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Gel permeation chromatography of dextrans in parenteral solutions: Calibration procedure development and method validation

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Abstract

We describe development and validation of a gel permeation chromatographic (GPC) method for dextrans in parenteral solutions. The GPC method was adopted from USP monographs on Dextran 40 and Dextran 70 raw materials. The method was optimized with a mobile phase flow rate of 1 mL/min and column temperature of 40 °C, to sharpen dextran and dextrose peaks. An easy-to-use, curve-fitting program capable of non-linear regression was developed in-house, using Microsoft Excel® and its Solver add-in to successfully meet the GPC calibration requirements for dextrans and dextrose, i.e., the experimental molecular weights within 100 ± 5% of the known molecular weights for dextrans and molecular weight of dextrose within 180 ± 2 Da. The GPC method was validated in terms of its stability indicating nature, robustness (column temperature of 40 ± 3 °C), accuracy (lack of effects of pH and concentration of dextrans or matrix components), and precision (repeatability and intermediate). Molecular weight distribution of dextrans were unchanged when the dextran containing test solutions were subjected to forced degradation using heat, light (daylight and UV light), extreme alkaline conditions or oxidative conditions. The method was capable of detecting changes in molecular weight distribution caused by degradation under extreme acidic conditions and heat, thereby confirming the stability indicating nature of the method. The concentration of Dextran 40 and Dextran 70 (75–125% of the nominal assay concentration), matrix components (108–111% of their nominal concentrations), and solution pH (pH 3–7 for Dextran 40 solutions and pH 4–7 for Dextran 70 solutions) did not affect the measured molecular weight distribution of Dextran 40 or Dextran 70. The method was precise with %R.S.D. of less than 1% for $\bar{M}_W$ values of Dextran 40 or Dextran 70.

Keywords: Dextran; Gel permeation chromatography; GPC; Validation; USP

1. Introduction

For many years Dextran, a glucose polymer, has been used in medicine as a blood plasma volume expander or blood flow improver [1,2]. In these glucose polymers the linkages between glucose units are almost entirely of the α-1:6 type. The weight average molecular weights of Dextran 40 and Dextran 70 are in the range of 35,000–45,000 and 63,000–77,000 Da, respectively [3,4]. For clinical purposes these heterogeneous dextran fractions should have narrow molecular weight distribution (MWD). Material with too small a molecular weight is rapidly lost from circulation and is therefore therapeutically ineffective. Material with too high a molecular weight can interfere with normal coagulation process of the blood [2]. A large number of dextran injections containing 0.9% NaCl, 5% dextrose, or both as the matrix components are commercially available [1].

Aqueous gel permeation chromatography (GPC) based methods have been reported in the literature for the determination of MWDs of dextrans [2–5]. The USP monographs on Dextran 40 and Dextran 70 describe a GPC method, which is calibrated using dextran standards of known molecular weights ranging from 4000 to 250,000 Da. The monographs state that the iterative procedure described by Nilsson and Nilsson [5] is a suitable method to obtain parameters of an exponential 3rd order equation (see Eq. (2)) used for the GPC calibration. The monographs also state that a curve-fitting program capable of non-linear regression may be used. The EP monographs allow performing the calibration by either plotting of the curve or by calculation of the curve [7]. Calculation of the curve is essentially performed using the same procedure as specified in the USP monograph.

Our attempts to perform GPC calibration per the USP procedure on Millennium®32 (Version 4.0, Waters Corporation,

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Milford, MA) were, however, unsuccessful. Since a software program for performing GPC calibration was not commercially available, we developed an accurate and easy-to-use Microsoft Excel® based procedure for GPC calibration. This development work, based on the approach described by Nilsson and Nilsson [5], employs the Newton method to minimize sum of squares of errors by optimizing parameters of the exponential equation. The Excel based procedure was then used in the subsequent validation of the GPC method.

The GPC method measures a fundamental property of the analyte (Dextran 40 or Dextran 70) and not its concentration. Validation of such method type is not presently covered in the International Conference on Harmonization (ICH) guidelines [6]. On a related topic, Kristensen et al. [8] have described validation of size exclusion chromatographic method for determination of molecular masses and MWD in low molecular weight heparin in which the validation parameters included precision (within run, between run and between laboratories) and robustness. A draft guidance document by FDA recommends evaluation of accuracy, repeatability, intermediate precision, and robustness for molecular size distribution GPC [9].

In the work presented here, the GPC method was adopted from USP monographs on Dextran 40 and Dextran 70 raw materials. The method was validated for determining MWDs of dextrans in parenteral solutions in terms of its stability indicating nature (matrix and test solutions stressed in terms of heat, pH, UV and daylight, and oxidative conditions), robustness (column temperature), solution stability, and the effects of dextran concentration, matrix components, and pH on accuracy and precision.

2. Experimental

2.1. Materials

Sodium sulfate used was ACS reagent having 99 + % purity. Dextran calibration reference standards, Dextran 4, Dextran 10, Dextran 40, Dextran 70, and Dextran 250, were purchased from USP. The known molecular weights for these reference standards were furnished by USP. Also obtained from USP were, the V_0 marker and Dextran 40 and Dextran 70 system suitability reference standards. Dextrose with ≥99.5% purity was purchased from Fluka Chemical Company (Hauppauge, NY).

2.2. HPLC apparatus and conditions

The validation runs were performed on two HPLC instruments. The first one was an Agilent 1100 liquid chromatography system (Agilent Technologies, Palo Alto, CA) with an Eldex CH-150 column heater (Eldex Laboratories, Napa, CA) and Waters (Waters Corporation, Milford, MA) 2414 Refractive Index (RI) detector. The second system consisted of a Hitachi L-7100 high-pressure pump (Hitachi High Technologies America Inc., San Jose, CA), Waters 717 plus autosampler, Eldex CH-150 column heater, and Waters 410 RI detector.

Several columns from Waters (Ultrahydrogel series) and Tosoh Bioscience (PW and PW_{XL} series, Tosoh Bioscience LLC, Montgomeryville, PA) were considered. These columns vary in porosities and particle size of the packing materials. Of all the columns evaluated, only Tosoh Bioscience PW series columns had dimensions of 7.5 mm i.d. and 300 mm length as stated in the USP monographs. Two of G5000PW columns (7.5 mm i.d. × 30 cm length) and one of G3000PW column (7.5 mm i.d. × 30 cm length) were connected in decreasing order of exclusion limits. Mobile phase contained 7.1 g of Na_2SO_4 per liter. The USP monograph describes constant temperature for the HPLC operation, and the mobile phase flow rate is not stated. With the optimized conditions, the mobile phase flow rate was set at 1 mL/min and the column temperature was kept at 40 °C. The injection volume was 50 μL. Instrument control and data acquisition were performed using Waters Millennium®32 Chromatography System, version 4.0.

2.3. Preparation of standards, marker solution, and system suitability solutions

Dextran standards were prepared by separately weighing 20 ± 2 mg USP Dextran 4 Calibration RS, USP Dextran 10 Calibration RS, USP Dextran 40 Calibration RS, USP Dextran 70 Calibration RS, and USP Dextran 250 Calibration RS into five separate volumetric flasks. The dextran in each flask was dissolved and diluted with 1.0 mL volume of the mobile phase. The dextrose standard was prepared by weighing 20 ± 2 mg of dextrose into a 5 mL volumetric flask, and then dissolved and brought to 5.0 mL volume with the mobile phase.

The marker solution was prepared by weighing 15 ± 2 mg dextrose and 15 ± 2 mg of USP Dextran V_0 RS Marker in a 5 mL volumetric flask. The chemicals were dissolved and brought to volume with the mobile phase.

The system suitability solutions were prepared by weighing 20 ± 2 mg of USP Dextran 40 or USP Dextran 70 system suitability RS in a 1.0 mL volumetric flask. The chemical was dissolved and brought to final volume with the mobile phase.

2.4. Method calibration and molecular weight distribution calculations

Initially GPC calibration and molecular weight distribution calculations were both performed using Waters Millennium®32 GPC software. A system developed using Microsoft® Excel 2000 or later version (Microsoft Corporation, Redmond, WA) was used to obtain optimized parameters of the exponential third-order equation (Eq. (2)). These parameters were then used to perform the molecular weight distribution calculations in Millennium®32 GPC software (Version 4.0, Waters Corporation, Milford, MA).

2.5. Test solutions for method validation

Test solutions were prepared to contain Dextran 40 or Dextran 70 at 80–120% of nominal concentration of 99 g Dextran 40/L or 59.4 g Dextran 70/L. These solutions contained matrix components as dextrose and NaCl for the Dextran 40 containing solutions and NaCl for the Dextran 70 solutions. The test solu-

Table 1
Comparison of results obtained with Millennium®32 GPC calibration and those obtained using in-house developed Excel system

	$\bar{M}_W$ known	Calibration using Millennium®32 GPC			Calibration using the Excel procedure		
		$\bar{M}_W$ calculated	% Difference	Pass/fail	$\bar{M}_W$ calculated	% Difference	Pass/fail
Calibration							
250	238200	166377	−30	Fail	238094	0	Pass
70	70300	65501	−7	Fail	70773	1	Pass
40	40900	39319	−4	Pass	40385	1	Pass
10	10450	10495	0	Pass	10582	1	Pass
4	3850	3720	−3	Pass	3821	1	Pass
Dextrose	180	183		Fail	180	0	Pass
Sys. Suit.[a] 40							
$\bar{M}_W$	39000–46000	41312	N/A[b]	Pass	43157	N/A	Pass
≥90%	6000–9000	7482	N/A	Pass	7602	N/A	Pass
≤10%	111000–135000	112190	N/A	Pass	126697	N/A	Pass
Sys. Suit.[a] 70							
$\bar{M}_W$	65000–74000	62671	N/A	Fail	68827	N/A	Pass
≥90%	7000–11000	9396	N/A	Pass	9495	N/A	Pass
≤10%	180000–240000	166881	N/A	Fail	208728	N/A	Pass

[a] Sys. Suit.: system suitability solution.
[b] N/A: not applicable.

tions representing the nominal concentration (TS-100) solutions were pH adjusted in the range of 3–7 to encompass maximum possible pH variations of these samples.

For the GPC analysis, test solutions were diluted by pipetting 5.0 mL of Dextran 40 or 9.0 mL of Dextran 70 containing solutions into a 25-mL volumetric flask. A 5.0 mL of mobile phase concentrate (35.5 g Na_2SO_4/L) was added to the flask and brought to the final volume with distilled water. The diluted test solutions would then contain Na_2SO_4 at the same concentration of the mobile phase with Dextran 40 or Dextran 70 at 20 mg/mL.

2.5.1. Forced degradation of test solutions

The TS-100 solutions and the matrix components were exposed to forced degradation conditions with respect to heat, light and oxidative conditions.

Heat and Acid/Base: The Dextran 40 solutions (inherent pH, pH 3, and pH 7) and dextrose/NaCl matrix solutions were steam sterilized in glass ampoules at 121 °C for 15 min while the Dextran 70 solutions (inherent pH, pH 3, and pH 7) and NaCl matrix solution were steam sterilized at 121 °C for 30 min.

Light. A 20 mL aliquot of each of the TS-100 solutions were exposed, in parallel, to each of the two light conditions (near UV and daylight) generated in a light chamber. The light sources produce 20.00 klx from 400 to 750 nm (daylight) and 20.00 W/m^2 from 315 to 400 nm (near UV). Temperature and humidity settings were 25 °C and 60% RH, respectively. Samples were exposed to the daylight source for 120 h (approximately 2.4 million lx/h) and the near UV light source for 20 h (approximately 400 W h/m^2). The daylight exposure of 120 h and UV exposure of 20 h corresponds to 200% of the ICH requirements [10]. A 20 mL aliquot of each test solution, wrapped in aluminum foil and stored in the same chamber, was used as controls.

Oxidative. A 15.0 mL aliquot of each TS-100 solutions was treated with 0.3 mL of 30% H_2O_2. The oxidative reaction was allowed to proceed overnight. The reaction was quenched with 300 μL of catalase beads (Sigma–Aldrich, Milwaukee, WI), previously washed with water. The residual H_2O_2 was determined using Macherey–Nagel Quantofix Test strips for peroxide (product number 91319, lot number 319350). The residual H_2O_2 ranged from 0.5 to 25 mg/L H_2O_2 after treatment with catalase compared with an estimated initial concentration of 6000 mg/L. A water blank was prepared in a similar manner.

2.6. System suitability for the GPC method

To judge suitability of the GPC system for the analyses of samples, system suitability criteria were established for the injections of marker solution, calibration accuracy, and for the molecular weight distribution values for the Dextran 40 and Dextran 70 system suitability solutions. A marker solution consisting of USP V_0 marker and dextrose were injected in replicate ($n = 5$). The system suitability criteria for these injections were (a) elution profile for each injection shows two peaks, one due to V_0 marker and the second one due to dextrose, (b) the tailing factor for the dextrose peak is not more than 1.3, and (c) the %R.S.D. (relative standard deviation) for the ratio of V_0/V_T is not more than 1%. Each of the dextran calibration standards and dextrose were injected once. The b_1 through b_5 values were obtained such that the weight average molecular weight values for dextrans and dextrose meet the criteria listed in Table 1. The Dextran 40 and Dextran 70 system suitability solutions were injected, in triplicate or greater for any given validation run. For each injection, various molecular weight distribution criteria (Table 1) had to be met, and also the %R.S.D. for $\bar{M}_W$ values for Dextran 40 or Dextran 70 could not be more than 2%.

3. Results and discussion

In implementing the USP method for Dextran 40 and Dextran 70 for determining the MWD of the Dextrans in the parenteral

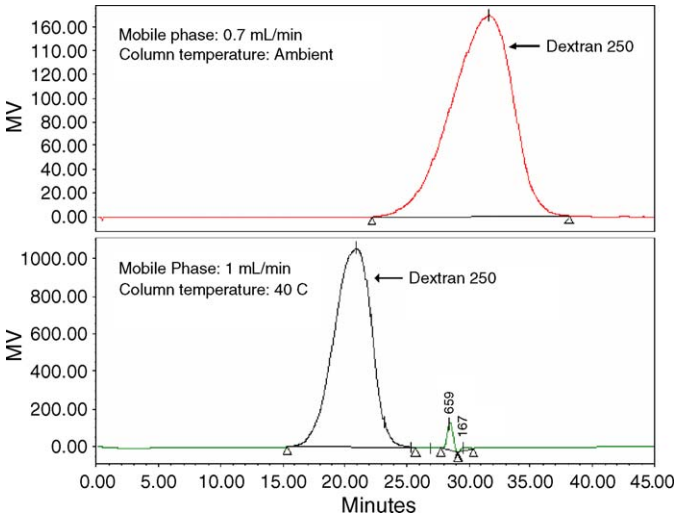

Fig. 1. Comparison of chromatograms for Dextran 250 standard as affected by mobile phase flow rate and column temperature. Chromatographic conditions: mobile phase, 7.1 g of Na_2SO_4/L, Columns, two of G5000 PW_{XL} and one of G4000 PW_{XL} (7.8 mm i.d. × 300 mm long from Tosoh Bioscience); injection volume, 20 μL. Similar sharpening of peaks was also observed for the other four dextran standards.

solutions, developmental work included optimizing the HPLC parameters and developing an iterative procedure for GPC calibration. The ICH guidelines were followed to validate the GPC method.

3.1. GPC method development

Sharpening of Dextran 250 peaks is presented in Fig. 1 when the mobile phase flow rate was increased from 0.7 to 1 mL/min, and column temperature was increased from ambient conditions to 40 °C. Similarly peaks for other dextran standards were sharpened at flow rate of 1 mL/min at column temperature of 40 °C. Based on this information, the HPLC method was operated with mobile phase flow rate of 1 mL/min and column temperature of 40 °C.

Per the USP monographs, the V_0 and V_T are the retention times of V_0 marker and dextrose peaks, providing exclusion volume and permeation volume of the GPC system, respectively. The values for the ratio of V_0/V_T were calculated per USP and used in system suitability test. The value for V_T was, however, not used as a permeation volume since doing so would have resulted in losing half of the dextrose slices in calibrating the GPC method. The permeation volume was instead assigned as the end of dextrose peak in the first injection of solution containing V_0 marker and dextrose.

The Millennium®32 GPC software was then applied to GPC calibration runs performed using these optimized conditions. The obtained calibration data, however, failed to meet the requirements (Table 1) since the calculated molecular weights were not within 100 ± 5% of the known values. Also, the Dextrans 40 and 70 system suitability injections failed to meet the molecular weight distribution requirements (Table 1).

In the monograph method, using a non-linear regression technique, parameters of an exponential third order equation are optimized such that $\bar{M}_W$ values of dextran standards are accurate within ±5% of the known values and $\bar{M}_W$ value for dextrose is 180 ± 2 Da, i.e., ±1.1%. The accuracy requirement of ±1.1% for dextrose is much tighter for dextrose than that for dextrans (±5%) although (a) dextrose calibration standard is not included in the monograph method and (b) dextrose molecular weight is well below the smallest molecular weight standard, i.e., Dextran 4 (nominal molecular weight of 4000 Da). The dextrose accuracy requirement, however, could not be met even when dextrose standard was included in the Millennium®32 GPC calibration.

3.2. Developing Excel based procedure for GPC calibration

The failure in GPC calibration was observed when the dextran peaks were broader with the mobile phase flow rate of 0.7 mL/min and ambient column temperature or also when the peaks were sharpened with the flow rate of 1 mL/min and column temperature of 40 °C. Further examination of the Millennium®32 GPC software showed that it lacked the option of using an iterative procedure to minimize the sum of squares of errors (as defined in Eq. (5)) by adjusting the exponential equation parameters as specified in the USP monographs.

An iterative procedure using the Solver function, available in Microsoft® Excel, was developed (Fig. 2). The procedure included the following steps [3–5]:

- Calculation of K_i

  The peaks for dextran and dextrose calibration standards were integrated in Millennium®32. The slice data information, comprising of slice retention time and slice area, was exported as an ASCII file. The system performed a check to ensure that the peak was divided into a minimum of 60 slices as specified in the USP monographs:

$$K_i = \frac{v_i - V_0}{V_T - V_0} \quad (1)$$

  where v_i is the retention time of the i-th slice, V_0 is the exclusion volume (retention time of V_0 Marker), and V_T is the baseline end of dextrose peak in the first injection of marker solution.
- Calculation of M_i

$$M_i = b_5 + e^{b_4 + b_1 K_i + b_2 K_i^2 + b_3 K_i^3} \quad (2)$$

  where K_i is calculated per Eq. (1), and the values of b_1 through b_5 parameters are optimized through the iterative process to minimize the sum of squares of errors (Eq. (5)). Initial value of 1 was used for each of these parameters.
- Calculation of $\bar{M}_w$

$$\bar{M}_W = \frac{\sum_{i=1}^{a}(y_i \times M_i)}{\sum_{i=1}^{a} y_i} \quad (3)$$

  where y_i is the area at the i-th slice (obtained from the slice data information in the ASCII file) and a is the total number of slices. M_i is calculated per Eq. (2).

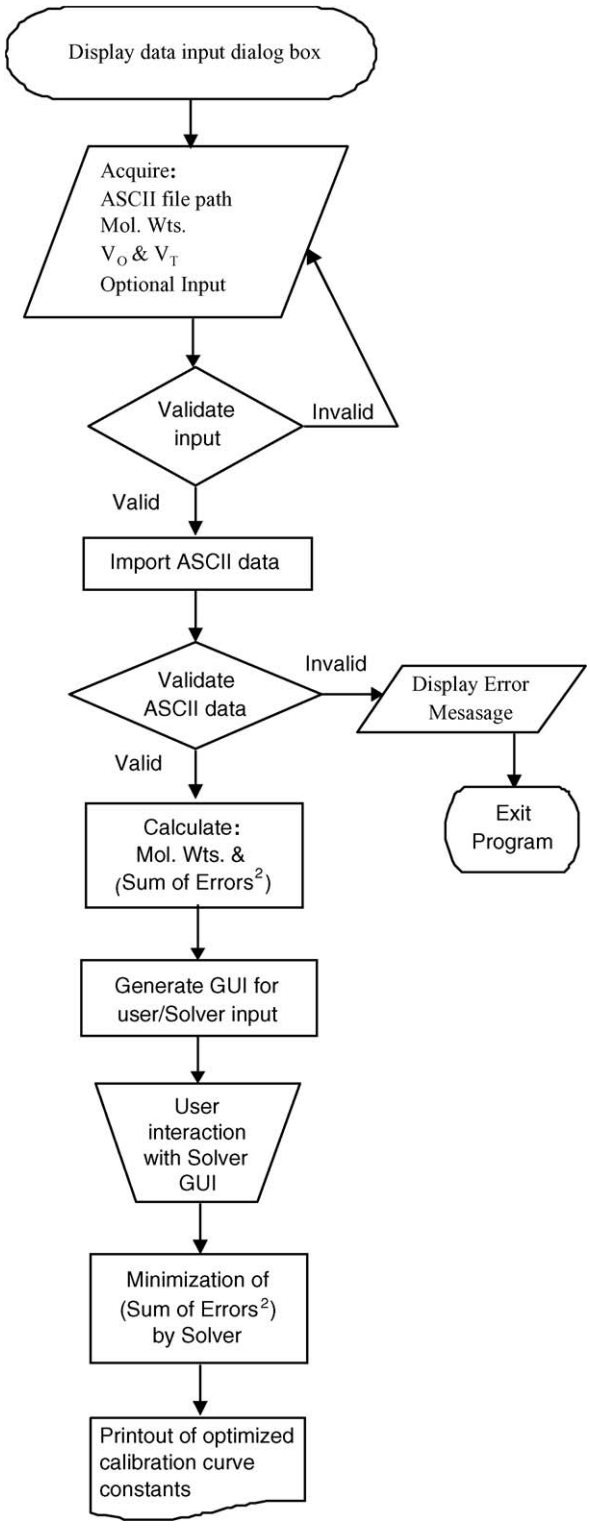

Fig. 2. Schematics describing a curve-fitting Excel based procedure for performing non-linear regression calibration of the GPC method.

- Calculation of error in $\bar{M}_w$ value

$$\text{error} = \frac{\bar{M}_W(\text{experimental}) - \bar{M}_W(\text{theoretical})}{\bar{M}_W(\text{theoretical})} \quad (4)$$

where $\bar{M}_W$ (experimental) is the value calculated per Eq. (3), $\bar{M}_W$ (theoretical) is the labeled value for the calibration standard. The error values were calculated for each of the five dextran standards and dextrose.

- Calculation of sum of squares of errors (SSE)

$$\text{SSE} = \sum (\text{error})^2 \quad (5)$$

where errors for each dextran standard and dextrose as determined per Eq. (4). The Solver function would minimize the SSE by optimizing b_1 through b_5 parameters in Eq. (2).

- Error %

This formula was used to determine if calculated values for molecular weight meet the USP criteria:

$$\text{error}(\%) = \text{error} \times 100\% \quad (6)$$

Values of the parameters, b_1 through b_5, were then transcribed into Millennium®32 GPC to calculate MWDs of unknown samples.

3.2.1. Comparison of GPC calibration using Millennium®32 GPC with that using the Excel procedure

Table 1 compares the calibration results obtained using the Millennium®32 chromatography system with the results generated by the Excel based procedure for the same chromatographic run. The data shows that unlike Millennium®32 GPC, the Excel procedure furnished passing results for all calibration standards for dextrans and dextrose. The results presented in Table 1 illustrate that, using the optimized b_1 through b_5 parameters from the Excel procedure, the various MWD values for Dextrans 40 and 70 system suitability injections were also well within the USP criteria. The Excel procedure for GPC calibration was then used in the method validation.

3.3. GPC validation

The validation parameters, experiments conducted, acceptance criteria, and obtained results are summarized in Table 2. A typical chromatogram for Dextran 40 and Dextran 70 containing solutions is presented in Fig. 3.

3.3.1. Stability indicating nature

The experiments on stability indicating nature of the method evaluated (a) ability of the GPC method to separate peaks for Dextran 40 and Dextran 70 from the peaks that may be formed due to the forced degradation in the stressed solutions (e.g., 5-HMF is a known dextrose degradant), and (b) effects of these potentially present peaks on molecular weight distribution of Dextran 40 and Dextran 70.

Test solution containing 5-HMF did not contain peaks that would interfere with either Dextran 40 or Dextran 70 (data not shown). The MWDs of dextrans were unchanged when the dextran containing test solutions were subjected to forced degradation using heat, light (daylight and UV light), extreme alkaline conditions or oxidative conditions (Tables 3 and 4 for Dextran 40 and Dextran 70, respectively). The GPC method was found to be capable of detecting changes in molecular weight

Table 2
Validation parameters, acceptance criteria, and obtained results

Item	Experimental plan	Acceptance criteria	Validation results
Stability indicating nature	1. Injections of matrix solutions (containing NaCl and dextrose) and injections of test solutions containing DEHP, 5-HMF, Ca, or Zn	1. Peaks, if present, in injections of matrix solutions do not interfere with Dextran 40 or Dextran 70 peaks	1. Matrix solutions did not contain peaks that would interfere with either Dextran 40 or Dextran 70 peaks
	2. Injections of Dextran 40 and Dextran 70 containing solutions exposed to heat, acid, base, light (daylight and UV light), and oxidative degradation conditions	2. Report values for $\bar{M}_W$, low fraction, and high fraction for Dextran 40 and Dextran 70 peaks in the stressed solutions. Additional peaks, if any, present in injections of stressed solutions do not interfere with Dextran 40 or Dextran 70 peaks	2. The values for $\bar{M}_W$ are presented in Table 3. No additional peaks were found that would interfere with Dextran 40 or Dextran 70 peaks
Robustness	Minor variations in column temperature (nominal $\pm 3\,^{\circ}C$) with bracketing nominal conditions. Injections of calibration solutions, system suitability solutions, and duplicate injections of test solutions were made	1. Meet calibration and system suitability requirements at each column temperature setting	1. The requirements were met at each column temperature settings
		2. The average values for $\bar{M}_W$ and $\bar{M}_n$ are $100 \pm 5\%$ and $100 \pm 10\%$, respectively, compared with those of the mean nominal conditions	2. Pass, compared with mean nominal conditions: average $\bar{M}_W$: 99.2–100.4% and average $\bar{M}_n$: 99.0–100.3%
		3. The low and high fraction values meet the USP requirements, and the values are within ±2000 and ±20,000, respectively, versus the mean nominal conditions	3. Pass, low and high faction values were within the USP limits, and these values were within ±200 and ±600, respectively, versus the mean nominal conditions
Stability of standards	Calibration standards were injected at three intervals and analyzed with freshly prepared calibration standards	The $\bar{M}_W$ values for dextrans are $100 \pm 5\%$ versus the original preparation, and that for dextrose is 180 ± 2 Da	Pass, compared with the original preparation, the $\bar{M}_W$ values for standards stored at ambient conditions for 125 h were from 98.2–102.3% for dextrans, and 180–182 for dextrose
Stability of sample preparation	The diluted samples were periodically analyzed against freshly prepared calibration standards	1. The $\bar{M}_W$ values are $100 \pm 5\%$ versus the original preparation and the $\bar{M}_n$ values are $100 \pm 10\%$ versus the original preparation	1. Pass, compared with the original preparation, the diluted samples stored at ambient conditions for 127 h had $\bar{M}_W$ values of 99.4–100.3% and $\bar{M}_n$ values of 98.6–100.0%
		2. The low and high fraction values meet the USP requirements, and the values are within ±2000 and ±20,000, respectively, versus the original preparation	2. Low and high fraction values within USP limits and within ±300 and ±2000, respectively, versus the original preparation
Accuracy	Triplicate injections of all test and control solutions by the primary analyst	1. The $\bar{M}_W$ values are $100 \pm 5\%$ versus the mean control solutions and the $\bar{M}_n$ values are $100 \pm 10\%$ versus the mean control solutions	1. Pass, compared with control, $\bar{M}_W$ values were within $100 \pm 2\%$ and $\bar{M}_n$ values are within $100 \pm 3\%$
	Triplicate injections of control and test solutions prepared at nominal concentration by secondary analyst	2. The low and high fraction values meet the USP requirements, and the values are within ±2000 and ±20,000, respectively, versus the mean control solutions	2. The low and high fraction values were within USP limits, and they were within ±300 and ±3000, respectively, versus the mean control solutions
Precision—repeatability	Results from the accuracy experiments	1. For $\bar{M}_W$, % R.S.D. values ≤3% 2. For $\bar{M}_n$, %R.S.D. values ≤ 5%	1. Pass, for $\bar{M}_W$, %R.S.D. values ≤ 1% 2. For $\bar{M}_n$, %R.S.D. values ≤ 1%
Precision—intermediate precision	Results from the accuracy experiments, test solutions prepared at nominal concentration	The requirements specified in the repeatability section are met by each analyst individually ($n = 3$) and also for the pooled results ($n = 6$)	Pass Individually 1. For $\bar{M}_W$, %R.S.D. values ≤ 1% 2. For $\bar{M}_n$, %R.S.D. values ≤ 1% Pooled results 1. For $\bar{M}_W$, %R.S.D. values ≤ 1% 2. For $\bar{M}_n$, %R.S.D. values ≤ 2%

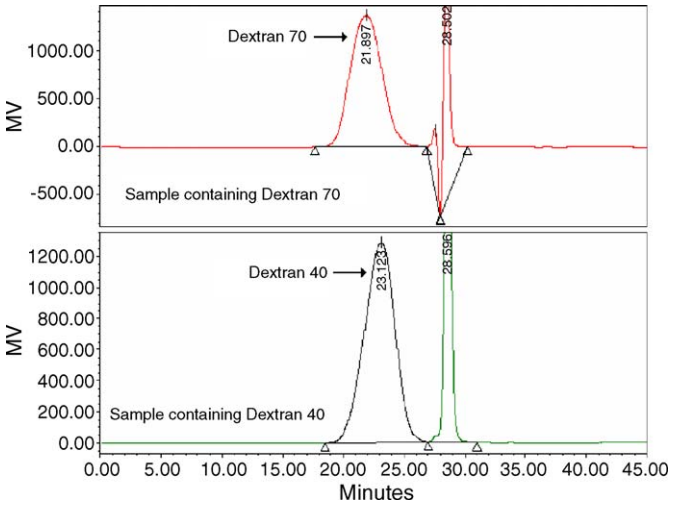

Fig. 3. Typical chromatograms for Dextran 40 and Dextran 70 containing solutions. Chromatographic conditions: mobile phase, 7.1 g of Na_2SO_4/L at 1 mL/min; columns, two of G5000 PW and one of G4000 PW (7.5 mm i.d. × 300 mm long from Tosoh Bioscience); column temperature of 40 °C; injection volume, 20 μL.

distribution caused by degradation under acidic conditions and heat, thereby confirming the stability indicating nature of the method.

3.3.2. Robustness

At each of the column temperature settings (37 and 43 °C), calibration and system suitability criteria were met. Also, the criteria on $\bar{M}_W$, $\bar{M}_n$, low fraction and high fraction values were met at the two settings (Table 2). The method was, therefore, considered robust when the column temperature is within 40 ± 3 °C.

3.3.3. Stability of standard and sample preparation

The standards are considered stable for at least 125 h when stored at ambient conditions. The diluted samples are considered stable for at least 127 h when stored at ambient conditions. Actual results are summarized in Table 2.

Table 3
Molecular weight distribution of Dextran 40 solutions as affected by stressed conditions

Stressed condition		$\bar{M}_W$
Condition	pH	
Daylight	Inherent, 5.5	37616
	pH 3	37922
	pH 7	38389
UV light	Inherent, 5.5	37922
	pH 3	37578
	pH 7	37798
Steam	Inherent, 5.5	37062
	pH 3	28609
	pH 7	37612
Oxidative	Inherent, 5.5	36655
	pH 3	36740
	pH 7	37839
Control	Inherent, 5.5	37016
	pH 3	36553
	pH 7	36438

Table 4
Molecular weight distribution of Dextran 70 solutions as affected by stressed conditions

Stressed condition		$\bar{M}_W$
Condition	pH	
Daylight	Inherent, 5.8	65783
	pH 3	66226
	pH 7	66385
UV light	Inherent, 5.8	66186
	pH 3	66187
	pH 7	65298
Steam	Inherent, 5.8	65330
	pH 3	62321
	pH 7	65215
Oxidative	Inherent, 5.8	64541
	pH 3	64580
	pH 7	64033
Control	Inherent, 5.8	65579
	pH 3	65109
	pH 7	65546

3.3.4. Accuracy

Compared with the corresponding mean control solutions, all test solutions containing Dextran 40 or Dextran 70 had $\bar{M}_W$ and $\bar{M}_n$ values that were within the acceptance criteria of 100 ± 5% and 100 ± 10%. The results also met the acceptance criteria for low and high fraction values (Table 2). The method is, therefore, considered accurate for Dextran 40 and Dextran 70 in the parenteral solutions.

3.3.5. Precision

Repeatability. For both Dextran 40 or Dextran 70 containing test solutions, the %R.S.D. values were within the acceptance criteria of ≤3% and ≤5% for $\bar{M}_W$ and $\bar{M}_n$ values, respectively. The method, therefore, met the repeatability criteria.

Intermediate precision. Two analysts individually met the acceptance criteria of ≤3% and ≤5% for $\bar{M}_W$ and $\bar{M}_n$, respectively. The pooled results ($n = 6$) also met the acceptance criteria (Table 2).

4. Conclusion

An accurate and easy-to-use curve-fitting program capable of non-linear regression was developed, in-house, using the Microsoft Excel® for GPC calibration. The molecular weight distribution results obtained using the curve-fitting program met the calibration requirements for dextrans and dextrose. The GPC method was validated for determining molecular weight distribution of Dextrans in the parenteral solutions. The method is accurate, precise, stability indicating, and robust.

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