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Quantitative capillary electrophoresis–ion-trap mass spectrometry determination of methylphenidate in human urine

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Abstract

A quantitative capillary electrophoresis–mass spectrometry method has been demonstrated for the determination of methylphenidate in human urine over a dynamic range of 640. The samples were prepared by liquid–liquid extraction using 4 ml of human urine. The extracts were analyzed using a Finnigan LCQ ion-trap mass spectrometer in a two-event, positive-ion full-scan MS and tandem MS selected reaction monitoring mode. The lower level of quantitation was determined to be 1.5 ng/ml methylphenidate in human urine. The intra-assay precision had a relative standard deviation less than 6.8%. The intra-assay accuracy was less than ±14.7% bias from the nominal concentration for the full-scan MS analysis, and less than ±26.5% bias for the tandem MS analysis. Six incurred human urine samples containing methylphenidate were analyzed and a simple pharmacokinetic curve is presented. © 1998 Elsevier Science B.V.

Keywords: CE–MS; Ion-trap mass spectrometry; Methylphenidate

1. Introduction

Analytical methods that are used for the quantitative determination of drugs and metabolites in biological fluids play a significant role in evaluation and interpretation of pharmacokinetic data. Pharmacokinetic data are generally presented in terms of concentration of the drug or metabolites of interest versus time after administration of the drug. It is necessary to have the ability to collect phamacokinetic data to monitor the levels of drugs and metabolites in biological fluids in order to ensure maintenance of effective therapeutic ranges and avoidance of toxicity. The determination of methylphenidate in human urine is used as an example in this work to explore the analytical potential of capillary electrophoresis coupled to mass spectrometry (CE–MS), and CE tandem MS (CE–MS^2), using the LCQ ion-trap mass spectrometer, for determination of pharmacokinetic data.

The use of CE for separation of lower-molecular-mass compounds is becoming more widely accepted. The technique is attractive because of its high separation efficiency, short analysis times and low sample consumption [1]. The benefits of high quality electropherograms combined with mass spectrometric detection include high sensitivity and selectivity. Quantitative CE–MS was first demonstrated by Cai and Mordehai [2]. Sheppard and Henion [3] developed the first validated CE–MS^2 method for analyte quantitation.

The ideal mass spectrometer for use as a CE–MS detector for validated quantitative methods is one which scans rapidly enough to provide accurate

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sampling of the narrow peaks characteristic of CE, provides the highest possible sensitivity, and is commercially available at relatively low cost. The Finnigan LCQ ion-trap mass spectrometer was believed to meet these criteria. We found the duty cycle of the LCQ to be capable of employing both a full-scan MS event, (m/z 200–320), and an MS^2 precursor–product ion scan event, (m/z 234→84), specific to methylphenidate. This provided the ability to screen for the presence of other drugs and metabolites while simultaneously doing both full-scan MS and MS^2 quantitation of methylphenidate.

Methylphenidate (Ritalin; methyl α-phenyl-2-piperidineacetate hydrochloride) is used to treat children and adults with attention deficit disorder (ADD) [4]. ADD is thought to be present in approximately 20% of the US population. Methylphenidate is a potent inhibitor of re-uptake of the neurotransmitter dopamine from the gap (synapse) between two neurons [5]. A deficit of neuronal dopamine has been correlated with excessive behavioral inhibition. An excess of neuronal dopamine has been correlated with a high degree of ADD [4]. Methylphenidate inhibits neuronal re-uptake of dopamine from the synapse and thereby decreases the concentration of dopamine metabolite in the cerebrospinal fluid [4]. Fluoxetine, [Prozac; *N*-methyl-3-phenyl-3-(4-trifluoromethylphenoxy)propylamine)], is commonly co-administered with methylphenidate. Fluoxetine inhibits the re-uptake of the neurotransmitter serotonin from the synapse, and is prescribed as an antidepressant. Norfluoxetine is a metabolite of fluoxetine. The structures of methylphenidate, fluoxetine and norfluoxetine are shown in Fig. 1.

Quantitative determination and separation of (+)- and (−)-methylphenidate in human urine using GC–MS with derivatization has been reported over the range of 1 ng/ml to 500 ng/ml by Aoyama et al. [6]. Quantitative determination and separation of (+)- and (−)-methylphenidate in human urine using GC–electron-capture detection (ECD) has been reported by Srinivas et al. [7]. They did not report correlation coefficients or relative standard deviations (R.S.D.s) for their method. An unpublished validated method for quantitative determination and separation of (+)- and (−)-methylphenidate in human plasma by triple quadrupole LC–MS^2 without derivatization has been reported by Bugge et al. [8]. The range of their calibration curve was 100 pg/ml to 20 ng/ml with correlation coefficients of 0.996 or better and R.S.D.s in the range of 2.6 to 8.5%. The ability to resolve enantiomers using cyclodextrin bonded to the inner surface of a fused-silica CE capillary has been demonstrated by Mayer and Schurig using CE–UV [9], and is currently being extended to CE–MS in our laboratory (unpublished results).

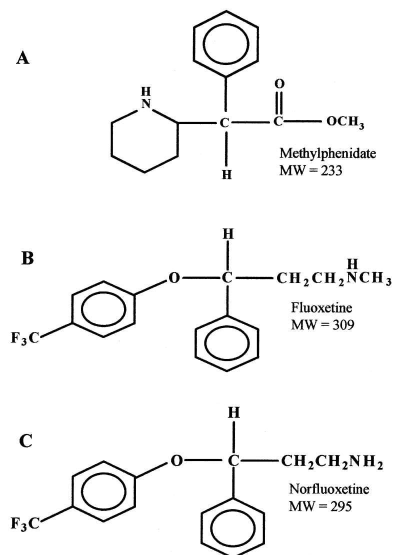

Fig. 1. (A) Structure of methylphenidate, (B) structure of fluoxetine, (C) structure of norfluoxetine.

Analytical methods and techniques are constantly being changed and improved. The use of ion-trap mass spectrometers for quantitative MS and MS^2 analyses is a new cutting edge technology for which no validated CE–MS or MS^n methods have been published. The ion-trap mass spectrometer is approximately 1/3 the price of the triple quadrupole mass spectrometer which was used in the validated method for LC–MS^2 determination of methylphenidate by Bugge et al. [8]. The ion-trap mass spectrometer also has the capability to do MS^n analysis, while the triple quadrupole can only do up to MS^2. Prior to that work only GC–MS and GC–ECD methods were used for determination of methylphenidate.

Coupling CE to the mass spectrometer for quantitative analysis is also a cutting edge technology for which several features, (such as analyte injection and ease of use), are under development. Currently the field is moving toward fabrication of microdevices

which couple electrophoretic flow separations to electrospray. These devices represent a new generation of sample preparation, analyte separation and electrospray on a single microdevice, which is analogous to the development of integrated circuits in the early 1970s. Development of quantitative analytical techniques for standard CE such as that done in this work should be easily transferable to the microdevice technology. Methylphenidate was chosen as an example compound for the described method of quantitative CE–MS and CE–MS^2 determination.

2. Experimental

2.1. Chemicals

Methylphenidate HCl was purchased from Sigma (St. Louis, MO, USA) and methylphenidate-D_3 was purchased from Isotec (Miamisburg, OH, USA). Fluoxetine HCl and norfluoxetine HCl were generously provided by Eli Lilly (Indianapolis, IN, USA). Ammonium formate and 1 *M* sodium hydroxide were obtained from Fluka (Ronkonkoma, NY, USA). HPLC-grade acetonitrile was obtained from J.T. Baker (Phillipsburg, NJ, USA) and 30% ammonium hydroxide was obtained from Fisher (Pittsburgh, PA, USA). Deionized water was taken from an in-laboratory Barnstead Nanopure Ultrapure water system (Dubuque, IA, USA). Sodium tetraborate was obtained from Sigma. Siliconized polypropylene tubes were obtained from Eagle-Picher (Miami, OK, USA) and siliconized pipette tips were obtained from Phenix Research Products (Hayward, CA, USA).

2.2. Capillary electrophoresis

A commercial Hewlett-Packard ^{3D}CE system was used for this work. The bare fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA), and was 65 cm×50 μm I.D.× 190 μm O.D. CE was carried out by applying 20 kV to the anode and 4 kV to the cathode. The cathode was the electrospray tip on the LCQ. Sample injection was done electrokinetically at the anode for 20 s during which 20 kV was applied to the anode and 0 kV to the cathode. Separation of the drugs was achieved by using a running buffer of 100% aqueous 40 m*M* ammonium acetate, which was adjusted to pH 9.0 with 30% ammonium hydroxide.

2.3. Mass spectrometry

MS was performed on a Finnigan LCQ ion-trap (San Jose, CA, USA). The standard Finnigan electrospray interface was used in the positive ion mode with a sheath gas pressure of 20 p.s.i. (1 p.s.i.= 6894.76 Pa), and with 100% ACN sheath liquid delivered at 7 μl/min. Four kV was applied to the electrospray needle. The electrospray power supply was decoupled from the CE by attaching a 40 MΩ resistor between the sprayer tip and ground. This was done to ensure that current generated by the spray process was drawn from the electrospray power supply rather than from the CE anode. The 40 MΩ resistor was not supplied with the LCQ and was not generally necessary; however, it ensured constant current was drawn from the electrospray power supply and therefore maintenance of constant voltage between the electrospray tip and CE anode. During the course of these experiments it was determined that methylphenidate, fluoxetine, and norfluoxetine thermally degraded in the heated capillary interface between the electrospray tip and the ion-trap. The temperature of the heated capillary was therefore maintained at 110°C to preclude the thermal degradation.

A two-event scan was used. The first event was a full-scan MS (m/z 200–320). The second event was carried out under selected reaction monitoring (SRM) conditions which monitored the precursor–product ion transition between the protonated precursor ion at m/z 234 (isolation width of 3 u for optimal sensitivity, collision energy 20% of maximum), and product at m/z 84. We chose to use SRM over full-scan MS^2 for the second event because there were no product ions other than m/z 84. The LCQ scan duty cycle was not fast enough to add an extra MS^2 event for the methylphenidate-D_3 internal standard while maintaining a minimum of ten points sampled across the relatively narrow electrophoretic peaks observed in this work. Therefore, measurement of ion current for the D_3 internal standard was done in the CE–MS mode. The maximum automatic gain control (AGC) ion storage time was 200 ms. The

full-scan event required 0.30 s while the SRM scan event required 0.18 s. The two scan events combined resulted in a total time of 0.48 s. Two microscans per scan were done to optimize the observed ion current stability, which resulted in a total scan time of 0.96 s.

2.4. Standard preparation

Serial dilution standards were prepared from two different 100% aqueous 1 mg/ml stock solutions made from separate weighings of methylphenidate. Human urine samples were fortified with 1.25, 2.5, 50, 200, 400 and 800 ng/ml methylphenidate for the urine calibration standards and 5, 100 and 600 ng/ml for the urine QC samples. A 1 mg/ml stock solution of methylphenidate-D_3 internal standard in ACN purchased from Isotec was diluted to 10 ng/μl in ACN. Four hundred ng of internal standard was added to each of the calibration standards and QC samples to give a concentration of 100 ng of internal standard per ml of urine.

2.5. Extraction procedure

One ml of saturated sodium tetraborate, (1 *M*, pH 9.3), and 40 μl of the 10 ng/μl internal standard solution were added to 4 ml of urine containing the methylphenidate standard in a 12-ml glass centrifuge tube. The procedure was performed on ice to prevent hydrolysis of the methylphenidate [5]. The analytes were extracted into 4 ml of cyclohexane by mixing on a roto-rack at the maximum speed of 40 rpm for 10 min followed by centrifugation at 268 *g* for 10 min. The cyclohexane layer was transferred to a siliconized polypropylene tube, evaporated to dryness under N_2 at ambient temperature on a Pierce Reacti-Therm III heating/stirring module (Rockford IL, USA), and reconstituted to 200 μl in water.

During the course of this research it became evident that methylphenidate, fluoxetine and norfluoxetine were adsorbing to the polypropylene test tubes that were used in the sample preparation. The extraction recoveries were much lower when non-siliconized polypropylene tubes were used.

3. Results and discussion

Fluoxetine and its metabolite, norfluoxetine, [3-phenyl-3-(4-trifluoromethylphenoxy)propylamine)], were spiked into the urine samples that were used to generate the methylphenidate calibration curves. Fluoxetine was spiked into the urine samples at the same concentrations as methylphenidate. Norfluoxetine was spiked into each urine sample at the level of 200 ng/ml with the intent of being used as an internal standard to fluoxetine. This was done to test the capability of simultaneous full-scan CE–MS detection of fluoxetine and norfluoxetine and quantitative CE–MS and CE–MS^2 determination of methylphenidate. A calibration curve for fluoxetine was generated in addition to the methylphenidate calibration curves (unpublished results).

Fig. 2A shows an example of a full-scan LCQ mass spectrum of only the ACN sheath liquid infused at 5 μl/min. Fig. 2B shows an example of a full-scan LCQ mass spectrum of an infused urine extract which was used for the calibration curve, with the same *y*-axis as shown in Fig. 2A. Fig. 2B shows a number of additional components in the mass spectrum that are derived from the urine matrix and concurrently excreted drugs, which makes separation and compound-specific detection necessary. The ions at m/z 234, 296 and 310 observed in Fig. 2B correspond to the protonated methylphenidate, norfluoxetine and fluoxetine molecules, respectively. Since CE is well-suited for the separation of small basic drugs, and CE–MS and CE–MS^2 can provide a unique combination of separation and identification ability, we selected this application to demonstrate the utility of the CE–MS and CE–MS^2 techniques for analyzing complex samples such as the urine extract shown in Fig. 2B.

3.1. Extraction recovery

The amount of methylphenidate recovered from the urine was estimated by preparing a blank urine extract fortified with 100 ng/ml of internal standard. Four blank extracts were prepared. Two were spiked with 20 ng of methylphenidate and two with 2400 ng of methylphenidate after evaporation to dryness.

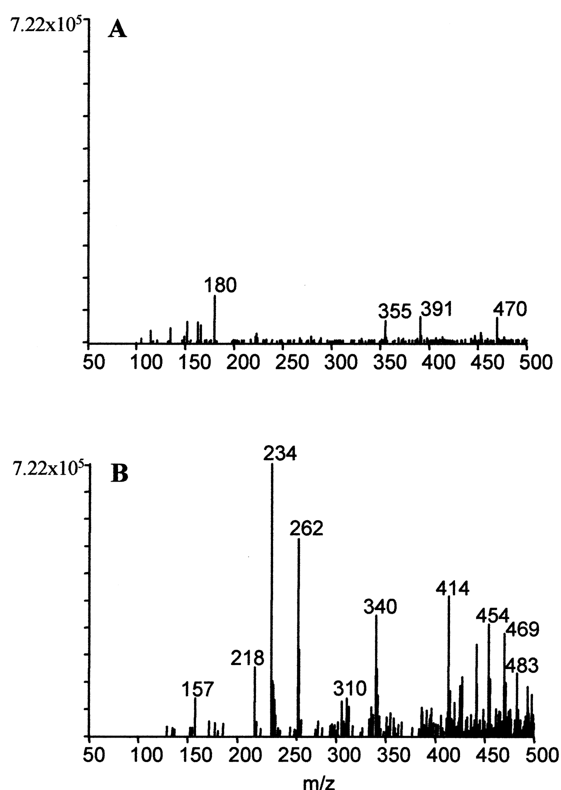

Fig. 2. Demonstration of need for separation and compound specific detection: (A) full-scan (m/z 50–500) LCQ mass spectrum of ACN sheath liquid infused at 5 μl/min, (B) full-scan (m/z 50–500) LCQ mass spectrum of an infused urine extract spiked with 600 ng/ml methylphenidate and fluoxetine. (A) and (B) are plotted on the same scale to show the relative intensity of the additional components from the urine matrix and concurrently excreted drugs, which makes separation and compound specific detection necessary.

These amounts corresponded to 5 and 600 ng of methylphenidate per ml of urine. The final solutions were diluted to 200 μl with water. The full-scan CE–MS and CE–MS^2 area ratios of methylphenidate to methylphenidate-D_3 from the post-extract spiked samples were compared to the pre-extract spiked QC samples at the 5 and 600 ng/ml levels. The results showed that the sample extraction procedure recovered approximately 25% of the added methylphenidate from the urine matrix at both the 5 and 600 ng/ml levels.

3.2. Method linearity

The CE–MS peak area ratios were calculated by dividing the methylphenidate CE–MS peak area by that of the methylphenidate-D_3 internal standard peak area. Two replicates of each standard were prepared and analyzed in the range of 1.5 to 800 ng/ml. Five replicates of each QC sample were prepared at 5, 100 and 600 ng/ml and analyzed by CE–MS. The calibration plots for full-scan CE–MS and MS^2 SRM of methylphenidate are shown in Figs. 3 and 4. The data from the QC runs were plotted to show how they relate to the calibration curve, but they were not used in the curve-fitting calculation. The $1/x$ weighted linear regression results showed that the slope of the full-scan CE–MS curve was 0.0127 and the y-intercept was 0.0037. The slope of the CE–MS^2 SRM curve was 0.0031 and the y-intercept was −0.0017. The correlation coefficients for the CE–MS and CE–MS^2 curves were 0.999 and 0.996, respectively. It is interesting to note that another full-scan CE–MS methylphenidate calibration curve was generated five months prior to this work under different conditions in our

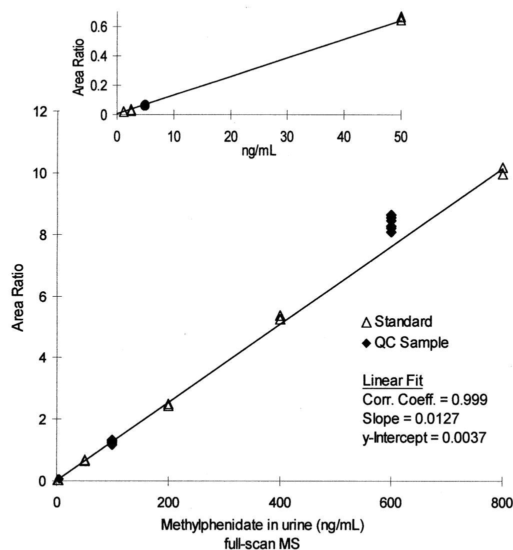

Fig. 3. Calibration curve for full-scan CE–MS quantitation of methylphenidate in human urine.

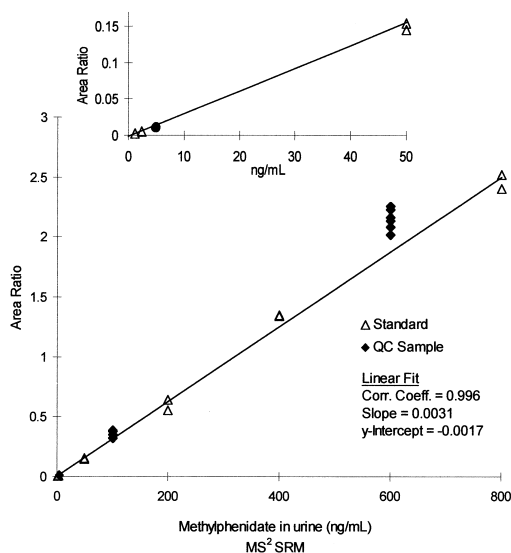

Fig. 4. Calibration curve for CE–MS^2 SRM quantitation of methylphenidate in human urine.

laboratory, and the linear regression results showed a slope of 0.0120 and an intercept of zero [10]. The linear regression results from this work showed a slope of 0.0127 and an intercept of 0.0037. This is an indicator of the fairly consistent sensitivity of the CE–MS ion-trap method.

3.3. Precision and accuracy

Six replicates of each QC sample at the 5, 100 and 600 ng/ml level were prepared. The purpose of the QC samples was to test the accuracy and precision of the analytical method for both the full-scan CE–MS and MS^2 SRM analysis of methylphenidate. The intra-assay precision was determined at the 5, 100 and 600 ng/ml levels by calculating the R.S.D. of the experimentally determined concentrations. The R.S.D. was obtained by dividing the standard deviation by the mean and multiplying by 100. The intra-assay accuracy was calculated as the percent bias of the experimentally determined concentrations of the 5, 100 and 600 ng/ml QC replicates. The percent bias from the nominal concentration was calculated by subtracting the nominal concentration from the found concentration, dividing by the nominal concentration and multiplying by 100.

The intra-assay accuracy and precision results are summarized in Table 1. The intra-assay precision was 6.2 and 6.7% for the 5 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The intra-assay precision was 4.4 and 6.8% for the 100 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The intra-assay precision was 2.6 and 4.2% for the 600 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The intra-assay accuracy ranged from −14.6 to −2.2% bias and from −26.4 to −12.8% bias for the 5 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The intra-assay accuracy ranged from −7.7 to 3.3% bias and from 3.1 to 23.2% bias for the 100 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The intra-assay accuracy ranged from 5.7 to 13.1% bias and from 7.3 to 20.1% bias for the 600 ng/ml full-scan CE–MS and CE–MS^2 data, respectively. The lowest accuracy data points were those from the CE–MS^2 SRM data. One possible explanation for the lower accuracy of the CE–MS^2 data is that non-linear loss of MS^2 product ions was observed at the low levels of quantitation relative to the high levels. A standard $1/y$ weighted linear fit to the MS^2 calibration curve gave better

Table 1
Summary of intra-assay accuracy and precision results for six replicate analyses of QC samples at low-, mid- and high-range

$n=6$	Nominal concentration (ng/ml)	Mean (ng/ml)	% Nominal	% R.S.D.	% Bias range
MS^1	5	4.57	91.40	6.23	−14.60–−2.2
MS^2	5	3.95	79.00	6.66	−26.40–−12.80
MS^1	100	8.35	98.35	4.42	−7.62–3.32
MS^2	100	115.70	86.43	6.76	3.12–23.18
MS^1	600	656.44	91.40	2.61	5.65–13.11
MS^2	600	684.23	87.69	4.20	7.31–20.05

accuracy for the 100 and 600 ng/ml QC data points, and worse accuracy for the 5 ng/ml QC data points. A $1/x$ weighted linear fit was chosen to make the accuracy consistent across the entire dynamic range.

A blank and double blank were run after each concentration level to test for carryover of the methylphenidate and methylphenidate-D_3. The blank urine samples were spiked only with the methylphenidate-D_3 internal standard, and the double blanks were not spiked with either the D_0 or D_3 compounds. Slight CE–MS carryover of methylphenidate was detected after injections at the 400 ng/ml level and higher. Carryover of methylphenidate-D_3 was not detected since it is present in each sample at the level of 100 ng/ml. The methylphenidate carryover was eliminated by rinsing the capillary for 3 min between runs with 1 *M* NaOH followed by a 3 min rinse with water. The LCQ electrospray interface probe assembly was pulled back from the spray shield, and the heated capillary was covered with a septum during the rinsing procedure to prevent salt deposition and contamination.

The lower level of quantitation (LLQ) is defined to be the lowest level concentration with acceptable accuracy and precision, or a signal that is 10-times the noise [3]. For this assay the LLQ was determined to be 2.5 ng/ml. The lower level of detection (LOD) is defined as an analyte signal that is at least 3-times the average noise level [3]. For this assay the LOD was determined to be 1.25 ng/ml. Figs. 5 and 6 show representative CE–MS electropherograms from the LLQ (1.25 ng/ml) and the 600 ng/ml urine standards, respectively. Fig. 5A–E show the corresponding CE–MS and CE–MS^2 ion current profiles for the LLQ (1.25 ng/ml) urine sample. Fig. 6A–E

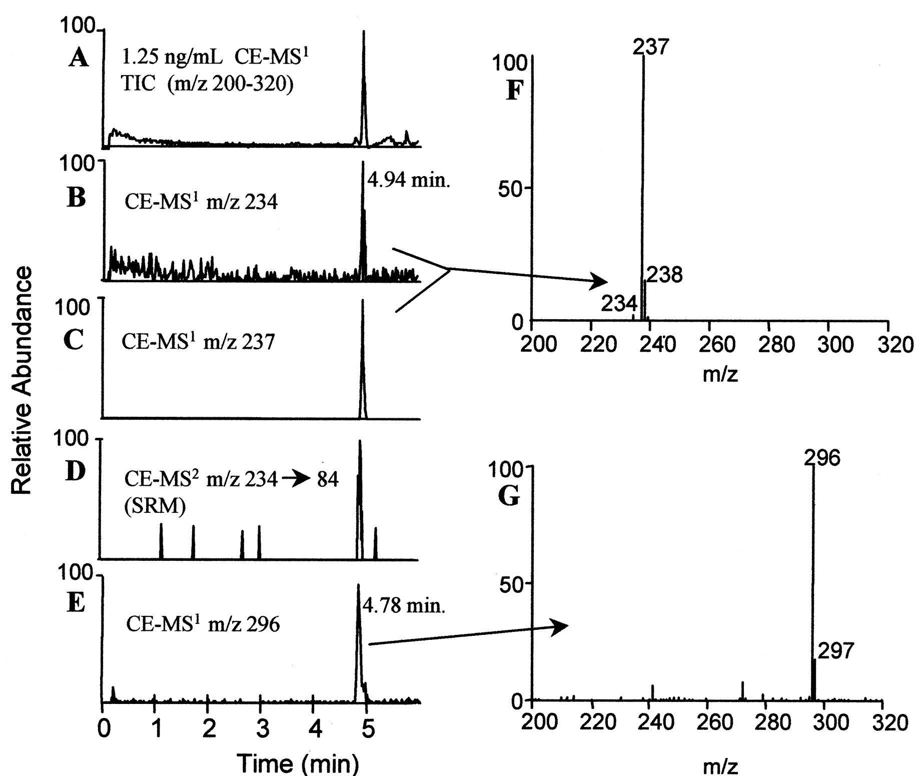

Fig. 5. Representative CE–MS electropherograms from the LLQ, (1.25 ng/ml; ~10 fg injected), standard: (A) full-scan CE–MS TIC, (B) full-scan CE–MS extracted ion current profile for protonated methylphenidate, (C) full-scan CE–MS extracted ion current profile for protonated methylphenidate-D_3, (D) methylphenidate CE–MS^2 SRM transition of m/z 234 fragmenting to m/z 84, (E) full-scan CE–MS extracted ion current profile for protonated norfluoxetine.

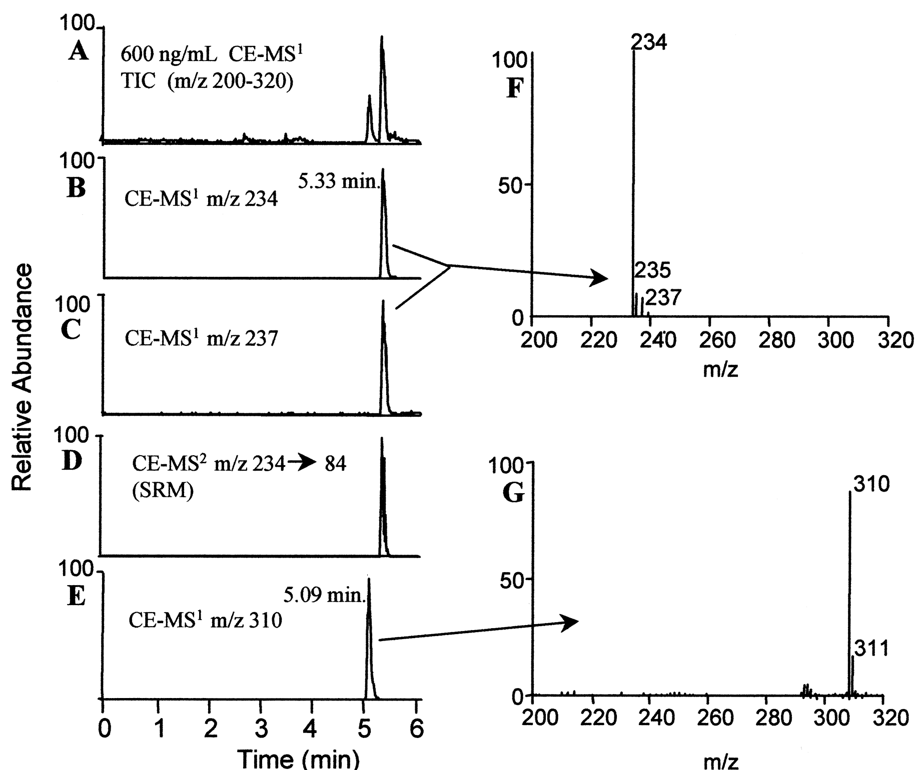

Fig. 6. Representative CE–MS electropherograms from the 600 ng/ml standard, (~5 pg injected): (A) full-scan CE–MS TIC, (B) full-scan CE–MS extracted ion current profile for protonated methylphenidate, (C) full-scan CE–MS extracted ion current profile for protonated methylphenidate-D_3, (D) methylphenidate SRM CE–MS^2 transition of *m/z* 234 fragmenting to *m/z* 84, (E) full-scan CE–MS extracted ion current profile for protonated fluoxetine.

show the corresponding CE–MS and CE–MS^2 ion current profiles for the 600 ng/ml urine sample.

3.4. Determination of methylphenidate in incurred human urine samples

Five samples of human urine were analyzed to determine the rate of excretion of methylphenidate. The samples were taken from a healthy human subject who has been taking 30–35 mg of methylphenidate daily for over ten years. The freshly collected urine samples were immersed into a dewar of liquid N_2 and frozen. This was done to minimize the hydrolysis of methylphenidate in the warm urine. The samples were stored in the laboratory freezer at −20°C and extracted the following day at ice bath temperature. The first urine sample was taken early in the morning, which was 10 h after ingestion of the last 5 mg tablet from the previous day. The next sample was taken 0.5 h after ingestion of the first daily 10 mg tablet. Subsequent samples were taken hourly during the next 3 h. The last point for the pharmacokinetic curve was at 3.5 h after ingestion of the first daily tablet because at that time the subject ingested the second daily 10 mg tablet. A blank and double blank standard were run following CE–MS analysis of the calibration curve samples and before analysis of the human samples to ensure there was no carryover from the higher calibration standards. Duplicate extracts from each time interval urine sample were prepared and analyzed.

Representative CE–MS electropherograms from the incurred urine samples with the lowest and highest concentrations of methylphenidate are shown in Fig. 7. Interpolation from the calibration curve indicated that the methylphenidate levels ranged

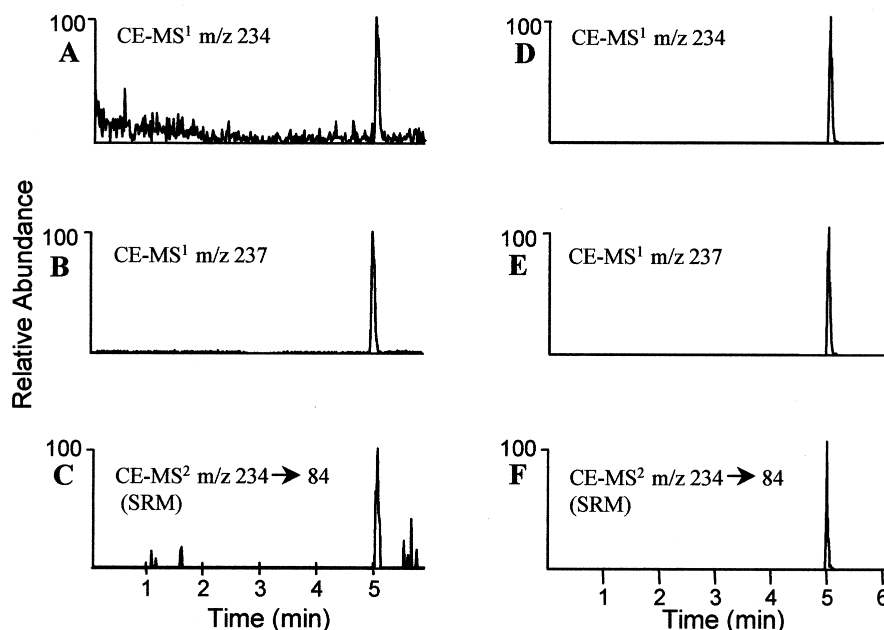

Fig. 7. Representative electropherograms from the incurred human urine samples with the lowest and highest concentrations of methylphenidate: (A and D) full-scan CE–MS extracted ion current profile for protonated methylphenidate, (B and E) full-scan CE–MS extracted ion current profile for protonated methylphenidate-D_3, (C and F) methylphenidate SRM CE–MS^2 transition of m/z 234 fragmenting to m/z 84.

from approximately 5 ng/ml at the pre-dose time to approximately 70 ng/ml at the 1.5 h post-dose time. A simple pharmacokinetic profile for methylphenidate excretion is shown in Fig. 8. Each time point is the average of the two analyzed replicate extracts. The results for both full-scan CE–MS and CE–MS^2 SRM are plotted on the same graph (Fig. 8). The deviation between the CE–MS and CE–MS^2 results were higher at the lowest concentrations. The 0- and 0.5-h samples had deviations of 26.5% and 18.3% between the MS and MS^2 data, respectively. Deviations between the MS and MS^2 results were 2.0%, 0.95% and 4.18% for the 1.5-, 2.5- and 3.5-h samples. This is consistent with the lower accuracy QC data points observed from the low QC CE–MS^2 SRM data as shown in Table 1. The intent of the pharmacokinetic study was to show the validity of this type of scan by demonstrating that quantitation can be done for both the full-scan MS and MS^2 SRM events within a single microscan, using CE coupled to the LCQ. While the results between the full-scan MS and MS^2 data were not quantitatively compared, this work demonstrates that both the MS and MS^2 events produce uniquely valid data over the same dynamic range, and therefore each event may be used to obtain unique analytical information.

4. Conclusions

The results of this work confirm that CE coupled to the LCQ mass spectrometer may be used as a quantitative technique for determination of methylphenidate in human urine over at least a dynamic range of 640. The LCQ ion-trap mass spectrometer is capable of doing both a full-scan event, (over a range of approximately 100 Da), and an MS^2 SRM scan event within a single duty cycle of 1 s per scan. This provides the capability to screen for the presence of other compounds while simultaneously doing quantitative MS^2 SRM on one target. This analysis method is not possible with any other type of mass spectrometer. The LCQ was easy

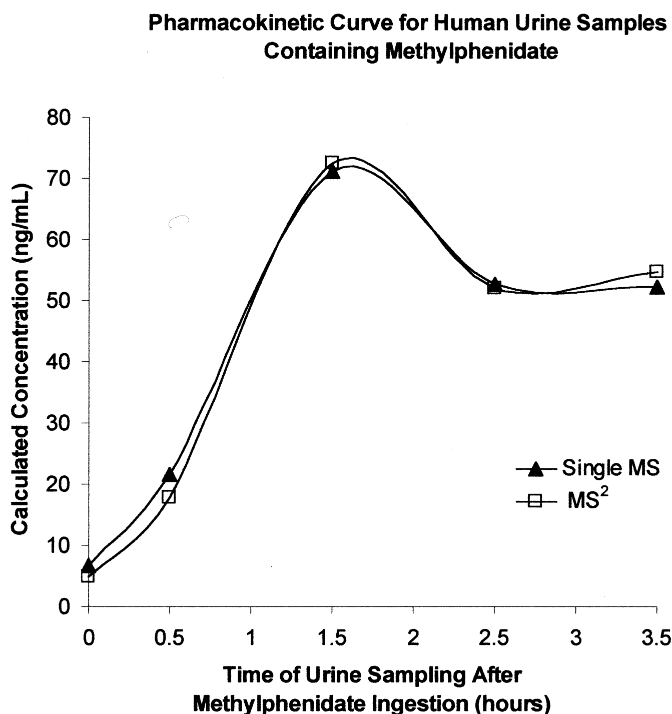

Fig. 8. Pharmacokinetic profile for methylphenidate excretion. The results for both full-scan CE–MS and CE–MS^2 SRM are plotted.

to use for this CE–MS application and showed relatively consistent performance when the experiments were repeated over a five-month period.

It is predicted that further improvements to CE–MS techniques may allow the quantitative determination of methylphenidate and other small molecule drugs in human urine and plasma to be comparable in sensitivity, accuracy and precision to current LC–MS techniques. LC–MS currently provides better bioanalytical measurements than CE–MS techniques with regard to sensitivity and enantiomer resolution. Improvement in the area of CE sample stacking to allow larger injection volumes and simultaneous compatibility with both the CE–MS interface and the mass spectrometer is necessary to increase the sensitivity of the technique [11]. Development of the cyclodextrin-bonded fused-silica capillaries for use with CE–MS to resolve enantiomers would also extend the utility of CE–MS techniques. Finally, improvements in the ease of operation of CE–MS experiments would presumably increase the acceptance and hence the application of this important alternative to LC–MS sample analysis.

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