Determination of Total Polyvinylpyrrolidone (PVP) in Ophthalmic Solutions by Size Exclusion Chromatography with Ultraviolet-visible Detection

Panagiotis Tavlarakis[1,2], John J. Urban[1], and **Nicholas Snow[2,*]**

[1]Global R&D Operations, Analytical Development, Johnson & Johnson Group of Consumer Companies, 185 Tabor Road, Morris Plains, NJ 07950 and [2]Department of Chemistry and Biochemistry, Center for Academic Industry Partnership, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079

Abstract

A straightforward size exclusion chromatography (SEC) method was developed and validated for the determination of total polivinylpyrrolidone (PVP) in ophthalmic solutions using the unusual combination of size exclusion chromatography (SEC), ultraviolet-visible detection and quantitation of an analyte peak that elutes in the total exclusion volume of the column. Samples of opthalmic solutions are diluted with water and injected onto a TSKgel G1000PW, 7.5 mm i.d. × 30 cm, 12 µm column at 50°C, with 80:20 0.1M sodium acetate–methanol mobile phase and UV detection at 220 nm. Validation was successful for a stability indicating pharmaceutical method, with parameters including specificity, accuracy, linearity, and precision within typical pharmaceutical acceptance criteria. A stress study with acid, base, peroxide, heat, and light indicates that there is no interference from drug, product, or excipients.

Introduction

Povidone (Polyvinylpyrrolidone, PVP) is a chain polymer of 1-vinyl-2-pyrrolidone, developed in the late 1930's (1). PVP is obtained by a multistep synthesis that concludes by polymerization of vinylpyrrolidone in aqueous solution in the presence of hydrogen peroxide (2). A wide range of molecular weights, from a few thousand to a few million Daltons can be obtained by controlling the degree of polymerization. PVP is a white hygroscopic powder and unlike many synthetic polymers is soluble in a variety of traditional solvents such as water, chlorinated hydrocarbons, alcohols, amides, and amines (3). In our studies, PVP with a molecular weight of approximately 50,000 with a K-value of 30, typical in ophthalmic solutions, were used (4). Figure 1 shows the structure of povidone.

PVP originally was used as a plasma substitute and in a variety of applications. Its hygroscopic properties, film formation, and adhesion to different materials have made PVP widely used in pharmaceuticals, cosmetics, and industrial production. The interactions between the carbonyl groups in PVP and the hydroxyl group in polyphenols are well known and have been reported in the literature. Due to these interactions PVP is used to isolate polyphenols and as a colloidal stabilizer in beers by selective removal of tannoid polyphenols (5,6). PVP formulations have been used to produce desired solution viscosity, allowing the deposition of a uniform coating thickness of a photoresist in the manufacture of high resolution display screens (7). In ophthalmic solutions, PVP is used as a demulcent or moisturizer and is generally present at approximately 1% concentration in an aqueous matrix also containing other excipients and active pharmaceutical ingredients. It has been shown in combination with polyethylene glycol 400 and dextran 70 to be effective for the temporary relief of minor irritations, for protection of the eye against further irritation from the wind or sun, and relief from eye dryness (8).

Many chromatographic methods have been reported in the literature for the determination of PVP, either qualitative determination of the molecular weight range of the polymer or quantitative determination in formulations and products, with most focusing on the qualitative aspects such as the form of povidone present and whether materials with same K-value are structurally the same (9,10). A variety of capillary electrophoresis (CE) methods for the characterization and determination of povidone have been reported, including capillary zone electrophoresis (CZE) and capillary gel electrophoresis (CGE) (11–14). Povidone has been determined in several pharmaceutical matrices with solid-phase microextraction. The fibers were polypyrrole (PPy) and desorption was performed at the inlet of a gas chromatograph equipped with a nitrogen phosphorous detector (15). PVP has also been used as stationary phase materials or extractant for both extractions and chromatography (16–19). SEC determinations of povidone have focused on

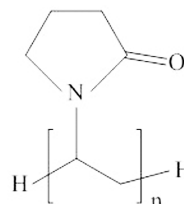

Figure 1. Chemical structure of polyvinylpyrollidone.

*Author to whom correspondence should be addressed: email nicholas.snow@shu.edu.

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molecular weight characterization of the povidone itself or the use of povidone as a molecular weight calibrator for other determinations (20–28).

SEC is not usually used in combination with UV detection; however, it is clearly applicable if the analytes and other compounds of interest in the analytical samples contain a chromophore. Some pharmaceutical applications of SEC with UV detection include a recent study of the mass balance in the oxidative degradation of rapamycin and the analysis of various proteins and biological polymers in formulations (29–31). Determination of total PVP present in the matrix of a pharmaceutical formulation using SEC and UV detection has not been previously reported. In this work, we describe a fast, straightforward, and new HPLC method for the analysis of total PVP in ophthalmic solutions. The new method is stability indicating and was successfully validated based on the International Conference on Harmonization guidelines for pharmaceutical quality assurance (32).

Materials and Methods

Reagents and chemicals

The raw material for PVP was purchased from BASF AG (Ludwigshafen, Germany). HPLC-grade methanol was obtained from Fisher Scientific Inc. (Fairlawn, New Jersey). ACS reagent-grade sodium acetate was purchased from Sigma Aldrich (Saint Louis, Missouri). Water was obtained using a Milli-Q (Millipore, Milford, MA) purification system located in our laboratory.

Laboratory formulations of typical opthalmic solutions were prepared in water at 0.5 mg/mL concentration of povidone. There were diluted by adding 10 mL of formulation to a 50 mL volumetric flask and diluting to the mark with water prior to HPLC analysis. The final working concentration of the sample and standard solutions was 0.1 mg/mL.

Instrumental Conditions

An Alliance HPLC system equipped with a 2695 separation module with 2487 UV and 996 photodiode array detectors was used for all experiments. The final method required the use of a variable wavelength UV detector by receiving laboratories; the photodiode array detector was used to confirm UV spectra of analytes and other compounds present during method development. Data collection and processing was performed using an Empower chromatographic data acquisition system (Waters Corporation, Milford, Massachusetts). The chromatographic column was a TSKgel G1000PW, 7.5 mm i.d. × 30 cm, 12 µm column (Tosoh Bioscience, Tokyo, Japan). The flow was kept at 1.0 mL/min during the length of the run, and the column temperature was 50°C. The UV detector wavelength was 220 nm and the injection volume was 25 µL. The mobile phase was premixed with 800 mL 0.1M sodium acetate and 200 mL methanol generating a mobile phase pH of about 10.

Method validation

Method validation was performed in accordance with ICH guidelines and internal standard operating procedures. The test parameters are presented in the same order as they were investigated during the method validation. Specificity was determined by exposing ophthalmic solution samples, placebos, and povidone standards to stress conditions of acid, base, peroxide, light, and heat and subsequently analyzing them according to the method. A photodiode array detector was used during validation to ensure that no interfering compounds co-eluted with PVP but is not necessary for the final method. Accuracy was measured by spiking placebos with PVP at three working levels 0.35, 0.50, and 0.80 mg/mL (70%, 100%, and 130% of the standard concentration). Six preparations were performed at each level and assayed as per method conditions. The average result from each individual level was compared to its respective theoretical concentration value to check for any potential bias.

To ensure that the method is linear in the working concentration range, five concentration level solutions of povidone, corresponding from 0.25–0.75 mg/mL (50–150% of the expected analyte concentration) were prepared and injected. In addition, the ability of the system to retain and carryover the analyte into subsequent injections were evaluated by injecting a blank solution (diluent only) in duplicate immediately after the 150% linearity level. To ensure performance of the system before and during the analysis, system suitability parameters, as defined in USP/NF, were established as a direct result of ruggedness and robustness experiments (33).

System precision was determined using six replicate measurements of a 100% theoretical concentration standard solution (0.5 mg/mL PVP concentration) containing povidone, with an acceptance criterion of the RSD being less than 2.0%. Repeatability was determined by six identical sample preparations of the same lot. To determine agreement among test results obtained from multiple samplings of the same lot of samples on different days using different instruments, columns, and analysts, six identical samples from the same lot were prepared and analyzed.

Robustness was determined by examining small variations in: wavelength (± 4 nm), flow rate (± 0.1 mL/min), column temperature (± 5°C), and mobile phase preparation (± 10%). These parameters were changed one at a time. System suitability and sample runs were both conducted with unchanged method parameters and modified parameters. In addition, a quantitative comparison study was performed between the raw material that was used to prepare the batch and other raw materials of povidone, including a USP reference standard. One sample preparation was run and quantitated with five different standards as per the method. Further, to assess sample stability, standard and sample solutions were stored at room temperature and tested at initial, 24 h, 96 h, and 192 h. The solutions were tested against a freshly prepared standard at each time point.

Results and Discussion

Method development

The physical and chemical properties of povidone have been well established, since its discovery in 1930 (1–4). UV detection was selected for this work because PVP has a chromophore in the

ultraviolet range, with a maximum at 213.5 nm. The UV spectrum of PVP is shown in Figure 2. In the final method, 220 nm was selected for the UV detector wavelength. Blank spectra obtained of the mobile phase showed excessive background absorbance and noise at wavelengths below ~ 216 nm, precluding the use of the spectral maximum at 213.5 nm. The lowest wavelength that did not exhibit these issues was 220 nm. Although it is generally advisable to avoid performing quantitative analysis at a wavelength that appears on the slope of the analyte's UV spectrum, due to losses in sensitivity and precision versus quantitating at a spectral maximum, both sensitivity and precision were acceptable in this case. While the UV detector is possibly the most versatile and useful detector in HPLC, it is not as widely used in SEC because many polymers do not absorb electromagnetic radiation in the UV range. Other detectors such as refractive index or light scattering are more commonly used, but quantitation and method validation are often challenging with these (34). Thus, the combination of SEC with UV detection is especially suited to this application and would be suitable for other polymer analysis in which a chromophore is present.

The main goal during method development was to have an isocratic method that separates PVP from other compounds in the formulation or degradation products. Usually in SEC method development for unknowns, the molecular weight of the analyte polymer must be independently determined to aid in column selection. In this study that was not necessary because the molecular weight range of PVP, approximately 50kDa, was provided by the vendor. Initially, several size exclusion columns from different manufacturers were screened and a TSK-gel G1000PW, 7.5 mm i.d. × 30 cm × 12 µm column was selected based on the resulting symmetrical peak shape of PVP. TSK-gel columns in general consist of hydrophilic polymethacrylate spherical beads with sizes ranging from 12 µm to 17 µm. These types of columns are suitable for the analysis of water soluble polymers and more specific the G1000PW is suitable for small (1000 Da and less) molecular weight polymers.

As seen in Figure 3, in our method the TSK-gel G1000PW column is used in an unusual context: the analyte is of much higher molecular weight than would normally be analyzed on this column, eluting before the baseline disturbance resulting from injection, and fully excluded from the stationary phase. Other compounds present, with molecular weights less than 1000, elute within the separation range of the column. While uncommon, quantifying a peak eluting before the baseline disturbance has been recently reported in a similar context for the group assay of polyvinylsulfonic acid impurities in 2-(N0morpholino)-ethanesulfonic acid (35). Figure 3 shows a very symmetrical peak for PVP, clearly demonstrating satisfactory tailing for system suitability and the validation data presented below demonstrate adequate precision.

SEC separation is based on molecular size of the analyte relative to the pore size of the packing material. Mobile phase selection is important to avoid enthalpic interactions between the analyte and the packing material. There are a variety of solvents compatible with TSK-GEL columns, so the selection process depends on the chemical structure and ionic nature of the analyte. In this study, because a UV detector was used, the ideal mobile phase should have a low UV absorbance as well. Methanol has a low wavelength UV absorbance cutoff of ~ 205 nm and low background absorbance combined with good solubilizing properties for povidone, which made methanol the organic solvent of choice for this method. For simplicity, a premixed aqueous buffer mixture of 0.1M sodium acetate with methanol (80:20% v:v) was selected as the mobile phase (36). Sodium nitrate was also tested but exhibited poor peak shape for the PVP, possible due to protonation of the electron pair on nitrogen in PVP. The acetate buffer generates a mobile phase pH of approximately 10, which assists in ensuring rapid and efficient transport of PVP through the column by ensuring that the electron pair on nitrogen in PVP does not protonate while the hydroxyl groups on the surface of the TSK-GEL column are slightly deprotonated, generating additional repulsion between the stationary phase and the analyte.

Temperature adjustment can reduce the analysis time and improve chromatographic performance. More specific as the temperature increases, the viscosity of the mobile phase decreases and the diffusivity of the analyte increases. Fast size exclusion chromatography has been discussed in the literature and temperature is one of the primary parameters adjusted to achieve faster analysis times (37,38). Optimum chromatographic performance was obtained in this method with column temperature at 50°C.

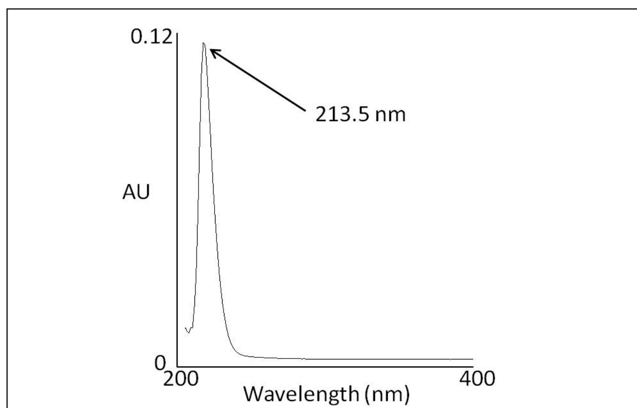

Figure 2. UV-vis spectrum of polyvinylpyrollidone. Note the maximum at 213.5. Quantitation in this paper was performed on the slope at 220 nm.

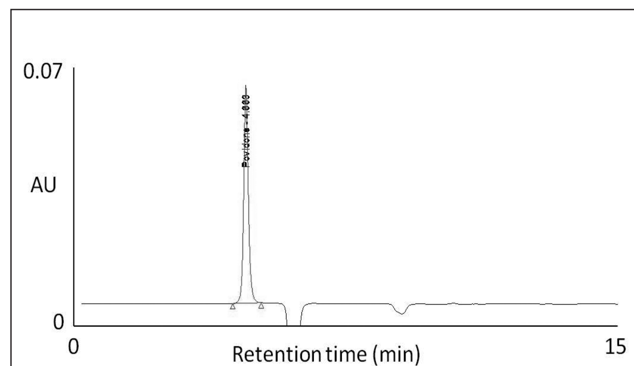

Figure 3. Chromatogram of polyvinylpyrollidone standard. Note the symmetrical peak eluting before the negative baseline disturbance in the total exclusion volume of the column.

Method validation

Figure 4 shows the analysis of PVP in a formulation (A), standard (B), and placebo (C). PVP does not appear in the placebo which is simply a formulation prepared without the analyte, demonstrating that compounds other compounds that commonly appear in pharmaceutical formulations will not interfere with the method. This chromatogram clearly demonstrates the quantification of a larger molecular weight compound using a column designed for small molecular weight analytes. PVP elutes first, followed by the baseline disturbance from the injection, followed by other components. In each case, chromatographic performance, observed by the lack of tailing, and symmetrical peak shape, is adequate.

Figure 5A shows chromatograms of several PVP polymer formulations in combination with a vinyl pyrollidone monomer. In these chromatograms, the PVP polymer is seen eluting before the baseline disturbance, totally excluded from the stationary phase pores, and the monomer eluting as expected within the analytical range of the column. The materials with masses between 10–50 kDa all elute at the same time with similar peak shapes. The extremely large materials with masses up to 360 kDa are seen eluting slightly earlier. In this analysis, PVP is expected to have a molar mass of 30 kDa or less, with degradants or scission products that may be contained in the polymer co-eluting as desired with the main peak, as the analysis is for total PVP in the samples. The method is not intended to be selective for the various molecular weights of the PVP that may be encountered. Material with a molecular weight greater than approximately 1000 Da should be included in the main peak. In Figure 5B, a chromatogram of the PVP used in this study for ophthalmic formulations in combination with its monomer which has very strong absorbance at 220 nm is shown, demonstrating the selectivity of this system in separating the high molecular weight polymer from lower molecular weight components with similar or interfering UV absorbance that may be present in formulations with it.

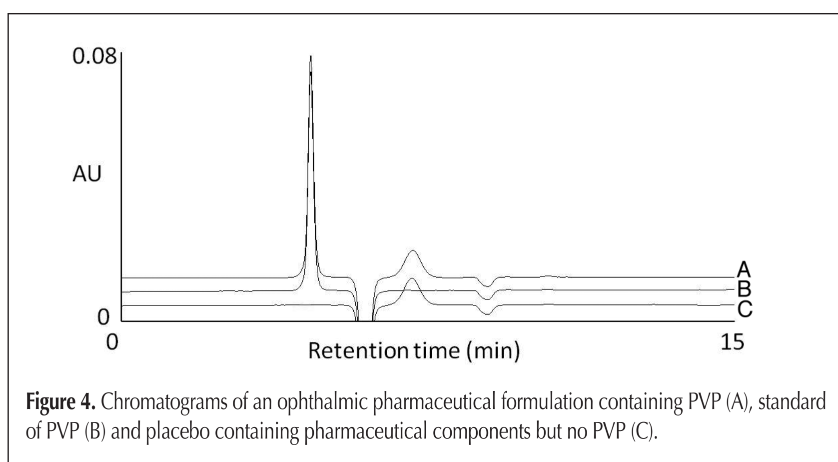

Figure 4. Chromatograms of an ophthalmic pharmaceutical formulation containing PVP (A), standard of PVP (B) and placebo containing pharmaceutical components but no PVP (C).

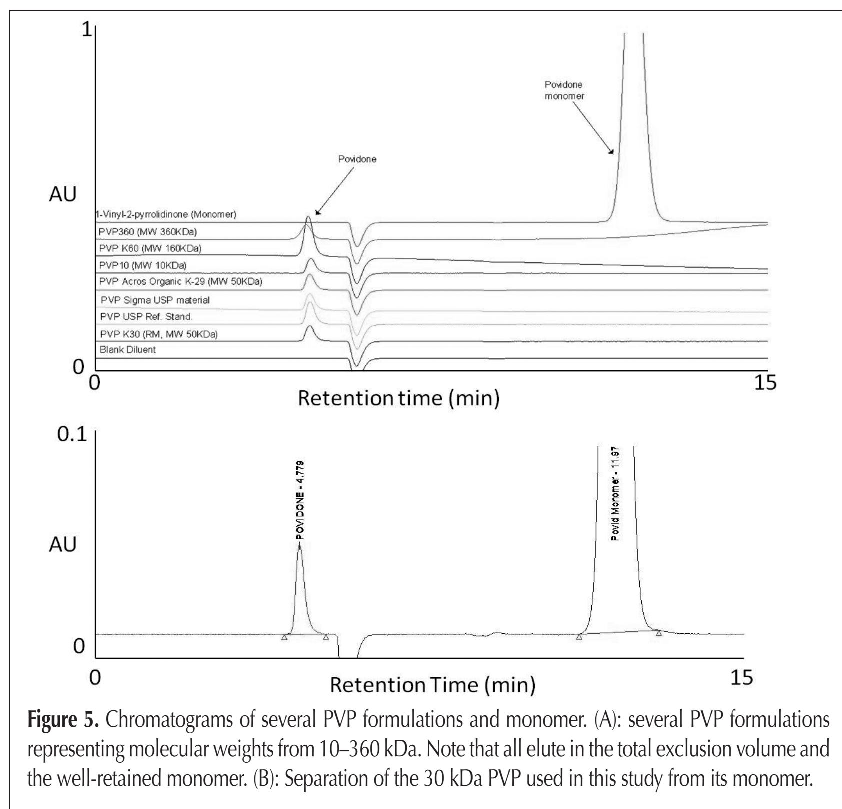

Figure 5. Chromatograms of several PVP formulations and monomer. (A): several PVP formulations representing molecular weights from 10–360 kDa. Note that all elute in the total exclusion volume and the well-retained monomer. (B): Separation of the 30 kDa PVP used in this study from its monomer.

The specificity of the method was tested and no interference was observed for the povidone peak from placebo and forced degradation samples. During method development, it was confirmed that all peaks were pure by double checking the full UV spectrum for all peaks using a photodiode array detector. In this study, all peaks encountered were pure, according to peak purity determination using Empower.

Linearity of the method was established by injecting five standard concentrations of povidone and preparing a calibration curve by plotting PVP response versus concentration. The solutions covered a range of 0.5–1.5% concentration. The method was linear in this range with R^2 values of 0.9999. No carryover was observed into blank injections immediately after the highest level linearity standard, ensuring independence of the samples.

System precision was established by six replicate measurements of a 1% standard solution of povidone. The %RSD for povidone was found to be 0.1%. The repeatability of the method was evaluated by six identical sample preparations of a homogeneous batch and the results were found to be within the specifications. The percent relative standard deviation of the six preparations for povidone was found to be 0.3%. In order to further validate the results, the experiment was conducted again on a different day using a different instrument, column and analyst. In addition the work was repeated at a different work site, using different instruments and different columns. The experimental mean agreement for povidone between the two days and sites was found to be 0.1 and 0.5 respectively and is within the acceptance criteria. Results are displayed in Table I.

The accuracy of the method was established by assaying three different concentration levels 70%, 100%, and 130% of the theoretical concentration. Six preparations of placebo, at each level, were spiked with standard of PVP and were injected into the HPLC system. Results are reported in Table II with PVP mean recovery values varied from 100.0 to 101.8 % of the prepared standard concentration. No bias was observed for PVP, since the results for the mean recovery from all three accuracy levels were not significantly lower or higher (± 1.5%) than the theoretical value. The range in which the method is shown to be linear and accurate for povidone is between 70–130% of theoretical concentration.

The method was unaffected by small, deliberate variations in chromatographic parameters and mobile phase preparation. The parameters tested were detector wavelength (± 4 nm), mobile phase flow rate (± 0.1 mL/min) and temperature (± 5°C). As expected for an unretained peak, retention time and peak shape were not affected by these parameters. The variation in results that was observed between the normal method conditions and the changed parameters were from 0.1 to 1.6% for povidone. The stability of the standard and sample solutions for povidone was also evaluated. No significant change in PVP response was observed over a period of 192 h.

Table I. Intermediate Method Precision and Reproducibility Results

	% Label Claim of Povidone (%LC)			
	Intermediate Precision		Reproducibility	
Replicate	Analyst 1/Lab 1	Analyst 2/Lab 1	Lab 1	Lab 2
1	102.0	102.0	102.0	102.4
2	102.3	101.9	102.3	102.9
3	102.4	102.1	102.4	102.9
4	102.8	103.3	102.8	103.1
5	101.9	101.4	101.9	102.7
6	102.6	102.7	102.6	102.8
Mean	**102.3**	**102.2**	**102.3**	**102.8**
%RSD	**0.3**	**0.7**	**0.3**	**0.2**
Mean Agreement	**0.1**		**0.5**	

Table II. Accuracy Results (Placebo Spiked with Povidone at Three Levels, 70, 100, and 130% of Theoretical)

	Povidone		
	Mean Recovery Value %LC		
Prep #	70% Level	100% Level	130% Level
1	100.5	101.0	100.6
2	98.9	100.7	100.1
3	99.9	100.8	101.0
4	100.2	101.1	99.5
5	99.1	100.3	100.9
6	101.0	101.1	100.9
Mean	**100.0**	**100.8**	**100.5**
%RSD	**0.81**	**0.3**	**0.6**

Conclusions

A new method for the determination of total povidone in ophthalmic solutions was developed using SEC-UV with quantitation of the analyte peak eluting in the exclusion volume. This unique combination separated povidone from other excipients and allowed a simple isocratic method. Validation for this method was performed according to ICH guidelines and met all acceptance criteria. The method is precise (± 0.1%), accurate (± 1%), and linear at concentration ranges of 0.07 mg/mL to 0.13 mg/mL, typical of prepared ophthalmic solution samples for povidone. Several unusual chromatographic situations were used together successfully in this work: SEC with UV detection and quantitation of a chromatographic peak eluting in the total exclusion volume. This method provides a model for the analysis of a polymeric component in the presence of monomeric components in pharmaceutical formulations.

Acknowledgments

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