Application Note

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Molecular Weight Determination of Dextran According to USP/EP Monograph

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Abstract

This application note introduces the analysis of dextran 40/60/70 according to a USP/EP monograph using GPC/SEC. A specific e-workflow in Agilent WinGPC allows for calibration and molar mass determination for dextran 40, 60, and 70 as described by the United States Pharmacopeia (USP) and European Pharmacopoeia (EP).

Introduction

Dextrans are water-soluble, branched polysaccharides composed of glucose units. Dextran is used as a blood plasma volume expander or blood flow improver. Dextran molar mass is crucial for the success of the relevant 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. In pharmaceutical dextran applications, only dextrans of specific molar masses and dispersities can be used.[1]

The USP and EP recommend aqueous size exclusion chromatography (SEC) with a specific dextran calibration to determine molar mass information.

GPC/SEC calibration curves are constructed using five dextran standards of known molecular weights ranging from 4,000 to 250,000 g/mol, glucose (180 g/mol, total column volume Vt), and a value for the V0 (column void volume). In addition, a special fitting function is required to describe the relation between molar mass and elution volume.

Specific results for dextran samples include the weight average molar mass (Mw) for the whole dextran as well as for the fractions at 10% and 90%. USP also requires number-average molar mass (Mn) and the dispersity, Đ.

Experimental

Table 1. Instrument conditions.

	Conditions
Pump	Isocratic pump
Injection System	Autosampler **Loading:** as stated in EP/USP
Columns	As stated in EP/USP
Detectors	Refractive index (RI) detector
Software	Agilent WinGPC

Results and discussion

Calibration

The dextran calibration reference materials were prepared as recommended in the corresponding standard and the data were recorded. Baseline limits and integration limits were set for each calibrant using either interactive integration or WinGPC Quick Analysis. The traces of the five calibration standards, the glucose sample, and the peak to determine V0 (void marker) were added to the WinGPC Overlay.

The file path Options > Dextran Monograph > Calibration opens the Dextran Monograph Calibration dialogue to start the iterative nonlinear regression. As the monographs differ slightly in their requirements, users can select between the EP and USP method (Figure 1).

WinGPC reports whether 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.

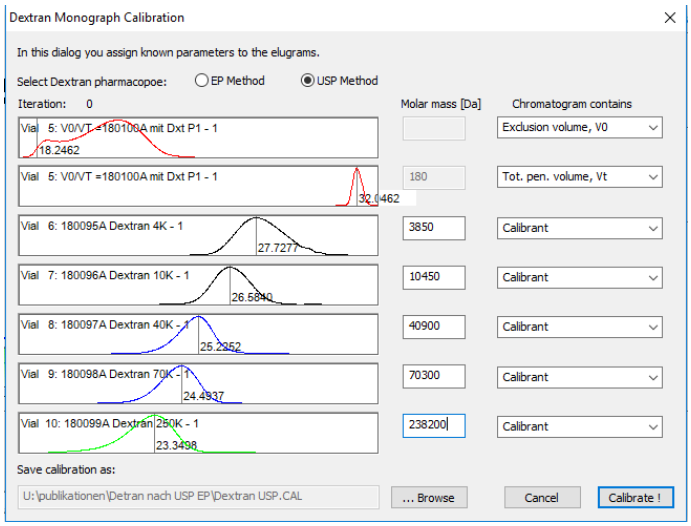

Figure 1. Agilent WinGPC Dextran Monograph Calibration window.

Verification samples and analysis of unknowns

Recorded data of the verification samples (system suitability and performance) and unknown dextrans can be evaluated using the calibration curve and the menu item Options > Dextran Monograph > Evaluate.

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

Results

The molar mass results for each of the three areas are determined, and a preconfigured WinGPC dextran report can be printed. The result report can be directed to printers, previewed, or opened as a PDF, Excel file, and many more. The results are automatically compared to the acceptance limits and a failed or passed status is assigned to each of them.

Conclusion

GPC/SEC is a standard technique in different pharmacopoeias (for example, USP, Pharm. Eur., British, Chinese, and Japanese monographs) for determining molecular weights and molecular weight distributions (MWDs) in pharmaceutical testing of dextrans. Agilent GPC/SEC systems can be used to perform these experiments.

Agilent WinGPC software has a specific e-workflow that comprises data capture, specific dextran calibration, data analysis, and compliance reporting. For regulated laboratories, FDA 21CFR11 support, including audit trails and electronic signatures, is available.

Reference

1. Isbister, J. P.; Fisher, M. Adverse Effects of Plasma Volume Expanders. *Anaesth. Intens. Care*, **1980**, *8*, 145.

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