

Molecular weight determination for Dextran 40/60/70 according to USP/EP Monograph

Application Note Pharmaceutical Analysis

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A specific e-workflow in PSS WinGPC MCDS allows for calibration and molar mass determination for Dextran 40, 60 and 70 as stated by USP and EP.

Introduction

Dextran is used as a blood plasma volume expander or blood flow improver. The Dextran molar mass is crucial for the success of the therapy. If the molar mass is too high there is a risk of interference with normal blood coagulation processes. Dextrans with a too low molar mass are therapeutically ineffective.

United States Pharmacopeia (USP) and European Pharmacopoeia (EP) recommend aqueous size exclusion chromatography, SEC, with a specific Dextran calibration to determine the molar mass information.

The SEC calibration curve is constructed using 5 Dextran standards of known molecular weights ranging from 4 000 to 250 000 Da, Glucose (180 Da, total column volume V_t) and a value for the V_0 (column void volume). A procedure as described by Nilsson and Nilsson is used to obtain parameters of an exponential 3rd order equation.

$$M_i = a + \exp(b + cV_i + dV_i^2 + eV_i^3)$$

Specific results for Dextran samples include the weight average molar mass, M_w , for the whole Dextran as well as for the fractions at 10% and 90%. USP additionally requires M_n and the Polydispersity Index, PDI.

System Requirements

	Conditions
Pump	PSS SECcurity ² GPC1260 isocratic pump
Injection system	PSS SECcurity ² GPC1260 Autosampler
Columns	as stated in EP/USP
Loading	as stated in EP/USP
Detectors	Refractive index PSS SECcurity ² 1260 RI
Software	PSS WinGPC UniChrom 8.3 SR2 WinGPC Report Designer Optional: Compliance Pack, ChromPilot,



Procedure, Results & Discussion

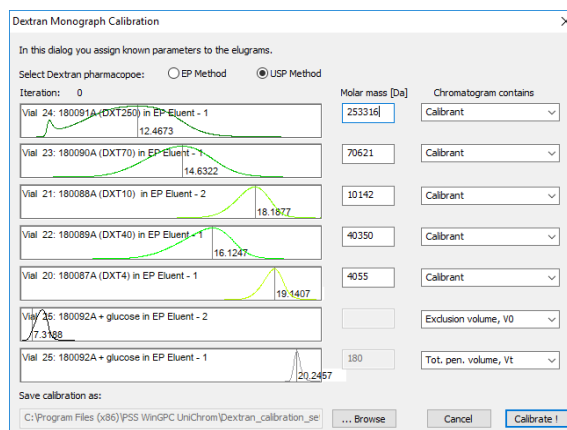
1) Calibration

The Dextran calibration reference materials are prepared as recommended in the corresponding standard and data are recorded.

Baseline limits and integration limits are set for each calibrant using either interactive integration or WinGPC Quick Analysis. The traces of the 5 calibration standards, the Glucose sample and the peak to determine V_0 are added to the WinGPC Overlay.

[Options] [Dextran Monograph] [Calibration] opens the *Dextran Monograph Calibration* dialogue to start the iterative non-linear regression fit based on the concept by Nilsson and Nilsson. As the monographs differ slightly in their requirements users can select here between EP method and USP method.

WinGPC reports if the data can be successfully fitted according to the requirements. If the calibration was successful a WinGPC calibration file is saved and a report is printed.



2) Verification samples and analysis of unknowns

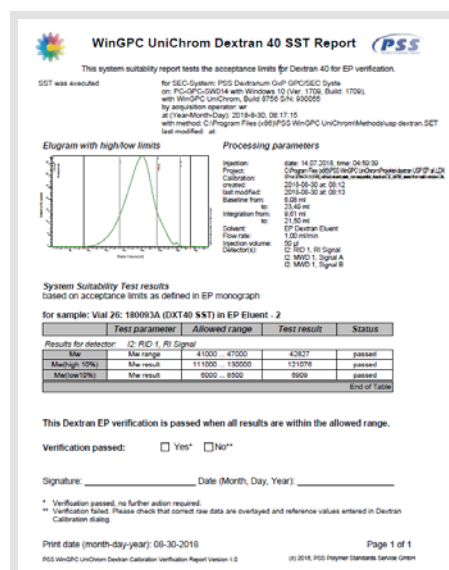
Recorded data of the verification samples (System Suitability, Performance) and unknown Dextran can then be evaluated using the calibration curve created above and the menu item [Options] [Dextran Monograph][Evaluate].

WinGPC will automatically determine 3 different areas for the full dextran, for 10% eluted mass and for 90% eluted mass.

3) Results

The molar mass results for each of the 3 areas are determined and a pre-configured WinGPC Dextran report can be printed. The result report can be directed to printers, preview, PDF, Excel and many more. Figure 2 shows an example report with results of a Dextran 40.

The results are automatically compared to the acceptance limits and the status failed/passed is assigned to each of them.



Summary

SEC is a standard technique in different pharmacopoeias (USP , Pharm. Eur., British, Chinese and Japanese monographs) to determine molecular weights and molecular weight distributions (MWDs) in pharmaceutical testing of Dextrans. Such experiments can be performed by PSS SECcurity and SECcurity² GPC/SEC systems.

PSS WinGPC Software has a specific e-workflow implemented comprising data capture, specific dextran calibration, data analysis and compliant reporting. Regulated laboratories should opt for the WinGPC UniChrom Compliance Pack for FDA 21CFR11 support including audit trails and electronic signatures.

Additional Resources



PSS e-book 1: GPC/SEC
Theory and Background



PSS White Paper: Is
WinGPC right for me?



Video Interview Analytica
2018: PSS WinGPC MCDS

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