Development and Validation of a Novel Reversed Phase High Performance Liquid Chromatography with Refractive Index Detector Method for Assay of Polyvinyl Alcohol in an Ophthalmic Solution

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Abstract

Polyvinyl alcohol (PVA), a polymer, is in demand due to its usage in different applications such as pharmaceutical, biomedical and textile, paper, food industries. A new sensitive reversed phased high pressure liquid chromatography (RP-HPLC) method with refractive index detector (RID) was developed for determination of PVA in an ophthalmic solution containing dexpanthenol and PVA as active substances and it was validated according to ICH guideline. Chromatographic separation was achieved on a Chiral-AGP (150mm× 4.0 mm, 5 µm) column kept at 30°C with an isocratic flow at a flow rate of 1.0 ml/min. The detector temperature was 30°C, the retention time of PVA was around 1.0 min and the total run time was 5 minutes. The proposed method showed linearity, accuracy, precision, specificity, robustness, solution stability, and system suitability results within the acceptance criteria.

Keywords: Polyvinyl Alcohol, RP-HPLC, RID Detector, Method Development, Validation, Pharmaceutical

1. Introduction

Polyvinyl alcohol (PVA) is a linear hydrophilic polymer synthesized via partial or full hydrolysis of polyvinyl acetate.1 Because of properties such as inertness, stability, resistance against organic solvents, non-toxicity, biocompatibility and elasticity, aqueous solubility, PVA is used in many treatments.

PVA homopolymer or copolymers have been used in pharmaceutical dosage forms such as tablets, transdermals, ophthalmics and implants as an excipient. PVA hydrogels have been used as tissue replacement material, soft contact lens material, artificial heart linings, artificial cartilages, catheters, skin, and pancreas membranes, drug delivery systems in oral, transdermal, buccal, intramuscular, rectal routes of administration.[2]

Literature researches show that there are not many spectroscopic methods for determination PVA. Some methods in the literature for determination or quantification of PVA are complexation with iodine followed by spectrophotometric detection,[3-7] gel filtration liquid chromatography with refractometric,[8] adsorptive stripping voltammetry,[9] evaporative light scattering (ELS)[10] and static laser scattering[11] detection. Most of these methods are used in textile or paper industry. Almost none of the methods are suitable for pharmaceutical use. They are not highly specific, insufficient for recovery and most of them require expensive equipments.

The aim of this study is to develop and validate easily available method in the pharmaceutical field. This method is also fast, sensitive, reliable and low cost in ophthalmic dosage form. This method was validated according to ICH guideline.[12]

2. Materials and Methods

2.1 Instrumentation

Waters E2695 HPLC system was used for liquid chromatography method development and validation (Singapore, Asia), equipped with a model code SM4 and serial number D13SM4641A, a Chiral-AGP (150 mm × 4.0 mm) 5 µm column and the detector was RID detector, model code was 214 and serial number was C13214522N (USA). Empower 3 Software was used for data processing and evaluation.

2.2 Chemicals and Reagents

A pharmaceutical grade sample of PVA CRS was purchased from EP (assigned purity 100.0%) and PVA raw material was obtained from Nippon Gohsei (purity 99.4%). The ophthalmic solution containing 30 mg/mL dexpanthenol and 14 mg/mL PVA was the product which has been improving by the World Medicine Pharmaceutical Industry and Trade Inc. (Istanbul, Turkey). Potassium dihydrogen phosphate was purchased from Merck. HPLC grade water (0.05 µc) was produced by the Sartorius Stedim Biotech system.

2.3 Chromatographic Conditions

The mobile phase was prepared by dissolving 2.72 g. potassium dihydrogen phosphate in 2000 ml water. Prior to use the mobile phase was filtered through 0.45 µm membrane filters and degassed by sonication for 10 min. The analysis was carried out on an Waters E2695 HPLC system. The analytes were conducted on an analytical column Chiral-AGP, 5 µm, 150 × 4.0mm. The column and detector temperatures were 30°C and sample temperature was 25°C. The injection volume was 100 µL, and the flow rate was maintained at 1.0 mL/min. The run time was 5 minutes.

2.4 Preparation of Standard Solution

A standard solution of PVA was prepared by dissolving an accurately weighed amount of 7.0 mg PVA in 10 mL volumetric flask using mobile phase by sonicated for 30 minutes. After the solution reaches to room temperature, complete to volume with mobile phase. Filtered through 0.45 µm PTFE filter.(c_{PVA}: 0.70 mg/mL)

2.5 Preparation of Sample Solution

From the PVA ophthalmic solution, 1.0 mL of the test sample (equivalent to 7.0 mg PVA) was transferred into a 10 mL volumetric flask, sonicated with mobile phase for 30 minutes. After the solution reaches to room temperature, made up to volume with the same solvent. Filtered through 0.45 µm PTFE filter.

2.6 Method Validation

The developed method was validated according to ICH guideline. The validation parameters included specificity, linearity, accuracy, precision and intermediate precision, solution stability and robustness.

3. Results and discussion

3.1 Specificity

The specificity of the developed method was examined by injecting solutions of standard, sample, placebo+ PVA raw material, blank, and placebo separately. No another peak was observed at the retention time of PVA proved the specificity of the method. Chromatograms were given in Supporting Information (Table S-1, Figure S-1–S-5).

3.2 Linearity

Analytical method linearity is described as the quality of the method to obtain results that are directly comparative to the analyte concentration, within a determine ratio. As shown in the Figure 1, the results of linearity gave linear relation with certain concentrations. Seven concentrations were prepared (0.07-0.98 mg/mL), %100 concentration (0.70 mg/mL) was injected six times and the other concentrations were injected three times. From the regression analysis, a linear equation was obtained: y=632688.936037x+925.4189860 and the correlation coefficient (r2) was found 0.9995. Chromatograms and Relative Standard Deviations (RSDs) were given in Supporting Information (Table 1, Figure S-6 – S-30).

Table 1. Linearity results of the method.

%	Concentration (mg/mL)	Area 1	Area 2	Area 3	Average Area	Calculated Area	Relative Standard Deviation (RSD)
10	0.0702	44439	44446	43964	44283	45220	0.62
20	0.1403	89641	89219	89339	89399	89553	0.24
40	0.2806	178383	177935	177577	177965	178221	0.23
80	0.5612	359204	359541	359648	359464	355555	0.06
100	0.7015	448891	446431	447443	*447558	444222	0.18
120	0.8418	522580	523300	522472	522784	532889	0.09
140	0.9821	626321	625320	625647	625763	621556	0.08

* The average area of %100 concentration was taken from the results of six replications of the injection. The RSD result was calculated according to this result.

3.3 Accuracy

Under the optimized conditions, accuracy was evaluated by recoveries. Solutions at known concentrations (80%, 100% and 120%) were prepared and analyzed in triplicate. Percentage of recoveries ranged from 100.119% to 100.757%. The results of percentage recovery were within the accepted limits from 98.0% to 102.0% and %RSD was not more than 2.0% as shown in Table 2. (All chromatograms were given in Supporting Information Figure S-31 – S-57).

3.4 Precision and Intermediate Precision

The system precision (repeatability) and method precision of the developed method were evaluated by determining several measurements of standard and sample solutions both intra- and inter-day. The system precision (intra-day precision) was performed with six repeated injections of standard solution prepared at 100% concentration. The method precision was calculated with six different sample solutions prepared at 100% concentration.

Table 2. Recovery results of the method.

%	Replicate Number	Area 1	Area 2	Area 3	Average Area	Concnt. Added (mg/mL)	Concnt. Found (mg/mL)	Average % Recovery
80%	1	358249	358046	358628	358308	0.561	0.565	100.757
	2	356948	357309	357311	357189	0.561	0.563	100.353
	3	356991	357748	357428	357389	0.560	0.563	100.588
100%	1	444238	444481	444315	444345	0.699	0.701	100.296
	2	444539	444583	444479	444534	0.699	0.701	100.267
	3	447973	446741	446014	446909	0.700	0.705	100.733
120%	1	533799	534838	533566	534068	0.841	0.843	100.147
	2	533722	533545	533545	533604	0.841	0.842	100.119
	3	537715	536612	535903	536743	0.842	0.847	100.591
						Mean % Recovery		100.428
						% RSD		0.24

The intermediate precision (inter-day precision) was established by both six measurements of standard solution and six different sample solutions at the 100% concentration on a different day by a second analyst and second equipment The RSD values were calculated to estimate all results (Table 3-4).

Table 3. The system precision (repeatability) results of the method.

Standard 100% Concentration	Area	Retention Time
1	446431	1.145
2	447443	1.145
3	447775	1.143
4	447480	1.143
5	447330	1.143
6	448891	1.143
Mean	447558	1.144
SD	795.6	0.001
RSD %	0.18	0.09

The results of the method precision were calculated according to repeatability results. Similarly, intermediate precision results were determined by the six measurements of standard solution studied on the same day (Table S-3 and Table S-4). All chromatograms for precision and intermediate precision were given in the Supporting Information (Figure S-58 – S-81). A summary of the RSD values were given in Table 5.

Table 4. The method precision results of the method.

Sample 100% *Conc.	Conc. Added (mg/mL)	Area	Conc. Found (mg/mL)
1	0.04917	446349	14.228
2	0.04932	445452	14.158
3	0.04946	451681	14.314
4	0.04943	450880	14.298
5	0.04940	450209	14.285
6	0.04937	450118	14.291
Mean		449115	14.262
SD		2567.9	0.059
RSD %		0.57	0.41

*** Conc. = Concentration.**

Table 5. The precision and intermediate precision results of the method.

RSD for Standard Solutions (n=6)	Day 1	0.18
	Day 2	0.41
RSD for Sample Solutions (n=6)	Day 1	0.41
	Day 2	0.39
RSD (n=12)	Day 1-2	0.50

3.5 Robustness

To validate the robustness of the development method, small changes were made in the method parameters. Robustness was tested by studying the effect of changing detector temperature ±2ºC, column temperature ±2°C and using different column. Results were compared and %

variations were calculated. Variations were less than % 2.0 (Table S-5, Figure S-82 – S-87).

3.6 Stability of Solution

Solution stability was also evaluated by monitoring the peak area response. Standard and test solutions were analyzed right after its preparation 6, 24, and 48 hours after at 5°C and at room temperature. Results were compared and % variations were calculated. Variations were less than % 2.0 (Table S-6, Figure S-87 – S-100).

3. Conclusions

In conclusion, a rapid, simple, sensitive and selective HPLC method with refractive index detector was developed and validated and it was proved to be linear, specific**,** precise, accurate, robust and solution stabile. This method provides an alternative method for PVA assay in pharmaceutical dosage forms. The advantages of the method are short run time, simple mobile phase preparation, low cost and applicability. All results and chromatograms can be found in the Supporting Information.

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Supplemantary Data

Supplementary data associated with this article can be found, in the online version, at

REFERENCES

[1] Maribel I. Baker, Steven P. Walsh, Zvi Schwartz, Barbara D. Boyan. A Review of Polyvinyl Alcohol and Its Uses in Cartilage and Orthopedic Applications. *J Biomed Mater Res Part B*, **2012**, 00B: 000–000, DOI: 10.1002/jbm.b.32694.

[2] Srinath Muppalaneni and Hossein Omidian, Polyvinyl Alcohol in Medicine and Pharmacy: A Perspective, *Journal of Developing Drugs,* **2013**, 2:3. http://dx.doi.org/10.4172/2329-6631.1000112.

[3] Aleksandrovich, I.F. and Lyubimova, L.N. Spectrophotometric Determination of the Polyvinyl Alcohol Content of Sizing Compositions. *Fibre Chem*, **1993**, 24, 156-157.

[4] Finley, J.H. Spectrophotometric Determination of the Polyvinyl Alcohol in Paper Coatings. *Anal. Chem*. **1961**, 33, 1925-1927.

[5] Joshi, D.P.; Lan-Chun-Fung, Y.L.; Pritchard, J.G. Determination of Poly(vinyl alcohol) Via Its Complex With Boric Acid and Iodine. *Anal. Chim. Acta*. **1979**, 104, 153-160. https://doi.org/10.1016/S0003-2670(01)83825-3

[6] Pritchard, J.G.; Akintola, D.A. Complexation of Polyvinyl Alcohol With Iodine Analytical Precision and Mechanism. *Talanta*, **1972**, 19, 877-88.

[7] Morishima, Y.; Fujisawa K.; Nozakura, S. Sequence Length Required for Poly(vinyl acetate)-Iodine and Poly(vinyl alcohol)-Iodine Color Reactions. *Polym.J*., **1978**, 10(3), 281-285.

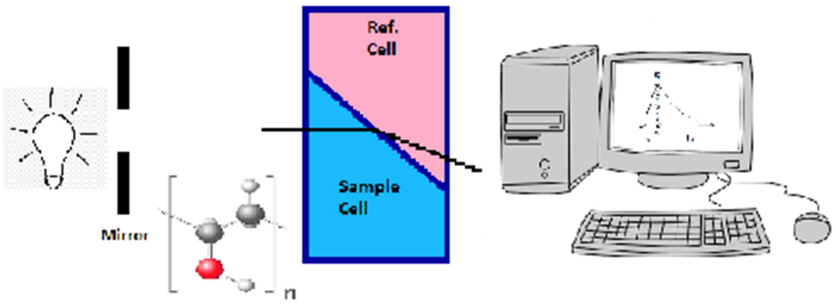
[8] Jones, S.A.; Brown, M.; Martin, G.P. Determination of Polyvinyl Alcohol Using Gel Filtration Liquid Chromatography *Chromatographia*, **2004**, 59, 43-46.

[9] Song, M.; Ma, H.M.; Huang, Y.X.; Liang, S.C. Determination of Poly(vinyl alcohol) by Adsorptive Stripping Voltammetry Based on Its Copper(II) Complex *Anal. Chim. Acta*. **1997**, 338, 103-108. https://doi.org/10.1016/S0003-2670(96)00498-9

[10] Dunn, K.D. Determination of Polyvinyl Alcohol in a Poly(DL-lactide-co-glycolide) Matrix by Size Exclusion Chromatography Using Evaporative Light Scattering Detection. *J. Pharm. Biomed. Anal*. **2001**, 25 (3-4), 539-43.

[11] H. Hashim, F. El-Mekawey, H. El-Kashef, R. Ghazy. Determination of Scattering Parameters of Polyvinyl Alcohol by Static Laser Scattering. *In: Beni-Suef University Journal of Basic and Applied Sciences*, Elsevier, **2014**, 3, 203-208.

[12] ICH Guideline, "Validation of analytical procedures: text and methodology," in Proceedings of International Conference on Harmonization, Topic Q2 (R1), Geneva, Switzerland, November 2005.



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