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Short communication

Determination of polyvinylpyrrolidone using high-performance liquid chromatography

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Abstract

Polyvinylpyrrolidone (PVP) is a versatile polymer with innate surface activity. It is very difficult to accurately assay due to its wide molecular weight range and amphiphilic nature. This study evaluated a reversed-phase HPLC method to separate and quantify PVP K15. The assay used a Hicrome C_{18} 150 mm × 3 μm HPLC column in combination with an 80/20 propanol-1-ol: deionised water, 0.01% TFA mobile phase, which resolved the polymer as a single peak, $t_R = 10.69 \pm 0.17$ min ($n = 120$) at 243 nm. The column's performance was constant throughout the study, N (theoretical plates) = 1729 ± 22 and the peak symmetry remained good (A_s ranged from 0.74 to 0.92, $n = 10$ over the calibration range). The developed assay proved to be accurate, sensitive and capable of recovering PVP K15 from pharmaceutical formulations. The limits of quantification and detection were calculated statistically as 2.40 and 0.72 mg ml^{-1}, respectively. Assay reproducibility assessed at five concentrations gave an average coefficient of variance <3.5% and the accuracy of the analytical method was 102.62 ± 2.04%. The recovery of PVP K15 from directly compressed tablets and Refresh eye drops™ was 98.02 ± 2.73 and 108.35 ± 6.52%, respectively.

Keywords: Polyvinylpyrrolidone; High-performance liquid chromatography; Ultraviolet detection; Formulation

1. Introduction

Polyvinylpyrrolidone (PVP) is a water-soluble synthetic polymer. It is an amphiphilic large molecular weight compound containing highly polar amide group's in conjunction with apolar methylene and methane (CH) moieties. The molecule exists as a loose random coil in solution and is soluble in a wide range of organic solvents [1]. PVP is physiologically inactive leading to its initial medical use as a plasma substitute, although this has now been discontinued due to accumulation of large molecular weight fractions in the body [1]. It is now classically used in a pharmaceutical context as a binder [2,3], although more novel formulations include the polymer as a taste masking additive [4], controlled release excipient [2,5] and transdermal penetration enhancer [6]. Analytical methods previously used to quantify PVP include refractometry, colorimetry, infrared and fluorimetric spectroscopy [1,7]. However, these techniques are limited in terms of sensitivity and do not have the capability to separate the polymer from other

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excipients within a formulation. PVP is compatible with a wide range of solvents and has the ability to form hydrophobic interactions, hence reversed-phase high-performance liquid chromatography (HPLC) could be a useful tool in the analysis of the molecule. Compared to other analytical techniques described above HPLC would afford advantages in terms of speed and accuracy of analysis. Hence, the purpose of this work was to develop a HPLC method for the determination of polyvinylpyrrolidone.

2. Experimental

2.1. UV absorbance maximum

A series 2000 liquid chromatography system (Perkin-Elmer, Beaconsfield, UK) coupled with a diode array detector (Perkin-Elmer, Beaconsfield, UK) was used to detect the UV absorbance maximum of PVP in the mobile phase. The solvent was pumped through the system isocratically at 0.1 ml min^{-1} without a column inline and a single injection of 100 μl was performed. The UV spectrum was collected between 200 and 700 nm.

2.2. HPLC method

A 20 mg ml^{-1} polyvinylpyrrolidone K15, M_w: 10,000 (average molecular weight as quoted by Sigma–Aldrich, Gillingham, UK) stock solution was made up in deionised water. Five serial dilutions were used to produce calibration standards in the range of 0.08–20 mg ml^{-1}. These were run on a liquid chromatography system consisting of an isocratic Pu 980 Pump (Jasco, Great Dunmow, UK) set at 0.6 ml min^{-1}, an AS 950 auto-sampler fitted with a 100 μl injection loop (Jasco, Great Dunmow, UK), a CI-10B integrator (LDC/Milton Roy, Stone, UK) and a chart printer (LDC/Milton Roy, Stone, UK). PVP was detected using a 975 UV-Vis detector (Jasco, Great Dunmow, UK) set at 243 nm. A C_{18} 150 mm × 3 μm column (Hichrome, Theale, UK) was used in combination with a C_{18} guard column (Phenomenex, Macclesfield, UK). The mobile phase consisted of 80/20 deionised water: propan-1-ol (HPLC grade, Merck labs, Darmstat, Germany) and 0.01% TFA (Sigma–Aldrich, Gillingham, UK). The calibration standards were used to determine the assay sensitivity, reproducibility and accuracy.

2.3. Formulation recovery

Tablets consisting of 94.5% spray dried lactose (Zeprox 202, Borculo Domo Ingredients, Zwolle, the Netherlands), 5.0% PVP K15, M_w: 10,000 (Sigma–Aldrich, Gillingham, UK) and 0.5% magnesium state (Sigma–Aldrich, Gillingham, UK) were compressed using a single punch tablet press (F-press, Manesty, Merseyside, UK). The tableting machine was set-up with 12 mm flat faced tooling and run at a speed of 3600 tablets per hour with a compaction force of 20 kN to achieve a target tablet weight of 550 mg. Five tablets (sampled from the beginning middle and end of the batch) were allowed to dissolve in water over 24 h at room temperature. The resultant liquid was passed through a 0.2 μm PVDF filter (Whatman, Maidstone, UK) and the PVP was analysed using the developed HPLC method. In addition, the binding of PVP K15 to the filters was simultaneously determined by passing a standard 10 mg ml^{-1} solution of PVP K15 through three different filters. The pre and post filtration samples were compared using the HPLC method.

Refresh eye drops™, a commercially manufactured preparation, was spiked with a 3.33 mg ml^{-1} PVP K15 sample used in the calibration curves. This mixture was injected directly onto the HPLC column and the PVP assayed using the developed method (described above).

2.4. Calculations

2.4.1. Peak symmetry (A_s)

$$A_s = \frac{W_{0.05}}{2d} \quad (1)$$

where $W_{0.05}$ is the width of the peak at one-twentieth of the peak height and d the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

2.4.2. Accuracy or recovery

$$\text{Accuracy} = \text{recovery} = \frac{A}{T} \times 100 \quad (2)$$

where T is the theoretical concentration of analyte and A the actual concentration of analyte.

2.4.3. Limits of detection and quantification (LOD, LOQ) [8]

$$\text{LOD} = y_B + 3s_B \quad (3)$$

$$\text{LOQ} = y_B + 10s_B \quad (4)$$

where s_B is the standard error of the y estimate and y_B the intercept from the regression equation.

2.4.4. Theoretical plates

$$N = 5.54\left(\frac{t}{W_{h/2}}\right)^2 \quad (5)$$

where $W_{h/2}$ is the width of the peak at half the peak height and t the retention time of the peak

2.4.5. Statistical analysis

Results were compared using ANOVA on Minitab® (Minitab Inc, USA).

3. Results and discussion

3.1. System suitability, linearity and sensitivity

The UV absorbance maximum for PVP K15 in mobile phase was 243 nm, which was used as the detection wavelength throughout the study. A single peak was eluted in the HPLC chromatogram with a retention time of 10.69 ± 0.17 min ($n = 120$). The peak shape was good (A_s ranged from 0.74 to 0.92 ($n = 10$)) and the column efficiency remained constant throughout the study, $N = 1729 \pm 22$ ($n = 12$, three from each of the four calibration runs). Calibration curves were linear over the concentration range of 0.08–20 mg ml^{-1} ($r^2 > 0.99$). Limits of quantification and detection were determined statistically using a method developed by Miller [8] as, 2.40 and 0.72 mg ml^{-1}, respectively.

3.2. Precision

Inter- and intra-day variation was compared at five concentrations ($n = 5$). Two calibration curves were run on a single day to determine the intra-day variance; this data combined with two additional calibration curves on two further days determined the inter-day variance. There was no significant difference between the four calibrations curves at any of the five concentrations ($P < 0.05$). A summary of the intra- and inter-day coefficient of variation is presented in Table 1. The data illustrates that precision of the peak area measurement was very similar irrespective of concentration. The assay proved to be less precise compared to limits usually applied to HPLC for small molecular weight molecules i.e. coefficient of variance <2% [9]. However, taking into account the added complexity of analysing large molecular weight polymers and the inherent variability of the raw material, the method was considered to have acceptable precision although outside these limits.

Table 1
Variance of the HPLC PVP assay over the calibration range

Concentration (mg/ml)	CV of the intra-day (%) ($n = 10$)	CV of the inter-day (%) ($n = 15$)
20.00	0.66	1.93
10.00	1.71	2.52
4.00	2.88	4.64
0.40	4.37	3.55
0.08	3.32	3.82
Mean	2.59	3.29

3.3. Assay accuracy

The accuracy of the assay was compared using two quantification methods, peak height and peak area at a range of three concentrations, 18.326, 7.255 and 12.700 mg ml^{-1} ($n = 3$). The average accuracy was 102.62 ± 2.04% using peak area for quantification and 102.90 ± 1.98% using peak height. There was no significant difference between the two quantification methods ($P > 0.05$) and as such peak area was used throughout the remainder of the study.

3.4. Formulation recovery

Refresh eye drops™ (that contain 1.5% PVP) injected straight onto the column did not produce any separated peaks in the chromatogram ($n = 3$, Fig. 1a), implying that the formulation did not contain PVP K15. However, it was possible to recover 108.35 ±

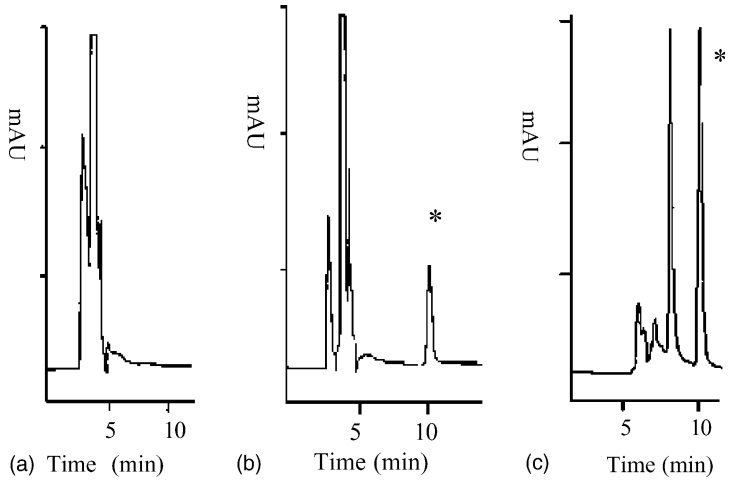

Fig. 1. (a) Refresh eye drops™ 100 μl injected directly onto the column. (b) Refresh eye drops™ spiked with 3.33 mg ml^{-1} PVP K15. (c) PVP K15 recovered from the directly compressed tablets. Asterisk (*) indicates PVP K15 peak.

6.52% ($n = 4$) of a 3.33 mg ml^{-1} spiked PVP K15 sample from the eye drop formulation. The PVP was resolved into a single peak, $t_R = 10.20 \pm 0.18$ (Fig. 1b, this t_R was not significantly different to the PVP K15 standard's elution time).

PVP K15 did not to bind to the PVDF syringe filters, $100.05 \pm 0.57\%$ ($n = 3$) of the polymer was recovered after filtration. HPLC analysis of the directly compressed tablets produced two peaks in the chromatogram: t_{R1}, lactose; t_{R2}, PVP K15 (Fig. 1c). The PVP K15 peak was identified using retention time (t_R was not significantly different to the PVP K15 standard's elution time) and quantification using peak area gave an average recovery of $98.02 \pm 2.73\%$ ($n = 5$) from the tablets.

4. Conclusion

HPLC was shown to be a suitable technique to analyse polyvinylpyrrolidone K15. It successfully separated the polymer into a single peak within 12 min using an isocratic reversed-phase method. At present PVP is commonly quantified using iodine as an adduct, however, the developed HPLC method provides an excellent alternative to this colourimetric method, with improved precision and accuracy [10]. Furthermore, HPLC analysis has the capability to separate PVP K15 from other excipients within a formulation. Further work should be performed to develop an HPLC technique with greater resolution that can potentially be used to separate and quantify mixtures of PVP grades differing in molecular weight.

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