Journal of Chromatography A, 1218 (2011) 739–745

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Rapid and sensitive drug metabolism studies by SU-8 microchip capillary electrophoresis-electrospray ionization mass spectrometry

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ARTICLE INFO

Article history:
Received 23 September 2010
Received in revised form
29 November 2010
Accepted 6 December 2010
Available online 13 December 2010

Keywords:
Electrophoresis
Epoxy photoresist SU-8
Mass spectrometry
Metabolism
Microfluidics

ABSTRACT

Monolithically integrated, polymer (SU-8) microchips comprising an electrophoretic separation unit, a sheath flow interface, and an electrospray ionization (ESI) emitter were developed to improve the speed and throughput of metabolism research. Validation of the microchip method was performed using bufuralol 1-hydroxylation *via* CYP450 enzymes as the model reaction. The metabolite, 1-hydroxybufuralol, was easily separated from the substrate (R_s = 0.5) with very good detection sensitivity (LOD = 9.3 nM), linearity (range: 50–500 nM, r^2 = 0.9997), and repeatability (RSD_{Area} = 10.3%, $RSD_{Migration\ time}$ = 2.5% at 80 nM concentration without internal standard). The kinetic parameters of bufuralol 1-hydroxylation determined by the microchip capillary electrophoresis (CE)-ESI/mass spectrometry (MS) method, were comparable to the values presented in literature as well as to the values determined by in-house liquid chromatography (LC)-UV. In addition to enzyme kinetics, metabolic profiling was demonstrated using authentic urine samples from healthy volunteers after intake of either tramadol or paracetamol. As a result, six metabolites of tramadol and four metabolites of paracetamol, including both phase I oxidation products and phase II conjugation products, were detected and separated from each other within 30–35 s. Before analysis, the urine samples were pre-treated with on-chip, on-line liquid-phase microextraction (LPME) and the results were compared to those obtained from urine samples pre-treated with conventional C18 solid-phase extraction (SPE, off-chip cartridges). On the basis of our results, the SU-8 CE-ESI/MS microchips incorporating on-chip sample pre-treatment, injection, separation, and ESI/MS detection were proven as efficient and versatile tools for drug metabolism research.

1. Introduction

One of the major reasons for the termination of the development of potential drug candidates is their poor adsorption, distribution, metabolism, and excretion (ADME) properties. To prevent expensive terminations in late clinical stages, much effort needs to be expended to investigate the ADME characteristics as early in the drug discovery process as possible [1]. Metabolic profiling of new drug candidates is particularly important and includes not only identification of metabolites, but also screening of their properties, such as stability and toxicity [2]. Also screening of drug–drug interactions and determining the kinetic parameters of the drug metabolism are equally important [2]. In these types of analyses, the key issue is high throughput and therefore the analytical method should be as fast as possible. Sometimes the amounts of the metabolites are extremely low and thus, highly specific and sensitive analytical methods are also required.

Presently, most metabolism assays are performed by gas (GC) – or liquid (LC) chromatography combined with mass spectrometry (MS) or by nuclear magnetic resonance (NMR) spectroscopy [3,4]. Since a vast majority of metabolites are polar and ionic, capillary electrophoresis (CE) is another approach for the separation of metabolites, in addition to LC analysis. Microchip CE in particular provides significant improvement in terms of fast analysis times and enables direct coupling to electrospray ionization (ESI)/MS which eventually results in high sensitivity and good selectivity. Although microchip CE with electrochemical [5,6] or optical [7–9] detection has occasionally been applied to metabolism research, the use of MS detection is still rare. Even though most microchip methods provide considerable increase regarding speed of analysis, the time-consuming (off-chip) sample pre-treatment often prolongs the total analysis time. Typically, complex biological matrices,

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doi:10.1016/j.chroma.2010.12.010

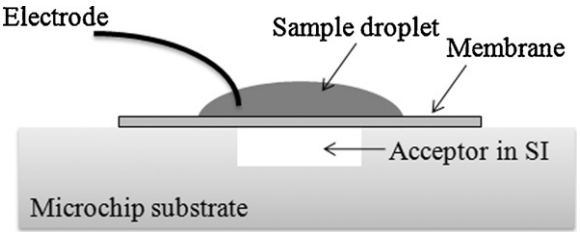


Fig. 1. Schematic illustration of the on-chip droplet-membrane-droplet LPME system on top of an SU-8 CE-ESI/MS microchip. The sample solution was alkaline (30 mM NaOH, pH 11.4) urine. The acidic (0.1% formic acid, pH 2.7) acceptor solution contained 100 μM verapamil as an internal qualifier. SI = sample inlet.

such as urine, require extensive and laborious clean-up before analysis. Thus, integration of sample pretreatment with injection, separation and detection on a single microchip increases sample throughput and approaches the μTAS (micro total analysis systems) concept [10].

Today, modern lithographic and adhesive bonding techniques enable mass production of very complex and accurately defined microstructures on highly integrated devices [11]. Recently, the epoxy-based negative photoresist SU-8 has shown to be a very suitable material for microchip production [12–15]. It is easily patterned by standard photolithography, it has excellent thermal and mechanical properties, and it is also stable against many acids, bases and solvents. In this work, highly integrated CE-ESI/MS microchips were fabricated from SU-8 polymer so that all critical structures were simultaneously patterned by photolithography. SU-8 microfabrication technology relies on photolithography and wafer-level bonding, an approach that enables production of tens of identical chips on one wafer [12,16]. Also reproducibility from wafer-to-wafer and batch-to-batch is very good [17]. Here, the applicability of the microchips to metabolism research was first demonstrated by determining the kinetic parameters of the cytochrome P450 (CYP) mediated bufuralol 1-hydroxylation by microchip CE-ESI/tandem MS (MS/MS). In addition, authentic urine samples were screened for metabolites of tramadol and paracetamol by microchip CE-ESI/MS after on-chip liquid-phase microextraction (LPME). To the best of our knowledge, CE-ESI/MS microchips have not been previously used in drug metabolism research, which is highly demanding because of the need for very low detection limits and highly reproducible and quantitative determination of the produced metabolites. Instead, most of

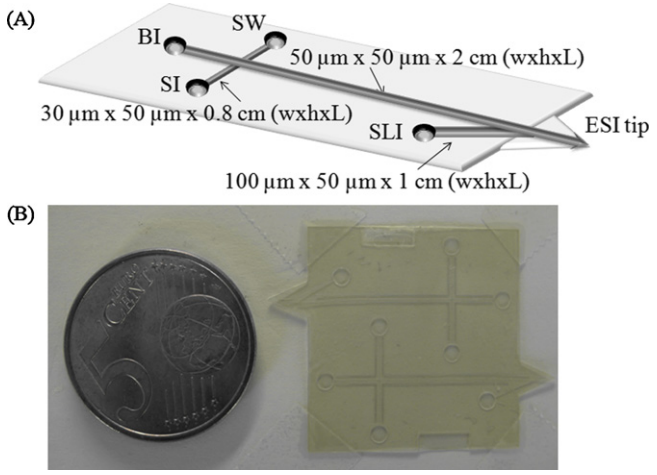


Fig. 2. (A) Schematic view of the fluidic design of the SU-8 CE-ESI/MS microchip (dimensions not to scale) and (B) photograph of the SU-8 microchip. BI = buffer inlet, SI = sample inlet, SW = sample waste, SLI = sheath liquid inlet.

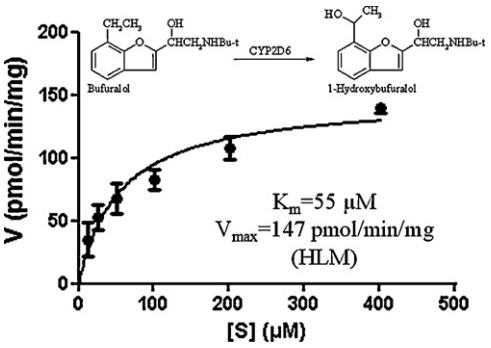


Fig. 3. Michaelis–Menten kinetics of the CYP450 mediated bufuralol metabolism to 1-hydroxybufuralol in HLM determined by microchip CE-ESI/MS in SRM mode. The separation was performed under electric field strength of 750 V/cm in 30 mM ammonium acetate with 50% methanol as BGE and 80% methanol–20% water with 1% acetic acid as sheath liquid. The substrate concentration ranged from 12.5 to 400 μM and all incubations were done in duplicate at each substrate concentration.

the previous work has gone into development of microchips for qualitative protein analysis [18–20].

2. Materials and methods

2.1. Chemicals

Bufuralol was obtained from Roche (Basel, Switzerland), 1-hydroxy bufuralol was from Ultrafine Chemicals (Manchester, England), paracetamol was from Orion Pharma (Espoo, Finland), paracetamol glucuronide and verapamil hydrochloride were from Sigma–Aldrich (Steinheim, Germany), and tramadol as well as the metabolites *O*-desmethyltramadol (M1), *N*,*O*-didesmethyltramadol (M5) were kindly donated by the Department of Forensic Medicine, University of Helsinki, Finland. Human liver microsomes (HLM) were purchased from BD Gentest™ (Erembodegem, Belgium) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) was from Sigma–Aldrich. Acetic acid, formic acid, ammonium formate and 1-methoxy-2-propyl acetate (PMA) were all purchased from Sigma–Aldrich, sodium hydroxide and methanol were from J.T. Baker (Deventer, Holland), acetonitrile was from VWR (Espoo, Finland), hydrochloride and sodium phosphate from Riedel de Haen (Seelze, Germany), and 1-octanol and ammonium acetate were from Fluka (Buchs, Switzerland). Water was purified with a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Enzyme incubations

The kinetic parameters of the bufuralol 1-hydroxylation in HLM were determined by monitoring the CYP mediated reaction during incubation at 37 °C for 60 min. The incubation conditions were optimized in-house (data not shown) and six different substrate concentrations were used, i.e., 12.5, 25, 50, 100, 200 and 400 μM (two replicates of each). The incubation mixture (100 μL) contained bufuralol (12.5–400 μM), 50 mM sodium phosphate buffer (pH 7.4), HLM (0.8 mg/mL) and NADPH (1 mM), and the reaction was terminated by the addition of 100 μL of ice-cold acetonitrile. After removal of the proteins by centrifugation (5 min, 13,000 rpm), the supernatant was analyzed without further treatment. Blank samples were prepared without NADPH, HLM or substrate as well as with zero incubation time. GraphPad Prism 5.01 was used for data processing.

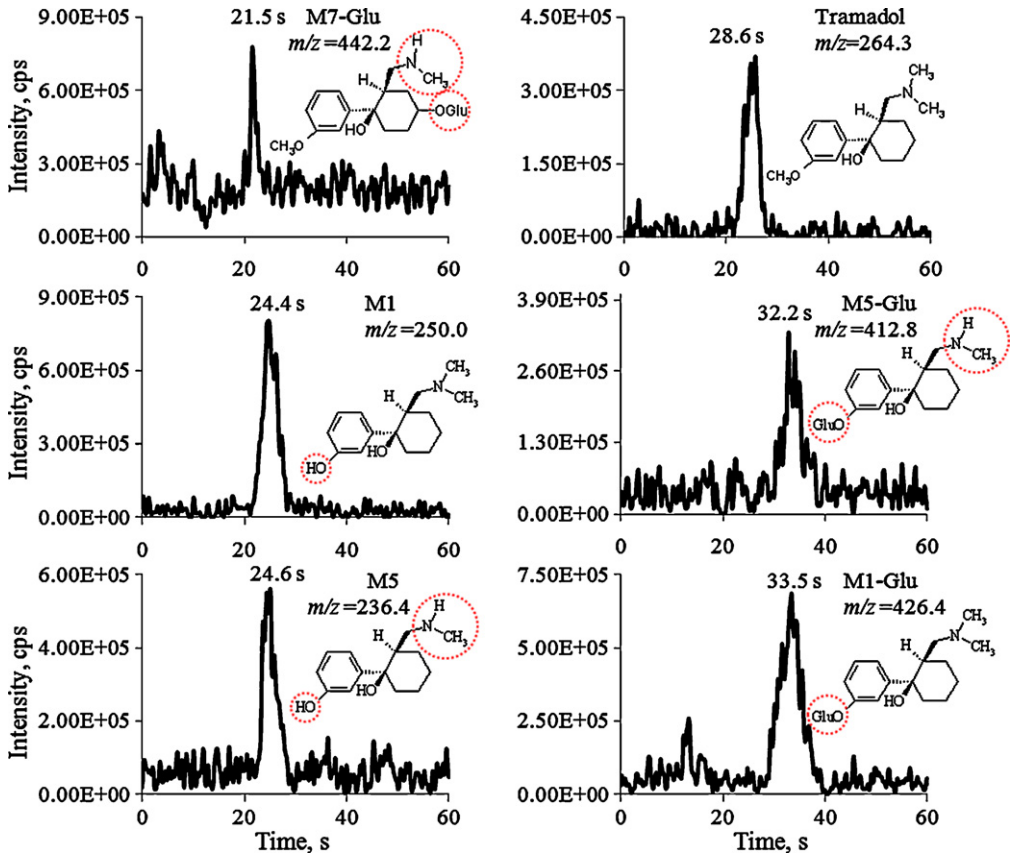


Fig. 4. Extracted ion electropherograms (EIE) of tramadol and its metabolites detected from a human urine sample and separated by microchip CE-ESI/MS after SPE (5-fold preconcentration). The BGE was 30 mM ammonium acetate with 50% methanol and the sheath liquid was 80% methanol–20% water with 1% acetic acid. The electric field strengths during injection (20 s) and separation were 1000 and 800 V/cm, respectively.

2.3. Urine samples

Urine samples were collected from two healthy volunteers 4 h after paracetamol (500 mg) intake or 12 h after tramadol (50 mg) intake. Urine samples were stored frozen at −20 °C until use and pretreated either by off-chip solid-phase extraction (SPE) or by on-chip LPME before analysis.

2.4. Off-chip solid phase extraction

Two different SPE extraction sorbents were used for the urine samples containing paracetamol or tramadol metabolites. For paracetamol samples, an Isolute MF C18, 100 mg reversed phase cartridge (International Sorbent Technology Ltd., Mid Glamorgan, U.K.) was conditioned with 1 mL methanol and balanced with 1 mL of 50 mM HCl in 2% methanol. One milliliter of paracetamol urine was acidified with 50 μL of 1 M HCl and slowly loaded onto the cartridge and washed with 1 mL 10 mM HCl. The analytes were eluted with 1.5 mL of methanol. For tramadol urine samples an Oasis HBL, 30 mg cartridge (Waters, Milford, MA, USA) was conditioned with 1 mL of methanol and 1 mL of water. The tramadol urine sample (1 mL) was slowly loaded onto the cartridge and washed with 1 mL of water. Elution was performed with 1 mL of methanol. The extracts of both the tramadol and paracetamol urine were evaporated to dryness under nitrogen and the residues were reconstituted in 200 μL of 10 mM ammonium acetate containing 50% methanol (5-fold concentration).

2.5. On-chip liquid-phase-microextraction

On-chip LPME was performed as previously described [21] and as illustrated in Fig. 1. An aliquot of 2 μL of 0.1% formic acid (pH 2.7) with 100 μM verapamil as an internal qualifier (acceptor solution) was applied to the sample inlet (SI) of the SU-8 CE-ESI/MS microchip (Fig. 2). A 5 mm × 5 mm piece of a Celgard 2500 microporous polypropylene membrane (Celgard, Charlotte, NC, USA) with a 25 μm thickness, 55% porosity, and 0.21 μm × 0.05 μm pores was wetted with 1-octanol and placed on top of the acceptor solution. Finally, 4 μL of alkaline tramadol urine sample (30 mM sodium hydroxide, pH 11.4, donor solution) was applied on top of the membrane to initiate extraction. After 5 min, the polypropylene membrane was removed and a platinum electrode was placed in the acceptor solution in the SI. The injection voltages were immediately applied and the injection was performed in pinched mode for 60 s before application of the CE separation voltages.

2.6. Microchip capillary electrophoresis

The microchips comprising a monolithically integrated injection and separation unit, a sheath flow interface and an ESI emitter were fabricated entirely of epoxy photoresist SU-8 using photolithography and adhesive bonding techniques as reported earlier [12,13]. The effective separation length of the CE chip was 2 cm. Other microchannel dimensions are given in Fig. 2. Before use, poly(dimethylsiloxane) (PDMS) sheets with 2 mm inlet holes were attached on top of the SU-8 chips to increase the inlet volumes. On the microchips that were used for the LPME experiments, the SI (Fig. 2) was left uncovered.

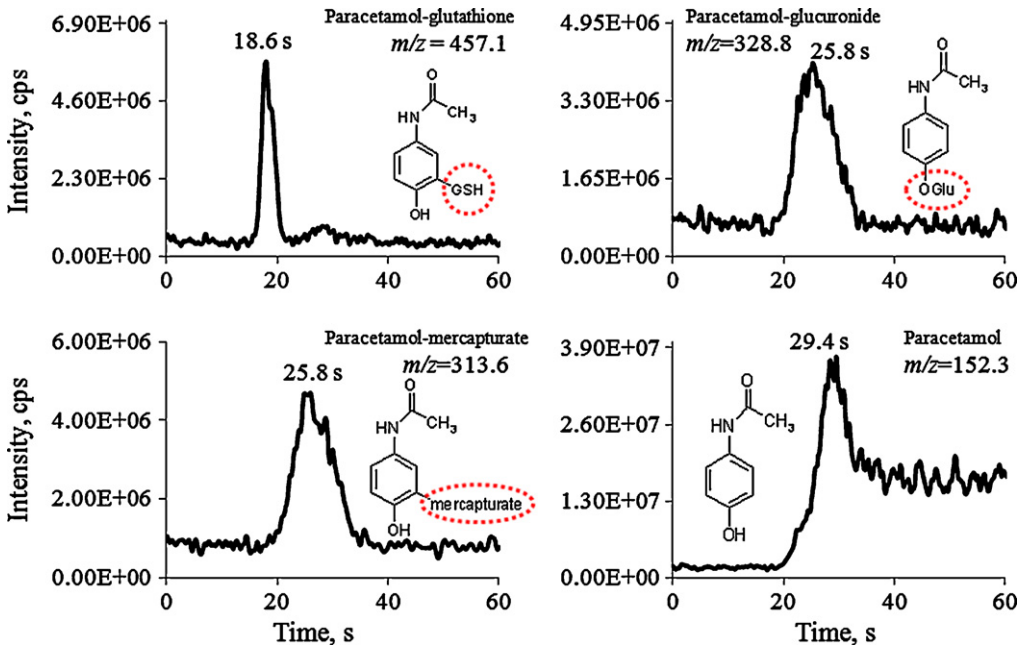
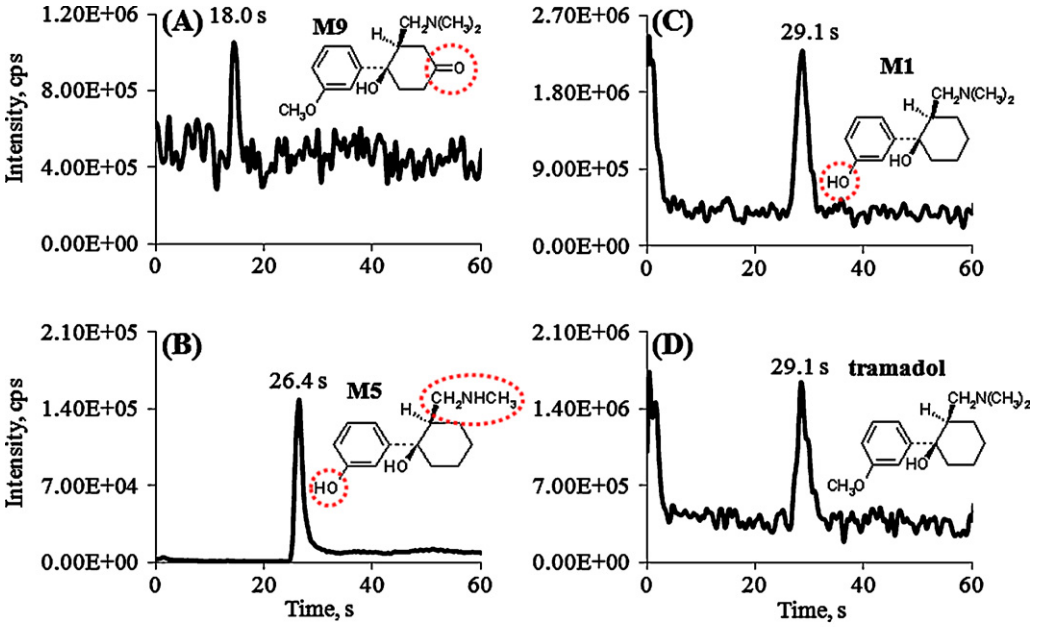


Fig. 5. Extracted ion electropherograms (EIE) of paracetamol and its metabolites detected from a human urine sample and separated by microchip CE-ESI/MS after SPE (5-fold preconcentration). The BGE was 30 mM ammonium acetate with 50% methanol and the sheath liquid was 80% methanol–20% water with 1% acetic acid. The electric field strengths during injection (20 s) and separation were 1000 and 500 V/cm, respectively.



Metabolite		m/z	MS mode*	pK$_a$		logD	
				Amine	Phenol	pH 11	pH 3
(A)	M9	278.6	EIE	9.28	-	1.26	-2.23
(B)	M5	236.1→44.2	SRM	10.02	9.22	0.34	-1.32
(C)	M1	250.5	EIE	8.97	9.62	0.74	-1.20
(D)	tramadol	264.3	EIE	9.23	-	2.44	-1.05

*EIE=extracted ion electropherogram, SRM=selected reaction monitoring

Fig. 6. Extracted ion electropherograms (EIE, M9, M1 and tramadol) and selected reaction monitoring (SRM) electropherogram (M5) of tramadol and its metabolites detected from a human urine sample and separated by microchip CE-ESI/MS or MS/MS after on-chip LPME (2-fold preconcentration). The LPME was performed from alkaline urine (NaOH 30 mM, pH 11.4) into acidic acceptor solution (0.1% formic acid, pH 2.7) for 5 min followed by injection (60 s, 1000 V/cm) and separation (750 V/cm) in 30 mM ammonium formate with 50% methanol. The sheath liquid consisted of 80% methanol–20% water with 1% formic acid. Log D and pK_a values were derived from the online MarvinSketch chemical editor at www.chemaxon.com.

The samples were injected electrokinetically (20–60 s) in pinched injection mode with an electric field strength of 1000 V/cm applied between the SI and the sample waste (SW). Simultaneously, a small focusing potential was applied to the buffer inlet (BI) to avoid sample leakage into the separation channel (Fig. 2). The sheath liquid inlet (SLI) was left floating during injection so that no spray was produced. The CE separations were performed in cathodic mode using electric field strengths of 500–800 V/cm between the BI and SLI. In addition, small push-back voltages were applied to the SI and SW. The background electrolyte (BGE) consisted of 30 mM ammonium acetate (pH 7.0) or ammonium formate (pH 6.4) with 50% methanol, while the sheath liquid was methanol:water 80:20 (v/v) with 1% acetic or formic acid, respectively.

2.7. Mass spectrometry

The microchips were placed on an *xyz*-aligning stage in front of an API3000 or an API365 triple–quadrupole MS (Perkin-Elmer Sciex, Concord, ON, Canada). An external power supply (Micralyne Inc., Edmonton, Canada) was used for application of the injection, separation and electrospray (ES) voltages through platinum wires placed in the liquid filled inlets. The MS was operated in positive ion mode with an ES voltage of 3.6 kV (relative to MS) applied through the SLI. This voltage also served as the counter voltage for the CE separation. The separation current was divided at the sheath flow intersection into the ES and the auxiliary channel from where the excess current was led to ground through a 50 MΩ resistor coupled in parallel with the ES voltage power supply. Data were recorded in full-scan MS mode (metabolic profiling) with a dwell time of 300 ms per scan and a mass range of m/z 50–500 or in selected reaction monitoring (SRM) mode (enzyme kinetics) with a dwell time of 50 ms per selected precursor/product ion pair. Analyst 1.4 software was used for data acquisition and processing.

2.8. High performance liquid chromatography (a reference method)

In addition to microchip CE-ESI/MS, the kinetic parameters of the bufuralol 1-hydroxylation were determined based on conventional high performance liquid chromatography (HPLC)-UV analysis. The HPLC instrument was an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler and a UV multiple wavelength detector. The HPLC separation was performed by reversed phase chromatography using a Zorbax Eclipse Plus C18 column (5 μm, 150 mm × 4.6 mm, Agilent Technologies, Palo Alto, CA, USA). The injection volume was 20 μL and the eluent consisted of 0.1% formic acid (A) and methanol (B) with a gradient profile from 20% to 90% B in 6 min followed by 90% B for 5 min. The flow rate was 1 mL/min and the UV detection wavelength was 250 nm.

3. Results and discussion

The performance of the SU-8 microchips in quantitative CE-ESI/MS analysis was validated using 1-hydroxy bufuralol as a standard. The limit of detection (LOD, S/N = 3) and limit of quantitation (LOQ, S/N = 10), as per the ICH guidelines, were 9.3 and 31.2 nM, respectively. This corresponds to sample amounts of only 0.42 and 1.4 attomol for LOD and LOQ, respectively, as per injection volume of 45 pL (determined by the volume of the intersection of the separation and the injection channels). The regression coefficient (r^2) in the concentration range 50–500 nM was 0.9997 indicating good linearity. At best, the relative standard deviations (RSD, at 80 nM, $n = 5$) of the peak area and the migration time were 10.3% and 2.5%, respectively, which indicated relatively good quantitative performance of the microchip-based system even without the use of an internal standard. All values were determined using SRM mode with the selected precursor (protonated 1-hydroxybufuralol)/product ion pairs of m/z 278.1 → 242.0 ($[M+H\text{-}2H_2O]^+$) and 278.1 → 186.1 ($[M+H\text{-}2H_2O\text{-}C(CH_3)_3]^+$).

3.1. Enzyme kinetics

Bufuralol is a fairly specific substrate of the CYP2D6 enzyme and thus bufuralol 1-hydroxylation is often used as a model reaction to determine the activity of this CYP isoenzyme [22,23]. However, few other CYP enzymes, of which the two most important are CYP1A2 [24] and CYP2C19 [25], also exhibit minor bufuralol 1-hydroxylase activity in addition to CYP2D6. In this study, we determined the kinetic parameters (K_m and V_{max}) of bufuralol 1-hydroxylation in HLM in order to demonstrate the applicability of the SU-8 microchips to enzyme kinetics studies. The kinetic parameters of the bufuralol 1-hydroxylation in HLM were determined by the developed microchip CE-ESI/MS/MS method. As a result, the CYP mediated metabolism was shown to follow Michaelis–Menten kinetics with K_m and V_{max} values of 55 μM and 147 pmol/min/mg protein, respectively (Fig. 3). These kinetic parameters compare very well with the values determined by in-house HPLC-UV (K_m = 31 μM, V_{max} = 185 pmol/min/mg protein) and also with the literature values for K_m of 50–250 μM [24] and for V_{max} of 60–240 pmol/min/mg protein [25]. In contrast to conventional HPLC analysis, the SU-8 CE-ESI/MS microchips offer significantly improved speed of analysis and lower sample consumption. Here, the migration time of 1-hydroxybufuralol and bufuralol were only 20.6 s and 22.5 s, respectively, while the corresponding retention times with the in-house HPLC-UV method were 5.0 min and 6.5 min. In addition, the microchip CE-ESI/MS consumes only few tens of picoliters of sample (here approximately 45 pL) while a typical injected volume in HPLC is tens of μL (here 20 μL). Instead, the actual volumes of sample needed for the microchip CE-ESI/MS analysis (here 3 μL applied to the sample inlet) and the HPLC-UV analysis (here 30–40 μL in the sample vial) largely depend on the injector geometry and typically differ from each other by an order of magnitude.

3.2. Analysis of urine samples

In addition to enzyme kinetics, microchip CE-ESI/MS was used in the screening of metabolites of tramadol and paracetamol from human urine samples. The metabolic profiles of these two pharmaceuticals are very different and their broad spectrum of potential metabolites with variable chemical and physical properties make them particularly suitable reference compounds for validating the microchip CE-ESI/MS method for metabolic profiling. Namely, the main urinary metabolites of paracetamol are the phase II conjugation products, e.g., glucuronides [26] while tramadol is extensively converted to several phase I metabolites (oxidation products) by CYP enzymes. The main urinary metabolites of tramadol are *O*-desmethyl-tramadol (metabolite M1) and *N*-desmethyl-tramadol (metabolite M2) [27]. In addition, traces of several other metabolites, both phase I and phase II (conjugation products) are possible [28].

Before analysis, the urine samples were pre-treated either by off-chip SPE (5-fold concentration) or by on-chip LPME (2-fold concentration). SPE is extensively used in metabolic profiling because of its versatility and the possibility of extracting a broad range of metabolites from urine samples, for example. In this study, six tramadol metabolites, including both phase I and phase II products, as well as tramadol itself were detected and separated from each other by the microchip-based analysis following off-chip SPE (Fig. 4). In case of paracetamol, mainly phase II conjugation products (glu-

curonide, glutathione and cystein) were detected in addition to paracetamol itself which was detected as a very intense, slightly tailing peak in the electropherogram (Fig. 5). The biotransformation of xenobiotics is a very complex process and can greatly vary between individuals. Here, the content of unmetabolized paracetamol in urine was seemingly much higher than those of the metabolites. Taking into account the relatively high dose of paracetamol (500 mg), it is likely that this was the reason for the observed tailing of the paracetamol peak in Fig. 5. All tramadol and paracetamol metabolites migrated within approximately 30–35 s. These metabolite findings are in good accordance with the previously published reports [29,30].

In addition to SPE, the urine samples containing metabolites of tramadol were pre-treated using on-line LPME prior to microchip CE-ESI/MS. LPME offers selectivity in the analysis of less polar phase I metabolites, which may be hard to detect if the SPE conditions are optimized for the extraction of very polar phase II metabolites. In LPME, target analytes are extracted from the biological matrix, through a hollow fiber wetted with an organic solvent, into a suitable acceptor solution [31]. The acceptor solution is then injected into a chromatographic or electrophoretic separation system. LPME is easily downscaled to low μL volumes by replacing the hollow fiber with a flat polypropylene membrane, which also facilitates its implementation to lab-on-a-chip systems [21]. The experimental set-up used in this study is shown in Fig. 1. For extraction of the tramadol metabolites, the urine pH was adjusted to 11.4 with NaOH (30 mM) in order to convert tramadol as well as the expected basic metabolites into their neutral form (Fig. 6). In the acceptor droplet, the pH was adjusted to 2.7 with formic acid (0.1%) to ensure full protonation. In brief, the analytes in their neutral form were extracted from 4 μL of alkaline donor solution into the organic phase (octanol wetted polypropylene membrane) from which they were distributed and concentrated (2-fold) into 2 μL of acidic acceptor solution in their protonated form. The pH gradient between the donor and acceptor solution served as the only driving force for extraction, since no stirring was applied to promote mass transfer in the system. Fig. 6 lists the metabolites of tramadol detected using the LPME set-up. All compounds except the metabolite M5 could be easily detected even in full scan MS mode. For reference, the estimated detection limits (S/N = 3) of the tramadol M1 metabolite, for example, were 2 μM and 4 nM in full-scan and SRM modes, respectively. The metabolite M5 was only observed by using the more specific and sensitive detection in SRM mode, which was likely because of its poorer extraction efficiency (i.e., zwitterionic nature and relatively low log D value at pH 11, which lower its theoretical mass transfer from the donor solution into the octanol wetted membrane).

A closer examination of the SPE and LPME treated samples clearly shows the differences between the two methods. The phase I metabolites M1 and M5 as well as tramadol itself were detected by both SPE (Fig. 4) and LPME (Fig. 6). As expected, the phase II metabolites were detected by SPE only, whereas LPME provided increased selectivity with respect to the less polar phase I metabolites so that one additional metabolite, M9, was detected by LPME only. A further advantage of the LPME set-up is the possibility of performing on-line sample clean-up and preconcentration prior to analysis. In this work a preconcentration factor of two was used, but depending on the applied chip material and fabrication method, the depth of the sample inlet (i.e., acceptor side) can be reduced to only a few tens of micrometers which correspond to acceptor volumes in the nL range. Thereby, multi-fold sample preconcentration can easily be achieved with on-chip LPME in the same way as with off-chip SPE. In addition, on-chip LPME offers advantages in terms of speed of analysis. For example the time for sample preparation by LPME is only a few minutes (here a 5 min extraction time was used), while the time for sample preparation by conventional off-chip SPE columns is tens of minutes.

4. Conclusions

Sensitive and efficient analysis of drug metabolism products was demonstrated by using the SU-8 CE-ESI/MS microchips. The microchip method including rapid CE separation of parent drugs and their metabolites followed by MS detection in full-scan MS or SRM mode was validated by using 1-hydroxybufuralol, a CYP metabolism product of bufuralol, as the model compound. The enzyme kinetic parameters determined for the bufuralol 1-hydroxylation compared very well between the microchip method and a standard HPLC-UV method. The microchips were also applied to the analysis of authentic urine samples from which metabolites of tramadol or paracetamol were detected. Before microchip CE-ESI/MS analysis, the urine samples were pre-treated with either off-chip SPE or on-chip LPME. Comparison of these sample pre-treatment methods evidenced that LPME increases selectivity for the less polar phase I metabolites, while SPE is capable of extracting a broad range of phase II metabolites. However, the small sample volumes required in LPME together with the possibility of very simple on-line coupling to the separation microchips offer advantages in terms of speed of analysis and performing on-line sample concentration prior to analysis. Most importantly, the highly reproducible, low cost fabrication of SU-8 microchips by photolithography and wafer-level adhesive bonding enables mass production of microchips with accurately defined microstructures and identical features from chip to chip. In addition to the very fast analysis times and the reported high sensitivity, this is the main advantage of the fully integrated SU-8 CE-ESI/MS microchips over other thus far published CE-ESI/MS microchips.

Acknowledgements

The Academy of Finland (121882) and the Aalto University Graduate School in Electrical and Communication Engineering provided financial support.

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