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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		

3. Results and discussion

3.1. Method development

An extensive screening of columns and chromatographic conditions was conducted to find a suitable column and chromatographic condition for the separation. A general gradient elution from 90%/10% to 10%/90% water/acetonitrile (v/v) in either 20 or 30 min or various isocratic elutions with 90%/10% to 10%/90% water/acetonitrile (v/v) were used. The ELSD parameters were set as follows: gas flow rate = 1.5 SLM; nebulizer temperature = 80 °C; evaporator temperature = 90 °C. Out of the seven columns screened initially using HPMCAS as a test compound, there was no peak eluted out from two Discovery Zirconia-based columns (Zr-Carbon or Zr-PS). The peak eluted out from Asihipak ODP had bad tailing, so did the peak from Luna C18. Jupiter C18 gave a peak with fair shape. The peak from both YMC Pack Polymer C18 and Shodex RS Pak columns looked better than that from other columns. It was then decided to further test the performance of three columns (Jupiter C18, 5 μm, 250 mm × 4.6 mm; YMC Pack Polymer C18, 6 μm, 250 mm × 4.6 mm; and Polymer X RP-1, 5 μm, 250 mm × 4.6 mm) at various isocratic conditions using both HPMC and HPMCAS as test compounds. When the mobile phase was 25%/75% water/acetonitrile (v/v) with 0.1% formic acid added to both water and acetonitrile, it was seen that HPMC eluted out before or at the solvent fronts in all three columns. For HPMCAS polymers, the peak started to elute out at the solvent front but the peak tailed badly in Jupiter C18 column. Putting two YMC Pack Polymer C18 columns in series made the HPMCAS chromatograms more like those when a Polymer X RP-1 column was used. For the three grades of HPMCAS polymer in Polymer X RP-1 column, there was a sharp peak followed by a broad peak before the solvent front. There were some subtle differences in the chromatograms of different grades of HPMCAS (position of the broad peak and the ratio of the sharp peak to the broad peak), but they were generally overlapped with each other. Putting two Polymer X RP-1 columns in series pulled the broad peak in HPMCAS chromatograms further away towards the solvent front for L and M grades and pass the solvent front for H grade.

In summary, it was found that Polymer X RP-1 (5 μm, 150 mm × 4.6 mm) in combination with a gradient elution separates most cellulose ether and ester derivatives well. Polymer X RP-1, made by Phenomenex, consists of microporous (10 nm) polystyrene divinylbenzene (PSDVB) with no bonded surface ligands. It is commonly used for separating small proteins, peptides and biological molecules. Typical chromatograms for HPMCAS separation are shown in Fig. 1. If formic acid was not used in the mobile phases, HPMCAS polymers eluded in two well-defined peaks, one at the retention time when formic acid was used and the other at the solvent front. It was thought that without formic acid in the mobile phase, there was some degree of aggregation of HPMCAS polymer in the mobile phase that prevented its penetration into the pores of the column packing and, therefore, caused it to elute out at the solvent front. It was interesting to notice that adding formic acid effectively broke

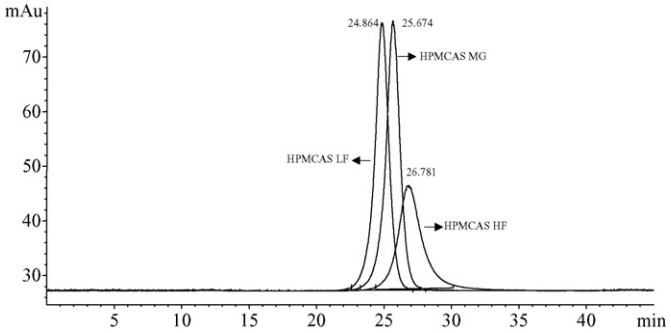

Fig. 1. Typical chromatograms for HPMCAS polymers at experimental conditions defined in Section 2 (*Y*-axis for signal intensity in mAU, *X*-axis for retention time in minutes).

the aggregation of HPMCAS polymer in the mobile phase, but the mechanism remains unclear at this time.

It appeared that cellulose ether and ester derivatives were separated on the Polymer X column by solubility difference as in the case of gradient polymer elution chromatography (GPEC) [4,5]. For example, HPMCAS MG polymer was run on the same gradient (with a hold time of 10 min for 100% solution A at the start) at two different temperatures (30 and 55 °C). There was little change in the retention time (35.5 min at 30 °C versus 36.0 min at 50 °C), which indicated that the interaction with the column was minimal.

When three grades of HPMCAS (L, M and H), which differed in the ratio and content of acetyl and succinoyl substitutions, were analyzed by the method (see Fig. 1), it was found that the retention times varied according to grades. In Fig. 2, two grades of HPMC (Methocel E3 and E15), which only differed in the molecular weights, were analyzed and had the same retention time. This suggested that the different grades of HPMCAS have different solubility, possibly due to the difference in the ratio and content of acetyl and succinoyl substitutions, while different grades of HPMC do not have much difference in solubility.

Using the same gradient conditions, different organic solvents were used in the mobile phase to investigate solvent effect. When methanol was substituted for acetonitrile in solution B, there was no elution of peaks in 45 min. If ethanol was substituted in solution B, there was overlap of peaks. When acetone was

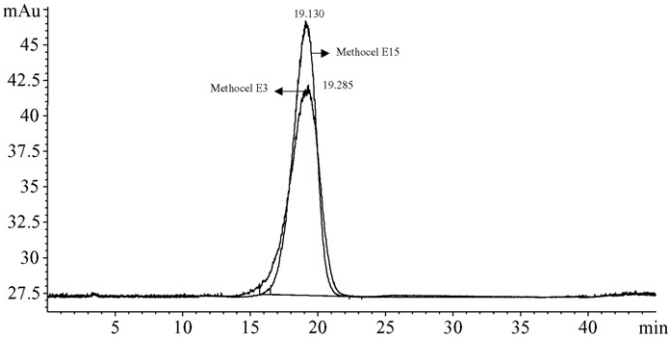

Fig. 2. Typical chromatograms for HPMC polymers at experimental conditions defined in Section 2 (*Y*-axis for signal intensity in mAU, *X*-axis for retention time in minutes).

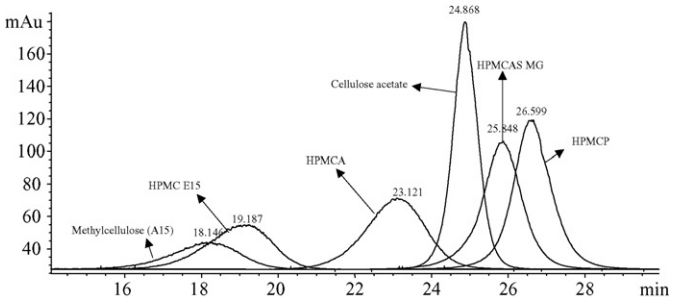

Fig. 3. Separation of six types of cellulose ether and ester derivatives at experimental conditions defined in Section 2 (*Y*-axis for signal intensity in mAU, *X*-axis for retention time in minutes).

substituted in solution B, the separation was almost identical to those when acetonitrile was used for solution B.

Other cellulose polymers were evaluated by this method. No attempts were made to adjust the gradient or to change mobile phases, which could be used to fine tune the separation for a specific cellulose derivative. The results in Fig. 3 demonstrated that methylcellulose, hydroxylmethylcellulose, hydroxylpropylmethylcellulose acetate, cellulose acetate, hydroxylpropylmethylcellulose acetate succinate and hydroxylpropylmethylcellulose phthalate all eluted at different retention times. This method can be used as a general screening method for analyzing various kinds of cellulose ether and ester derivatives in pharmaceutical formulations.

3.2. Evaluation of impact of ELSD parameter variations

The operation of an evaporative light scattering detector comprises three steps: (a) nebulization, in which the chromatographic eluent is nebulized using nitrogen or air to produce an aerosol of minute droplets; (b) mobile phase evaporation, in which the aerosol is introduced to a heated drift tube, where the mobile phase is evaporated and leaves behind particles of the analytes; (c) detection, in which the particles of the analytes scatter light and produce a response in proportional to the intensity of the scattered light. ELSD requires use of volatile mobile phase, is compatible with gradient elution and produces more uniform detection sensitivity for most analytes, regardless of their physical and chemical properties. The major drawback of ELSD is that the response (intensity of the scattered light) is non-linearly related to the analyte concentration, so multilevel standards are needed for calibration and quantitation. Since particle formation is a multi-step process and is influenced by several instrument parameters, optimization and proper control of these parameters are required for optimal signal and acceptable baseline noise [12,13].

Three ELSD instrument parameters, gas flow rate, nebulizer temperature and evaporator temperature, were tunable for optimizing detector response. The impact of the three parameters on detector response was investigated by a statistical design of experiments (DOE) [14]. The detail of the design is shown in Table 1. A sample prepared as described in Section 2 was injected five times under each of the ELSD settings. The experimental data were analyzed by Minitab (v14) statistical software. The

Table 1
Experimental design for evaluating impact of ELSD parameters to the detector peak response and signal to noise ratio (S/N)

Condition	Gas flow rate SLM	Nebulizer temp (°C)	Evaporator temp. (°C)
A	1.3	70	100
B	1.3	100	70
C	1.3	70	70
D	1.0	70	70
E	1.0	100	100
F	1.0	70	100
G	0.7	100	100
H	1.3	100	100
I	0.7	100	70
J	1.0	100	70
K	0.7	70	70
L	0.7	70	100

95% confidence intervals for the mean of log(area) at each combination of three parameters are plotted in Fig. 4 and the possible interactions of parameters to the values of log(area) are plotted in Fig. 5. In summary, the following conclusions were drawn from the DOE analysis:

(1) The gas flow rate had the highest impact on the peak area; the lower the flow rate, the larger the peak area.
(2) The nebulizer temperature also impacted the peak area; the higher the temperature, the larger the peak area.
(3) The evaporator temperature had no significant impact on the peak area.
(4) There were no interactions among the three parameters in terms of impacting the peak area, as the lines in Fig. 5 did not intercept.

On the contrary, statistical analysis revealed that the evaporator temperature was the only significant parameter that impacted the ratio of S/N. The results are shown in Fig. 6, where the 95% confidence intervals for the mean of S/N at each combination of three parameters were plotted. A reference line at S/N ratio of 30 was drawn for comparison. As can be seen, all low values of

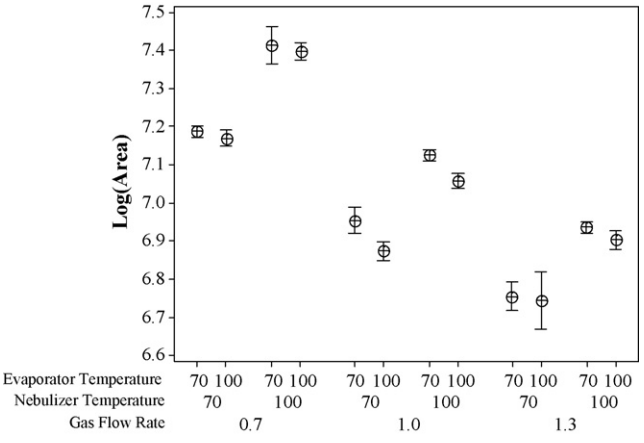

Fig. 4. Statistical analysis of peak area, as log(area) vs. ELSD parameters-interval plots (95% confidence interval).

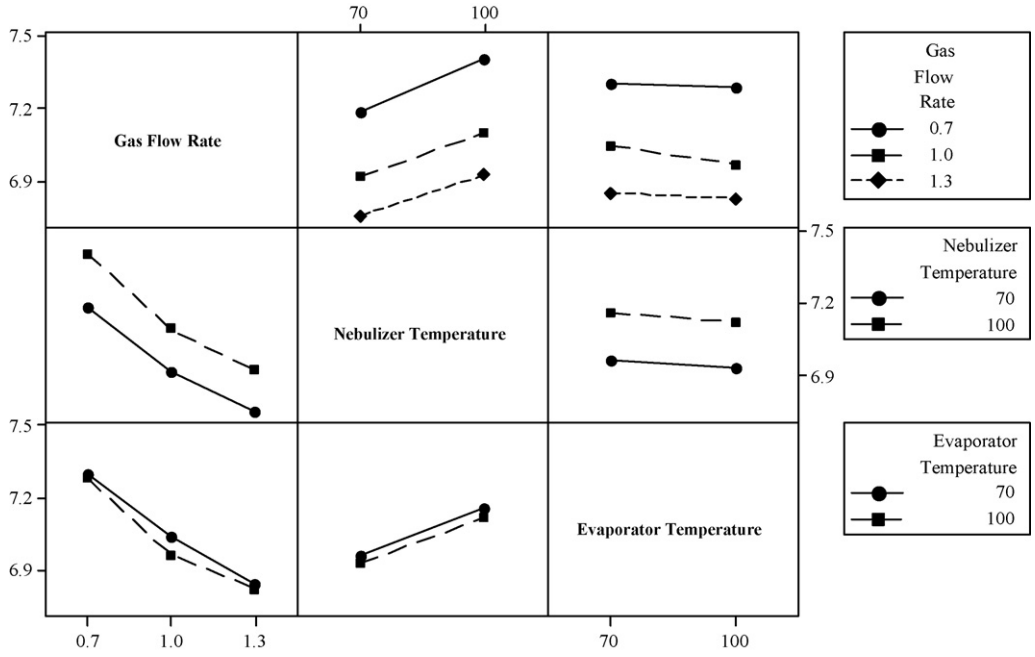

Fig. 5. Statistical analysis of peak area, as log(area) vs. ELSD parameters-interaction plots.

S/N ratio were associated with the low evaporator temperature (70 °C). When evaporator temperature was set at low (70 °C), noises were significantly higher, whereas the peak area were minimally impacted by the setting of evaporator temperature.

3.3. Method validation

The following aspects of the method have been studied in order to establish method validation [15] for assaying HPMCAS polymer in a drug formulation. The results are summarized in Table 2.

3.3.1. Selectivity

The assay has been demonstrated as specific for HPMCAS. HPMCAS polymer (eluted at 25.7 min) was baseline resolved from the testing drug (eluted at 32.1 min) with a resolution of 4.1. There was no interference peaks in the solvent blank.

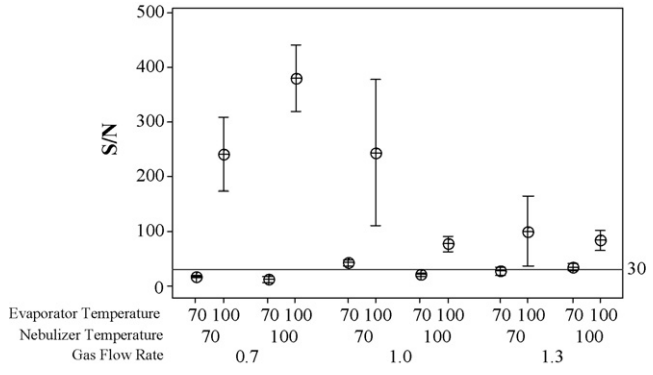

Fig. 6. Statistical analysis of peak signal to noise ratio (S/N) vs. ELSD parameters-interval plots (95% confidence interval).

3.3.2. Precision

The repeatability of the HPLC instrument and the ELSD was verified by running five replicate injections of the standard at nominal concentration (ca. 1200 ppm, see data for 3A in Table 2). The average area response was reproducible with a %R.S.D. of 1.2.

3.3.3. Linearity

Since area responses from the ELSD are not linearly correlated to the sample concentrations, logarithmic transformation is necessary in order to produce a linear calibration curve using external standards for quantitation.

$$\log(\text{area}) = m \times \log(C) + b$$

where C is the concentration (ppm) of HPMCAS polymer in the standards or sample; m is the slope of the calibration curve; b is the Y intercept of the calibration curve.

Two calibration curves for HPMCAS polymers were evaluated using standards in the absence and in the presence of the drug, respectively. In the absence of the drug, the calibration curve produced the following correlation:

$$\log(\text{area}) = 1.7488 \times \log(C) + 1.6379$$

With $R^2 = 0.9969$, a standard error of 0.0251 for the slope and a standard error of 0.0772 for the intercept.

In the presence of the drug, the calibration curve produced the following correlation:

$$\log(\text{area}) = 1.6817 \times \log(C) + 1.8098$$

With $R^2 = 0.9949$, a standard error of 0.0401 for the slope and a standard error of 0.1229 for the intercept. In both cases, the values of R^2 were larger than 0.99 and the %R.S.D. between the two slopes was 2.8.

Table 2
Analysis of validation results for precision, linearity and accuracy testing

Without the presence of the drug				With the presence of drug			
Sample	*C* (ppm)	Log (area)	Recovery (%)	Sample	*C* (ppm)	Log (area)	Recovery (%)
1A	665	6.5826	101.1	6A	644	6.5060	96.3
2A	902	6.8168	101.4	7A	929	6.8246	103.3
2B	910	6.8029	98.7	7B	851	6.7593	103.1
2C	979	6.8745	100.8	7C	880	6.7664	100.7
3A	1218	7.0276	99.1	8A	1252	7.0149	99.4
3A	1218	7.0203	98.2	8B	1225	7.0165	101.9
3A	1218	7.0145	97.5	8C	1183	6.9582	97.4
3A	1218	7.0244	98.7	9A	1473	7.1280	98.7
3A	1218	7.0266	99.0	9B	1456	7.1178	98.4
3B	1188	7.0084	99.1	9C	1466	7.1375	100.4
3C	1216	7.0411	101.1	10A	1872	7.3159	100.4
3D	1237	7.0503	100.6				
3E	1210	7.0406	101.5	At 80%	Average	102.3%	
4A	1506	7.1991	100.5		%R.S.D.	1.4	
4B	1492	7.2006	101.6				
4C	1475	7.1819	100.3	At 100%	Average	99.6%	
5A	1853	7.3593	100.9		%R.S.D.	2.3	
At 80%	Average	100.3%		At 120%	Average	99.2%	
	%R.S.D.	1.4			%R.S.D.	1.1	
At 100%	Average	99.4%					
	%R.S.D.	1.4					
At 120%	Average	100.8%					
	%R.S.D.	0.7					

3.3.4. Accuracy

The accuracy of the assay was assessed. The percent recoveries of the standards were determined by assaying them against the calibration curves in the absence and in the presence of the drug, respectively. The results are summarized in Table 2. In both cases, all recoveries were between 95% and 105%. Furthermore, the values of %R.S.D. of recoveries were all less than 3% at 80%, 100% and 120% levels in both cases. The data demonstrated that the assay was accurate and reproducible.

4. Conclusions

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. 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.

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