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Direct analysis of nitrocatechol-type glucuronides in urine by capillary electrophoresis–electrospray ionisation mass spectrometry and tandem mass spectrometry

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Abstract

Direct, quantitative capillary electrophoresis–electrospray ionisation mass spectrometric (CE–ESI-MS) and tandem mass spectrometric (CE–ESI-MS–MS) methods are described for the quantitation of 3-*O*-glucuronides of *E*- and *Z*-entacapone isomers (EEG and EZG) and tolcapone (TG) in urine. 3-*O*-Glucuronide of nitecapone was used as internal standard. Good separation of glucuronides was achieved with 20 m*M* ammonium acetate as separation solution at pH 6.84. Stacking was used to increase the sensitivity of the method by introducing samples in 5 m*M* ammonium acetate. CE–ESI-MS and CE–ESI-MS–MS methods are linear with correlation coefficients better than 0.9983 and 0.9982, and repeatable with relative standard deviations below 9 and 14%, respectively. The limit of detection (LOD) in CE–ESI-MS at signal-to-noise ratio 3 is 100 ng/ml for EEG and EZG and 250 ng/ml for TG. The CE–ESI-MS–MS method was the more sensitive; LOD was 7 ng/ml for all compounds, without any concentration of the sample.

Keywords: Glucuronides; Entacapone; Tolcapone

1. Introduction

Entacapone and tolcapone are catechol-*O*-methyltransferase (COMT) inhibitors used as adjuncts in the medical treatment of patients with Parkinson's disease (PD). Levodopa, the most commonly used drug in PD, is strongly inactivated by COMT, and adding entacapone to the medication of PD-patients enhances the bioavailability of levodopa [1]. In a study of the human metabolism of entacapone, the main metabolites of entacapone in human urine were determined to be 3-*O*-glucuronide conjugates of entacapone and its *Z*-isomer [2]. Tolcapone is similarly eliminated mainly by conjugation [3].

A variety of methods have been used in the analysis of glucuronide conjugates [4]. The glucuronides are thermolabile and methods such as gas chromatography that make use of thermal energy are inappropriate. Thus the major technique for the

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determination of glucuronides is liquid chromatography (LC) [4]. Connection of LC with mass spectrometry (MS) provides additional sensitivity and especially high selectivity. LC–MS has been successfully applied in the identification of glucuronides [5–11]. Quantitative direct LC–MS methods for glucuronides are still rare owing to the lack of commercially available standard compounds. Manini et al. [12] have developed a semi-quantitative LC–MS method with electrospray ionisation (ESI) for the analysis of *n*-hexane glucuronides in urine samples. An LC–MS system with atmospheric pressure chemical ionisation (APCI) for the quantitation of four commercially available toxicologically interesting glucuronides has been developed by the same authors [13]. Several quantitative LC–ESI-MS [14–16] and MS–MS methods [17,18] and also LC–APCI-MS methods [19] have been reported for the quantitation of morphine-3- and morphine-6-glucuronides in serum and plasma samples. Ethyl glucuronide, a minor metabolite of ethanol, has been quantitated by LC–ESI-MS with a synthesised standard [20]. Synthesised standards have also been used in a study of several steroid glucuronides by Bowers and Sanaullah [21]. A direct method for anabolic steroid conjugates in human urine was developed by Bean and Henion [22]. And a direct LC–ESI-MS–MS method for the quantitation of nitrocatechol-type glucuronides in urine was recently developed in our laboratory [23].

Capillary electrophoresis (CE) is a relatively new analytical technique with high separation efficiency. Both micellar electrokinetic capillary chromatography (MECK) [24–27] and capillary zone electrophoresis (CZE) [28–33] have been utilised in the analysis of glucuronides, which are ideal compounds for CE analysis owing to their ionic character. The sensitivity of CE, however, is sometimes insufficient for the determination of very low levels of metabolites [25,28]. The small diameter of the capillary allows injection volumes of only a few nanolitres and, especially in bioanalysis, the most commonly used ultraviolet (UV) detection through a detection window made to the capillary is not always sensitive enough. Sensitivity can be improved by using the mass spectrometer as a detector and/or by applying special concentrative injection techniques or on-line sample preconcentration.

Capillary electrophoresis–mass spectrometry (CE–MS) was first introduced in 1987 [34]. Combination of the instruments is usually achieved with an electrospray interface using coaxial sheath liquid [35] or a liquid junction coupling [36]. Sheathless coupling with nanospray interface may provide better limits of detection because dilution of samples is then avoided. It is probably not yet appropriate for long-period routine analysis, however. CE–MS is a powerful method for the bioanalysis of drugs thanks to the high separation power of CE and high specificity of MS. Althought the method has been applied in the bioanalysis of several drugs, the analysis of drug metabolites, especially glucuronide conjugates, is still rare [37]. Heitmeier and Blaschke [38] recently developed a screening method for the analysis of non-opioid analgesics and their metabolites in urine by CE–MS. Lausecker et al. [39] compared CE–MS and μ-LC–MS methods for the analysis of midazolam and three of its metabolites, one being glucuronide. The CE–MS method showed almost 10 times as high sensitivity for the glucuronide as did the μ-LC–MS method.

To our knowledge no direct quantitative CE–MS method for any glucuronide has been published. In this paper we describe direct CE–ESI-MS and CE–ESI-MS–MS methods for the quantitation of nitrocatechol-type glucuronides in urine using synthesised standard compounds. The most important factors affecting the separation, i.e. the pH and the ionic strength of the electrolyte solution are discussed, along with various instrumental parameters. The results of method validation including linearity, within-day and between-day repeatability, recovery, and the limits of detection are presented. The suitability of the method for the quantitation of 3-*O*-glucuronides of *E*- and *Z*-isomers of entacapone in urine samples is shown by the analysis of clinical patient samples.

2. Experimental

2.1. Materials

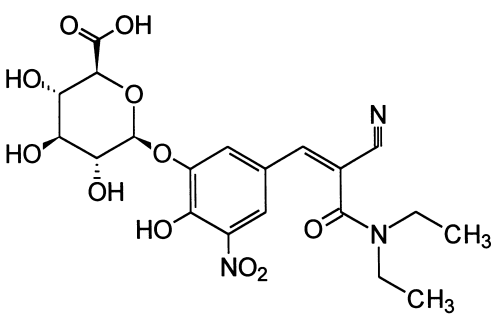
The 3-*O*-glucuronides of *E*- and *Z*-isomers of entacapone (EEG and EZG), nitecapone (NG) and tolcapone (TG) were enzymatically synthesised at

the Department of Pharmacy, University of Helsinki, Finland [40] (Fig. 1). All organic solvents and other chemicals were of analytical or chromatographic grade. Acetonitrile and acetic acid were purchased from Rathburn (Walkerburn, Scotland), ammonium acetate, ammonium hydroxide and hydrochloric acid were from Merck (Darmstadt, Germany) and sodium hydroxide was from Eka Nobel (Bohus, Sweden). Water was purified in a Milli-Q water purification system (Millipore, Molsheim, France). Blank urine was obtained from healthy volunteers.

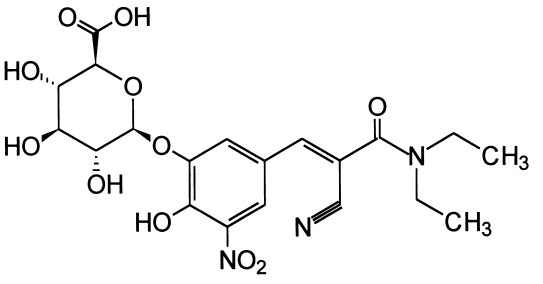
2.2. Instrumentation

A Beckman P/ACE System 2200 capillary electrophoresis system (Beckman Instruments Inc., Palo Alto, CA, USA) was used in the method development and for the MS coupling. During CE method development the instrument was controlled with System Gold software and UV detection at 335 nm was used. Fused-silica capillaries of 50 μm I.D.× 186 μm O.D. or 75 μm I.D.×375 μm O.D. were supplied by Composite Metal Services Ltd. (Hallow, UK) and Polymicro Technologies Inc. (Phoenix, AZ, USA). The total length of the capillary in method development was 67 cm and the length to the detector was 60 cm. Longer (85 cm) capillary was obligatory in MS coupling. Samples were introduced with pressure injection (3.45 kPa, 80 s). Voltage of 25 kV was set to the anode.

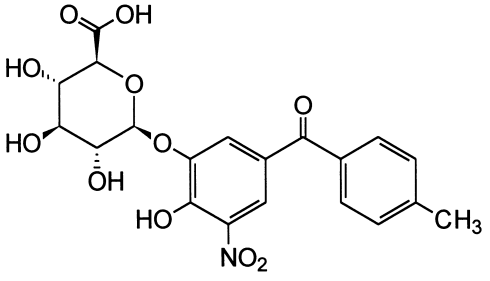
New capillaries were conditioned by rinsing with 1 *M* sodium hydroxide solution for 20 min, with 0.1 *M* sodium hydroxide for 20 min, with water for 2 min and finally with running buffer for 20 min.



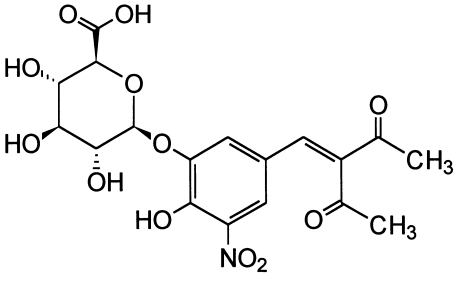
Z-Entacapone glucuronide (EZG)



E-Entacapone glucuronide (EEG)



Tolcapone glucuronide (TG)



Nitecapone glucuronide (NG)

Fig. 1. Structures of the studied nitrocatechol glucuronides.

Between runs the capillary was rinsed with running buffer solution for 3 min. All solutions and water were filtered (Durapore membrane filters HV, 0.45 μm, Millipore corp., Cork, Ireland). Direct coupling of CE with MS was made with a sheath liquid coaxial coupling. Composition of the sheath liquid was acetonitrile −20 m*M* ammonium acetate (1:1, v/v). A micro-syringe pump (Harvard Apparatus Inc., Holliston, MA, USA) was used for the sheath liquid delivery at a flow-rate of 5 μl/min.

The mass spectrometer was a Sciex API300 triple quadrupole equipped with an electrospray interface (Sciex, Toronto, Canada). Data were collected using a Macintosh 8500/180 computer and PE Sciex API 100/300 software (version 1.2). High-purity air (99.998%) was used as a nebulising gas and nitrogen generated with a Whatman 75-720 (Whatman Inc., Haverhill, MA, USA) nitrogen generator was used as curtain and collision gas in MS–MS experiments. The instrument was operated in negative-ion mode (ion spray voltage −4.8 kV). Interface and ion source tuning were done by continuous feed of EZG solution (20 μg/ml) via the CE capillary (voltage of 29.8 kV over the capillary) to produce a maximum intensity and stability of deprotonated molecule.

Collision conditions in tandem mass spectrometry were optimised by recording product ion spectra of deprotonated glucuronides. The scan range was m/z 50–500 (1 s/scan). The maximum intensity of the product ions chosen for multiple reaction monitoring (MRM) in CE–ESI-MS–MS was obtained at the collision energy of 25 eV. Optimisation of mass spectrometric parameters was discussed in detail in our earlier study [41].

2.3. Samples

Stock solutions of the analytes and internal standard (I.S.) were prepared in water. Samples for CE method development were prepared by diluting aqueous stock solutions with water or electrolyte solution. When the stacking effect was used, samples were prepared in 5 m*M* ammonium acetate solution. Spiked urine samples were prepared by adding appropriate amounts of standard solutions to blank urine to obtain standards with concentrations of 2–50 and 0.05–1 μg/ml for MS and MS–MS analysis, respectively. Internal standard was added to give a concentration of 30 μg/ml in MS experiments and 2 μg/ml in MS–MS experiments.

All urine samples were purified by solid-phase extraction (SPE) with Sep-Pak C18 cartridges (Waters, Milford, MA, USA). SPE was carried out with a Baker-10 SPE System (J.T. Baker, Phillipsburg, NJ, USA) vacuum manifold. Cartridges were conditioned with 1 ml of methanol and 1 ml of 50 m*M* hydrochloric acid in 2% methanol solution. Urine samples (1.0 ml) were acidified with 50 μl of 1 *M* hydrochloric acid, loaded to the SPE cartridges and washed with 1 ml of 5 m*M* hydrochloric acid and 1 ml of water. Analytes were eluted with 1.5 ml of methanol and evaporated to dryness at 35°C under nitrogen stream (Techne DB-2A, Techne (Cambridge) Ltd., Duxford, UK). Residues were reconstituted in 1.0 ml of 5 m*M* ammonium acetate. Blank urine and patient samples were treated similarly. All samples were filtered (Millipore HV, 0.45 μm, Nihon Millipore, Yonezawa, Japan) and sonicated before use.

3. Results and discussion

The on-line coupling of capillary electrophoresis with mass spectrometry was was achieved with a self-made coaxial sheath-flow interface pioneered by Smith et al. [35]. The parameters affecting CE separation and the operating parameters of MS were optimised separately before the coupling of the instruments. EEG and EZG cannot be distinguished by MS due to their similar mass spectrometric behaviour [23], and thus optimisation of CE separation was performed using these compounds.

3.1. Optimisation of separation in CE

Ionic strength, pH, and the composition of the electrolyte solution are among the most important factors influencing CE separation. Electrolytes that can be used in CE–ESI-MS are few because only volatile buffers are compatible with electrospray ionisation. Non-volatile buffers cause contamination of the instrument in long-term use and may even lead to a total plugging of the system. Improvements in interfaces, such as orthogonal sprayers creating an off-axis ion pathway [42,43] and a self-cleaning interface [44], have been developed to minimise this

problem. In our experience, however, non-volatile buffers also increase the background noise markedly. For these reasons, volatile ammonium acetate was chosen as electrolyte for CE–MS experiments.

Ionic strength, which has an effect on electroosmotic as well as electrophoretic mobility, was optimised between 10 and 50 m*M* (Table 1). Migration times increased with buffer concentration, because the electroosmotic flow (EOF) from the anode towards the cathodic end decreased less than the electrophoretic mobility in opposite direction. Better resolutions were obtained at higher buffer concentrations, probably because of the better suppression of electrophoretic dispersion. Fast analysis and sufficient resolution were achieved with 20 m*M* ammonium acetate, which was chosen for the analysis.

The EOF and the dissociation state of the analyte, and thus migration times, are controlled by pH. The influence of pH on resolution was studied at pH 5.0, 6.0, 6.84, 7.0 and 8.0 (Table 1). The pH of the 20 m*M* electrolyte solution was adjusted with ammonium hydroxide or acetic acid. At pH 5.0, EOF was slow and the migration times long. At higher pH the migration was faster and peaks sharper. The resolution was best at pH 6.0, but almost 2 min faster separation and still good resolution were obtained with 20 m*M* ammonium acetate at pH 6.84. This solution was chosen for the analysis in order to keep the sample preparation as simple and fast as possible.

Instrumental parameters of CE – applied voltage and injection time – were optimised after the selection of electrolyte composition. The optimal voltage was determined by plotting the applied voltage versus the resulting current. No deviation in linearity was observed and the use of highest voltage (+30 kV) was possible (data not shown).

Injection volume in CE is usually just a few nanolitres, which keeps the sensitivity of the method low. A larger amount of sample can be injected into the column by using sample stacking, as introduced by Mikkers et al. [45]. In our experiments, stacking conditions were optimised with samples dissolved in water, and in 2, 2.5, 4, 5 and 20 m*M* buffer solution. Owing to the too low electrical conductivity, no peaks were detected when the buffer concentration was below 4 m*M*. Stacking effect was demonstrated by injecting 34 nl of standards (12.5 μg/ml) in 5 and 20 m*M* buffers. The 5 m*M* buffer gave a high and sharp peak, while the 20 m*M* buffer showed a very broad and flat peak. The effect of injection volume (17, 25, 34 and 42 nl) on the signal height and resolution was tested with standards (12.5 μg/ml) dissolved in 5 m*M* ammonium acetate. The peak height increased with injection volume, as expected, while the resolution decreased only slightly (Fig. 2). Injection volume of 34 nl (80 s, 3.45 kPa) with acceptable resolution was chosen for the analysis.

Injections were made at the anodic end of the capillary. Negatively charged analytes migrated to the cathodic end in order of decreasing molecular weight, which usually is proportional to the radius. EEG (MW 481), having the lowest electrophoretic mobility to the anode, migrated first, then EZG of the same molecular weight, TG (MW 449) and NG (MW 441).

3.2. Mass spectrometry

Negative-ion mode MS and MS–MS conditions were similar to those optimised for the compounds earlier [41]. Each compound has two acidic groups capable of serving as deprotonation site: carboxylic

Table 1
Effect of ionic strength and pH of the electrolyte solution upon migration of the analytes

Effect of ionic strength				Effect of buffer pH			
Ionic strength (mM)	Migration time of EEG (min)	Migration time of EZG (min)	Resolution	pH	Migration time of EEG (min)	Migration time of EZG (min)	Resolution
10	9.60	9.82	1.28	5.00	17.14	17.64	1.97
20	11.10	11.40	1.76	6.00	13.83	14.31	2.23
30	12.67	13.10	2.16	6.84	12.24	12.63	2.12
40	14.12	14.68	2.32	7.00	12.22	12.62	1.94
50	15.55	16.26	2.74	8.00	9.81	10.06	1.39

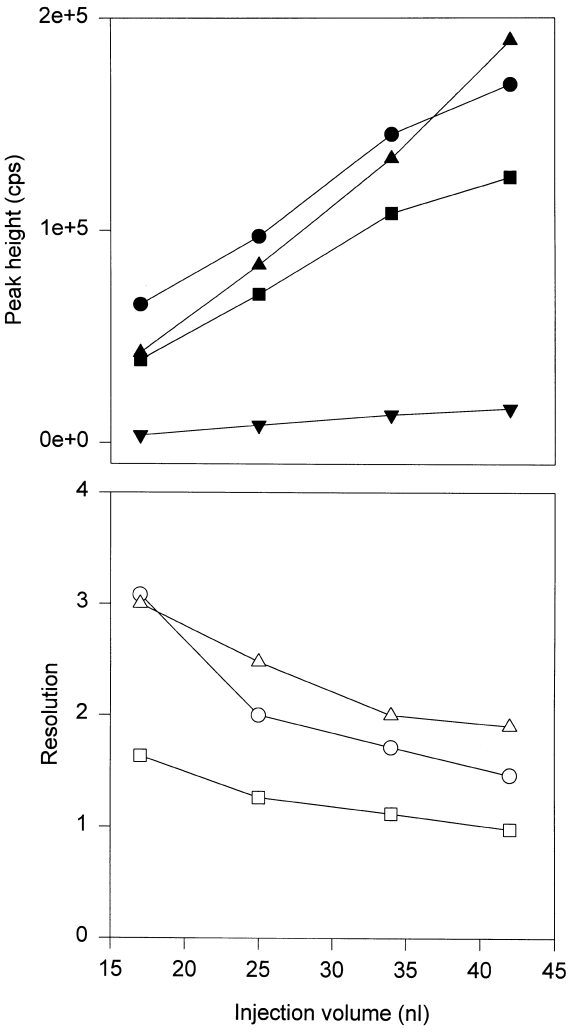


Fig. 2. Effect of injection volume on (a) peak height and (b) resolution of the compounds. ●, *E*-entacapone glucuronide (EEG); ■, *Z*-entacapone glucuronide (EZG); ▲, tolcapone glucuronide (TG); ▼, internal standard (IS); △, TG/IS; □, EZG/TG; ○, EEG/EZG.

acid in the glucuronide part and the phenolic hydroxyl group in the aglycone part. The pK_a value of the carboxylic acid in the glucuronide moiety has been determined to be 3.2 [46] and thus the compounds are deprotonated in neutral pH. The most abundant peaks in the negative-ion mode ESI-MS spectra of the compounds are deprotonated molecules, $[M-H]^-$, and these were chosen for the multiple ion monitoring (MIM) used in CE–ESI-MS. The ions were m/z 480 for EEG and EZG, m/z 448 for TG and m/z 440 for NG.

The only product ion in the negative-ion mode ESI-MS–MS spectra of the compounds is deprotonated aglycone $[M-H-Glu]^-$ formed by the loss of the glucuronide moiety after the cleavage of the glycoside bond [41]. Negatively charged ether oxygen is retained on the aglycone part and the resulting product is extremely stable due to the resonance stabilisation. No other ions exist in the negative product ion spectra and thus these product ions were chosen for MRM in CE–ESI-MS–MS experiments. Monitored reactions were m/z 480→304 for EEG and EZG, m/z 448→272 for TG and m/z 440→264 for NG.

3.3. Capillary electrophoresis–mass spectrometry

Flow-rates in CE are too low for proper operation of standard ESI source. In this study a mixture of acetonitrile and 20 m*M* ammonium acetate (1:1) was used as sheath liquid. This composition produced a stable and reproducible ESI signal at a flow-rate of 5 μl/min. Acetonitrile was chosen over methanol because of the smaller background noise.

One of the key parameters to be optimised in CE–ESI-MS and CE–ESI-MS–MS analysis was the location of the sprayer tip. If the tip was located too close to the curtain plate of the MS instrument, the stability and intensity of the signal declined noticeably, owing to the charge discharge occurring at the tip of the electrode tube. The problem is avoided by keeping the distance between the tip of the capillary and the curtain plate long enough. Another factor likely to affect the stability of the signal was the length of the fused-silica capillary outside the electrode tube at the ESI interface. Maximum intensity and stability of the analytes were obtained when the tip of the fused-silica capillary protruded less than 1 mm from the stainless steel capillary. Perkins and Tomer [47] and the Tomlinson group [48] have presented similar results.

3.4. Quantitative analysis

The suitability of the methods for the quantitative analysis was studied by testing the linearity, the

Table 2
Calibration data obtained in the analysis of EEG, EZG and TG

	CE–ESI-MS (n=8) 2–150 μg/ml			CE–ESI-MS–MS (n=6) 0.05–1 μg/ml		
Compound	EEG	EZG	TG	EEG	EZG	TG
Slope	0.1172	0.0807	0.0648	4.6623	5.0958	6.1214
Intercept	−0.0515	0.0340	0.0055	−0.0278	−0.0124	−0.0302
Correlation coefficient	0.9983	0.9985	0.9986	0.9995	0.9994	0.9982

repeatability and the limits of detection with spiked urine samples. Although CE has been used for the analysis of biological samples with no or minimal sample pre-treatment, Lehtonen et al. [24] considered pre-treatment to be necessary since clinical patient samples might include endogenous compounds (e.g. proteins due to kidney disfunction), which are normally not found in the urine of a healthy person. Endogenous compounds also affect the selectivity and sensitivity of the method. The samples were thus purified by SPE before analysis in order to avoid clogging of the capillary and contamination of the instrument in long-term analysis. The recovery of TG was determined at concentrations of 2, 50 and 150 μg/ml and was 102.7, 97.3 and 95.8%, respectively. The recovery of EEG, EZG and NG with use of a similar SPE method has been studied earlier in our laboratory [24].

3.5. Linearity

The concentration range studied was 0.05–150 μg/ml. Concentration levels of *E*-entacapone and *Z*-entacapone glucuronides in real patient samples vary between 2 and 150 μg/ml, while the average concentration of entacapone glucuronide after oral administration of 200 mg of entacapone is over 30 μg/ml [24]. In CE–ESI-MS method the calibration curves were drawn from 2 to 150 μg/ml and the curves showed good linearity in the studied range with correlation coefficients 0.9983–0.9986. Some peak broadening, probably due to overloading of the capillary, was noticed at concentration levels over 75 μg/ml. The linearity in tandem mass spectrometric analysis was studied at concentration range of 0.05–1 μg/ml and found to be good (r=0.9982–0.9995) for all compounds (Table 2).

3.6. Repeatability

Repeatability of the CE–ESI-MS method was studied at 25 μg/ml, which is close to the average real level of EEG in patient samples. Repeatability was tested as within-day and between-day repeatability (Table 3). Relative standard deviations (RSD) within-day and between-day were below 8.3 and 15%, and acceptable for biological samples. Repeatability of CE–ESI-MS–MS method was

Table 3
Within-day and between-day repeatability of the methods

	CE–ESI-MS (25 μg/ml)					CE–ESI-MS–MS (0.2 μg/ml)				
	EEG	EZG	TG	n	Days	EEG	EZG	TG	n	Days
Within-day repeatability										
Mean	2.97	2.47	2.15	6	1	0.838	0.739	1.12	6	1
RSD (%)	8.34	8.10	7.26			5.02	13.6	5.99		
Between-day repeatability										
Mean	2.96	2.44	2.10	15	7	0.816	0.882	1.12	11	6
RSD (%)	13.2	15.0	14.9			14.8	11.3	8.88		

Table 4
Repeatability of relative migration times

	CE–ESI-MS (25 μg/ml)				
	EEG	EZG	TG	*n*	Days
Within-day repeatability					
Mean	0.935	0.960	0.974	6	1
RSD (%)	0.373	0.242	0.184		
Between-day repeatability					
Mean	0.933	0.958	0.973	15	7
RSD (%)	0.462	0.281	0.237		

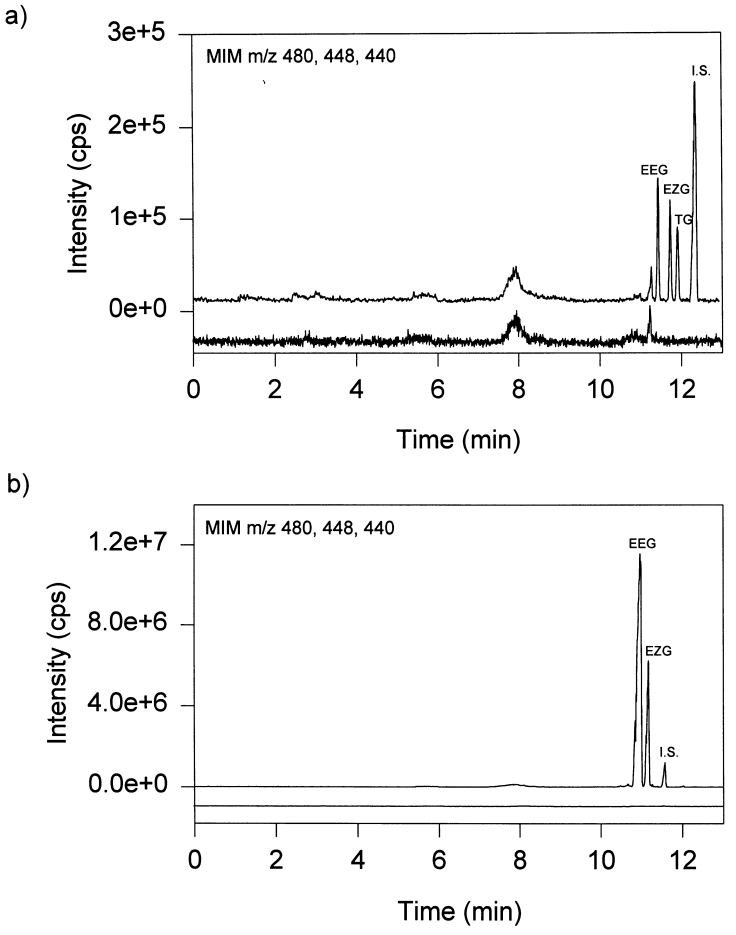


Fig. 3. Electropherograms obtained in the CE–ESI-MS analysis of (a) urine sample spiked with EEG, EZG, TG (2 μg/ml) and internal standard (30 μg/ml) and (b) a real patient sample. Blank urine traces are shown below. Abbreviations: see Fig. 1.

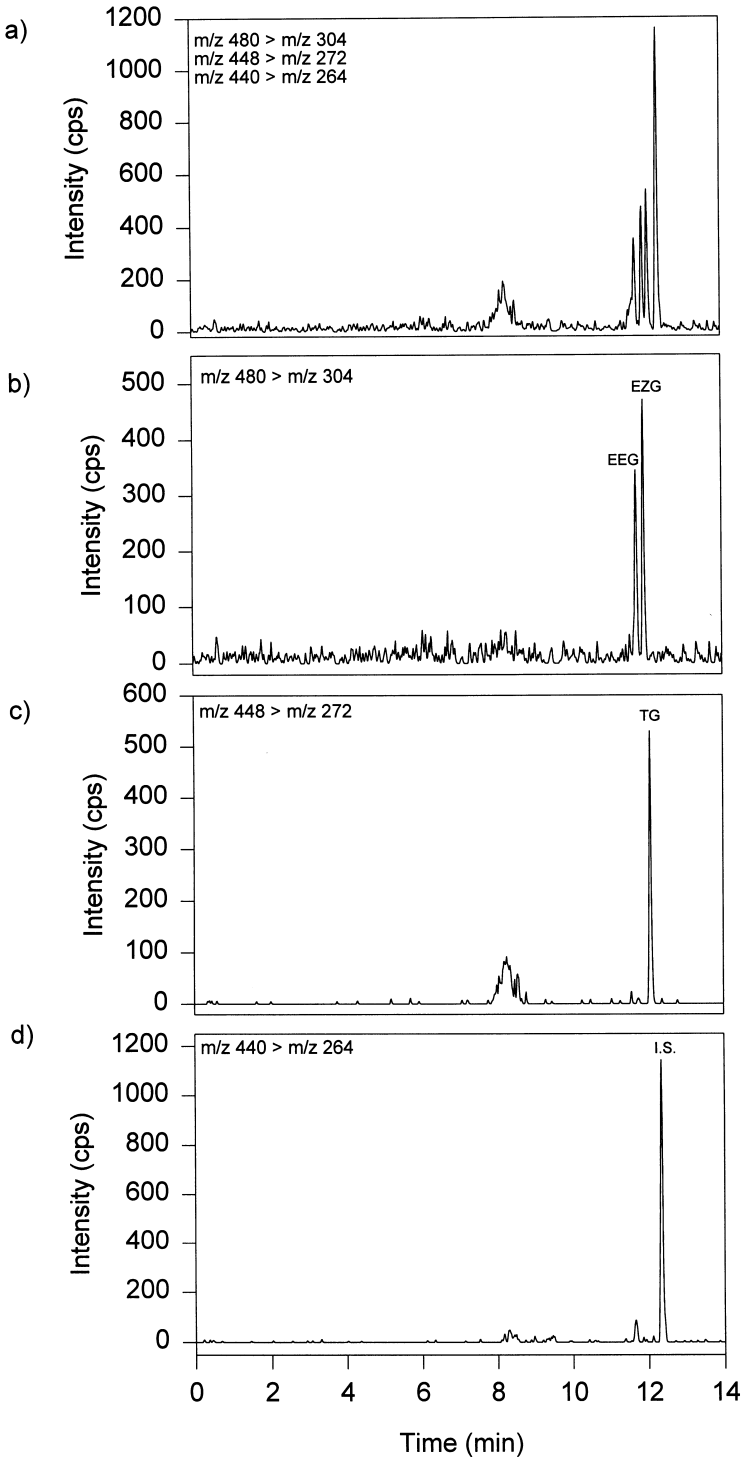


Fig. 4. Total and selected ion electropherograms obtained in the CE–ESI-MS–MS analysis of a urine sample spiked with EEG, EZG, TG (0.08 μg/ml) and I.S. (2 μg/ml). Abbreviations: see Fig. 1.

studied at a concentration of 0.2 μg/ml, which is in the lower part of the calibration curve. MS–MS method was also repeatable, RSDs being within-day and between-day below 14 and 15%, respectively, for all compounds. The results obtained with MS–MS are not as good as those obtained with MS because the concentration of the sample in MS–MS was less than one tenth that in MS.

Study was also made of the migration times. The effect of different rinsing procedures on the repeatability of the migration times has been thoroughly examined. Faller and Engelhardt [49] found a rinse with 0.1 *M* hydrochloric acid and buffer to lead to a highly stable series of migration times. Using alkaline preconditioning procedures, Ehmann et al. [50] came to the conclusion that the multistep preconditioning step is rarely superior to simple flushing with electrolyte. In our study, only a 3-min rinse with electrolyte solution was done between runs. When the repeatability of the migration times was calculated, the RSD was about 2%. This figure is high, partly because the runs were started manually owing to the non-commercial connection of the instruments. When relative migration times were calculated using I.S. as a migration standard, RSDs were below 0.37% within-day and below 0.46% between-day. The results of the study are presented in Table 4.

3.7. Limits of detection

The limit of detection in CE–ESI-MS was 100 ng/ml for EEG and EZG and 250 ng/ml for TG ($S/N=3$). Owing to the background and chemical noise (data not shown), these values are relatively high. Better LODs were obtained by CE–ESI-MS–MS with its higher selectivity: the limit of detection was 7 ng/ml for all compounds. Since the injected volume was 34 nl, the amounts of analytes detected were only a few femtomoles. All results were obtained without sample preconcentration.

Fig. 3a shows an electropherogram from the CE–ESI-MS analysis of urine sample spiked with EEG, EZG and TG (2 μg/ml). As can be seen from the figure, the resolution between EEG and EZG is good enough with baseline separation. In fact, baseline separation of entacapone glucuronide isomers and tolcapone glucuronide is not necessary since patients with Parkinson's disease receive only one of the drugs. Electropherograms obtained in the analysis of blank urine and a real patient sample are shown in Fig. 3b and c. Concentrations of EEG and EZG in a urine sample collected during 6 h after 3×200 mg oral dose of entacapone were 29.2 and 6.81 μg/ml, respectively. No interfering peaks due to other medication of the patient appear in the electropherogram. Sensitivity is noticeably improved with CE–ESI-MS–MS owing to the higher selectivity (Fig. 4). High selectivity and sensitivity are essential in kinetic studies when the metabolic fate of a drug is being determined. The metabolites occur in complex matrices and the concentration may be extremely low, especially at the end of the series.

4. Conclusions

The direct CE–ESI-MS and CE–ESI-MS–MS methods described proved suitable for the quantitation of the glucuronide conjugates of entacapone and tolcapone in urine. The methods are simple and rapid, allowing analysis in just 15 min. Samples are purified by solid-phase extraction, separated in CE with 20 m*M* ammonium acetate as electrolyte solution, and analysed in negative-ion mode by MS and MS–MS. Both methods show good linearity and acceptable repeatability. Especially in MS–MS mode, the methods are highly selective and sensitive and can be utilised in metabolic studies when low concentrations of the metabolites must be analysed in complex matrices.

Acknowledgements

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