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Developing a versatile gradient elution LC/ELSD method for analyzing cellulose derivatives in pharmaceutical formulations

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Abstract

Liquid chromatography combined with evaporative light scattering detection is a powerful tool for analyzing polymeric excipients used in pharmaceutical formulations. A versatile, gradient elution liquid chromatographic method utilizing evaporative light scattering detection (ELSD) has been developed for analyzing several types of cellulose ether and ester derivatives in pharmaceutical formulations. This single method was proven to be capable of differentiating six types of cellulose ether and ester derivatives. The influence of ELSD instrument parameters on the detector response and sensitivity has been studied by a statistical design of experiments. It was found that lowering gas flow rate increased peak area response significantly. Increasing nebulizer temperature also increased peak area response. In contrast, evaporator temperature has very minor impact on peak area response, but had a significant impact on noise level. Thus, signal to noise ratio was significantly lower for low evaporator temperature setting. Despite the logarithmic relationship between peak area responses versus concentrations, sufficient selectivity, precision and accuracy were achieved. The method has been validated for assaying hypromellose acetate succinate (HPMCAS) polymer in a pharmaceutical formulation.

Keywords: Evaporative light scattering detector; ELSD; HPLC; Cellulose ether derivative; Cellulose ester derivative; HPMCAS; HPMC

1. Introduction

Cellulose ether and ester derivatives are functional polymers that have various degrees of ether and ester functional groups substituted for the hydroxyl groups in the chains [1]. They are extensively used in pharmaceutical industry as functional excipients in solid tablet dosage form manufacturing [2]. For example, methylcellulose is used as a binding agent. Hypromellose (HPMC) is used as a binder in immediate-release tablets, in film-coating and as a matrix in extended-release tablet. Hypromellose acetate succinate (HPMCAS) and hypromellose phthalate (HPMCP) are used in enteric-coatings for delayed-release tablets [3]. The final properties of the tablets are not only dependent on the properties of the active pharmaceutical ingredient (API), but also highly dependent on the types of excipients chosen, the amount and the interaction of them with the active pharmaceutical ingredient and each other. Monitoring excipients in drug formulations is important during drug development process to ensure bioavailability, performance, quality and stability of the drug product.

Cellulose ether and ester derivatives are made from natural macromolecules. The behavior of macromolecules such as the cellulose derivatives is different in several aspects from the behavior of the small molar mass molecules. Cellulose derivatives are made from the repeat unit of anhydroglucose. Derivatization of the cellulose is hardly homogeneous, thus, there is heterogeneity in chemical structures among the repeat units. Furthermore, large molar masses (molecular weights) invariably have a distribution caused by the source of the cellulose and chemical process (degradation for process ability and chemical derivatization). The long chains from large molar masses make the chains flexible and are prone to physical entanglement. Besides having significantly slower diffusion in solution, large chains with chemical heterogeneity along and among the chains make the macromolecules have a very limited solubility as compared with the low molar mass molecules. All these factors make the HPLC separation of macromolecules such as cellulose derivatives very challenging. For example, in a typical reverse phase HPLC separation, the partitioning between the mobile phase and the stationary phase of the column for small molecules is very quick. In a typical HPLC analysis timeframe (<60 min), there are numerous adsorption–desorption interactions of the small molecule analytes with the stationary phase in a

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HPLC column. The strength of the interactions and the residence time in the stationary phase are dictated by the analyte chemical structure, leading to separation of the analytes by their difference in chemical structure. Chemical structure difference is the only important parameter in small molecule separation and the molar mass do not play any significant role. This is not the case for the separation of macromolecules. First, in a giving mobile phase there may be numerous simultaneous interactions of the many repeat units in a macromolecular chain with the stationary phase of a column. Second, these numerous simultaneous interactions persist in a wide range of mobile phase choices, due to heterogeneity in chemical structure and molecular weight distribution. This makes the retention of the macromolecular chains to the stationary phase almost permanent (in the practical sense of a typical HPLC analysis timeframe) in a wide range of mobile phase choices. Only when a very strong solvent is used as mobile phase to overcome all these interactions, will all the macromolecular chains be dissolved and elute out "instantaneously". The elution is caused by a sufficient difference in solubility for the macromolecular chains in the mobile phase and in the stationary phase of the column. This is the basis for gradient polymer elution chromatography (GPEC) [4,5]. Isocratic separation of macromolecules based on the partitioning between the mobile phase and the stationary phase of the column is hardly practical. Practical HPLC separation of macromolecules is often achieved by a gradient elution and is usually governed by mixed mechanisms such as solubility difference, size exclusion, ion exclusion, etc.

As most cellulose derivatives have no chromophores, an alternative detector other than the UV detector is often needed for liquid chromatographic separation. In most cases, a mass spectrometer cannot be use because the polymeric excipients have high molar masses. Electrochemical detection and refractive index detection are possible only for isocratic conditions, but cannot be used in gradient conditions. Evaporative light scattering detection (ELSD) is increasingly used for non-volatile analytes. It is compatible with a variety of volatile mobile phases and gradient elution. Compared with spectroscopic detectors such as UV detector, ELSD produces quasi-universal detector response, regardless of the analytes' physical and chemical properties. It is the detector of choice for applications such as analyzing polymers, lipids and carbohydrate [6–10]. There is a reported method for simultaneous determination of a drug (ibuprofen) and a cellulose derivative (hypromellose, HPMC) using HPLC with ELSD [11]. It used a GlucoSep N column (250 mm × 4.6 mm) and an isocratic elution (40/60, v/v, water/methanol) at 1 mL/min. The HPMC peak eluted out at the retention time ca. 1.7 min. Based on void volume of the column, it seemed that the HPMC eluted out before or at the solvent front. While it is acceptable for its specific application, it is desirable to have some retention for an analyte beyond the void volume for a robust method.

In this paper, we report our work to develop, optimize and validate a gradient elution liquid chromatographic method with ELSD for analyzing cellulose ether and ester derivatives in pharmaceutical formulations. This single method is suitable as a screening method for differentiating six types of cellulose derivatives, and is validated as an assay method for HPMCAS polymers in a pharmaceutical formulation.

2. Experimental

2.1. Solvents and chemicals

HPLC grade acetonitrile, methanol, ethanol and acetone were purchased from J.T. Baker (Phillipsburg, NJ). Water was purified through MILLIPORE (Billerica, MA) MilliQ system and filtered through a 0.22 μm Millipak filter. Formic acid (>96%, reagent grade) was purchased from ACROS. HPMCA polymer (hypromellose acetate) was custom synthesized for research propose. Various lots of four grades of HPMCAS polymer (hypromellose acetate succinate, AQUOAT®, LF, MF, HF and MG) and one grade of HPMCP polymer (hypromellose phthalate, HP55) were purchased from Shin Etsu Chemical Co. Ltd. (Tokyo, Japan). Three lots of HPMC (hypromellose, Methocel premium LV, E, E5 and E15) and one lot of methylcellulose (Methocel premium LV, A15) were purchased from Dow Chemicals (Midland, MI). Cellulose acetate (CA-398-10NF) was purchased from Eastman Chemicals Co. (Kingsport, TN). A Pfizer proprietary experimental drug was used in the validation experiments.

2.2. Sample preparation and chromatographic conditions

A mixture of water/acetonitrile at 20/80 (v/v) was used as the dissolving solvent. All solutions of the cellulose derivatives with and without the drug were prepared by weighing appropriate amount of the cellulose derivative, the drug if needed and the solvent (10 mL) into a 20-mL vial. The solutions were stirred to complete dissolution for at least an hour. The concentration of the cellulose derivative was given in ppm from the ratio of the weight of the cellulose derivative dissolved divided by the total weight of the solution. The HPLC instrument used in this study was LC-1100 from Agilent Technologies and the ELSD was PL-ELS 1000 from Polymer Laboratories.

The chromatographic conditions are as follows:

Mobile phase	Solvent A = 1000 mL water + 1 mL formic acid Solvent B = 1000 mL acetonitrile + 1 mL formic acid		
Gradient	Time (min)	% Solvent A	% Solvent B
	0.00	100	0
	20.00	20	80
	30.00	20	80
	35.00	100	0
Flow rate	0.5 mL/min		
Injection volume	25 μL		
Column	Polymer X RP-1 (5 μm, 150 mm × 4.6 mm), at 30 °C		
Detection	Evaporative light scattering detector (ELSD)		
ELSD settings	Gas flow rate = 1.0 SLM (standard liter per minute); nebulizer temperature = 85 °C; evaporator temperature = 85 °C		
Run time	45 min		