RESEARCH PAPER

Development and Validation of a Simple UV Spectrophotometric Method for the Estimation of Povidone-Iodine at 291 nm

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Abstract
Objectives: Povidone-iodine (PVP-I) is a widely used antiseptic that is increasingly being incorporated into new dosage forms and materials, yet published analytical work on it is limited and mostly concerns trace iodine in plasma or another drug measured in the presence of PVP-I. This study aimed to develop and validate a simple, direct, single-wavelength UV spectrophotometric method for estimating PVP-I.
Methods: Absorbance was recorded at 291 nm. The method was validated for linearity (300–500 µg/mL), accuracy (recovery at 80%, 100% and 120% of 400 µg/mL), precision (six replicates), robustness (wavelength varied by ±4 nm) and ruggedness (two analysts).
Results: The calibration curve gave a correlation coefficient of 0.9951 ($r^2 = 0.9902$). Mean recoveries were 99.68%, 97.56% and 97.25% at the three levels, each with a %RSD of 0.17% or less. Repeatability showed a %RSD of 0.31%, and ruggedness %RSD values ranged from 0.47% to 1.61%. Robustness %RSD values ranged from 1.54% to 7.33% and were higher at the lower concentrations, indicating that the detection wavelength must be set with care.
Conclusion: The method is rapid, inexpensive and precise, and is suitable for assay-level estimation of PVP-I over 300–500 µg/mL when the wavelength is tightly controlled. Specificity and application to marketed products should be examined in future work.
Keywords: Povidone-iodine, UV spectrophotometry, Method validation, Linearity, Robustness
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1. Introduction

Povidone-iodine (PVP-I) is a complex of iodine with polyvinylpyrrolidone. It has been used as an antiseptic for more than 60 years, it acts on bacteria, fungi and viruses, no resistance to it has been reported, and it is on the World Health Organization list of essential medicines [1]. In laboratory tests, PVP-I products brought the infectivity of SARS coronavirus below the detectable level after two minutes of contact [2]. The antiseptic action depends on how the polymer holds iodine. Structural work suggests that the iodine in PVP-I is present as a triiodide anion that is bound ionically to a proton shared between two pyrrolidone carbonyl groups [3]. Later spectroscopic and computational work found that PVP-I holds both molecular iodine and polyiodides, that these are linked by weak halogen bonds, and that iodine is released through an equilibrium that moves with temperature [4]. The released iodine species also decide how strong the effect is. For example, silver ions raise the share of hypoiodous acid, a stronger bactericidal species, in PVP-I solutions [5].

Because of this broad activity, PVP-I is being placed into many formulations. A diluted extemporaneous eye preparation stayed stable for 28 days but caused mild irritation even at 0.5% w/v [6]. Other workers made bio adhesive vaginal tablets that released complexed iodine for up to 8 h [7], collagen–hydroxypropyl methylcellulose scaffolds [8] and chitosan hydrogels [9] for wound care, bigels for hand antisepsis [10], plasma-polymer coatings for dressings and devices [11], polystyrene resins for water decontamination [12], and a combination with Tridax procumbens extract [13]. The way PVP-I is prepared also affects its quality, since yield and iodine content were found to depend on pH, stirring speed, temperature and time [14]. Solid complexes of iodine with hydroxypropyl cyclodextrins have been suggested as other stable iodophors [15]. Skin irritation differs between similar PVP-I solutions and can be measured [16], and non-chemical options such as UV-C light are now compared with PVP-I for preoperative disinfection [17]. In each of these uses the amount of PVP-I in the product has to be known, so a dependable assay is needed.

The analytical methods available for PVP-I are fewer than its uses would suggest. An ion-pair RP-HPLC method measured total free iodine in rabbit plasma after conversion to iodide, over 0.005–2 µg/mL [18]. A first-order derivative UV method estimated metronidazole in the presence of PVP-I over 5–45 µg/mL [19]. For ophthalmic suspensions that contain dexamethasone and PVP-I, the work has centred on the steroid, using RP-HPLC with LC-MS/MS and white analytical chemistry metrics [20] and a USP type-IV dissolution method [21]. A validated UV method was used to measure the iodine content of

cyclodextrin–iodine solids [15], and iodide has been sensed with silver nanoparticles in water, serum and urine [22]. Simple spectrophotometry is also well accepted for routine control of other drugs, for example cefixime estimated through a potassium iodide–iodate reaction [23]. A recent letter further points out that many drug products still lack published analytical methods [24].

UV–visible spectrophotometry is one of the oldest and simplest tools of pharmaceutical analysis. It needs one instrument, uses little solvent, gives a result within minutes and can be run by a trained technician without a long method-development period. Earlier workers have used it for iodine-based assays, such as the cefixime method mentioned above [23], and for the iodine content of cyclodextrin solids [15]. A derivative form of it has also been used to resolve overlapping spectra when PVP-I is present together with another drug [19]. PVP-I, however, is not a single chemical species in solution. Molecular iodine, polyiodides and the polymer-bound complex exist together in an equilibrium [3, 4], and this equilibrium can shift with temperature and with other components of the medium [4, 5]. A direct UV reading therefore has to be tested with care. Its linearity, its recovery, its repeatability and, above all, its sensitivity to small changes in working conditions must all be known before it can be trusted for routine use.

From these reports, we see a clear gap. Within the literature considered for this work, PVP-I is measured either as trace free iodine in plasma [18] or as the co-formulated partner while another drug is the analyte [19–21]. The UV method for iodine in cyclodextrin solids was built for those solids and not for PVP-I itself [15]. We did not find a simple, direct, single-wavelength UV method for PVP-I as the analyte that is validated for linearity, accuracy, precision, robustness and ruggedness together. Such a method would suit quality control laboratories that have no chromatography facility, and it would save solvent and cost. Therefore, this study aimed to develop and validate a UV spectrophotometric method for PVP-I at 291 nm and to report honestly where the method performs well and where it needs care.

2. Materials and Methods

2.1 Principle of the method and choice of technique

The method rests on the absorption of ultraviolet radiation at 291 nm by the iodine species held in the PVP-I complex. When the path length is fixed, the Beer–Lambert law states that absorbance is directly proportional to the concentration of the absorbing species:

$$A = \varepsilon \times b \times C \qquad (1)$$

Here A is the absorbance, ε is the absorptivity, b is the path length and C is the concentration. The amount of PVP-I in an unknown solution can therefore be read from a calibration line made from standards of known strength. In PVP-I, iodine is held partly as molecular iodine and partly as polyiodides, including triiodide [3, 4]. A reading at one wavelength therefore reflects the combined response of these species and not that of a single pure compound. This is acceptable when the whole complex is assayed, but it is also the reason why the wavelength and the temperature of the solutions must be kept steady. A direct single-wavelength method was chosen in preference to a derivative or chromatographic approach because it is the simplest one to transfer to a routine quality control laboratory.

2.2 Instrument, chemicals and solutions

Absorbance measurements were performed using a Shimadzu UV-1900i double-beam UV–Visible spectrophotometer equipped with matched 1-cm quartz cuvettes. Analytical-grade povidone-iodine (PVP-I) was used for all experiments, with solutions prepared in purified water and phosphate buffers (pH 6.8 and pH 7.4). A primary stock solution of PVP-I (1000 µg/mL) was prepared and diluted appropriately to yield working calibration standards of 300, 350, 400, 450, and 500 µg/mL. Spectral acquisition across the 200–400 nm wavelength range identified the maximum absorption wavelength λmax for PVP-I at 291 nm

2.3 Validation of the method

The method was validated for the parameters that earlier UV and HPLC reports on PVP-I-containing products have used [19, 21, 23]. These were linearity, limit of detection and quantitation, accuracy, precision, robustness and ruggedness.

For linearity, the five working standards (300–500 µg/mL) were measured and absorbance was plotted against concentration. The slope, intercept, correlation coefficient (r) and coefficient of determination (r^2) were found by least-squares regression. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the same data as $3.3\sigma/S$ and $10\sigma/S$, where σ is the residual standard deviation of the regression and S is the slope.

For accuracy, a recovery study was done at three levels, 80, 100 and 120% of the target concentration of 400 µg/mL. That is, 320, 400 and 480 µg/mL were taken, each in triplicate. The amount found was calculated from the measured absorbance, and the percentage recovery, mean, standard deviation (SD) and percentage relative standard deviation (%RSD) were worked out for each level.

For precision, six separate readings were taken from a solution of 400 µg/mL under the same conditions, and the mean, SD and %RSD were calculated. This is a measure of repeatability.

For robustness, the effect of a small deliberate change in wavelength was tested. Each of the five standards was read at 287 nm (λ1, −4 nm), 291 nm (λ2) and 295

nm (λ3, +4 nm), and the %RSD of the three readings was calculated for every concentration.

For ruggedness, the same five standards were measured by two analysts, and the mean, SD and %RSD of the two sets were calculated.

2.4 Acceptance criteria and data treatment

Each parameter was judged against reference values taken from earlier validated methods for PVP-I-containing products and for iodine-based assays as shown in Table 1. No single limit suits every type of sample, so these values are used as points of comparison and not as a claim of regulatory compliance. The studies cited give no separate limit for robustness and ruggedness, so the 2% limit used for precision in them [19, 21] was applied to both.

Table 1. Validation parameters, how each was evaluated and the reference value used for comparison

Parameter	How it was evaluated	Reference value
Linearity	Five levels, 300–500 µg/mL; least-squares regression of absorbance on concentration	$r^2 > 0.999$ [18, 21]
Accuracy	Recovery at 80, 100 and 120% of 400 µg/mL, three determinations per level	98.5–101.5% [21]
Repeatability	Six readings of a 400 µg/mL solution	%RSD < 2% [19, 21]
Ruggedness	Two analysts, five concentrations	%RSD < 2% [19, 21]
Robustness	Wavelength changed by −4 and +4 nm, five concentrations	%RSD < 2% [19, 21]
LOD and LOQ	3.3σ/S and 10σ/S from the calibration data	29.94 and 90.74 µg/mL for a Povidone iodine UV method [23]

The results were calculated with the following equations, where σ is the residual standard deviation of the regression and S is its slope:

LOD = 3.3 × σ ÷ S (2)

LOQ = 10 × σ ÷ S (3)

% Recovery = (Amount found ÷ Amount added) × 100 (4)

% RSD = (SD ÷ Mean) × 100 (5)

The overall experimental strategy for method validation is depicted in Figure 1. Standard solutions of povidone-iodine (PVP-I) across the working range of 300–500 µg/mL were prepared, and their absorbance values were measured at 291 nm. The resulting photometric data were applied to evaluate five essential validation criteria:

- **Linearity:** Evaluated across 300–500 µg/mL, including the determination of limit of detection (LOD) and limit of quantitation (LOQ).
- **Accuracy:** Determined via triplicate measurements at three spike levels (80%, 100%, and 120% of the nominal concentration).
- **Repeatability:** Assessed through six consecutive readings at a target concentration of 400 µg/mL.
- **Robustness:** Examined across all five concentration levels under intentional wavelength shifts (291 ± 4 nm).
- **Ruggedness:** Verified across all five concentration levels by two independent analysts.

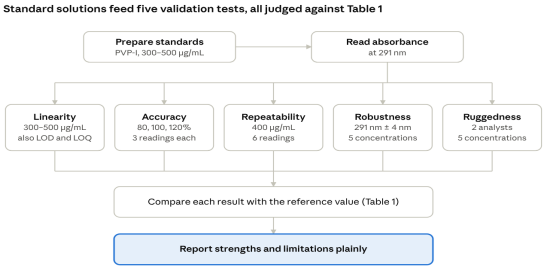


Figure 1. Schematic diagram illustrating the sequential workflow for the analytical validation of the proposed UV spectrophotometric method for PVP-I.

All experimental outcomes were benchmarked against predefined acceptance criteria (Table 1) to establish the analytical performance, practical utility, and operational boundaries of the method

3. Results and Discussion

3.1 Linearity, LOD and LOQ

Calibration data for povidone-iodine (PVP-I) standard solutions as showing absorbance response across concentrations from 300 to 500 µg/mL at 291 nm as shown in table 2.

Table 2. Linearity data at 291 nm

Concentration (µg/mL)	Absorbance
300	0.1714
350	0.2686
400	0.3783
450	0.5155
500	0.6723

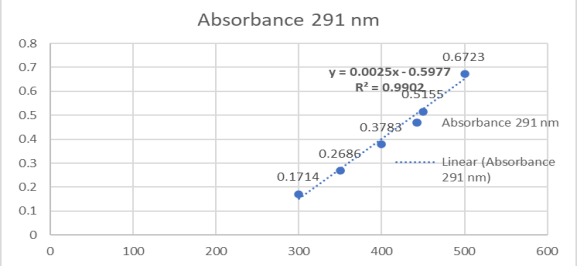


Figure 2. Calibration data for PVP-I at 291 nm.

Figure 2 shows the calibration data with the fitted line A proportional increase in optical absorbance was observed across the evaluated concentration range of 300–500 µg/mL (Table 2). Linear regression analysis yielded the calibration equation A = 0.0025C - 0.5977, demonstrating a strong linear relationship with a correlation coefficient (r)of 0.9951 and a coefficient of determination (r2) of 0.9902. The standard deviation of the regression residuals was calculated to be 0.0227. Based on the residual standard error, the limit of detection (LOD) and limit of quantitation (LOQ) were established as 29.94 µg/mL and 90.74 µg/mL, respectively.

3.2 Accuracy

Method accuracy was established through recovery experiments performed in triplicate at three spiking concentrations corresponding to 80%, 100%, and 120% of the nominal 400 µg/mL standard level (Table 3). Mean recovery values were found to be 99.68%, 97.56%, and 97.25% for the 320, 400, and 480 µg/mL concentration levels, respectively. The method demonstrated high truthfulness and exceptional closeness among measurements, as reflected by low standard deviations (SD ≤ 0.16) and %RSD values remaining at or below 0.17% across all tested ranges.

Table 3. Accuracy (recovery) study

Level	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery	Mean % recovery	SD	%RSD
80 %	320	318.96, 319.20, 318.80	99.68, 99.75, 99.63	99.68	0.06	0.06
100 %	400	390.40, 389.52, 390.80	97.60, 97.38, 97.70	97.56	0.16	0.17
120 %	480	466.72, 467.40, 466.32	97.23, 97.38, 97.15	97.25	0.11	0.12

3.3 Precision

The six readings of the 400 µg/mL solution were 0.3743, 0.3729, 0.3755, 0.3748, 0.3761 and 0.3739. The mean absorbance was 0.3746, the SD was 0.0011 and the %RSD was 0.31%.

Table 4. Precision study

Replicate	Concentration (µg/mL)	Absorbance	Mean	SD	%RSD
1	400	0.3743	0.3746	0.0011	0.31
2	400	0.3729			
3	400	0.3755			
4	400	0.3748			
5	400	0.3761			
6	400	0.3739			

Six replicate measurements of the 400 µg/mL PVP-I solution at 291 nm gave absorbance values between 0.3729 and 0.3761, with a mean of 0.3746 and a standard deviation of 0.0011. The %RSD was 0.31%, well below the commonly accepted limit of 2%, which shows that the method has good repeatability

3.4 Robustness

Robustness evaluation of the proposed UV spectrophotometric method for povidone-iodine

(PVP-I) under deliberate wavelength shifts (291 ± 4 nm) across 300–500 µg/mL.

Table 5. Robustness: effect of wavelength change

Concentration (µg/mL)	287 nm (−4)	291 nm	295 nm (+4)	Mean	SD	%RSD
300	0.1912	0.1817	0.1652	0.1794	0.0132	7.33
350	0.2621	0.2525	0.2326	0.2491	0.0150	6.04
400	0.3673	0.3614	0.3374	0.3554	0.0158	4.46
450	0.4678	0.4646	0.4419	0.4581	0.0141	3.08
500	0.6842	0.6901	0.6697	0.6813	0.0105	1.54

Method robustness was assessed by introducing intentional wavelength fluctuations of ±4 nm (287 nm and 295 nm) around the detection wavelength of 291 nm across five standard concentrations (Table 5). Across all tested levels, %RSD values ranged between 1.54% and 7.33%, showing an inverse relationship with sample concentration. Relative variance was most pronounced at the lower bound (7.33% at 300 µg/mL) and progressively diminished toward the higher end (1.54% at 500 µg/mL). This pronounced sensitivity at lower concentrations underscores the operational necessity of strict wavelength control during analysis.

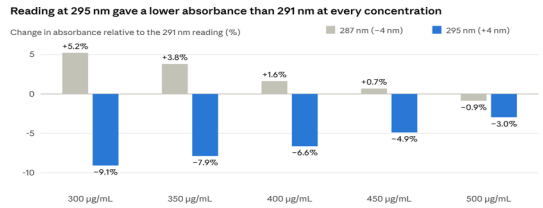


Figure 3. Effect of a 4 nm wavelength shift on absorbance.

The effect of wavelength variation on absorbance stability is depicted in Figure 3, illustrating the percentage change at 287 nm (−4 nm) and 295 nm (+4 nm) relative to the nominal wavelength of 291 nm. Shifting the wavelength upward to 295 nm consistently produced negative deviations in absorbance across all concentrations, with the magnitude of reduction decreasing from −9.1% at 300 µg/mL to −3.0% at 500 µg/mL. Conversely, shifting downward to 287 nm generated positive deviations across 300–450 µg/mL (ranging from +5.2% to +0.7%) before shifting slightly to −0.9% at 500 µg/mL. Overall, higher concentrations demonstrated greater resilience against spectral drift, whereas lower concentrations proved considerably more sensitive to wavelength offsets.

3.5 Ruggedness

Inter-analyst ruggedness assessment of the UV spectrophotometric method for povidone-iodine (PVP-I) across 300–500 µg/mL.

Table 6. Ruggedness: results of two analysts

Concentration (µg/mL)	Analyst 1	Analyst 2	Mean	SD	%RSD
300	0.1833	0.1803	0.1818	0.0021	1.17
350	0.2538	0.2481	0.2510	0.0040	1.61
400	0.3623	0.3694	0.3659	0.0050	1.37
450	0.4722	0.4691	0.4707	0.0022	0.47
500	0.6921	0.6874	0.6898	0.0033	0.48

Method ruggedness was evaluated by comparing absorbance measurements obtained independently by two analysts across five concentration levels from 300 to 500 µg/mL (Table 5). The inter-analyst variability remained consistently low across all evaluated samples, with standard deviation (SD) values between 0.0021 and 0.0050. The resulting %RSD ranged from 0.47% to 1.61%, well within acceptable analytical thresholds. These metrics demonstrate that the proposed spectrophotometric procedure exhibits high reproducibility and resilience against operator-dependent variations.

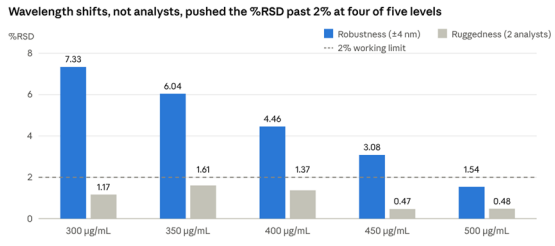


Figure 4. The %RSD of the robustness and ruggedness tests at each concentration.

A direct comparison between method robustness (±4 nm spectral shift) and inter-analyst ruggedness is

presented in Figure 4. Ruggedness testing displayed excellent reproducibility across all concentration levels, maintaining %RSD values between 0.47% and 1.61%, which comfortably met the 2% performance threshold. In contrast, deliberate wavelength variations during robustness testing led to elevated variability, pushing %RSD values beyond the 2% limit (3.08% to 7.33%) across four of the five evaluated concentrations (300–450 µg/mL). Only the highest concentration (500 µg/mL) remained within limits at 1.54% %RSD. These findings confirm that spectral alignment—rather than analyst performance—serves as the critical operational variable influencing measurement precision.

Place of the method among existing work. HPLC methods [18, 20, 21] are more selective and more sensitive, but they need costly equipment, columns and solvents. The present UV method needs only a spectrophotometer and one wavelength, so it suits routine quality control of PVP-I at assay level in laboratories with limited resources. It adds a directly validated option for PVP-I to the methods that exist for PVP-I-containing combinations [19–21] and iodine-containing solids [15], and it responds to the call for methods for products that are poorly covered in the literature [24].

4. Conclusion

A direct UV spectrophotometric method at 291 nm was developed and validated for estimating povidone-iodine (PVP-I) over 300–500 µg/mL. The method was precise: repeatability gave a %RSD of 0.31%, and two analysts obtained %RSD values of 0.47–1.61%. Mean recoveries of 97.25–99.68% show that results were close to the amounts added. However, the recoveries at the 100% and 120% levels (97.56% and 97.25%) were slightly below the 98.5–101.5% reference range. Linearity was acceptable ($r^2 = 0.9902$). The estimated LOD and LOQ (30.03 and 91.01 µg/mL) show that the method is not suitable for trace-level work. Results were also sensitive to the wavelength setting. A shift of ±4 nm gave %RSD values of 1.54–7.33%, which exceeded 2% at four of the five concentrations. The wavelength and temperature should therefore be fixed, and samples should be calibrated at concentrations close to the working level. Under these controls, the method offers a rapid, low-cost option for assay-level estimation of PVP-I in laboratories without chromatography. Specificity studies and tests on marketed products are needed before it is used in routine quality control.

Abbreviations

A: Absorbance, HPLC: High-performance liquid chromatography, LC-MS/MS: Liquid chromatography-tandem mass spectrometry, LOD: Limit of detection, LOQ: Limit of quantitation, PVP-I: Povidone-iodine, r: Correlation coefficient, r^2: Coefficient of determination RP-HPLC: Reversed-phase high-performance liquid chromatography, RSD (%RSD): Relative standard deviation (as a percentage), SARS: Severe acute respiratory syndrome, SD: Standard deviation, USP: United States Pharmacopeia, UV: Ultraviolet, UV-Vis: Ultraviolet-visible, UV-C: Ultraviolet C, w/v: Weight per volume

Symbols and units

ε: Absorptivity, b: Path length, C: Concentration, λmax: Wavelength of maximum absorbance λ1, λ2, λ3: Wavelengths used in robustness testing (287, 291 and 295 nm),σ: Residual standard deviation of the regression, S: Slope of the calibration line,µg/mL: Micrograms per millilitre nm: Nanometre, h: Hour, cm: Centimetre

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