

A New Simple and Rapid Method for the Determination of Sodium Hyaluronate in Active Pharmaceutical Ingredient and Ophthalmic Formulations by DP5 Photorode

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Abstract A new, simple, rapid and reliable turbidimetric routine method for the determination of sodium hyaluronate in active pharmaceutical ingredient and ophthalmic solution is described by using DP5 Phototrode. The turbidity of an aqueous ophthalmic sample solution was measured using a DP5 Phototrode™ for photometric indicated titration. Near the equivalence point, a precipitate between titrant and analyte is formed, and the solution becomes turbid. The method involves measurement of the equivalence point at minimum light transmission through the sample, containing cationic quaternary ammonium compound, cetylpyridinium chloride (CPC) solution as a dispersing agent, is measured at 520 nm. The results obtained for in-house prepared formulation and other marketed ophthalmic solution are compared with those obtained by the published HPLC method. A calibration curve was obtained from 0.08 to 0.122 mg mL⁻¹ ($r > 0.9998$). Within-day % RSD was 1.08 and between-day % RSD was 1.10. Specificity/ selectivity experiments revealed the absence of interference from excipients, recovery from spiked samples for sodium hyaluronate was 98.3–100.9%. The developed method was applied to the determination of sodium hyaluronate in pharmaceutical drug substance and drug product.

Keywords: sodium hyaluronate, hyaluronic acid, ophthalmic solution, turbidimetric, Phototrode

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1. Introduction

Sodium hyaluronate (SH) is a glycosaminoglycan composed of disaccharide units and a molecular weight of 2 to 6 x 10⁶ Daltons. It is produced by fibroblasts throughout the body. An increase of SH concentration is due to synovial inflammation and reduced catabolism caused by hepatic failure. It is unique among glycosaminoglycans in that it is nonsulfated, forms in the plasma membrane instead of the Golgi, and can be very large, with its molecular weight often reaching the millions. Sodium hyaluronate is used as a viscosupplement, administered through a series of injections into the knee, increasing the viscosity of the synovial fluid, which helps lubricate, cushion and reduce pain in the joint [1]. It is generally used as a last resort before surgery [2] and provides symptomatic relief, by recovering the viscoelasticity of the articular fluid, and by stimulating new production from synovial fluid [3]. Use of sodium hyaluronate may reduce the need for joint replacement [4]. Injections appear to increase in

effectiveness over the course of four weeks, reaching a peak at eight weeks and retaining some effectiveness at six months, with greater benefit for osteoarthritis than oral analgesics [5] SH. It may also be effective when used with other joints [6].

Sodium hyaluronate may also be used in plastic surgery to reduce wrinkles on the face or as filler in other parts of the body [7]. It is also in ophthalmology to assist in the extraction of cataracts, the implantation of intraocular lenses, corneal transplants, glaucoma filtration, retinal attachment and in the treatment of dry eyes [8]. Sodium hyaluronate is also used to coat the bladder lining in treating interstitial cystitis.

Unlike collagen, is able to penetrate the skin's upper layers to improve and benefit the skin when applied topically [9]. Sodium hyaluronate is a major component of skin, where it benefits tissue repair and protection [10]. When applied in an HA cream or serum, sodium hyaluronate forms an air permeable layer and penetrates into the dermis, thus boosting the elasticity and hydration of the skin. The protective barrier on the skin locks in moisture, which gives the skin a youthful appearance.

Sodium hyaluronate in the past was primarily derived from rooster combs, but given the increasing concern about animal derived ingredients, a method was developed in 1989 to extract sodium hyaluronate from vegetable sources such as soybean or corn, using a fermentation process involving bacteria. This process creates a very high quality Sodium hyaluronate that is safe, has excellent anti-aging properties and is vegetable sourced.

Determination of sodium hyaluronate raw material has been described in British Pharmacopoeia 2013 by spectrophotometer using disodium tetraborate sulfuric acid, carbazole as derivatising agent. Derivatization is carried out at 100°C constant temperature. After derivatization absorbance is measured at wavelength 530 nm [11].

Gao Qihe Zou Zhihong (2007) has published method for determination of concentration sodium hyaluronate of eye drops by Colorimetry. The method involves reaction between sodium hyaluronate with sulfuric acid carbazole at high temperature to produce the color product and determined by colorimetric. The detection wavelength was (530±2)nm.

Asteriou T, et al., 2001 reported that hyaluronan concentration and hyaluronidase activity can be assayed by using different techniques including turbidimetry, viscosimetry, ELISA, chromatography, and colorimetry. The most popular colorimetric method is that of J. Reissig et al. (1955, J. Biol. Chem. 217, 959-966), in which the color results from a reaction between the Ehrlich's reagent (DMAB) and the N-acetyl-d-glucosamine reducing end of each hyaluronan chain. Nevertheless, there are problems with this method when proteins are present in the medium. They proposed a new interpretation of the Reissig signal for estimating such reducing ends in media containing enzymes or other proteins. This interpretation was based on the fact that the absorbance obtained by using the Reissig method results from two factors: turbidity due to the formation of polysaccharide-protein complexes and a color resulting from the action of DMAB on the reducing end of the polysaccharide chains. The turbidity at 585 nm, the wavelength at which the color intensity is maximal, may be estimated by curve fitting the spectrum between 450 and 650 nm. Subtracting the turbidity from the absorbance gives the colorimetric intensity which represents the concentration of polysaccharide chains.

Both pharmacopoeias USP and BP have not described the assay procedure for ophthalmic solution. Also sodium hyaluronate raw material and finished product is not official in USP.

In comparison other published method, turbidimetric methods have the advantages of reducing analysis time, enhancing sensitivity and flexibility and lowering the cost of the instruments and maintenance [12]. One of the biggest disadvantages of derivatization has been lack of stability. The reaction products are not stable and have short half life possibly because of a spontaneous intermolecular rearrangement [13]. Another disadvantage of derivatization is that it reacts with only few functional groups.

The literature survey shows that several methods [14-30] like enzymatic, Carbowax PA1 chromatography, Chemiluminescence, digestion, Gas-liquid chromatographic, on-line HPLC/ESI-MS; HPLC UV-Vis methods have been reported for the determination of

sodium hyaluronate with derivatization. Most of the reported methods are by derivatization, gel permeation chromatography or digestion. These methods and the official methods may not be suitable for assay of sodium hyaluronate in ophthalmic solutions due to complexity, sensitivity, risk and flexibility issues involved into it. However, as per bibliographical revisions performed, no turbidimetric analytical method has been reported for direct (without derivatization) determination of sodium hyaluronate.

The present study was aimed at developing simple, rapid, accurate and precise turbidimetric method for the determination of sodium hyaluronate in commercially available and in-house prepared pharmaceutical formulations. Basic principle involved, measuring the loss of intensity of transmitted light due to the scattering effect of particles suspended in it. Light is passed through a filter creating a light of known wavelength which is then passed through a sample cup containing a solution. A photoelectric cell collects the light which passes through the solution. At the equivalence point, maximum turbidity is reached, and a minimum in light transmission is measured with a DP5 Phototrode™.

The method can be for use in routine quality control applications. The proposed method for the determination of sodium hyaluronate in pharmaceutical formulations by turbidimetric titration is first of its kind without involving costly instruments. The method is very cost effective, saves solvents, column, time and environmentally friendly and no need to use size exclusion chromatography and GPC software.

The issues with the GPC software are that it gives analysis data output as:

Relative molecular weight values (Mn, Mw, Mz and Mp), molecular weight distribution: MWD and polydispersity: Mw/Mn. Procedure involves lengthy and tedious column calibration. In regards to polymers, the molecular masses of most of the chains will be too close resulting in eluting broad peaks in the GPC separations.

A turbidimetric titration methods principle was based on titration reaction. Sodium hyaluronate was precipitated by addition of cationic surfactant cetylpyridinium chloride (CPC). The solution becomes turbid at the equivalence point. A comparative titration with sodium hyaluronate solution was first performed giving a calibrations factor indicating the sample amount titrated by 1mL of titrant (mg/mL). The factor was used in a second titration method (sample titration) where sodium hyaluronate in ophthalmic solution was estimated. The calibration factor and the titrant consumption define the amount of sodium hyaluronate in the sample.

The proposed automated method could be of use to industries that deal with sodium hyaluronate and need to determine its content without having to invest into other costliest instruments and less laborious, simple and fast.

2. Experimental

2.1. Instrumentation

Auto titration systems T-50 from Mettler-Toledo (Analytical, Switzerland) consisted of DP5 phototrode allowing switching between five different wavelengths

(520,555,590,620 and 660 nm), terminal with brilliant touch screen, expandable with dispensing burette drives, comprehensive communication connections. The XSE 205DU analytical balances from Mettler-Toledo (Analytical, Switzerland), were used. Titration curve, burette reading were recorded on terminal with brilliant touch screen and printed through this.

2.2. Reference Substances, Reagents and Chemicals

Sodium hyaluronate was obtained from Yantai Dongcheng Biochemicals, China. Cetylpyridinium chloride (CPC), Dodecyltrimethyl-ammonium bromide (DTAB), Benzalkonium chloride (BZK) was purchased from Sigma Aldrich, Germany and potassium dihydrogen phosphate was purchased from Panreac Quimica (Barcelona) Espana. Distilled water was obtained from a Milli-Q system Millipore, Milford, MA, USA. All the chemicals and reagents were of analytical or reagent grade. Reference standards of sodium hyaluronate were obtained from British Pharmacopoeia Commission Laboratory, London. The excipients sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were obtained from Merck, Germany. Ophthalmic formulations containing Sodium hyaluronate were developed and manufactured in our research and development laboratory.

2.3. Experimental Conditions

The auto titrator and DP5 photorode were turn on. Allowed to stand DP5 Photrode for 10-15 minutes in order to get a stable light intensity. The transmission signal of the photrode were checked in the deionised water and set at 1000 mV by turning the knob on the top of Photrode. Formations of bubbles during titration were avoided by selecting appropriate stirring speed, since they disturb photometric indication. The consumption v/mL, potential signal % transmission, time/s and equivalence point were recorded by terminal with brilliant touch screen. Method parameters for calibration factor and sample determination were set as below:

A: Method parameters for calibration factor:

Instrument:	Autotitrator, Model T50
Sensor:	Phtrode DP5
Unit:	% Transmittance
Wavelength:	520 nm
Stirring speed:	25%
Stirring duration:	30 seconds
Titrant:	CPC 1 mg/ml
Predispense:	2 ml
Increments:	0.1 ml
Tendency:	Negative
Threshold:	20 % T/ml
Maximum volumne:	10 ml

Calculation R1 : VEQ (ml of titrant consumed)

Calculation R2 : Q (mmol)

Calculation R3 : $m \cdot (H1/VEQ) \cdot H2$ (mg/ml)

Where

$m = 5$ mL 1 mg/mL Sodium hyaluronate standard solution.

H1 = Sodium hyaluronate standard solution concentration (1 mg/mL)

H2 = Purity of Sodium hyaluronate, e.g. 99.9% = 0.999

The calibration factor R3 is stored as auxiliary value H3.

B: Method parameters for sample analysis:

Instrument:	Autotitrator, Model T50
Sensor:	Phtrode DP5
Unit:	% Transmittance
Wavelength:	520 nm
Stirring speed:	25%
Stirring duration:	30 seconds
Titrant:	CPC 1 mg/ml
Predispense:	2 ml
Increments:	0.1 ml
Tendency:	Negative
Threshold:	20 % T/ml
Maximum volumne:	10 ml
Calculation R1 : VEQ (ml of titrant consumed)	

$$\text{Calculation R2 : } \frac{100 \cdot H3 \cdot \text{VEQ (ml)}}{5}$$

$$\text{Calculation R3 : } \frac{R2 \cdot 5}{100}$$

Where

100 = for percentage

H3 = Calibration factor (mg/mL)

2.3.1. pH 7.0 Phosphate Buffer

Phosphate buffer were prepared by weighing and transferring accurately about 297 mg of monobasic potassium Phosphate, 492 mg of dibasic potassium phosphate and 250 mg of polysorbate 80 into a 1000 mL volumetric flask. A 400-500mL portion of deionized water was added and mixed. pH was adjusted to 7.0 ± 0.2 with either 1M potassium hydroxide or phosphoric acid The solution was diluted to volume with the deionized water and mixed.

2.3.2. Titrant Cetylpyridinium Chloride Monohydrate ($C_{21}H_{38}NCl \cdot H_2O$):

Titrant was prepared by transferring accurately 1.0 g of cetylpyridinium chloride monohydrate (CPC), into a 1000 mL volumetric flask. A 400-500mL portion of deionized water was added and mixed. The solution was diluted to volume with the deionized water and mixed. The concentration of this solution was $1 \text{ mg/mL} = 0.003 \text{ mol/L}$

2.3.3. Sodium Hyaluronate Standard Solution 1 mg/mL

Standard solutions were prepared by transferring accurately about 100.0 mg of sodium hyaluronate (100/purity of reference standardx100) reference standard to a 100 mL volumetric flask. A 50-60 mL portion of deionized water was added and the solution was stirred until sodium hyaluronate was dissolved. The solution was diluted to volume with the deionized water and mixed. The standard solution was taken around 6-10 hrs to dissolve completely with continues stirring. Further this solution was taken for determination of calibration factor.

2.3.4. Calibration Factor Determination

5 mL of above sodium hyaluronate standard solution was transferred into 50 mL volumetric flask; 25 ml of buffer solution was added into the flask and mixed. The solution was diluted to volume with deionized water. Entire portion of this solution were transferred into titration beaker. The titration for the calibration factor was started. The result is stored as auxiliary value H3.

2.4. Samples

Test samples were ophthalmic solution prepared in-house and purchased from the local and international market with different composition of sodium hyaluronate. Other test samples used were accelerated stability samples with similar composition

2.4.1. Estimation from Formulations

Contents of five containers of ophthalmic solutions, containing sodium hyaluronate 2.0 mg/mL, were transferred to a 100 mL beaker. From this, a 2.5 mL portion of analyte was transferred into a 50 mL volumetric flask, 25 mL of buffer solution was added into this flask and mixed. The solution was diluted to volume with water and mixed. Complete solution was transferred to titration beaker. The solution was titrated against titrant cetylpyridinium chloride monohydrate and the consumption v/mL, potential signal % transmission, time/s and equivalence point were recorded by terminal with brilliant touch screen.

2.5. Calculation by Software

2.5.1. Calibration Factor Determination

$$R1 = VEQ(mL)$$

$$R2 = Q(mmol)$$

$$R3 = m * (H1/VEQ) * H2(mg/mL)$$

Where

m = 5 mL 1 mg/mL Sodium hyaluronate standard solution.

H1 = Sodium hyaluronate standard solution concentration (1 mg/mL)

H2 = Purity of Sodium hyaluronate, e.g. 99.9% = 0.999

The calibration factor R3 is stored as auxiliary value H3.

2.5.2. Sample Determination

$$R1 = VEQ(mL)$$

$$R2 = 100 * H3 * VEQ(mL) / 5$$

$$R3 = (R2 * 5 / 100)$$

where

100 = For percentage

H3 = Calibration factor (mg/mL)

3. Results and Discussion

3.1. Selection of Titrant

Initially dodecyltrimethyl-ammonium bromide (DTAB), $C_{15}H_{34}BrN$, benzalkonium chloride (BZK), $C_6H_5CH_2N(CH_3)_2RCl$ and cetylpyridinium chloride (CPC), $C_{21}H_{38}ClN \cdot H_2O$ were used as titrant. The qualitative differences due to the titrant have been observed. These three titrants were compared with sodium hyaluronate. It was found that cetylpyridinium chloride monohydrate (CPC), was a better titrant for sodium hyaluronate as compared to BZK and DTAB.

Three titrants having concentrations 4mg/mL of DTAB, 25 mg/mL BAK and 1mg/mL of CPC were calibrated before using. Their performance was compared. DTAB, BZK and CPC were calibrated by aqueous solution 1 mg/mL of sodium hyaluronate. It was found that assay of sodium hyaluronate in DTAB; BZK was around 80 % in both the titrant DTAB and BZK respectively despite of higher concentration. Lower concentration 1mg/mL of DTAB and BZK resulted in no precipitation, so higher concentration was tried and used. The titration with CPC gave larger equivalence point, accurate concentration and result shown in Figure 1. Here we concluded that CPC is better titrant compared with DTAB and BKZ and was used for the sodium hyaluronate assay determination.

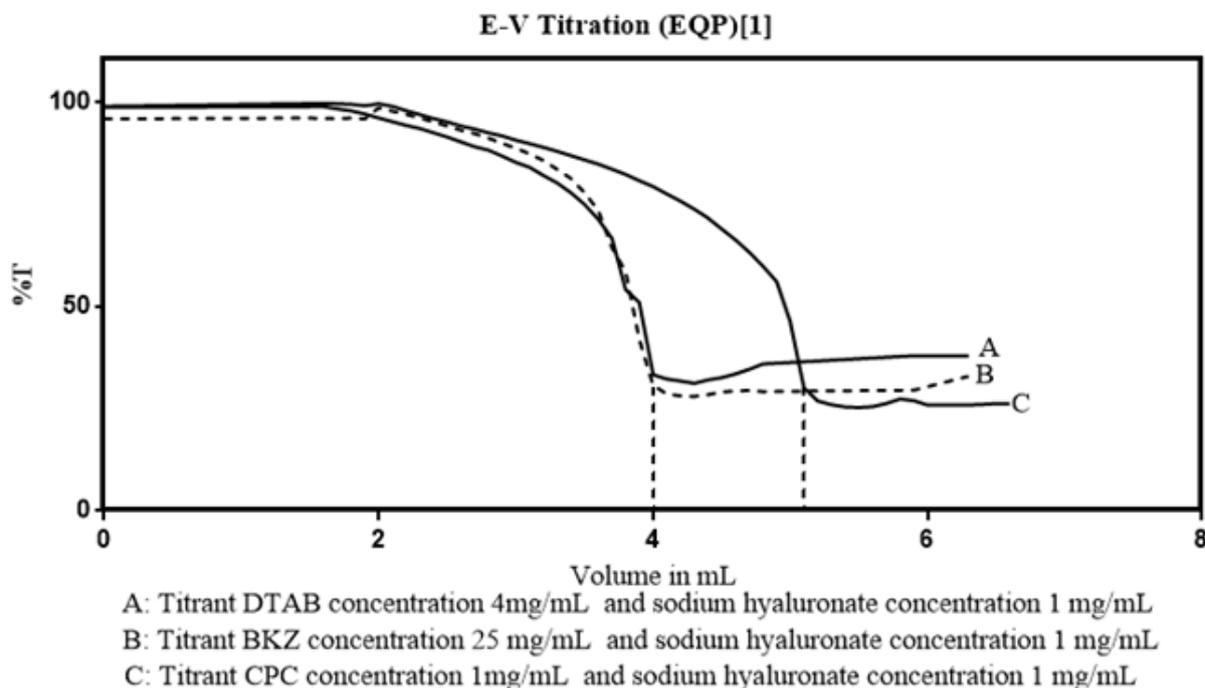


Figure 1. E-V titration curve (EQP) [1] showing three different titrant and out of these three titrant CPC gave larger equivalence point, accurate concentration and result. photorode wavelength was set at 520 nm

3.2. Organic Solvents

The Phototrode is solvent resistant, as it contains optical filter. The presence of sodium hyaluronate in the titration system can affect the titration results if it is not cleaned properly.

Methanol, ethanol, acetonitrile and propanol were used followed by deionised water as cleaning solvent for photorode. Drop in the percentage transmission to around 70 % was observed when Phototrode was cleaned with methanol, acetonitrile and propanol.

When cleaned with deionised water followed by ethanol and then again with deionised water percentage transmission to around 99 % led us to conclude that deionised water and ethanol are a better choice for cleaning the Phototrode.

3.3. Sample Size and Titrant

The Phototrode showed bigger equivalence point when the sodium hyaluronate concentration becomes more concentrated as showed in Figure 2. The sample and titrant was diluted within the range of 0.5 – 1.2 mg/mL.

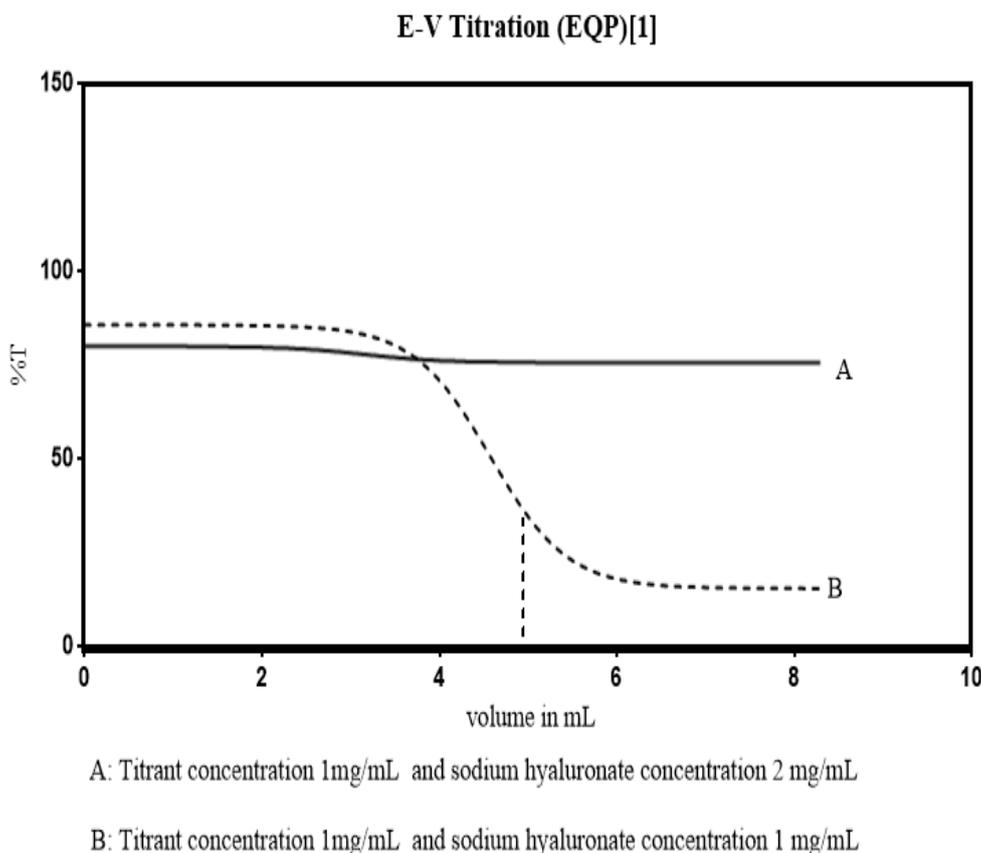


Figure 2. E-V titration curve (EQP) [1] showing effect of sample size and titrant. photorode wavelength was set 520 nm

3.4. Stirring

It was noted faster stir speeds showed increase in the percentage of transmission noise. It may be because of bubble formation in titration beaker containing sample solution. Significant error was encountered near the equivalence point. Stir speeds were optimized by running titration with different speed. Better equivalent point was obtained stirring when speed was set at 25 %. Hence throughout the experiment this speed was kept.

Further, in order to develop a simple and robust turbidimetric method for the determination of sodium hyaluronate by Phototrode the different wavelength and suitable stirring speed were employed to achieve the clear equivalence end point. Finally, the titrant consisting of 1mg/mL cetylpyridinium chloride monohydrate (CPC, pH 7.0 phosphate buffer pH adjusted to 7.0 ± 0.2 with either 1M potassium hydroxide or phosphoric acid and photorode wavelength set at 520 nm, was found to be appropriate, allowing good equivalence point of sodium hyaluronate as shown in Figure 3.

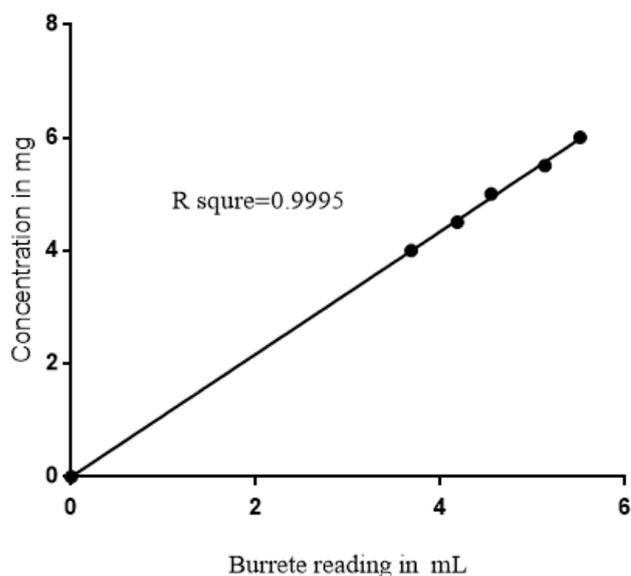


Figure 3. Linearity graph of sodium hyaluronate

3.5. Method Validation

Test method for the determination of sodium hyaluronate was validated to include the essential demands of International Conference on Harmonization (ICH) guidelines [29]. Parameters like specificity, linearity, accuracy, precision, range, robustness and system suitability were examined.

3.5.1. Specificity

No interferences were observed due to obvious presence of excipients like sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride.

3.5.2. Linearity

Burettes reading versus concentration in milligram per milliliter were plotted for Sodium hyaluronate at the concentration range between 80.0 to 120.0 percent of target level. Sodium hyaluronate showed linearity in the

range of 0.8-1.2 mg/mL., Slope, linear regression equations, and correlation coefficient (r^2) are provided below and shown in Figure 3:

$$\text{Slope} = 1.053 \text{ to } 1.118$$

$$Y_{\text{SODIUM HYALURONATE}} = 1.085 * X - 0.0009960 \quad (r^2 = 0.9995)$$

3.5.3 Accuracy

Accuracy of the proposed turbidimetric determination was evaluated from the assay results of the components. Accuracy was done by performing the assay of samples and calculated the equivalence point of different samples by recovery method.

Appropriate portions of stock solution were spiked into blank placebo matrix to produce concentration of 80.0 to 120.0 % of target level. Mean recovery of spiked samples was 99.30% for sodium hyaluronate as shown in Table 1.

Table 1. Accuracy data: analyte recovery (sodium hyaluronate)

Level	Theoretical amount mg	Theoretical (% of target level)	Determined amount (mg)	Determined (% of target level)	Recovery (%)	Bias (%)
1	4.007	80.00	3.987	79.60	99.50	+0.40
	4.007	80.00	3.940	78.66	98.32	-1.34
	4.007	80.00	3.932	78.50	98.13	-1.5
2	5.009	100.00	4.963	99.08	99.08	-0.92
	5.009	100.00	4.920	98.22	98.22	-1.78
	5.009	100.00	4.962	99.06	99.06	-0.94
3	6.001	120.00	5.952	118.82	99.02	-0.96
	6.001	120.00	5.916	118.10	98.42	-1.58
	6.001	120.00	6.000	119.78	99.82	-0.18

3.5.4. Precision

Instrumental precision was determined by six replicate determinations of standard solution; relative standard deviation was calculated and found to be 0.94% for sodium hyaluronate.

Method precision or intra-assay precision was performed by preparing six different samples from the same sample pool. Each solution titrated under same conditions and value of percentage assay for each solution were taken. The relative standard deviation of the sodium

hyaluronate in six sample solutions was calculated. Relative standard deviations obtained for sodium hyaluronate was 1.08%.

Intermediate precision was performed by analyzing the samples by two different analysts employing different instruments. Standard solution and six different samples at 100 percent target level were prepared by each analyst. Relative standard deviation obtained from 12 assay results by two analysts was 1.08 % for sodium hyaluronate. A typical titration curve was shown in Figure 4.

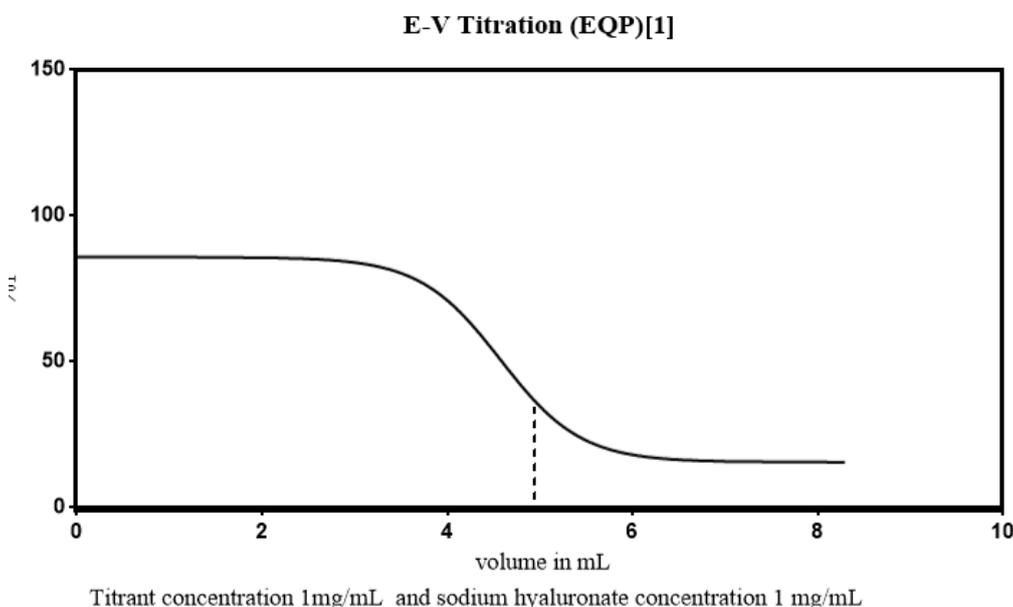


Figure 4. A typical E-V- titration (EQP) [1] sodium hyaluronate. Photrode wavelength was set at 520 nm

3.5.5. Range

Range of a method is defined as the lower and higher concentrations for which the method has adequate accuracy, precision and linearity. To demonstrate the range, five samples each of lower concentration (80 percent of target level) and higher concentration (120 percent of target level) were prepared similar to accuracy samples by spiking the drug substance into blank matrix (placebo). Each sample was analyzed in duplicate. At lower concentration, mean recovery of sodium hyaluronate was found to be 99.42%. Relative standard deviation obtained from these determinations was found to be 0.68% for sodium hyaluronate. At higher concentration, mean recovery of sodium hyaluronate was found to be 100.08%. Relative standard deviation obtained at the higher concentration level was found to be 0.49%

3.5.6. Robustness

Robustness of the proposed method was performed by keeping titration conditions constant with following deliberate variations.

I. Variation in buffer quantity.

II. Change in titrant concentration from 1.0 mg/mL to 0.8 and 1.2 mg/mL.

Standard solution was titrated six times in replicate for each minor change. System suitability parameters like titrant consumed and relative standard deviation were recorded for sodium hyaluronate found to be within acceptable limits.

Six test samples at the target concentration level were prepared and analyzed for each change. Recoveries and relative standard deviations were calculated for sodium hyaluronate during each change and found to be 98.90-100.52 % and less than 2.0 respectively. It was noted during the experiments that slight change in buffer quantity or change in titrant concentration does not affect

the method and produces results of similar system suitability.

3.5.7. System Suitability

System suitability tests were performed by titrating the standard six time and calculating the RSD of the mL of titrant consumed Results obtained from six replicate titration of standard solution as per the proposed method are summarized in Table 2.

Table 2. System suitability parameters of the proposed method

Number of titration	Titration consumed in mL	Concentration in mg/mL
1	4.737	1.057
2	4.787	1.046
3	4.817	1.040
4	4.703	1.065
4	4.706	1.064
6	4.737	1.057
RSD	0.956	0.943

3.6. Application of the proposed method

In-house prepared samples, marketed samples and samples stored at accelerated stability conditions (40°C/25%RH) were evaluated for assay of sodium hyaluronate. The method gave reproducible results of assay for all the samples tested for sodium hyaluronate. There was no interference observed in the estimation of test samples. The excipients in ophthalmic solution of sodium hyaluronate as a result of accelerated storage did not interfere with the estimation of the component. The assay of test samples (stored at room temperature and accelerated conditions) and market samples are summarized in Table 3.

Table 3. Application of the developed HPLC method for the determination of sodium hyaluronate in active pharmaceutical ingredient and ophthalmic solution

Manufacturer	Country of origin	Trade name	Conc.(mg/mL) ^a	Assay(%) A	Assay(%) B
Sun Pharma	India	Hyvisc	0.10	99.24 ^a	99.85 ^a
URASAPHARM	Germany	Hylo-comp	0.10	99.18 ^a	99.56 ^a
Moorfields Pharmaceuticals	United kingdom	Lubristil	0.15	98.21 ^a	99.42 ^a
Croma-Pharma GmbH	Germany	Olixia pure	0.15	99.14 ^a	99.46 ^a
TRB Chemedica (UK) Ltd	United kingdom	Vismed	0.18	98.64 ^a	98.75 ^a
Altacor Limited	United kingdom	Clinitas	0.40	99.88 ^a	100.2 ^a
Butterflies Healthcare Ltd	United kingdom	Ocusan	0.20	99.23 ^a	99.51 ^a
FDC Limited	India	Hymoist	0.15	99.62 ^a	99.26 ^a
Sun Pharma	India	Hyvisc Plus	0.18	99.11 ^a	98.23 ^a
In-house Room Temperature	Saudi Arabia	Hyfresh	0.20	99.06 ^a	99.75 ^a
In-house accelerated condition	Saudi Arabia	Hyfresh	0.20	99.43 ^a	99.64 ^a
Lot A ^b	China	Yantai Dongcheng Biochemicals	----	99.55 ^c	99.32 ^c
Lot B ^b	China	Yantai Dongcheng Biochemicals	-----	99.83 ^c	100.1 ^c

a: each value is average of two determinations (n=2); b: Active pharmaceutical ingredient; c= calculated on dry basis

A: Assay proposed method B: Assay by HPLC method

4. Conclusion

Sodium hyaluronate is an active pharmaceutical ingredient which is used in various ophthalmic preparations. The existing analytical methods for analysis of sodium hyaluronate are complex, require derivatization, time consuming and instruments like HPLC. It was aimed to develop a rapid, simple, accurate, precise method which

can be used to determine sodium hyaluronate in ophthalmic solutions and bulk drug. The present method utilizes the principles of turbidimetric titration by using DP5 phototrode of Mettler Toledo for the determination of sodium hyaluronate. The turbidimetric method was developed, validated and found to be rapid, simple, accurate (mean recovery 99.30%), precise (RSD < 2.0%) and linear (r²=0.9995) for determination of sodium hyaluronate in pharmaceutical formulations and bulk

drugs. The described method was applied successfully for routine quality control and stability studies.

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