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Note

A flow injection analysis/mass spectrometry method for the quantification of polyethylene glycol 300 in drug formulations

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Abstract

A direct flow injection analysis/mass spectrometry (FIA/MS) method was developed for the quantification of polyethylene glycol. The method was used for the evaluation of distribution uniformity and mixing homogeneity of polyethylene glycol 300 (PEG 300) as a component in drug formulation mixtures. In the method, five of the most intense ions of the PEG 300 oligomer were chosen for selected ion monitoring (SIM) by mass spectrometry. Standard calibration curves were established, using either single channel SIM or the summed intensity of all five SIM channels plotting against the standard concentrations. Both calibration approaches produced comparable results on quantification. The feasibility of the method was demonstrated using both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). The method provided fast and sensitive quantification of PEG 300 without tedious chromatographic separation or sample preparation. The method has been successfully adopted for the evaluation of the mixing process in drug formulations.

Keywords: PEG; Mass spectrometry; ESI; APCI

1. Introduction

There has been a continued interest in the study of PEG because of its wide application in pharmaceutical formulations and the cosmetic industry. For the safe and reliable utilization of PEG, researchers have always been in search of sensitive, undemanding and high throughput analytical methods for the characterization of PEG. There are reports on the quantification of PEG using different means, which include HPLC-UV (Leister et al., 1995; Lai, 1986; Rissler et al., 1993), HPLC-evaporative light scattering detector (ELSD) (Rissler et al., 1993; Rissler, 1994), gel permeation chromatography (GPC) (Sefisko et al., 1993), supercritical fluid chromatography (SFC) (Escott and Mortimer, 1991) and NMR (Vernooij et al., 1999). The

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broad bands of PEG from its oligomer distributions hamper the chromatographic analysis of PEG. In cases where certain oligomer fractions need to be quantified, it is unrealistic to have the oligomer fractions separated or well resolved for the quantification, due to the close similarity of homologs.

The purpose of this work was to develop an analytical method for the quantification of PEG 300, as a quality control methodology for monitoring PEG 300 mixing uniformity in the formulation process. A primary consideration is simplicity and high throughput of the assay, with minimal preparation and instrumentation. High throughput quantifications of drug components in complicated biological matrices have enjoyed tremendous success using LC/MS/MS on quadrupole mass spectrometers. In this study, a fast FIA/ SIM method has been developed for the quantification of PEG 300, the only polymer constituent added in drug formulations (5% sucrose, 20% PEG 300 and 10 mM histidine). Five major oligomer ions of PEG 300 were monitored by SIM concurrently. The quantifications were compared using single SIM channel and total intensities of all SIM channels. The method was also evaluated using both APCI and ESI.

2. Experimental section

2.1. Materials and equipment

PEG 300 used in this study was purchased from Aldrich (Milwaukee, WI) with an average molecular weight (Mn) of 300. The mobile phase was delivered by a Shimadzu HPLC 10AD vp binary pump with SCL-10A vp controller. Mobile phase A was water (Burdick & Jackson, Muskegon, MI) containing 0.1% formic acid (EM, Gibbstown, NJ) and mobile phase B was acetonitrile (Aldrich, Milwaukee, WI) containing 0.1% formic acid. An isocratic condition was employed delivering 20% A and 80% B. No chromatographic separation of oligomers was attempted. The mass spectrometer used in this study was an Applied BioSystems API3000 (Foster City, CA). All ions were monitored in unit resolution with a dwell time of 80 ms for each SIM ion. The nebulizer gas and curtain gas were set to 10, 10 in ESI, and 10, 12 in APCI. An ionspray voltage of 3500 V was used in both ionization modes. The HPLC flow rate was 1.0 mL/min. For ESI, 0.8 mL/min LC flow was split to the waste and 0.2 mL/min to the mass spectrometer, while no split was used for APCI. The sample injection was performed with a CTC HTS PAL auto sampler of Leap Technologies (Carrboro, NC). The injection volume was 10 μL for all the samples.

Table 1
Ions of PEG 300 for SIM

Repeating unit n in PEG 300	Calculated molecular mass	Observed $[M + H]^+$
5	238	239
6	282	283
7	326	327
8	370	371
9	414	415

2.2. Sample preparation and analysis

A series of PEG 300 standard solutions were prepared in HPLC grade water with other formulation constituents spiked similar to the formulated samples. The concentration range of the standards was from 136 to 6810 ng/mL. The QC samples were prepared at three concentration levels of low, middle and high in the aforementioned formulation matrix. Each level of the QC samples contained five individually diluted replicate samples. A full scan mass spectrometry analysis of the standard PEG 300 sample was performed and a typical "Gaussian Distribution" mass spectrum of the oligomers was observed (Fig. 1). Five of the most intense ions with the corresponding repeating units and molecular weights calculated from the generic formula $HO(-CH_2CH_2O-)_nH$ of PEG are listed in Table 1. The SIM chromatogram showing each ion monitored is presented in Fig. 2.

3. Results and discussion

The area under the SIM peak was subjected to regression analysis, and the actual PEG 300 concentration in each sample was determined by interpolation from the standard curve. The method was evaluated by the regression of the calibration standards, and precision, accuracy of the QC samples. The regression of analyte response was assessed by the coefficient of determination (R^2). The precision and accuracy was estimated by analysis of five replicate QC samples at each of three concentration levels.

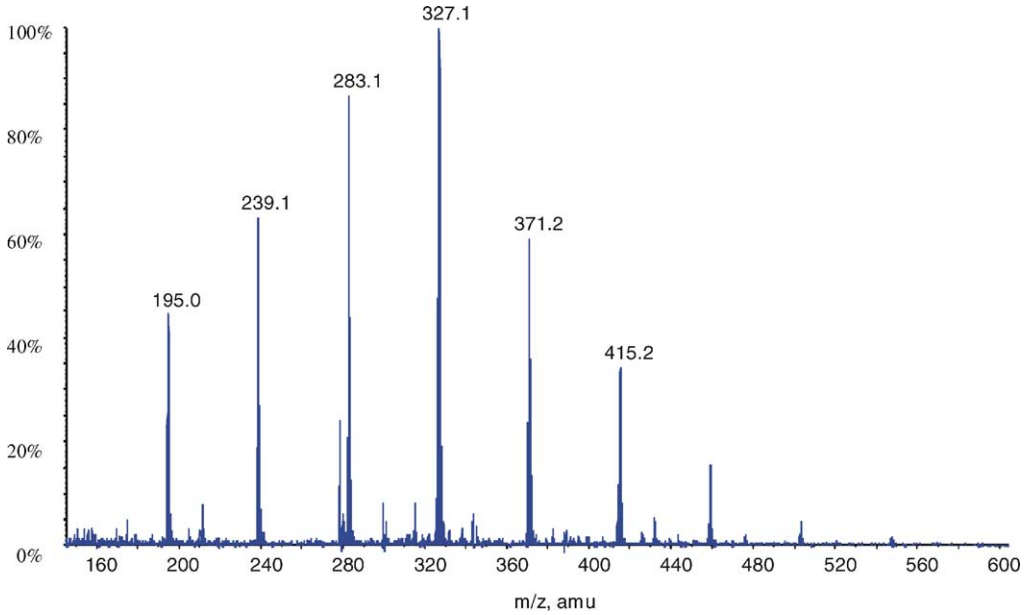

Fig. 1. Full scan mass spectrum of PEG 300 (from APCI).

The standards presented linear calibration in APCI, for both individual ion and summed ion intensities. The standards deviated from linear regression in ESI at high concentrations; and quadratic regression was found to fit the standard curve best for both individual ion and summed ion calibration. A weighting factor of $1/x$ was used for linear regression and none for quadratic regression. The standard curve statistics are listed in Table 2.

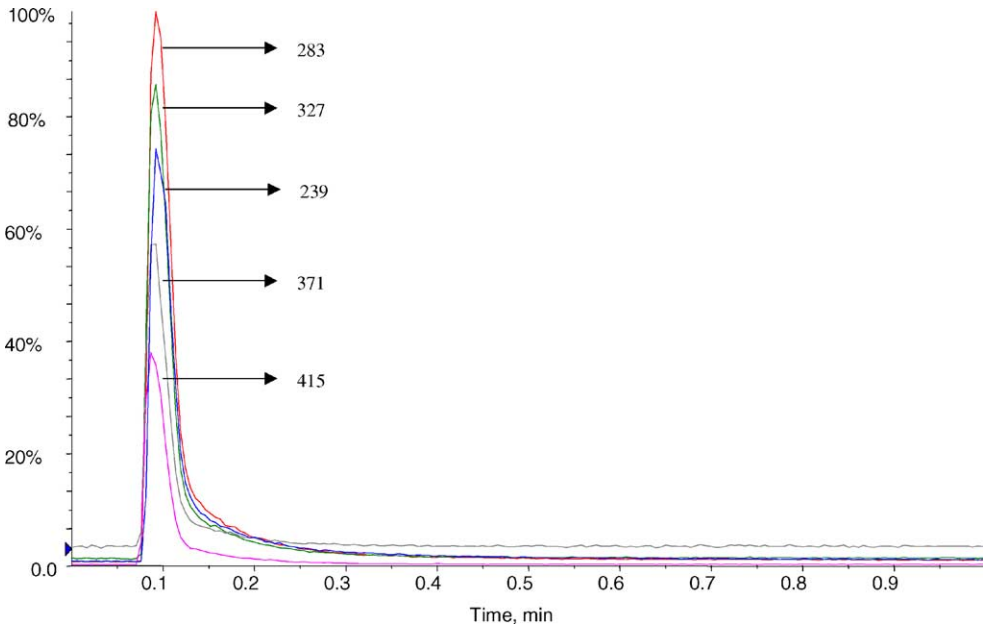

Fig. 2. SIM chromatogram of PEG 300 (from APCI).

Table 2
Statistics of calibration standards

Calibration with	Regression	R^2
APCI		
m/z (415)	$y = 1221.5x - 21{,}008$	0.9959
m/z (371)	$y = 1586.4x - 26{,}100$	0.9983
m/z (327)	$y = 2503.8x + 40{,}075$	0.9987
m/z (283)	$y = 3247.2x + 93{,}474$	0.9988
m/z (239)	$y = 2600.1x - 596.55$	0.9993
Sum ion intensity	$y = 11{,}147x + 148{,}561$	0.9989
ESI		
m/z (415)	$y = -0.1013x^2 + 1766.1x + 176{,}923$	0.9989
m/z (371)	$y = -0.1406x^2 + 2203.7x + 251{,}324$	0.9988
m/z (327)	$y = -0.1944x^2 + 3077.5x + 373{,}228$	0.9975
m/z (283)	$y = -0.2152x^2 + 3558.1x + 449{,}482$	0.9971
m/z (239)	$y = -0.1563x^2 + 2540.5x + 214{,}120$	0.9985
Sum ion intensity	$y = -0.8079x^2 + 13146x + 1E + 6$	0.9983

The relative errors (R.E., %) were within ±15% for all QC samples except for the low level QC in ESI by summed ion calibration (Table 3). The precision of the method was assessed by the coefficients of variations (CV, %) of the QC samples. The specificity of the method was examined by running two sets of QC samples of PEG 300, one set spiked with other formulation constituents and the other with no spiking.

Table 3
Statistics of QC samples

Item used for calibration	APCI			ESI		
	Mean (ng/mL)	CV (%)	Accuracy (%)	Mean (ng/mL)	CV (%)	Accuracy (%)
QC1, nominal concentration 136 ng/mL (n = 5), measured concentration						
SIM *m/z* (415)	127	7.8	93.4	171	4.9	115.0
SIM *m/z* (371)	147	3.8	107.8	148	6.4	108.8
SIM *m/z* (327)	149	9.3	109.3	160	5.4	113.2
SIM *m/z* (283)	161	5.7	108.8	149	3.7	109.3
SIM *m/z* (239)	164	8.8	115.3	149	3.9	109.7
Sum of SIM intensities	128	7.5	90.1	191	3.0	137.7
QC2, nominal concentration 1700 ng/mL (n=5), measured concentration						
SIM *m/z* (415)	1880	3.1	110.6	1852	1.0	108.9
SIM *m/z* (371)	1830	4.7	107.6	1802	1.9	106.0
SIM *m/z* (327)	1842	1.9	108.4	1854	1.6	109.1
SIM *m/z* (283)	1884	4.0	110.8	1880	1.2	110.6
SIM *m/z* (239)	1766	3.5	103.9	1782	1.7	104.8
Sum of SIM intensities	1833	3.0	107.8	1885	0.6	110.9
QC3, nominal concentration 6810 ng/mL (n = 5), measured concentration						
SIM *m/z* (415)	6548	2.3	96.2	7214	3.0	105.9
SIM *m/z* (371)	6484	4.5	95.2	6518	8.7	95.7
SIM *m/z* (327)	6474	4.7	95.1	7006	8.3	102.9
SIM *m/z* (283)	6404	4.6	94.0	6924	3.4	101.7
SIM *m/z* (239)	6502	2.3	95.5	7094	6.4	104.2
Sum of SIM Intensities	6517	3.6	95.7	7186	4.4	105.5

No significant discrepancy in the SIM intensities was observed in the two matrices.

The analysis of QC samples by single SIM or summed SIM in this study presented very similar results. For mixtures as the simple drug formulations in this study, without endogenous matrix interferences, the application of FIA for the quantification of PEG 300 was rapid and practical. It eliminates the time-consuming extraction or separation of PEG from non-PEG components. The run time for the FIA/SIM analysis of PEG 300 was just one minute. The increased throughput for the quantification is unparalleled by any of the previously reported methods; it is especially favorable for the screening quantification of PEG 300. Compared with conventional quantification methods for PEG, FIA/SIM provides much lower detection limits. A conventional HPLC method detected PEG1000 at 5 μg/mL (Rissler et al., 1993) and a colorimetric method at 1 μg/mL (Sefisko et al., 1993), while the FIA/SIM approach in this study had a lower limit of quantification (LLOQ, S/N > 5) of 136 ng/mL for all the ions selected. This detection limit can be further lowered for some of the selected ions with relatively higher oligomer abundance and stronger ionization.

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