.

6. LIMITATIONS AND FUTURE DIRECTIONS

Several limits should be kept in view. The published literature on favipiravir assays is young, with most reports appearing after 2019, and independent replication is scarce [7, 8, 20]. Many validation sections report excellent linearity and recovery but say little about degradation products, intermediate precision across analysts and days, or placebo interference from the exact excipients of marketed generics [11, 24, 26]. Because formulations differ between manufacturers, a method validated on one tablet may not transfer unchanged to another [29]. Reviews of other antivirals show the same pattern, in which fast-moving demand outruns the analytical literature [6, 11]. Method authors rarely publish negative findings, so the true failure rate of simple assays on real products is not visible in print [11, 24]. Reports from a single laboratory are useful starting points, but they are not yet a consensus.

Future work would be most useful where it is practical. Forced-degradation studies following the published approaches to stress testing would clarify when direct UV is acceptable [23, 24]. Interlaboratory comparisons would show how reproducible a simple method is outside the originating site. Pharmacopoeial monographs, when they appear, will set common expectations for wavelength, solvent and acceptance limits, and until then laboratories must justify their own choices against ICH principles [25, 27, 28]. Such work is not glamorous, but it is the kind that makes a simple method trustworthy [24, 26]. Open sharing of validation files and raw spectra would also make reviews like this one easier to write [25, 26]. Open data on real marketed tablets would be especially welcome.

7. CONCLUSION

The literature supports UV spectrophotometry as a plausible, economical way to determine favipiravir in bulk drug and in 200 mg tablets, because the molecule absorbs strongly in the near-UV and dissolves in common solvents [7, 8, 20]. The method is only as good as its validation. Linearity, accuracy, precision and robustness are easy to demonstrate.

Specificity is harder and should be addressed with placebo blanks and, where possible, comparison with chromatography [11, 25, 26].

For now, UV should be regarded as a fast routine assay for release testing of fresh product, with HPLC retained for stability studies and for confirmation when results are doubtful [7, 23, 24]. Analysts who adopt it should state the solvent, wavelength and sample preparation clearly, follow ICH and pharmacopoeial principles, and report the excipients tested [25, 27, 28, 29]. More independent, stability-oriented studies would strengthen the evidence.

8. ABBREVIATIONS

HPLC-High-performance liquid chromatography, ICH-International Council for Harmonisation, UV-Ultraviolet, RNA-Ribonucleic acid, COVID-19-Coronavirus disease 2019, USP-United States Pharmacopeia, FDA-Food and Drug Administration, C18-Octadecylsilane bonded phase

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Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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