OXFORD

*Journal of AOAC INTERNATIONAL*, 104(5), 2021, 1420–1429

**doi: 10.1093/jaoacint/qsaa177**
Advance Access Publication Date: 23 January 2021
Article



STATISTICAL ANALYSIS AND CHEMOMETRIC METHODS

# Spectrophotometric Determination of Polyvinyl Pyrrolidone in Pure and Pharmaceutical Dosage Form

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## Abstract

**Background:** Recently, functional polymers have attracted significant attention in the areas of pharmaceuticals and biomedical applications, so it is important to develop simple techniques to analyze functional polymers in their pharmaceutical dosage forms.
**Objective:** Three simple, accurate, and sensitive UV spectrophotometric methods have been developed and validated for determination of polyvinyl pyrrolidone (PVP) in the presence of benzalkonium chloride (BZ) and sodium lactate in ternary mixtures.
**Method:** Method A is a derivative ratio spectra zero-crossing (DRSZ) method which measures PVP peak amplitude at 303.1 nm. Method B is a double divisor ratio derivative (DDRD), used for determination of both PVP and BZ in the presence of sodium lactate at 272.6 and 271.5 nm, respectively. Method C is a double divisor ratio derivative-ratio difference spectrophotometric method (DDRD-RDSM), a new and hybrid method of double divisor and ratio difference that hasn't been applied before. It measures peak amplitude difference of the ratio spectra at $\Delta P_{278-252.4}$ and $\Delta P_{260.9-213}$ for PVP and BZ, respectively.
**Results:** Linear ranges for PVP (5.00–35.00, 10.00–40.00, and 10.00–40.00 μg/mL) was obtained by using DRSZ, DDRD, and DDRD-RDSM, respectively. While the linear range for BZ (5.00–60 μg/mL) was obtained by using both DDRD and DDRD-RDSM.
**Conclusions:** All results were statistically compared with reported methods. No significant differences were observed. The developed methods were applied to the analysis of the investigated drugs in pure and pharmaceutical dosage forms.
**Highlights:** The proposed methods are of great value, improving the efficiency of routine analysis of PVP and BZ in their pharmaceutical dosage forms.

Polyvinyl pyrrolidone (1-ethenylpyrrolidin-2-one; PVP) is a water-soluble synthetic polymer (Figure 1) which is a white to light yellow, hygroscopic, amorphous powder. It was used as a plasma substitute for victims of trauma as it is non-antigenic and requires no cross-matching. It has, however, been discontinued due to its accumulation inside the body (1). PVP is also used as an excipient (binder) in pharmaceutical formulations (2), as well as in eye lubricant (3). PVP added to iodine forms a complex called povidone-iodine that possesses disinfectant properties. It is known under the trade name Betadine. It is also used as an aid for increasing the solubility of drugs in liquid and semiliquid dosage forms (syrups and soft gelatin capsules) and as an inhibitor of recrystallization (4 ).

PVP is used as a stabilizer for liquid vitamins and minerals and prevents crystallization of liquid synthetic sweetener preparations. It is also used as a diluent and dispersant for food colorant and is used in cosmetics as a stiffener in hair lotions and improves the consistency of shampoos (5).

A literature review showed that PVP can be determined by colorimetric (6), fluorimetric (7, 8), HPLC (1, 9), and gas chromatographic methods (10).

**Received:** 1 October 2020; **Revised:** 15 November 2020; **Accepted:** 21 December 2020

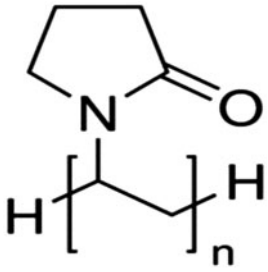

Figure 1. Structure of PVP.

The aim of our study is to present new, simple spectrophotometric methods for determination of the investigated drug in pure and pharmaceutical dosage form.

## Theory

### Derivative Ratio Spectra Zero Crossing Method (DRSZ)

In this method, the simultaneous determination of three compounds X, Y, and Z in a ternary mixture is realized by measuring the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra. The following equations illustrate the background of this method:

$$A_m, \lambda_i = \alpha_X, \lambda_i CX + \beta_Y, \lambda_i CY + \gamma_Z, \lambda_i CZ \quad (1)$$

where $A_m,\lambda_i$, = the absorbance of the mixture at wavelength $\lambda_i$ and $\alpha_X,\lambda_i$, $\beta_Y,\lambda_i$, and $\gamma_Z,\lambda_i$ are the absorptivities of X, Y, and Z, respectively. $C_X$, $C_Y$, and $C_Z$ represent the concentrations of the mixture compounds. If Equation (1) is divided by the spectrum of a standard solution ($C^0_X$) of one of the compounds in a ternary mixture, Equation (1) becomes:

$$A_m, \lambda_i/\alpha_{X,\lambda i}C^0{}_X = \alpha_{\lambda i}C_X/\alpha_{X,\lambda i}C^0{}_X + \beta_{\lambda i}C_Y/\alpha_{X,\lambda i}C^0{}_X + \gamma_{\lambda i}C_Z/\alpha_{X,\lambda i}C^0{}_X \quad (2)$$

If the first derivative of Equation (2) is taken, then we obtain the following:

$$d/d\lambda[A_{m,\lambda i}/\alpha_{X,\lambda i}C^0{}_X] = d/d\lambda[\beta_{\lambda i}/\alpha_{X,\lambda i}]C_Y/C^0{}_X + d/d\lambda[\gamma_{\lambda i}/\alpha_{X,\lambda i}]C_Z/C^0{}_X \quad (3)$$

In addition, if $d/d\lambda\ [\beta_{\lambda i}/\alpha_{X,\lambda i}]\ C_Y/C^0_X$ is equal to zero, for a given point corresponding to the wavelength λi, the Equation (3) would be obtained as:

$$d/d\lambda[A_{m,\lambda i}/\alpha_{X,\lambda i}C^0{}_X] = d/d\lambda[\gamma_{\lambda i}/\alpha_{X,\lambda i}]C_Z/C^0{}_X \quad (4)$$

Equation (4) shows that the derivative of the ratio spectra of a ternary mixture is dependent only on the concentration of $C_Z$ and ($C^0_X$) and is independent of the concentrations of $C_X$ and $C_Y$ of other compounds in the ternary mixture. The calibration graphs can be obtained by plotting $d/d\lambda\ [A_{m,\lambda i}/\alpha_{X,\lambda i}C^0_X]$ signals versus the concentration of $C_Z$. This procedure is repeated for other compounds in the ternary mixture.

### Double Divisor Ratio Spectra Derivative Method (DDRD)

This method is based on the use of the coincident spectra of the derivative of ratio spectra obtained by using a ''double divisor'' (sum of two spectra) and measuring at either maximum or minimum wavelengths.

$$A_{m,\lambda i} = \alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y \quad (5)$$

If Equation (1) is divided by Equation (5), corresponding to the spectrum of a standard solution of two components in the ternary mixture, the ratio spectrum is obtained in the form:

$$A_{m,\lambda i}/\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y = [\alpha_{X,\lambda i}C_X + \beta_{Y,\lambda i}C_Y]/[\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y] + [\gamma_{Z,\lambda i}C_Z]/[\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y] \quad (6)$$

The ratio of the sum of $\alpha_{X,\lambda i}C_X$ and $\beta_{Y,\lambda i}C_Y$ to the sum of $\alpha_{X,\lambda i}C^0_X$ and $\beta_{Y,\lambda i}C^0_Y$ is equal to the constant (k) with respect to λ, in a certain region or point of a wavelength. If the above constant (k) is replaced in Equation (6), we obtain:

$$A_{m,\lambda i}/\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y = k + [\gamma_{Z,\lambda i}C_Z]/[\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y] \quad (7)$$

However, if the standard concentrations of $C^0_X$ and $C^0_Y$ in Equation (5) are equal or very close to each other, we could write:

$$\alpha_{X,\lambda i}C^0{}_X + \beta_{Y,\lambda i}C^0{}_Y = C^0{}_X[\alpha_{X,\lambda i} + \beta_{Y,\lambda i}] \quad (8)$$

When Equation (8) is substituted into Equation (7), Equation (9) is obtained:

$$A_{m,\lambda i}/C^0{}_X[\alpha_{X,\lambda i} + \beta_{Y,\lambda i}] = k + [\gamma_{Z,\lambda i}]/[\alpha_{X,\lambda i} + \beta_{Y,\lambda i}] \times C_Z/C^0{}_X \quad (9)$$

Equation (9) is the mathematical foundation of multi-component analysis which permits the determination of the concentration of each of the active compounds in solution without the interference from other components of the ternary system. In practice, Equation (9), corresponding to the first derivative ratio spectrum of Z, is obtained by dividing the absorption spectrum of two of the ternary mixtures X, Y, and Z by the standard spectrum of two of the compounds in the ternary mixture. Also, in Equation (7), the derivative signal of the ratio spectrum of the ternary mixture is dependent only on the concentration values $C_X$ and $C_Y$ in the ternary mixture. In the developed method, the concentration $C_Z$ in the ternary mixture is proportional to the first derivative signals corresponding to a maximum or minimum point. A calibration graph is obtained by recording and storing the spectra of solutions of different concentrations of pure Z, and the spectrum of a solution of a binary mixture of pure X and Y of concentration $C^0_X$ and $C^0_Y$. The stored spectra of the solutions of pure Z are divided by the standard spectrum of the mixture of X and Y. The ratio spectra thus obtained are differential with respect to wavelength and the first derivative values at a given wavelength are plotted against $C_Z$, so that the calibration graph is obtained for $C_Z$. By using the calibration graph, the concentration of $C_Z$ is determined in the sample containing X, Y, and Z. The concentrations of X and Y are determined by analogous procedures.

### Double Divisor-Ratio Difference Method

This method is a hybrid method between double divisor (11) and ratio difference methods (12, 13). This method has never been applied before.

## Experimental

### Instrumentation

A SHIMADZU Dual-beam (Kyoto, Japan) UV-Visible spectrophotometer, model UV-1601 PC with 1 cm quartz cuvettes connected to an IBM compatible computer fitted with UV-PC personal spectroscopy software version 3.7.

A pH meter 3510 pH/mV/°C (Jenway, UK) with combined glass electrode was used for pH adjustments.

### Chemicals and Reagents

*Pure samples*.—PVP K25, BZ, and sodium lactate were kindly supplied by Egyptian International Pharmaceutical Industries Co. (EIPICO), Egypt. Purity was reported to be 100% (according to the standard certificate provided by the manufacturer).

*Pharmaceutical dosage forms*.—Orchatears® Plus eye drops, labeled to contain 50 mg/mL PVP K25, 0.1 mg/mL BZ, and sodium lactate as excipient, batch no. (0518108), was manufactured by Orchidia Pharmaceutical Ind., Egypt and was purchased from the market.

*Reagents and solvents*.—All chemicals and reagents used were of analytical grade. Bi-distilled water was used throughout and is indicated by the word "water". Phosphate buffer solution pH 8 was prepared by using potassium dihydrogen phosphate and disodium hydrogen phosphate (ADWIC, Egypt) with appropriate concentrations.

*Standard solutions*.—Standard stock solutions of PVP, BZ, and sodium lactate (100 μg/mL each) were freshly prepared in water. These solutions were also used as working standard solutions and diluted with phosphate buffer pH 8 to prepare serial dilutions. All stock solutions and working standard solutions were stored in a refrigerator at 4°C for further use and were stable for 1 month.

### Procedures

Aliquots of standard working solutions of PVP, BZ, and sodium lactate were diluted with phosphate buffer pH 8 to prepare concentrations of 10 μg/mL. Each was separately scanned over the range 200–400 nm using phosphate buffer pH 8 as a blank.

*DRSZ*.—Accurate volumes of standard working solutions of PVP, BZ, and sodium lactate were transferred into a set of 10 ml volumetric flasks and diluted to the mark with phosphate buffer pH 8 and then scanned in the range of 200–400 nm. By using sodium lactate (60 μg/mL) as a divisor, the amounts of PVP in the ternary mixture were determined by measuring the first derivative ratio amplitudes using $\Delta\lambda = 4$ and scaling factor = 10 at 303.1 nm (zero-crossing point for BZ).

*DDRD*.—In this method, the absorption spectra of PVP at different concentrations were recorded and divided by the sum of the absorption spectra of BZ and sodium lactate (10 μg/mL each) as a double divisor. Also, the absorption spectra of BZ at different concentrations were recorded and divided by the sum of the absorption spectra of PVP and sodium lactate (15 μg/mL each) as a double divisor, and then the first derivative ratio spectra was computed using scaling factor = 10 and $\Delta\lambda = 4$. The peak amplitudes of PVP and BZ were recorded at 272.6 and 271.5 nm, respectively and were plotted versus their corresponding concentrations. Then the regression equations were computed.

*DDRD-RDSM*.—The stored spectra of PVP were divided by the sum of spectra of 10 μg/mL of both BZ and sodium lactate while the spectra of BZ were divided by the sum of spectra of 15 μg/mL of both PVP and sodium lactate. The obtained ratio spectra were smoothed with $\Delta\lambda = 8$ nm then the peak amplitude differences of the ratio spectra $\Delta P_{278-252.4}$ and $\Delta P_{260.9-213}$ for PVP and BZ, respectively, were plotted verses the corresponding concentrations and the regression equations were computed.

### Laboratory Prepared Mixtures

Several laboratory prepared mixtures containing different ratios of PVP, BZ, and sodium lactate were prepared, and the corresponding concentrations were calculated using the regression equation by the above-mentioned methods.

### Pharmaceutical Dosage Forms

*Orchatears Plus eye drops*.—The contents of six bottles of Orchatears Plus eye drops were mixed together, then 1 mL was accurately transferred to a 250 mL volumetric flask and diluted to the mark with water. From this solution, 0.75 mL was transferred to a 10 mL volumetric flask, diluted to the mark with phosphate buffer pH 8 to prepare a concentration of 15 μg/mL of PVP. For BZ, 1 mL was accurately transferred from the Orchatears Plus eye drops to a 10 ml volumetric flask, diluted to the mark with phosphate buffer pH 8 to prepare a concentration of 10 μg/mL. The above-mentioned procedures were followed and the standard addition technique was adopted to check the validity of the proposed methods.

## Results and Discussion

Three simple and low-cost spectrophotometric methods were developed for rapid determination of PVP and BZ in ternary mixtures.

PVP, BZ, and sodium lactate are present together in multi-ingredient dosage forms (e.g., eye drops). PVP is used for eye lubrication and relieves pain in inflamed or irritated corneal epithelial cells as in the case of eye dryness. BZ is the most commonly used eye drop preservative that prevents any contamination during use. Sodium lactate is used as an electrolyte to maintain or lower tear osmolarity as high osmolarity products pull water from epithelial cells, interfering with corneal metabolism. It is therefore critical to analyze them together in their combined formulations.

Sodium lactate was found as an excipient in Orchatears Plus eye drops, its UV spectrum make sever overlapping with the UV spectra of PVP and BZ, that is why we analyze PVP and BZ in its presence.

Since traditional derivative techniques failed to resolve the severely overlapping spectra of the studied drugs (as in Figure 2), it became important to find new methods that could determine each of the studied drugs selectively in the presence of other interferents.

Analysis of PVP by UV spectrophotometry was challenging as this drug lacks conjugation and did not have significant peaks in the middle and near UV regions. The proposed

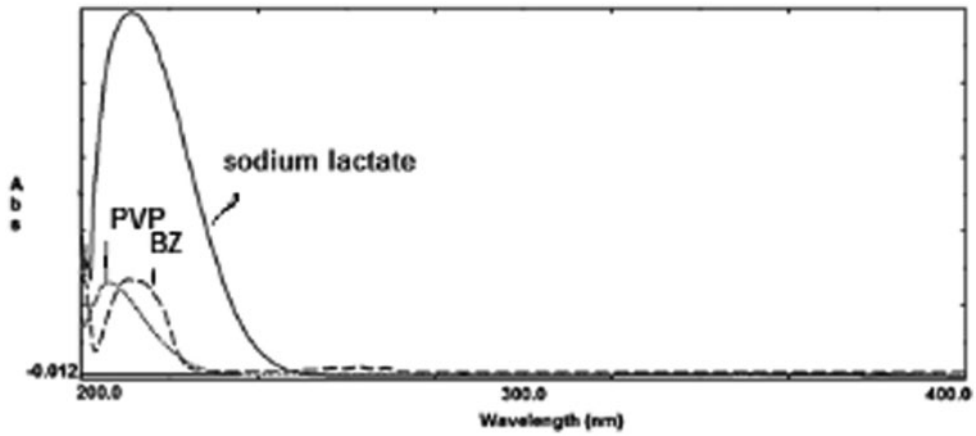


**Figure 2.** Zero order absorption spectra of PVP, BZ, and sodium lactate (10 μg/mL each) using phosphate buffer pH 8 as a blank.

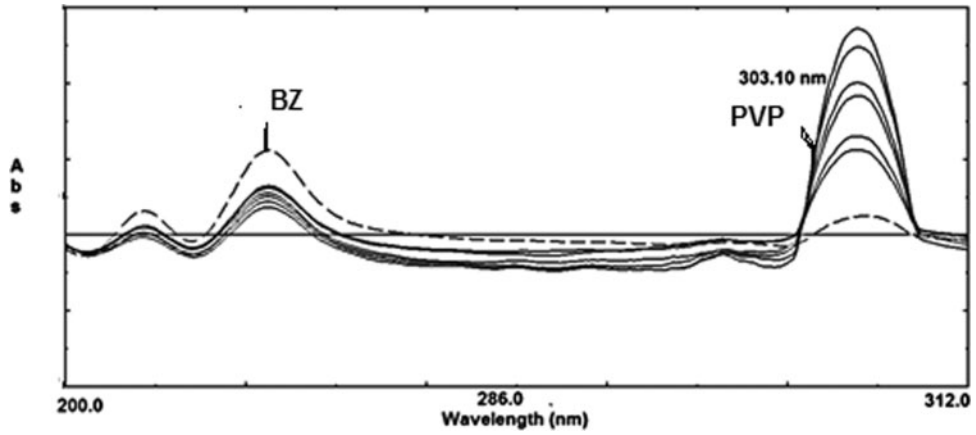


**Figure 3.** First derivative ratio spectra of PVP (5.00, 9.00, 18.00, 23.00, 30.00, 35.00 μg/mL) and BZ (40 μg/mL) using 60 μg/mL Na lactate as divisor at 303.1 nm in DRZC.

methods were the first UV spectrophotometric methods for analysis of this drug in its pure and pharmaceutical dosage form.

Many solvents and buffered solutions were tested in order to lengthen the wavelength of PVP. Phosphate buffer pH 8 was the solvent of choice as it made a bathochromic shift to the spectrum of PVP, making it possible to measure its absorbance in the UV region. The reason for this is that the carbonyl group of PVP may undergo keto/enol isomerization, which explains the bathochromic shift observed.

## PVP Ternary Mixture

*DRSZ.*—This method is achieved by measuring the amplitude at the zero-crossing point in the derivative spectrum of the ratio spectra (11).

The DRSZ approach was applied for determination of PVP in the presence of BZ and sodium lactate. PVP can be determined in this mixture by dividing the spectrum of the mixture by the absorption spectrum of sodium lactate (60 μg/mL) as a divisor. The amounts of PVP in the ternary mixtures were determined by measuring the first derivative ratio amplitude at 303.1 nm, where BZ exhibits zero absorbance (Figure 3).

For method optimization, it was observed that changing the concentration of the divisors had a significant effect on method selectivity. When the concentration of the divisor is increased or decreased, the resulting derivative values are proportionally decreased or increased, respectively, although the maxima and minima remain at the same wavelengths. Sodium lactate (60 μg/mL) as a divisor, together with $\Delta\lambda = 4$ and scaling factor = 10, assured the best compromise in terms of sensitivity, repeatability, and signal-to-noise ratio.

*DDRD.*—This method is based on the use of coincident spectra of the derivative of the ratio spectra obtained by using a double divisor (14).

For determination of PVP, the absorption spectra of PVP at different concentrations were recorded and divided by the sum of the absorption spectra of BZ and sodium lactate (10 μg/mL each) as a double divisor, then the first derivative ratio spectra were computed at 272.6 nm using scaling factor = 10 and $\Delta\lambda = 4$ (Figure 4).

In the same way, the absorption spectra of BZ at different concentrations were recorded and divided by the sum of the

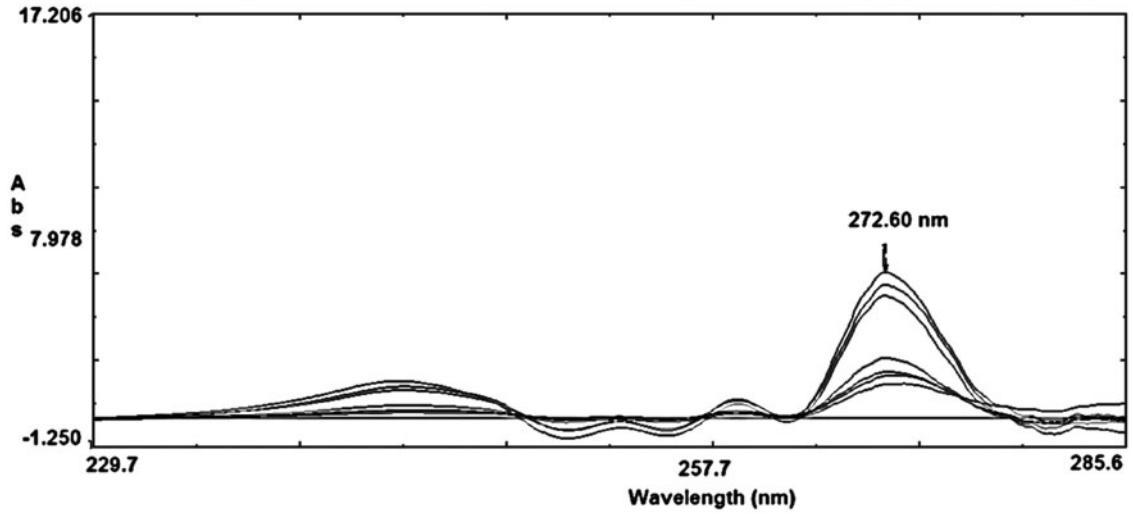


**Figure 4.** First derivative of ratio spectra of PVP (14.00, 16.00, 18.00, 20.00,22.00,35.00, 40.00 μg/mL) using 10.00 μg/mL each of BZ and sodium lactate as double divisor in phosphate buffer PH 8 in DDRD.

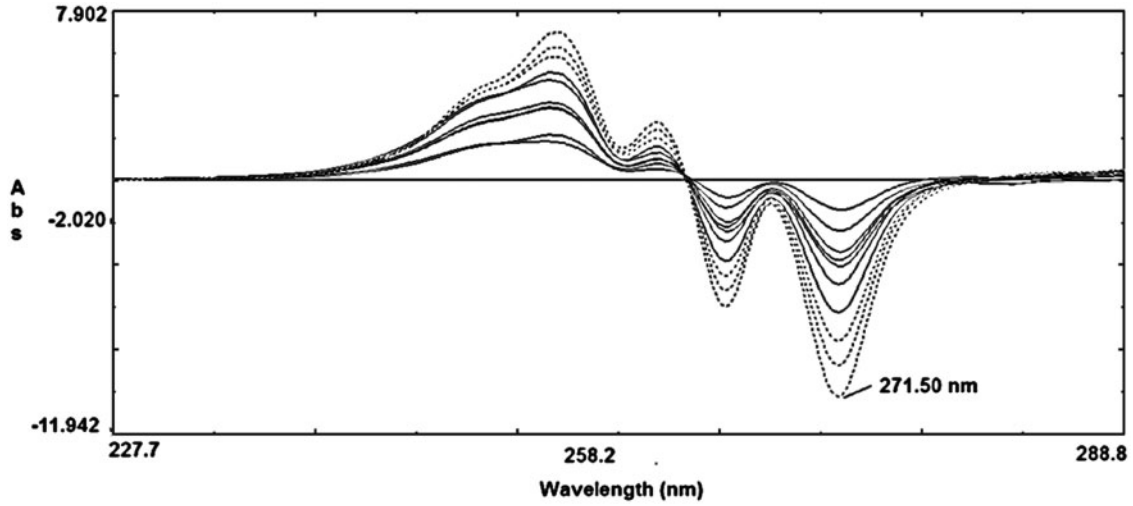


**Figure 5.** First derivative of ratio spectra of BZ (5.00,10.00, 14.00, 15.00, 16.00, 20.00, 25.00, 30.00, 35.00, 40.00 μg/mL) using 15.00 μg/mL each of PVP and sodium lactate as double divisor in phosphate buffer PH 8 at 271.5 nm in DDRD.

absorption spectra of PVP and sodium lactate (15 μg/mL each) as a double divisor and then the first derivative ratio spectra were computed at 271.5 nm (Figure 5).

To optimize this method, it was necessary to test the effect of the following variables:

(a) Divisor concentration: Different concentrations were tried as double divisor. The best sensitivity and response were obtained using BZ and sodium lactate (10μg/mL each), PVP and sodium lactate (15μg/mL each) as double divisor for determination of PVP and BZ, respectively.
(b) Different $\Delta\lambda$ values were tried where $\Delta\lambda = 4$ showed suitable signal-to-noise ratios and the resulting spectra showed good resolution.
(c) Different scaling factor values were tried, where a scaling factor of 10 was suitable to maximize the signal of the drugs and facilitate their measurement and decrease reading errors.

*DDRD-RDSM.*—This method is a hybrid of double divisor (14) and ratio difference methods (12, 13). This is the first time these two techniques have been hybridized and make a good use of this hybridization to selectively analyze our interest drug.

Where the stored spectra of PVP were divided by the sum of the absorbance of the spectra of BZ and sodium lactate (10 μg/mL each). The obtained ratio spectra were smoothed with $\Delta\lambda = 8$ nm Then the peak amplitude difference of the ratio spectra ($\Delta P_{278-252.4}$) was computed (Figure 6). While the spectra of BZ were divided by the sum of the absorbance of the spectra of PVP and sodium lactate (15 μg/mL each). The obtained ratio spectra were smoothed with $\Delta\lambda = 8$ nm Then the peak amplitude difference of the ratio spectra ($\Delta P_{260.9-213}$) was computed (Figure 7).

## Method Validation

The method validation was performed according to the ICH guidelines (15).

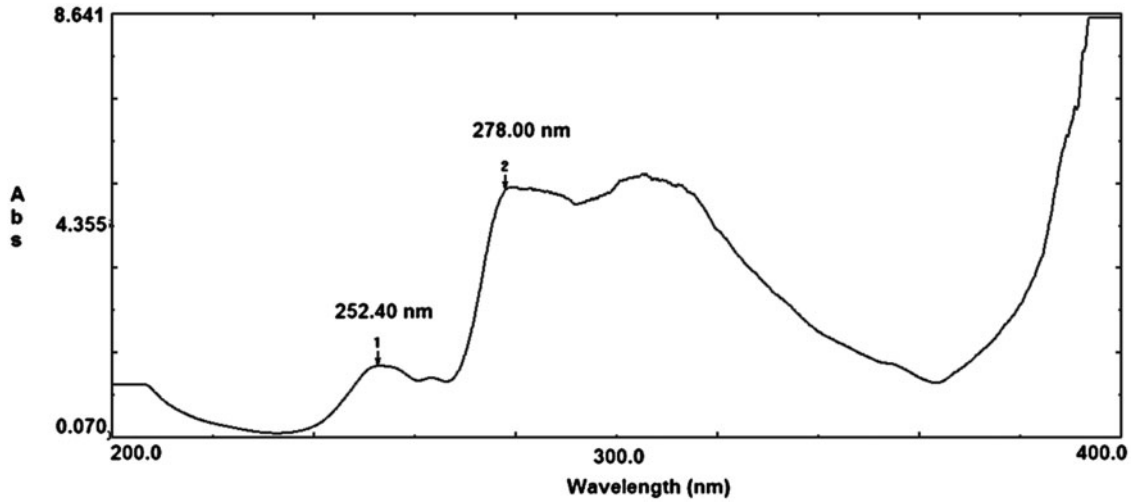


**Figure 6.** Ratio spectra of 40.00 μg/mL of PVP showing selected wavelength for DDRD-RDSM method using phosphate buffer pH 8 as blank after smoothing at $\Delta\lambda = 8$.

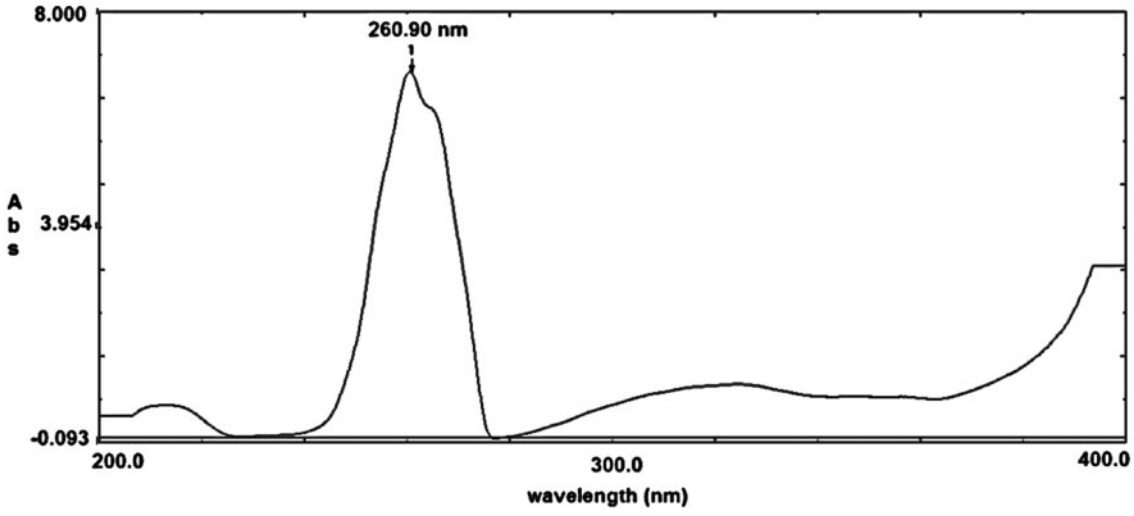


**Figure 7.** Ratio spectra of 35.00 μg/mL of BZ showing selected wavelength for DDRD-RDSM method using phosphate buffer pH 8 as blank after smoothing at $\Delta\lambda = 8$.

*Linearity*.—The linearity of the proposed methods were evaluated under different concentrations of standard solutions, with all the methods showing good correlation coefficients close to unity, indicating good linearity, as shown in Table 1, where DDRD and DDRD-RDSM shows wider linear range for PVP than that of DRSZ.

*Accuracy*.—The accuracy of the results was checked by applying the proposed methods for the determination of different samples of PVP and BZ. The concentrations were obtained from the corresponding regression equations, and the recoveries were calculated, as shown in Table 1, where DDRD shows better accuracy for PVP and BZ and also lower SD.

*Precision*.—Intraday and interday precision of three concentrations of PVP (18, 20, and 25 μg/mL) for DRSZ (16, 17, 22 μg/mL), DDRD, and DDRD-RDSM were checked. Intraday and interday precision of three concentrations of BZ (20, 45, 55 μg/mL) were checked by using DDRD and DDRD-RDSM. The obtained results for the three proposed methods showed good precision results due to low values of RSD, % as shown in Table 1, where DRSZ exhibits the lowest LOD for PVP, whereas DDRD exhibits the lowest LOD for BZ.

*LOD and LOQ*.—The LOD and LOQ were calculated as LOD = 3.3 (σ/S) and LOQ = 10 (σ/S), where "σ" represents standard deviation of the intercept and "S" is the slope of the calibration line as shown in Table 1.

*Specificity*.—Specificity was checked by analyzing PVP with BZ and sodium lactate in laboratory prepared mixtures with different ratios and was ascertained from the results represented in Table 2, where the DDRD method showed the highest specificity in terms of decreasing SD for both PVP and BZ.

*Application to pharmaceutical dosage form*.—The proposed methods were successfully applied to the pharmaceutical dosage form and the standard addition technique was performed as

**Table 1.** Calibration data for the determination of PVP and BZ in the presence of sodium lactate by the proposed methods[a]

| Method | Drug | Working λ, nm | Range, μg/mL | Regression equation | R | LOD, μg/mL | LOQ, μg/mL | Accuracy ± SD | Intraday RSD, % | Interday RSD, % |
|---|---|---|---|---|---|---|---|---|---|---|
| DRSZ | PVP | 303.10 | += 5.00–35.00 | Y = 0.0868C + 2.1018 | 0.9996 | 1.59 | 4.83 | 99.72 ± 1.11 | 1.05 | 1.94 |
| DDRD | PVP | 272.60 | 10.00–40.00 | Y = 0.1825C + 1.1409 | 0.9997 | 2.99 | 9.06 | 100.28 ± 0.33 | 1.40 | 1.56 |
| | BZ | 271.50 | 5.00–60.00 | Y = 0.2551C + 0.0163 | 0.9999 | 1.20 | 3.64 | 99.75 ± 0.58 | 0.85 | 0.95 |
| DDRD-RDSM | PVP | 252.40 and 278.00 | 10.00–40.00 | Y = 0.1057C–0.6758 | 0.9997 | 3.03 | 9.17 | 100.20 ± 0.63 | 1.23 | 1.50 |
| | BZ | 213.00 and 260.90 | 5.00–60.00 | Y = 0.1374C + 1.5250 | 0.9995 | 7<br>1.36 | 4.13 | 99.69 ± 0.59 | 1.30 | 1.59 |

[a]r = Correlation coefficient; C = Concentration of drugs in μg/mL.

**Table 2.** Specificity results for determination of PVP and BZ with sodium lactate in laboratory prepared mixtures using the proposed methods

| Ratio of mixture components (PVP K25: BZ: Na lactate) | Recovery, %[a] | | | | |
|---|---|---|---|---|---|
| | PVP | | | BZ | |
| | DRSZ | DDRD | DDRD-RDSM | DDRD | DDRD-RDSM |
| 2:1:2 | 100.00 | 100.5 | 101.00 | 99.00 | 98.60 |
| 6:1:2 | 101.20 | 100.3 | 98.30 | 100.80 | 100.40 |
| 3:2:1 | 100.43 | 98.37 | 98.13 | 101.90 | 101.70 |
| 4:3:1 | | 100.83 | 100.30 | 101.87 | 100.07 |
| 2.7:7.7:1 | 100.25 | 100.31 | 100.13 | 100.20 | 98.22 |
| 4.4:5.2:1 | 100.45 | | | | |
| Mean ± SD | 100.27 ± 0.45 | 100.06 ± 0.97 | 99.57 ± 1.28 | 100.75 ± 1.22 | 99.80 ± 1.41 |

[a]Mean of three determinations.

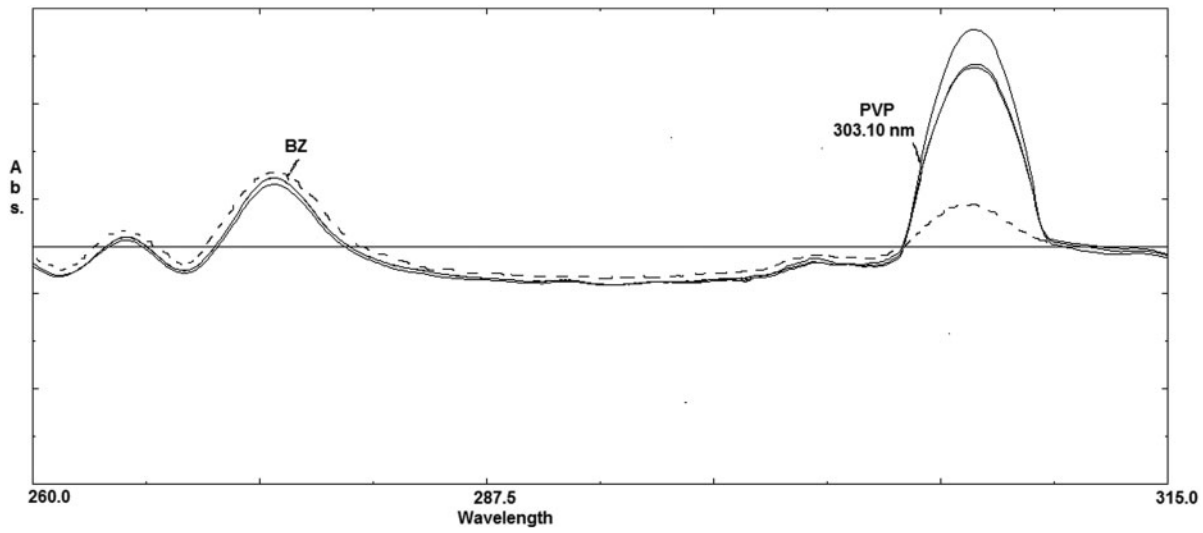


**Figure 8.** First derivative ratio spectra of PVP (15.00 μg/mL) and BZ (40 μg/mL) in Orchatears Plus eye drops using 60 μg/mL Na lactate as divisor at 303.1 nm in DRZC.

shown in Figures 8–12. The concentrations were calculated using the corresponding regression equations as shown in Table 3, where DDRD-RDSM showed the best recoveries for PVP and BZ and DDRD showed the lowest SD for PVP standard addition, whereas DDRD-RDSM revealed the least SD for standard addition of BZ.

*Statistical analysis.*—A statistical comparison of the results obtained by the proposed methods and the reference methods (7, 16) for determination of PVP and BZ was performed. The differences between the proposed and reference methods were tested by *F*-test and *t*-test (17) as shown in Table 4. The tests ascertained that there were no significant differences with

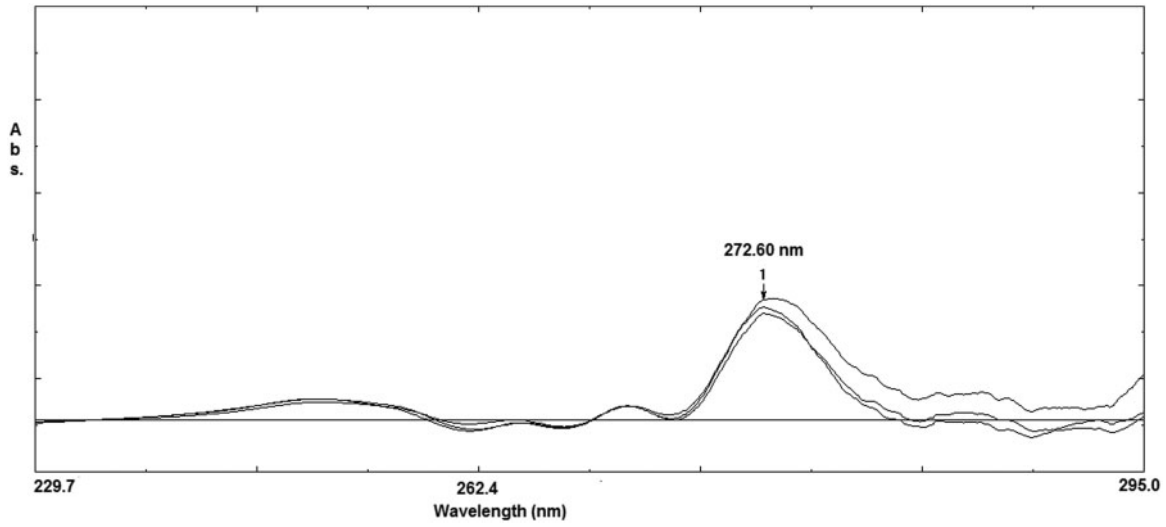


**Figure 9.** First derivative of ratio spectra of PVP (15.00 μg/mL) in Orchatears Plus eye drops using 10.00 μg/mL each of BZ and sodium lactate as double divisor in phosphate buffer PH 8 in DDRD.

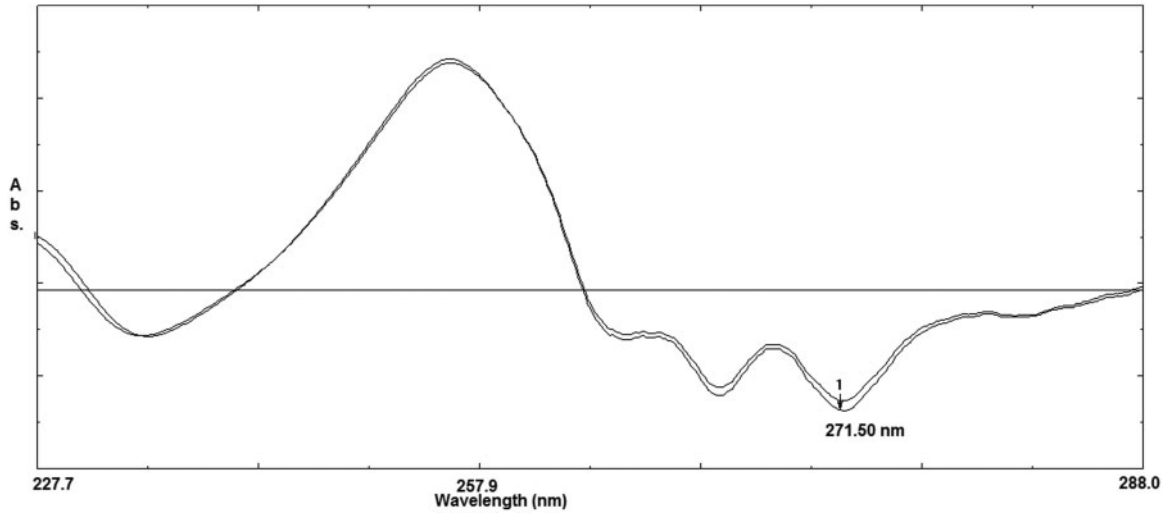


**Figure 10.** First derivative of ratio spectra of BZ 10.00 μg/mL in Orchatears Plus eye drops using 15.00 μg/mL of each of (PVP and sodium lactate) as double divisor in phosphate buffer PH 8 at 271.5 nm in DDRD.

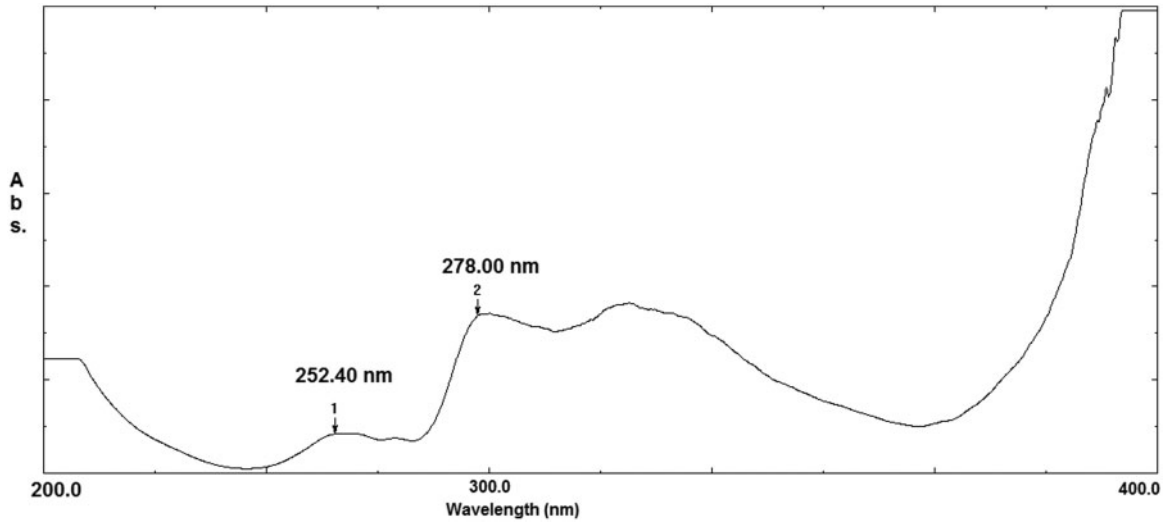


**Figure 11.** Ratio spectra of 15.00 μg/mL of PVP in Orchatears Plus eye drops showing selected wavelength for DDRD-RDSM method using phosphate buffer pH 8 as blank after smoothing at $\Delta\lambda = 8$.

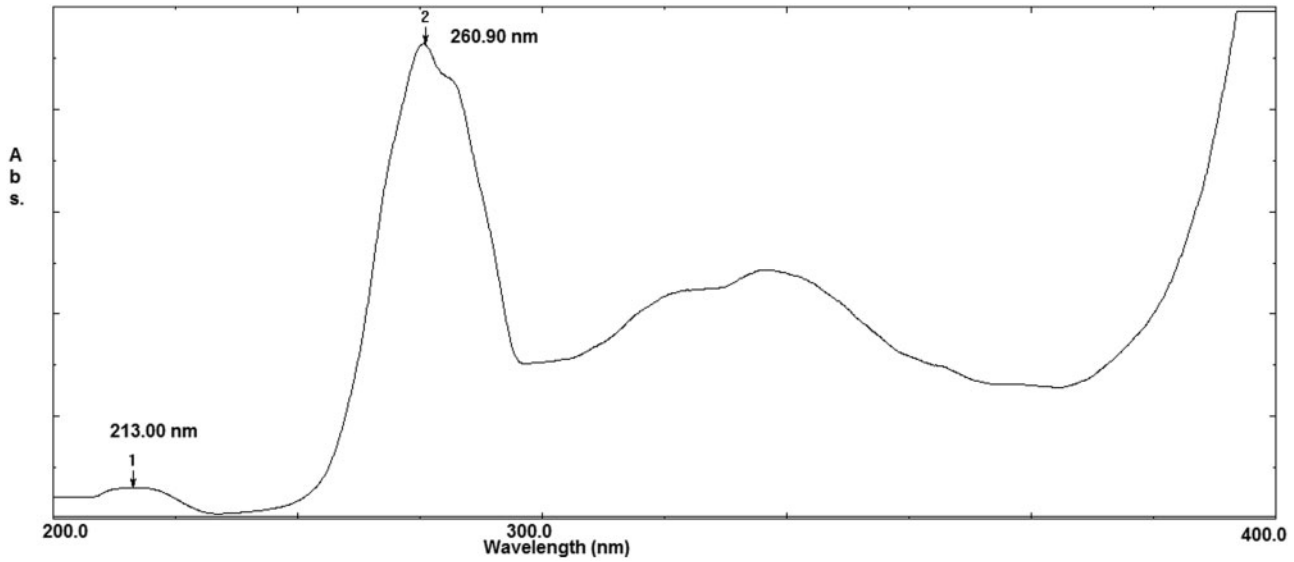


**Figure 12.** Ratio spectra of 15.00 μg/mL of BZ in Orchatears Plus eye drops showing selected wavelength for DDRD-RDSM method using phosphate buffer pH 8 as blank after smoothing at $\Delta\lambda = 8$.

**Table 3.** Application of the proposed method for determination of PVP and BZ in pharmaceutical dosage form

| Pharmaceutical formulation | Drugs | DRSZ | | | DDRD | | | DDRD-RDSM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Found, % ± SD[a] | Standard addition | | Found, % ± SD[a] | Standard addition | | Found, % ± SD[a] | Standard addition | |
| | | | Added, μg/mL | Recovery, % | | Added, μg/mL | Recovery, % | | Added, μg/mL | Recovery, % |
| Marketed Orchatears Plus Labeled to contain 50 mg/mL PVP K25 and 0.1 mg/mL BZ Batch no. 0518108 | PVP | 98.82 ± 0.45 | 10.00 | 99.10 | 98.89 ± 0.62 | 10.00 | 98.70 | 101.16 ± 0.71 | 10.00 | 100.40 |
| | | | 15.00 | 99.07 | | 15.00 | 99.33 | | 15.00 | 102.00 |
| | | | 20.00 | 99.90 | | 20.00 | 99.35 | | 20.00 | 99.05 |
| | | | Mean ± SD | 99.36 ± 0.47 | | Mean ± SD | 99.13 ± 0.37 | | Mean ± SD | 100.48 ± 1.48 |
| | BZ | | | | 99.47 ± 0.51 | 5.00 | 98.80 | 100.73 ± 0.90 | 5.00 | 98.60 |
| | | | | | | 10.00 | 98.70 | | 10.00 | 99.10 |
| | | | | | | 15.00 | 99.80 | | 15.00 | 98.27 |
| | | | | | | Mean ± SD | 99.10 ± 0.62 | | Mean ± SD | 98.66 ± 0.42 |

[a] Mean of three determinations.

**Table 4.** Statistical comparison between the proposed spectrophotometric methods and the reported methods for PVP and BZ, respectively

| | Reported method | | Proposed method | | | | |
|---|---|---|---|---|---|---|---|
| | | | PVP | | | BZ | |
| Statistical term | PVP[a] | BZ[b] | DRSZ | DDRD | DDRD-RDSM | DDRD | DDRD-RDSM |
| Mean | 99.79 | 100.17 | 99.71 | 100.29 | 100.20 | 99.74 | 99.69 |
| SD | 0.46 | 1.07 | 1.12 | 0.33 | 0.63 | 0.57 | 0.59 |
| RSD, % | 0.47 | 1.07 | 1.12 | 0.33 | 0.63 | 0.57 | 0.59 |
| *n* | 5 | 3 | 5 | 5 | 5 | 5 | 5 |
| Variance | 0.22 | 1.14 | 1.25 | 0.11 | 0.39 | 0.32 | 0.35 |
| *t* (test) | | | 0.15 (2.31)[c] | 2.00 (2.31)[c] | 1.18 (2.31)[c] | 0.76 (2.45)[c] | 0.84 (2.45)[c] |
| *F* (test) | | | 5.68 (6.39)[c] | 1.97 (6.39)[c] | 1.77 (6.39)[c] | 9.15 (9.94)[c] | 3.26 (9.94)[c] |

[a] Reported method = Fluorescence quenching reaction of PVP-Eosin *y* system for determination of PVP at 542 nm (7).
[b] Ratio subtraction method for determination of BZ at 208.00 nm (15).
[c] The values between parentheses are the corresponding theoretical values of *t* and *F* at the 95% confidence level.

respect to accuracy and precision between the proposed methods and the reported methods (7, 16).

## Conclusions

The proposed spectrophotometric methods for determination of PVP and BZ in the presence of sodium lactate could be successfully applied. The results show good selectivity, accuracy, and precision demonstrating that they are rapid, selective, sensitive, economic, and reproducible. The proposed methods are of great value, improving the efficiency of routine analysis of the cited drugs in their pharmaceutical dosage forms. Also, the proposed methods offer distinct advantages since they are the first developed spectrophotometric methods for analyses of this mixture and it is well known that spectrophotometric methods offer simplicity, ease of use, and low cost compared to other analytical methods (18).

## Funding

This research did not receive any specific grant from funding agencies in the public, commerical, or not-for-profit sectors.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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