



Straightforward Determination of Sodium Hyaluronate in Active Pharmaceutical Ingredient and Ophthalmic Formulations: Validation and Stability Study

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ABSTRACT

Objectives: A sensitive, selective and rapid HPLC method was developed and validated for sodium hyaluronate quantification. **Methods:** Chromatographic separation was achieved on SCHARLAU C₁₈ column (4.6 x 250 mm, 5 μ m) at 50°C. The mobile phase consisted of 50 mM phosphate buffer (pH = 7) (100 %, v/v) and at a flow rate of 0.5 mL/min. The column eluent was monitored at 205 nm. The retention time (R_t) of sodium hyaluronate was at 3.17 min. **Results:** The calibration curve was linear over the concentration range of 80 – 320 μ g/mL. The intra- and inter-day reproducibility studies demonstrated accuracy and precision according to ICH guidelines. **Conclusion:** The developed method can be applied to the analysis of sodium hyaluronate in Hycarenol[®] eye drops.

Keywords: Hycarenol[®] eye drops; HPLC; ICH guidelines; SCHARLAU; Sodium hyaluronate.

INTRODUCTION

Hyaluronic acid (HA) as shown in **Figure 1** is a naturally occurring non-sulfated glycosaminoglycan which is widely distributed throughout connective, epithelial, and neural tissues^{1,2}. Hyaluronic acid (HA), in the form of sodium hyaluronate, has many uses in pharmaceutical products as joint supplements, eye drops, nose spray, shampoos, face creams and other anti-aging products³⁻⁵.

Several techniques have been reported for determination of sodium hyaluronate such as planar electro migration separation (gel electrophoresis)⁶, enzymatic, Carbopac PA1 chromatography, chemiluminescence, digestion, DP5 Photrode^{6,7}, capillary electrophoresis (CE)⁸, and LC- size exclusion chromatography (SEC) coupled with UV detection^{6,8}. As well, contemporary instrumental tools, namely highly sensitive fluorescence detection (FL) or highly specific mass spectrometric (MS) detection are used⁹.

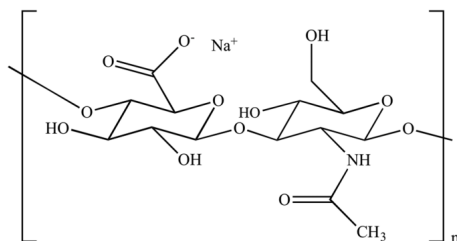


Figure 1. Chemical structure of sodium hyaluronate.

Most of the reported approaches for analysis of sodium hyaluronate are by derivatization, gel permeation chromatography or digestion. Consequently, it may not be appropriate for assay of sodium hyaluronate in eye products due to complexity, sensitivity, risk and flexibility matters⁶.

Indeed, reverse phase HPLC using ODS (C₁₈) column hyphenated with UV detector is the most practical technique for routine analysis of pharmaceutical products and presented as direct, non-derivatized, feasible and economic technique. Thus, in this work, it is planned to optimize a validated LC-ODS (C₁₈)-UV method for determination and quantification of sodium hyaluronate in Hycarenol[®] eye drops.

MATERIAL AND METHODS

Chemicals and reagents

Sodium hyaluronate was obtained from Sigma Aldrich (USA). Potassium dihydrogen orthophosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Scharlab S.L. (Spain). Hycarenol[®] eye drops were provided from Sanocare for Pharmaceutical industries with a composition of 2 mg/mL (0.2 % w/v).

Instrumentation

The analysis was carried out on KNAUER AZURA Integrated HPLC systems (KNAUER Wissenschaftliche Geräte GmbH, Germany) consisted of a quaternary pump, a high-speed auto-sampler, column oven, and a DAD detector. Chromatographic separation was performed on SCHARLAU C₁₈ stainless steel column (250 x 4.6 mm, 5 μ m) (Scharlab S.L., Spain) as a stationary phase.

Chromatographic conditions

Isocratic mobile phase consisted of 0.05 M phosphate buffer with a pH adjusted at 7 using 1 N sodium hydroxide. The mobile phase was filtered and degassed using 0.45- μ m membrane filter. A constant flow rate of 0.5 mL/min was employed throughout the analysis. The wavelength of detection was 205 nm and injection volume was 5 μ L. All the analysis was made at a temperature of 50°C.

Preparation of mobile phase buffer

A phosphate buffer solution, 0.05 M of pH = 7 was prepared by weighing 6.8 g of potassium dihydrogen orthophosphate dissolved in deionized water. The pH was adjusted with 1 N sodium hydroxide and then the total volume was completed to 1 L with deionized water.

Preparation of standard solutions

A stock standard solution of sodium hyaluronate was prepared in phosphate buffer to have a final concentration of 2000 μ g/mL. Working solution was prepared by successive dilution using the mobile phase. All sodium hyaluronate working solutions for intra-day and inter-day studies were prepared by further dilution with mobile phase.

Stability study

Different degradation conditions were used to test the stability of sodium hyaluronate; 5 N HCl, 5 N NaOH, 30 % H₂O₂ in water bath at 80°C for 3 hr and at oven of temperature 80°C for 8 hr. Sodium hyaluronate was injected to test its stability under acidic, alkaline, oxidative and thermal degradation conditions.

Application for pharmaceutical formulation

A 5 mL portion of Hycarenol[®] eye drops with a composition of 2 mg/mL (0.2 w/v) was transferred to a 25 mL volumetric flask, Initially 10 mL of mobile phase was added and shaken for few minutes to solubilize sodium hyaluronate and made to volume with mobile phase. The solution was filtered through 0.45- μ m membrane filter and 5 μ L was injected on to the column.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The development of a straightforward, practical HPLC method for sodium hyaluronate determination using a reversed phase C₁₈ column and UV detector is of great interest. Taking in account to eliminate the drawbacks of the previously reported methods⁶⁻⁹. The HPLC method was developed using a C₁₈ (250 x 4.6 mm, 5 μ m) column, mobile phase of 100 % phosphate buffer at pH = 7 and detection wavelength = 205 nm. The retention time of sodium hyaluronate is 3.15 min, which is a reasonable retention time for high throughput used in quality control laboratories as shown in **Figure 2**. The detection of sodium hyaluronate using the UV detector is very challenging as it is a low absorbing molecule. However, the use of a mobile phase of 100 % phosphate buffer was very helpful as water cut-off is 180 nm. Our present developed and validated method is simple, straightforward, cost-effective with no need for derivatization. This proposed method is suitable for quality control laboratories due to its simplicity and cost.

Table 1. Intra-day and Inter-day for determination of sodium hyaluronate

Nominal concentration ($\mu\text{g/mL}$)	Intra-day			Inter-day		
	Found ^a ($\mu\text{g/mL}$)			Found ^b ($\mu\text{g/mL}$)		
	Mean \pm SD	Accuracy (%)	RSD (%)	Mean \pm SD	Accuracy (%)	RSD (%)
120	120.85 \pm 0.443	100.71	0.358	121.57 \pm 0.488	101.31	0.402
200	207.48 \pm 1.858	103.74	0.895	198.06 \pm 1.039	99.03	0.525
320	323.58 \pm 1.028	101.12	0.318	322.55 \pm 1.541	100.79	0.478

^aMean of 3 determinations in one day

^bMean of 3 determinations in three consecutive days

Table 2. Analytical parameters of sodium hyaluronate quantitation

Parameter	Characteristic
Linearity range ($\mu\text{g/mL}$)	80 – 320
Slope	0.9975
Intercept	0.8198
Correlation Coefficient (r^2)	0.9997
LOD ($\mu\text{g/mL}$)	9.07
LOQ ($\mu\text{g/mL}$)	27.50

LOD: Limit of detection

LOQ: Limit of quantitation

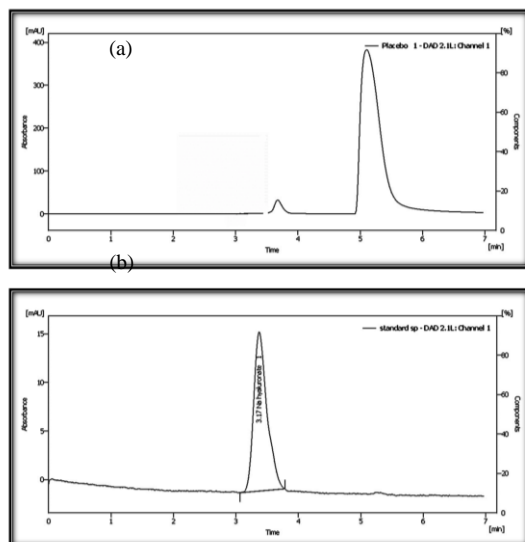


Figure 2. HPLC chromatogram of (a) Blank and (b) 200 g/mL sodium hyaluronate.

Method validation

The method for sodium hyaluronate determination was validated according to the International Conference on Harmonization (ICH) guidelines¹⁰. The following parameters were examined. The linearity of the developed method was investigated over the concentration range 80 – 320 $\mu\text{g/mL}$. Linear regression equation and correlation coefficient (r^2) are as follows:

$Y_{\text{sodium hyaluronate}} = 0.9975 x + 0.8198$ ($r^2 = 0.9997$) as shown in **Figure 3**.

The selectivity of the method was studied by injection of placebo (drug free) that showed no interfering peaks at the same retention time of sodium hyaluronate as shown in **Figure 2**.

Reproducibility was studied by injection of 200 $\mu\text{g/mL}$ of sodium hyaluronate six times using the optimized conditions. The results showed that RSD was 0.81 %, which ensure high precision of the present method.

Table 3. Stability Study

Mode of degradation	Condition	Assay (%)	% degradation	Purity angle	Purity threshold
Acidic degradation	5 N Hydrochloric acid for 3 hr	95.59	0.044	0.197	0.391
Basic degradation	5 N Sodium hydroxide for 3 hr	94.50	0.055	0.131	0.327
Oxidative degradation	30 % H ₂ O ₂ in water bath at 80°C for 3 hr	91.88	0.081	0.134	0.324
Thermal degradation	oven of temperature 80°C for 8 hr	96.66	0.033	0.124	0.315

$\% \text{ Degradation} = (C_0 - C) / C_0$

Table 3. Assay of Sodium hyaluronate Hycarenol® eye drops

Label Claim (mg)	2 mg/mL
Amount found (mg) ± SD	2.034 ± 0.071
% label claim	101.71
% RSD (n=3)	0.069

RSD = Relative standard deviation

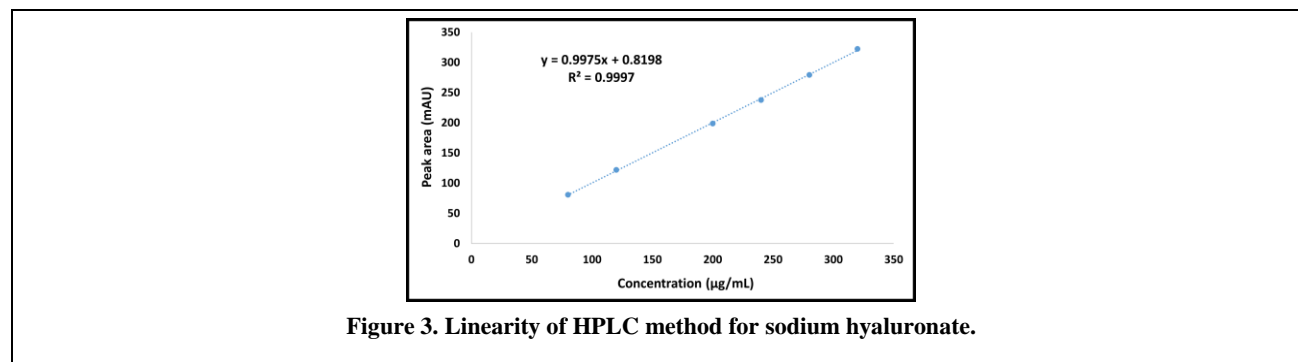


Figure 3. Linearity of HPLC method for sodium hyaluronate.

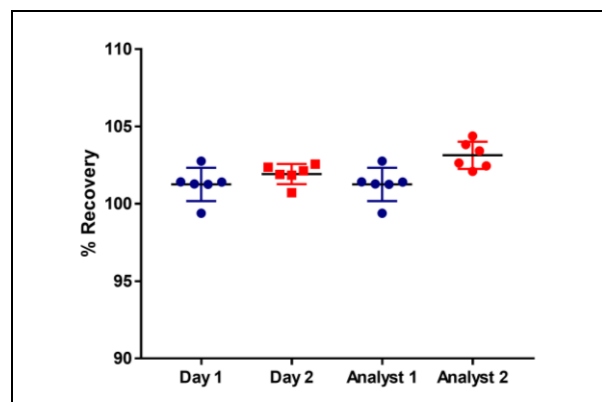


Figure 4. Ruggedness of HPLC method for sodium hyaluronate.

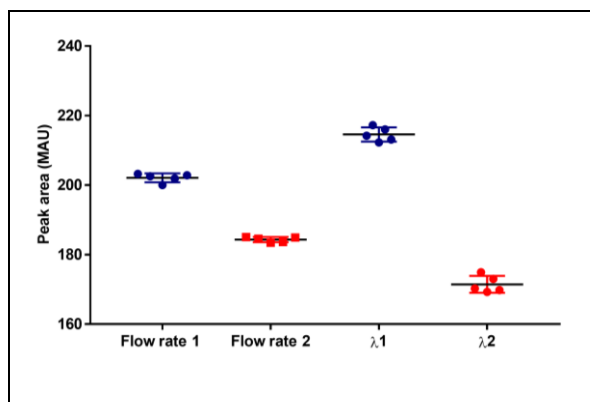


Figure 5. Robustness of HPLC method for sodium hyaluronate determination. (Flow rate 1= 0.475 mL/min, flow rate 2= 0.525 mL/min) and (λ1= 203 nm, λ1= 207 nm).

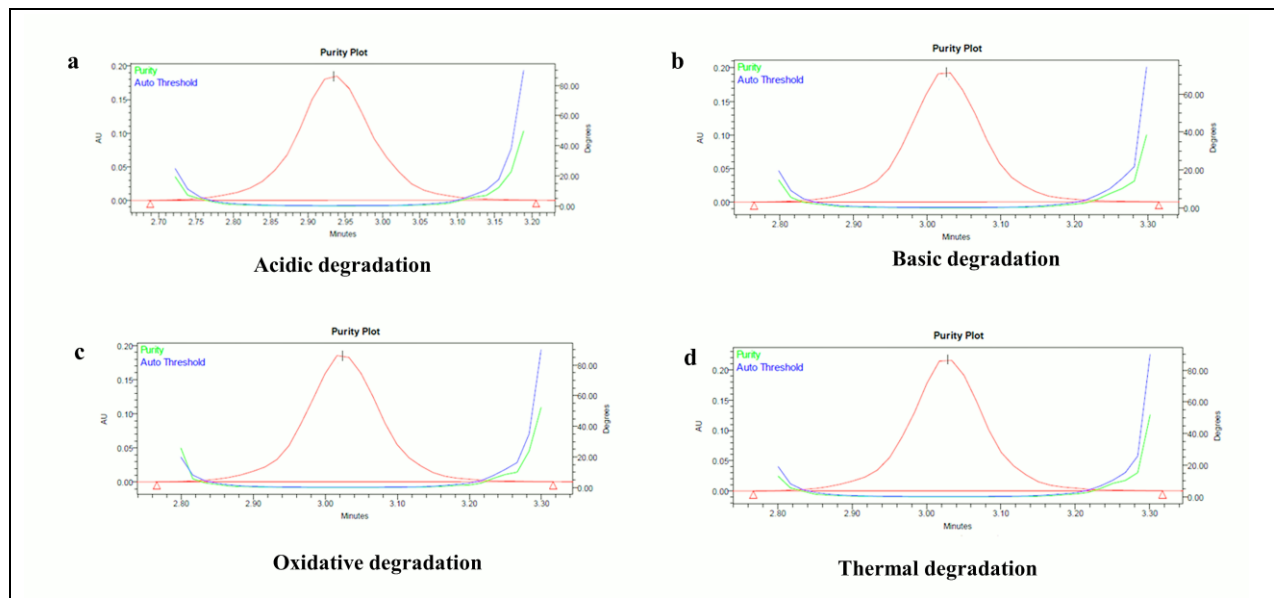


Figure 6. Purity plot of sodium hyaluronate after exposure to (a) Acidic, (b) Basic, (c) Oxidative and (d) Thermal degradation conditions

Standard addition method was used for solutions preparation at level of 80, 100 and 120 % level of the injected test concentration. Each concentration was prepared and injected three times at the same day and at three consecutive days, for intra- and Inter-day studies respectively. The results show that the accuracy are within the range 100.71- 103.74 % with RSD 0.318 – 0.895 % and 99.03 -101.31 % with RSD 0.402-0.525 %, for intra-and inter-day, respectively as shown in **Table 1**.

Stability study

Sodium hyaluronate was found to be stable to acidic, alkaline, oxidative and thermal degradation at 80°C. The degradation percentage of sodium hyaluronate is negligible and the chromatograms injected after the forced degradation studies contains no other than the drug peak. Peaks retention time values and peak purity plots prove that the peak of sodium hyaluronate is pure and free from any interference as shown in **Figure 6**. The peak purity data shows that sodium hyaluronate is homogenous and has no coeluting peaks and therefore, the HPLC method for sodium hyaluronate that is demonstrated in this work is considered as stability-indicating method. This data is shown in **Table 3**.

CONCLUSION

The developed method allows determination of sodium hyaluronate in eye drops by using UV detection at 205 nm. This study was validated successfully and able

to show linearity in the range 80 – 320 µg/mL. The method is considered a stability-indicating method as the degradation products are not interfering with the peaks of sodium hyaluronate. This method is a valid, accurate and precise method that can be used in routine analysis in quality control laboratories.

Acknowledgement

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Walker, K. Z.; Patrick M: Hyaluronic Acid. In *StatPearls [Internet]*; StatPearls Publishing, 2019.NBK482440PMID:29494047)
2. Mota, A. H. D.; Rosa; Carrasco, Marta P; Rijo, Patrícia; Ascensão, Lia; Viana, Ana Silveira; Rocha, João; Eduardo-Figueira, Maria; Rodrigues, Maria João; Custódio, Luísa. Combination of hyaluronic acid and PLGA particles as hybrid systems for viscosupplementation in osteoarthritis. *Inter. J Pharm.* **2019**, 559, 13-22.
3. Yazdani, M. S.; Aboulghassem; Jackson, Catherine Joan; Utheim, Tor Paaske. Hyaluronan-Based Hydrogel Scaffolds for Limbal Stem Cell Transplantation: A Review. *Cells* **2019**, 8, 245.

4. J. H. Poulson. Urine and tissue glycosaminoglycan and their interactions. *Dan. Med. Bull.* **1975**, *33*, 75–96.
5. Pereira, L. C. S., Claude; Moufarrij, Sara; Krähenbühl, Swenn M.; Favre, Julien; Gremion, Gerald; Applegate, Lee Ann; Jolles, Brigitte M. Gait analysis following single-shot hyaluronic acid supplementation: a pilot randomized double-blinded controlled trial. *Pilot and Feasibility Studies*, **2019**, *5*.
6. Ruckmani, K. S., Saleem Z.; Khalil, Pavne; Muneera, M. S.; Thusleem, O. A. Determination of sodium hyaluronate in pharmaceutical formulations by HPLC–UV. *J. Pharm. Anal.* **2013**, *3*, 324-329.
7. Ruckmani, K. Z. S., Saleem; Khalil, Pavne; Khatri, Aamer; Akmal, Javed. A New Simple and Rapid Method for the Determination of Sodium Hyaluronate in Active Pharmaceutical Ingredient and Ophthalmic Formulations by DP5 Photorede. *World J. Anal. Chem.* **2014**, *2*, 15-22.
8. Volpi, N. On-Line HPLC/ESI-MS Separation and Characterization of Hyaluronan Oligosaccharides from 2-mers to 40-mers. *Anal. Chemi.* **2007**, *79*, 6390-6397.
9. Jitka Kašparová, K. A.; Lucie Korecká; Lenka Česlová. Determination of hyaluronic acid in pharmaceutical products by spectrophotometry and HPLC coupled to fluorescence or mass spectrometric detection. Scientific Papers of the University of Pardubice, Series A; Faculty of Chemical Technology **2018**, *24*, 39–47.
10. International Conference on Harmonization (ICH, **2005**) Guidelines, Q2 (R1), Validation of analytical procedures: test and methodology.