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Relative quantification of polyethylene glycol 400 excreted in the urine of male and female volunteers by direct injection electrospray-selected ion monitoring mass spectrometry

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ABSTRACT

The use of polyethylene glycol 400 (PEG 400) as an excipient in oral formulations can have profound and differing effects on drug bioavailability in men and women; therefore an understanding of the pharmacokinetics of this excipient is required. A direct injection electrospray selected ion monitoring mass spectrometry methodology was developed and validated for the quantitation of PEG 400 excreted in human urine after oral administration. The most abundant ions corresponding to PEG 400 oligomers at *m/z* 365, 409, 453, 497, 541, and 585 were used for selected ion monitoring (SIM). Pre-dose urine of volunteers was spiked with various amounts of PEG 400 to generate calibration curves over the concentration range 2.5–90 μg/mL for all SIM channels. The relative standard deviations of intra- and inter-day analysis of PEG 400 in human urine were lower than 11.8% and bias percentage was less than 9.7%. This specific method for relative quantitation of PEG 400 was then used to analyse urine samples with minimal sample preparation. Urine samples of twelve healthy volunteers (six men and six women) who received 0.75 g and 1.5 g PEG 400 on two separate occasions were collected over 24 h. On average 36.5% of the orally administered dose of PEG 400 was recovered in the urine of the volunteers, with no significant difference observed between men and women.

1. Introduction

Polyethylene glycol 400 (PEG 400) is a polymer composed of repeating subunits of ethylene oxide and has low toxicity. PEG 400 is routinely used in the pharmaceutical industry as an enabling vehicle to enhance the solubility of poor water solubility drugs. Moreover, because it is not metabolised by intestinal bacteria and it is rapidly absorbed and excreted in urine, PEG 400 is also commonly used as a permeability probe to assess the barrier function of the gastrointestinal tract, specifically the paracellular pathway (Chadwick et al., 1977a; Ryan et al., 1992; Iqbal et al., 1995), especially in patients regularly subjected to non-steroidal anti-inflammatory drug treatment (NSAIDs), which can cause abnormal increased permeability (Krugliak et al., 1990).

Intestinal permeability is usually investigated with high doses of PEG 400, between 2 and 10 g (Chadwick et al., 1977a; Ryan et al., 1992; Iqbal et al., 1995; Ersoy et al., 1996; Fakt and Ervik, 1997). We have recently shown that PEG 400 stimulates intestinal motility and accelerates small intestinal transit at doses >2.5 g, and that lower doses of PEG 400, commonly found in pharmaceutical products, enhanced the bioavailability of the drug ranitidine in male subjects but not in females (Basit et al., 2001; Schulze et al., 2003; Ashiru et al., 2008). This gender effect was unexpected and could be due to differences in the absorption of PEG 400 between males and females. A method to quantify the pharmacokinetics of low doses of PEG 400 following oral ingestion is therefore desirable.

There are several general approaches to achieve the identification and quantification of PEG in urine: gas–liquid-chromatographic analysis (Bouska and Phillips, 1980), HPLC (Delahunty and Hollander, 1986; Rissler et al., 1993; Leister et al., 1995), gel permeation chromatography (GPC) (Sefisko et al., 1993), GC–MS (Fakt and Ervik, 1997) and NMR (Vernooij et al., 1999). The sample preparation for all these methods are tedious and time-consuming, often involving lyophilization of the samples prior to extraction or extensive sample pre-treatment with ion-exchange resins for the removal of interfering substances (Fakt and Ervik, 1997). Some of these methods also lack sensitivity and are only suitable to analyse higher doses of PEG 400. LC–MS methods to determine PEGs in biological samples have been reported (Hanton, 2001; Pelham et al., 2008; Barman et al., 2009). However, these methods require a tedious and time-consuming sample preparation and a long laborious LC–MS runs. The direct injection

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electrospray (ES), as the simplest form of rapid sample introduction into the mass spectrometer, has been used for the analysis of PEG 300 in non-biological samples (Zhang et al., 2004) and it can be a method of choice for the automation of analysis of large number of samples.

The aim of this study was to develop a method to quantify the amount of PEG 400 excreted in the urine of human volunteers after oral administration of low doses (0.75 and 1.5 g) by a direct injection ES combined with mass spectrometric detection utilising selected ion monitoring (SIM) mode and full scan mass spectral (MS) acquisition. The six most prominent ions corresponding to PEG 400 oligomers at *m*/*z* 365, 409, 453, 497, 541, and 585 were monitored using SIM to lower the probability of false positives that could arise due to the complex matrix of urine. Further benefits of SIM experiments are: (a) it provides lower detection limit and (b) greater speed than a full scan ES-MS analysis, since regions of the spectrum that are empty or have no ions of interest are not monitored.

Initially, pre-dose urine was spiked with known concentrations of PEG 400 and analysed by the direct injection ES-SIM-MS. The calibration curves were linear for all selected SIMs over the range 2.5–90 μg/mL. Encouraged by the success of this experiment, we quantified the level of orally administered PEG 400 in the urine from six male and six female volunteers.

2. Experimental

2.1. Reagents and chemicals

Polyethylene glycol 400 (PEG 400) used in this study with molecular formula $[H(C_2H_4O)_nOH]$, n is the number of ethylene oxide units ranging from 5 to 13 (Chadwick et al., 1977a) was obtained from Sigma–Aldrich, Dorset, UK. HPLC grade methanol and water were obtained from Fisher Scientific (Loughborough, UK). Formic acid was obtained from Sigma–Aldrich (UK).

2.2. Collection of urine samples and preparation for the analysis

Twelve volunteers (six males and six females) participated in this two-way crossover study after giving informed written consent. All volunteers were non-smokers, declared themselves healthy and had no history of gastrointestinal disease. The experimental protocol was approved by the Joint UCL/UCLH Committees on the Ethics of Human Research. The study was conducted in accordance to the Helsinki guidelines for ethics in research (1965) and its subsequent revisions.

The volunteers reported to the study centre after an overnight fast and each received a single dose of 0.75 g or 1.5 g PEG 400 in 150 mL water. A standard lunch consisting of two piece filled sandwich, packet of crisps and a juice drink (Calorie load – 750 kcal) was provided 4 h post dose, and water was available *ad libitum* from this point onwards.

Urine samples were collected at pre-dose (0 h) and at different time intervals as follows: 0–2, 2–4, 4–6, 6–12 and 12–24 h post-dose. Urine samples from each volunteer over 24 h were pooled, the volumes of urine obtained were measured, divided into aliquots (100 μL) in a 1.5 mL Eppendorf tubes, and frozen on dry ice. Samples were stored at −70 °C until analysis.

The frozen urine aliquots from volunteers were thawed at room temperature, and were dissolved 100 μL of human urine in 900 μL of methanol. Samples were vortexed for 2 min and then centrifuged at 13,000 × *g* for 15 min at 4 °C. The supernatant (50 μL) was injected into the quadrupole mass spectrometer. The samples were prepared in duplicate. The excretion ratio was calculated as follows: excretion ratio (%) = (PEG 400 concentration in sample × sample volume)/amount of PEG 400 administrated.

2.3. Direct-injection ESI-SIM-MS

Direct injection ESI-SIM-MS analysis was performed using a Waters Alliance 2695 Separation Module liquid chromatograph (Waters, UK) and Navigator quadrupole mass spectrometer (Finnigan, UK). A sample (50 μL, equivalent to 5 μL of urine) was injected via a Waters autosampler coupled through a polyether ether ketone (PEEK) tubing to the mass spectrometer. A PEEK sample loop (100 μL, Upchurch, UK) and a PEEK rotor seal were used in order to minimise carryover of PEG 400 between injections. The isocratic program was maintained with the mobile phase of methanol–water–formic acid (50:50:0.1, v/v/v), at a flow rate of 200 μL/min. The total run time was 5 min. Between samples, injector flushing and needle washing were performed using isopropanol to further minimise carryover of PEG 400 between injections. The LC effluent was continuously directed into the ES source of the Navigator mass spectrometer, which was operated in the positive-ion ES mode, under the following conditions: drying gas – 400 L/h, ion spray voltage 3.15 V, cone voltage 26 V, RF voltage 0.3 V, source heater 120 °C, LM and HM resolutions 12.5. All the source and instrument parameters for monitoring PEG 400 oligomers were optimised by standard solution of 4 μg/mL infused at 200 μL/min by a syringe pump. The mass spectrometer was set up to perform in MS-mode and to run in SIM-mode. The MS-mode was chosen for the identification of PEG 400 over the *m*/*z* range of 250–750 Da at a rate of 1 scan/s. The selected ion monitoring (SIM) mode was utilised for the quantification of PEG 400 in biological samples. In SIM mode, the most abundant and informative ions corresponding to PEG 400 oligomers were selected for SIM at *m*/*z* 365.1, 409.1, 453.1, 497.1, 541.1, and 585.1. The SIM mode involves the operation of the mass analyser to record signals corresponding to only selected *m*/*z* values, rather than scan continuously over the entire relevant *m*/*z* range. The dwell time of 0.3 s, cone voltage of 26 V, the inter-channel delay 0.02 s and span of 0.2 were chosen for all selected ions during the acquisition. During the 5 min run time, 7 scan events were repeated: a 1 s full mass scan (*m*/*z* range 250–750) followed by six SIM scans (0.3 s per each SIM scan with 0.02 s inter-scan time).

2.4. Preparation of standard and quality control (QC) solutions

A stock solution of PEG 400 was prepared by dissolving 1 mg of PEG 400 in 1 mL of methanol. This solution was diluted sequentially in 1.5 mL Eppendorf tubes with methanol to give a series of working solutions for preparing the standard curve and quality control (QC) samples. Each solution was divided into aliquots (100 μL) in 1.5 mL Eppendorf tubes and stored at 4 °C before use.

QC samples were prepared by adding the appropriate amount of PEG 400 solutions to pre-dose urine (100 μL) for lower limit of quantification (LLOQ 2.5 μg/mL), lower QC (LQC 3 μg/mL), middle QC (MQC 30 μg/mL) and higher QC (HQC 90 μg/mL). Pre-dose human urine from 12 volunteers (100 μL from each volunteer) were pooled and divided into aliquots (100 μL) and frozen on dry ice, and stored at −70 °C until analysis.

2.5. Method validation

The determination of PEG 400 was based on the external standard method. Experiments were carried out on pooled human urine collected at pre-dose (0 h). Calibration standards of PEG 400 were prepared by adding appropriate amounts of PEG 400 working solutions (100 μL each) to pre-dose urine samples (100 μL each) and further diluted with 800 μL of methanol in order to obtain concen-

trations of 2.5, 5, 10, 20, 30, 50, 70 and 90 μg/mL. Samples were vortex-mixed for 2 min, and centrifuged at 13,000 × *g* for 15 min at 4 °C to precipitate the proteins. An aliquot of 50 μL was directly injected into the mass spectrometer. A pre-dose urine was included with each calibration curve. Samples were analysed in triplicate. Peak areas were used in quantitative calculations and the average peak areas value was taken for the generation of the calibration curves.

The precision and accuracy of the assay were determined by the direct injection ES-SIM-MS analysis on the LQC (3 μg/mL of PEG 400), MQC (30 μg/mL of PEG 400) and HQC (90 μg/mL of PEG 400) spiked into pre-dose urine six replicates on three separate days. Precision was expressed as a percentage of relative standard deviation (% RSD), while the accuracy (%) was expressed as [(mean calculated concentration − nominal concentration)/(nominal concentration) × 100]. The accuracy was required to be within ±15%, and the intra- and inter-day precision values (% RSD) did not exceed 15% (Boyd et al., 2008). The limit of detection (LOD) was determined as the lowest concentration of PEG 400 that produced at least three times the baseline noise level, and the limit of quantification (LOQ) was determined at a signal-to-noise ratio of 10:1 (Boyd et al., 2008).

The assay selectivity was assessed by the direct injection ES-SIM-MS analysis of the pre-dose urine samples from twelve volunteers collected at pre-dose (0 h). No characteristic series of PEG 400's *m*/*z* species in the full scan ES-MS spectra were observed in any of the pre-dose urine samples evaluated. In addition, the "cross-talk" between SIM channels used for monitoring PEG 400's *m*/*z* species was assessed by separately injecting pre-dose urine samples and monitoring the response in the SIM channels at the sensitivity (*y*-axis) required for monitoring PEG 400 below the limit of detection. No "cross talk" was observed.

2.6. Analytical direct-injection ES-SIM-MS sequence

For the analysis of biological samples by the direct injection ES-SIM-MS, a typical analytical sequence consisted of injections of the calibration standards at the beginning of the analytical sequence followed by analyses of four urine samples. QC samples at three concentration levels were injected in the middle and the end of the analytical sequence. Each calibration standard, QC and biological sample was analysed by ES-SIM-MS in triplicate. Three injections of 100 μL of isopropanol were analysed between samples.

2.7. Data and statistical analyses

Data analysis was performed using MassLynx version 4.1 from raw mass spectral data. For the quantification of PEG 400 in urine, selected ion monitoring (SIM) chromatograms for all SIM channels over the duration of the direct injection ES-MS run were generated, and the area under the peaks were calculated. Peak integration parameters used were as follows: peak-to-peak amplitude 2000; the smoothing window size ±1; number of smooths 1. Peak detection parameters used were for baselines join valleys if peaks resolved to 1% above baseline and to reduce peak tailing until tailing edge is more than 100% wider then leading edge. Peak separation parameters used were to draw vertical if peak resolved to 5% above baseline and to detect shoulder peaks if slope is less than 1% of maximum. The independent sample *t*-test was performed on the PEG 400 urinary excretion data by SPSS® software to assess the effects of the different amounts of PEG 400 and to determine if there are any differences between gender.

3. Results and discussion

In this paper, we report a direct injection electrospray (ES) combined with mass spectrometric (MS) detection in selected ion monitoring (SIM) mode and full scan ES-MS acquisition. The direct injection ES-MS is based on the injection of the sample into a continuous flow of mobile phase. Automation is accomplished with the aid of an autosampler system. The injected sample formed a zone (plug), which is then transported towards the mass spectrometer that continuously acquire mass spectra over an experimental run time. The ultimate automation can be obtained by switching from MS to SIM mode over the run time. This method obviates the requirement to analyse the sample in MS mode to identify the PEG 400 and then to re-run the sample in SIM mode to acquire the SIM data. A disadvantage of the direct injection ES-MS is the electrospray signal suppression, which is usually high in biofluid analytes entering the mass spectrometer together because of the many competing analytes present and also because of salts in the biofluids (Griffiths, 2009). However, the use of PEG 400 in this work provides a major advantage to the recording of ES-MS spectra, since PEG 400 ions completely dominate the spectra, obscuring the ions of the matrix, due to its preferential ionisation under ES conditions at the studied PEG 400 concentrations. In fact, PEG polymers are widely used as ideal calibrants of the mass spectrometer *m*/*z* scale, because PEG polymers are heterogeneous with respect to carbon number, and give two-three ion series in ES (Griffiths et al., 2001).

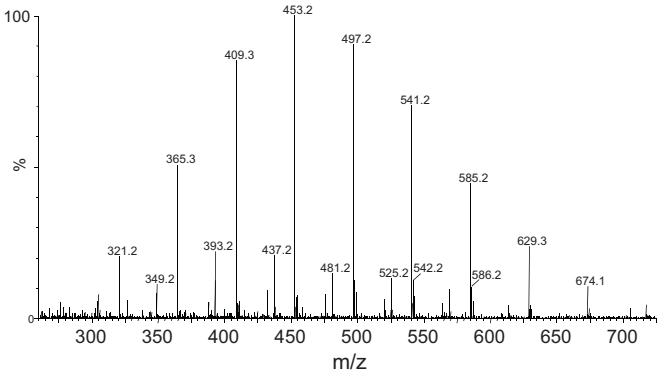

Fig. 1. Full scan ES-MS spectrum of PEG 400 at the concentration of 50 μg/mL in methanol recorded on a quadrupole mass spectrometer. Major peaks are associated with oligomer-K^+ adducts.

The strategy used for the quantification of PEG 400 in human urine has two stages: method development and validation, and its implementation. Initially, PEG 400 dissolved in methanol was analysed by direct injection ES-MS. Fig. 1 shows a full scan ES-MS spectrum obtained with PEG 400 at a concentration of 50 μg/mL in methanol at a rate of 0.2 mL/min. The full scan ES-MS spectrum shows a series of *m*/*z* species across a mass range from 300 to 700 and it is in good agreement with the literature data (Wong et al., 1988; Griffiths et al., 2001; Hanton, 2001). Major $[H(C_2H_4O)_nOH + K]^+$ ions at *m*/*z* 321, 365, 409, 453, 497, 541, 585, 629, and 674 and $[H(C_2H_4O)_nOH + Na]^+$ ions at *m*/*z* 349, 393, 437, 481, 525, 569, and 613 correspond to PEG 400 oligomers that were cationised by sodium or potassium, and minor $[H(C_2H_4O)_nOH + H]^+$ ions at *m*/*z* 327, 371, 415, 459, 503, 547, and 591 correspond to PEG 400 oligomers that were prononated. The formation of sodium and potassium adducts is a common process in positive-ion ES (Griffiths et al., 2001). The exact mass for any given member of an ion series can be readily calculated, e.g. $[H(C_2H_4O)_9OH + K]^+ = 497.3$ Da, and successive members differ in mass by 44.0 Da (C_2H_4O). These characteristic PEG ions can be targeted in SIM scans, which add an extra dimension of specificity to PEG 400 analysis. Therefore, six informative and most abundant ions corresponding to PEG 400 oligomers were chosen and then specifically monitored in rapid succession as a function of direct injection ES to form a SIM-MS (ES-SIM-MS) method.

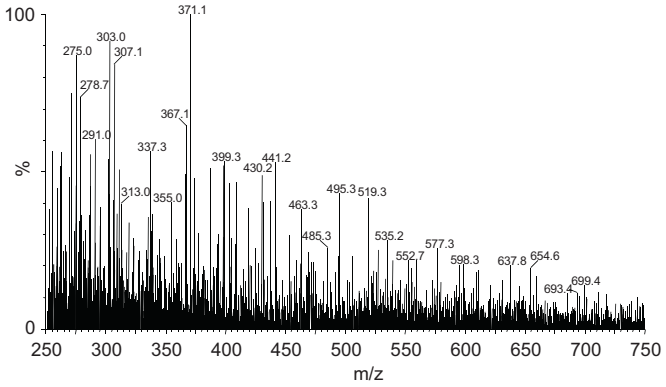

Fig. 2. Full scan ES-MS spectrum of a pre-dose human urine sample.

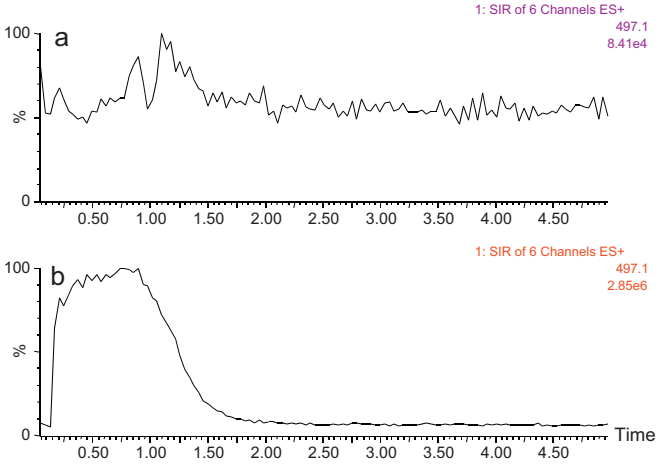

Fig. 4. SIM chromatograms for the SIM channel at *m*/*z* 497 corresponding to PEG 400 oligomer in (a) a pre-dose urine sample; (b) a sample of pre-dose human urine spiked with PEG 400 at 5 μg/mL.

Before applying the technique to unknown urine samples for the measurement of the amount of PEG 400 it was necessary to analyse control samples to validate the procedure. Urine is an aqueous solution of approximately 95% water, with the remaining percentages being metabolic wastes such as urea, dissolved salts, and proteins, hormones, and a wide range of metabolites. Sample preparation is a key procedure for the determination of analytes in biological samples. After several trials, a protein-precipitation using neat methanol was found to be appropriate for the determination of PEG 400 in human urine. The sample was directly injected into the mass spectrometer and ES-MS spectra were acquired to obtain qualitative information on PEG 400 in urine. The amount of sample injected was 50 μL corresponding to only 5 μL of human urine. The full scan ES-MS spectra of a pooled pre-dose urine sample and a pre-dose urine sample spiked with PEG 400 at 50 μg/mL are shown in Figs. 2 and 3. Fig. 2 shows the complexity of the pre-dose urine sample, its ES-MS spectrum has a number of peaks in the *m*/*z* range (250–750), which belong to components present in human urine. The full scan ES-MS spectrum recorded over *m*/*z* range (250–750) of the pre-dose human urine spiked with PEG 400 tend to offer maximum PEG 400 identification information, being dominated by characteristic PEG 400 ions corresponding to the PEG 400 oligomers (Fig. 3).

We also compared the full scan ES-MS spectra of a pooled pre-dose urine spiked with PEG 400 and PEG 400 in neat methanol at concentrations of 50 μg/mL (Figs. 1 and 3). Note that PEG 400 at this concentration completely suppresses the ionisation of the other components present in urine, giving identical mass spectra. In general, the use of PEG in many biochemical experiments can provide a major obstacle to the recording of ES spectra, in that PEG ions can completely dominate spectra, obscuring the ion of interest (Griffiths et al., 2001). However, this benefits the current study where the PEG 400 in the urine sample masks the presence of other urine components at the studied concentrations of PEG 400 and therefore the ionisation of PEG 400 not being affected by other components. Therefore, this allows qualitative and quantitative measurements of PEG 400 in human urine by ES.

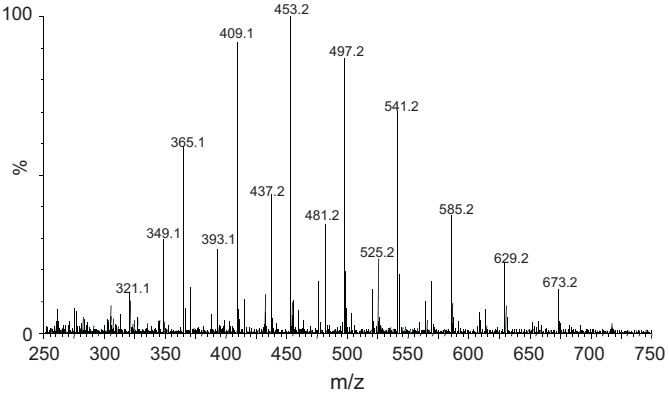

Fig. 3. Full scan ES-MS spectrum of an extract of a pre-dose human urine sample spiked with PEG 400 at 50 μg/mL.

We utilised a SIM-mode to determine the quantity of PEG 400 in human urine. The informative and abundant ions of PEG 400 oligomers recorded in the full-scan ES-MS spectra were selected for SIM at *m*/*z* 365, 409, 453, 497, 541, and 585. These SIM channels offer the twin advantages of sensitivity (only the specific ions are monitored) and selectivity (completely different ions involved in data acquisition) thereby avoiding cross-talk between reaction channels. We monitored six specific ions of PEG 400 oligomers to increase the specificity of the method and to reduce a false positive result of SIM experiments. SIM chromatograms were generated for all SIM channels. These extracted ion chromatograms generated for SIM channels also provide an extra dimension of specificity to the methodology. We examined the specificity of the ES-SIM-MS method by comparing the SIM chromatograms of pre-dose urine samples (the pre-dose urine samples from twelve volunteers) and spiked with PEG 400 samples. For example, Fig. 4 shows the SIM chromatograms generated for *m*/*z* 497 corresponding to the specific ion of PEG 400 oligomer in the pre-dose urine sample (Fig. 4a) and the pre-dose urine sample spiked with PEG 400 at 50 μg/mL (Fig. 4b). Note that the counts per second ($\sim 8.4 \times 10^4$ cps) are quite low relative to the counts expected in a typical PEG 400 analysis ($\sim 10 \times 10^5$ to 10×10^6 cps). No signals were observed in the SIM channels corresponding to other PEG 400 oligomers in pre-dose urine samples. It was also confirmed that no endogenous substances in the pre-dose urine samples from twelve volunteers interfere with PEG 400 analysis. This clearly demonstrates the possibility of PEG 400 quantitation in human urine by direct injection ES-SIM-MS.

A calibration curve was generated for each selected PEG 400 oligomer. Samples of pre-dose urine spiked with PEG 400 at various concentrations were analysed by the direct injection ES-SIM-MS. As discussed previously, this enabled us to take most of the matrix effect into account (potential ion suppression/enhancement) and to estimate the pre-dose level. The first point of the calibration curve was 2.5 μg/mL, as this level corresponds to the lower limit of quantitation (as discussed below). It is difficult to establish the upper limit of the working concentration range because there is no pre-

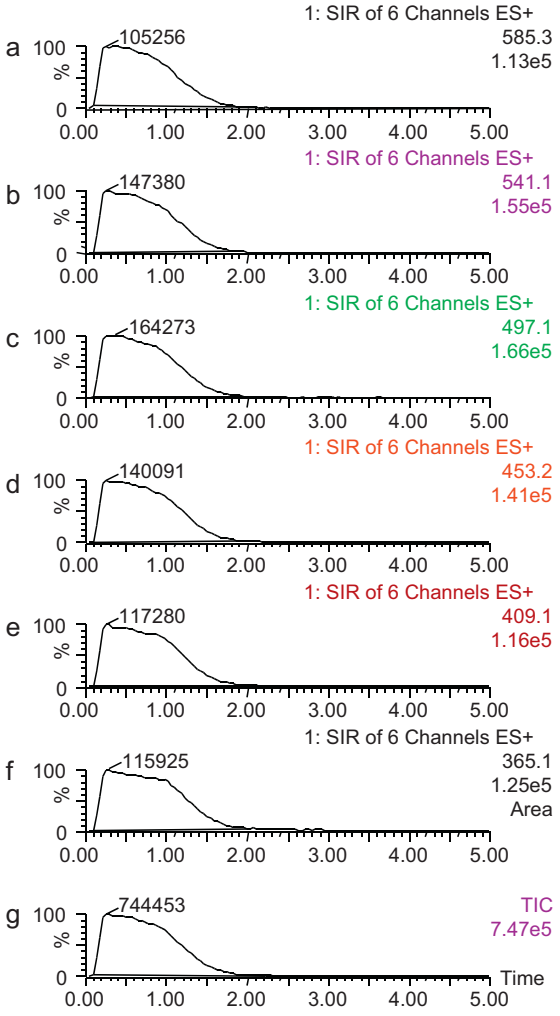

Fig. 5. SIM chromatograms generated for *m/z* (a) 365, (b) 409, (c) 453, (d) 497, (e) 541, and (f) 585 corresponding to PEG 400 oligomers. Area under the each SIM peak used to calculate the concentration of PEG 400 excreted. (g) Total ion chromatogram from the direct injection ES-SIM-MS analysis of a typical urine sample from a volunteer with post-administration of PEG 400.

cise established information regarding the PEG 400 excretion in urine over 24 h after administration of 0.75 g and 1.5 g. However, we assumed that possibly 70% of PEG 400 excretes in urine over 24 h after administration of 1.5 g. The upper limit of the calibration curve was set to 90 μg/mL. The SIM chromatograms for *m/z* 365, 409, 453, 497, 541, and 585 were generated and the area under each peak was determined (Fig. 5). The standard curve of peak area for all selected SIMs versus various concentrations of PEG 400 spiked into pre-dose urine was obtained in a linear range (R^2 from 0.974 to 0.998). The assay proved to be linear and acceptable. Regression analysis results were used to determine the actual PEG 400 concentration in volunteer human urine.

The intra-day and inter-day precision, accuracy, limit of detection and limit of quantification are reported in Table 1. The relative errors were within ±6% for all QC samples. The coefficients of variation for intra and inter day variability were less than 12%. In order to estimate the limit of quantification for the PEG 400 using the direct injection ES-SIM-MS analysis, the pre-dose urine spiked with PEG 400 was tested by decreasing PEG 400 concentrations (0, 0.5, 1.0, 2.5 and 5.0 μg/mL). SIM chromatograms for *m/z* 409 were constructed and a limit of quantification of 2.5 μg/mL with signal to noise ratio of 10 was measured for this set of data. The limit of detection was estimated at around 0.8 μg/mL using

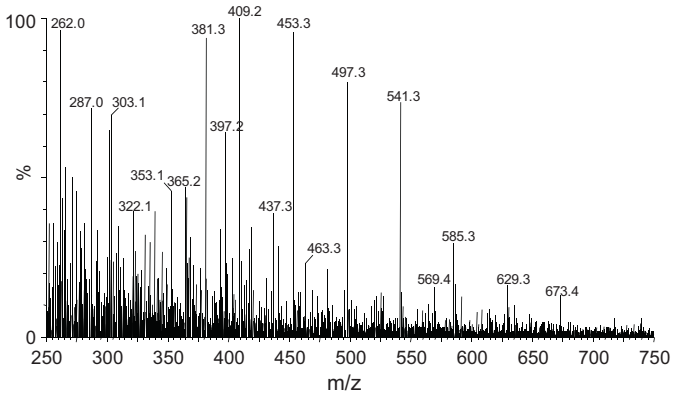

Fig. 6. Full scan ES-MS spectrum of a pre-dose urine sample spiked with PEG 400 at the low limit of detection (0.8 μg/mL).

a signal to noise ratio of 3:1; as demonstrated in the full scan ES-MS spectrum of pre-dose urine spiked with PEG 400 at the low limit of detection (Fig. 6). Note that at this level of PEG 400 in pre-dose urine the most abundant ions corresponding to PEG 400 oligomers are observed and distinguished from components present in human urine. The limit of quantification and the limit of detection of this method are lower than most of the previously published methods: gas–liquid-chromatography – 1 mg/mL (Bouska and Phillips, 1980); semi preparative HPLC – 0.73 mg/mL (Ryan et al., 1992); HPLC – 50 μg/mL (Young et al., 1990); Fourier transform-infrared spectrometry – 0.8 mg/mL (Ersoy et al., 1996); gel permeation chromatography – 1.15 mg/mL (Loret et al., 2004), but higher than LC–MS/MS – 30 ng/μL and flow injection ES-MS with SIM – 136 ng/mL (Zhang et al., 2004). However, the developed ES-MS analysis using an SIM experiment results in the highly specific, simplified method and allows rapid measurement of PEG 400 oligomers directly from extremely complex matrix. In the current study, the equivalent of 5 μL of urine was injected into the mass spectrometer. As the level of PEG 400 in urine was expected from 1 μg/mL to 100 μg/mL, this represents the lower limit of urine required for the quantitative determination of PEG 400.

The second stage of the PEG 400 strategy is its method implementation to measure the amount of a PEG 400 in human urine. The full-scan ES-MS spectra recorded for pre- and post-administration of PEG 400 to a volunteer are shown in Figs. 7 and 8 respectively, clearly demonstrated the absence and presence of PEG 400 oligomer peaks depending on the volunteer treatment. On average 34 and 35% of the orally administered 0.75 and 1.5 g PEG 400, respectively, were recovered in the urine of male volunteers (Table 2). In the female volunteers, the recovery was 37 and 40%,

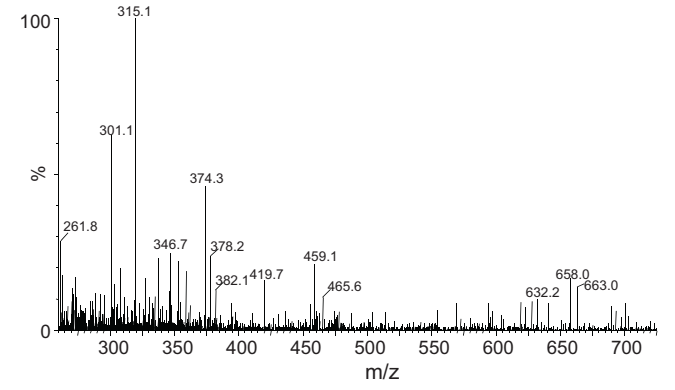

Fig. 7. Full scan ES-MS spectrum of a urine sample from a volunteer before administration of PEG 400.

Table 1
Accuracy, intra- and inter- day precision, limit of detection and limit of quantification of PEG 400 in human urine.

QC sample (μg/mL)	Calculated conc. (μg/mL)	Accuracy	Intra-assay C.V. (%)	Inter-assay C.V. (%)	LOD (μg/mL)	LOQ (μg/mL)
3	3.22 ± 0.19	105.3	6.0	5.6	0.8	2.5
30	31.0 ± 3.6	103.2	11.8	9.7		
90	81.7 ± 5.9	98.8	3.0	7.2		

Table 2
PEG 400 in urine samples collected over 24 h from healthy male subjects following the ingestion of 0.75 and 1.5 g of PEG 400.

Male subjects	Amount of PEG			
	0.75 g		1.5 g	
	Total excretion (mg)	% dose excreted	Total excretion (mg)	% dose excreted
1	326	43	613	41
2	180	24	343	23
3	240	32	593	40
4	254	34	604	40
5	171	23	344	23
6	377	50	661	44
Average	258 ± 81	34	526 ± 144	35

Table 3
PEG 400 in urine samples collected over 24 h from healthy female subjects following the ingestion 0.75 and 1.5 g of PEG 400.

Female subjects	Amount of PEG			
	0.75 g		1.5 g	
	Total excretion (mg)	% dose excreted	Total excretion (mg)	% dose excreted
1	255	34	846	56
2	436	58	661	44
3	179	24	308	21
4	234	31	450	30
5	321	43	858	57
6	239	32	463	31
Average	277 ± 90	37	597 ± 227	40

respectively (Table 3). Chadwick et al. investigated the amount of PEG 400 excreted in urine after human volunteers received 1, 5 or 15 g PEG 400 and determined that percentage absorption was independent of dose (Chadwick et al., 1977a). The amount of PEG 400 excreted by the volunteers over 24 h in that study was approximately 50%. Our results are in line with the observed results of PEG 400 excretion of 33.6 ± 3.2% in male volunteers after ingestion of 2 g PEG over 24 h (Parlesak et al., 1994) and excretion of 30.1 ± 3.87% after ingestion of 10 g PEG 400 (Oliva et al., 1994). Our study also reports that there are no statistically significant differences in PEG 400 excretion over 24 h between gender, and does not explain the significantly different bioavailability of ranitidine in the presence of this excipient (Ashiru et al., 2008).

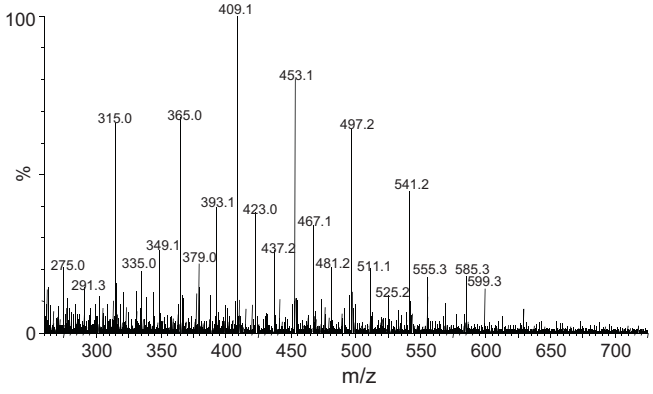

Fig. 8. Full scan ES-MS spectrum of a urine sample from a volunteer following administration of PEG 400.

4. Conclusion

The method developed is simple, convenient and reproducible, which is capable of quantifying PEG 400 in human urine. The assay was successfully applied to a volunteer study of both male and female subjects. While this assay does not explain the differences observed in the bioavailability of ranitidine between men and women in the presence of PEG 400 (Ashiru et al., 2008), it will be of use for clinicians who want to use lower doses of PEG 400 as permeability probes, and also in the pharmaceutical industry where PEG 400 is widely utilised in pharmaceutical products.

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