Two-dimensional capillary electrophoresis integrating on-line sample pretreatment and mass spectrometry detection for determination of cationic drugs and their cationic metabolites in human urine

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Peter Mikuš, Juraj Piešťanský, Katarína Maráková

Department of Pharmaceutical Analysis and Nuclear Pharmacy, Toxicological and Antidoping Center, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia
mikus@fpharm.uniba.sk

ABSTRACT

A powerful tool for the analysis of unpretreated or diluted biological samples containing ultra-trace concentration levels of ionizable drugs and their ionizable metabolic products, based on the two-dimensional isotachophoresis – capillary zone electrophoresis (ITP-CZE) technique on-line hyphenated with the electrospray ionization – tandem mass spectrometry (ESI-MS/MS, here triple-quadrupole mass spectrometry, QqQ), was developed in our work. Analytical and application potentialities of this new approach were demonstrated on the highly reliable determination of pg-ng/mL levels of various cationic drugs (varenicline VAR, pheniramine PHM, phenylephrine PHE) and identification of their cationic metabolites (2-hydroxyvarenicline, N-desmethyl pheniramine) in directly injected (unpretreated) human urine samples taken after the administration of a usual dose of the varenicline- or pheniramine-containing commercial drugs (Champix®, TheraFlu®). The success of the proposed method is linked with (i) the enhanced sample loadability of the used CE system, (ii) on-line electrophoresis sample pretreatment (preconcentration and sample clean-up) and separation, (iii) high compatibility of CZE and ESI electrolyte systems, (iv) mass spectrometry detection sensitivity and selectivity. The proposed ITP-CZE-ESI-QqQ method was approved by its favorable performance parameters such as the limit of detection, limit of quantitation, linearity, linear range, precision (intra-day, inter-day), recovery/accuracy, selectivity and robustness. Practical outcome of this study could drive advanced monitoring of target drugs, their metabolites and related biomarkers in biological samples, carried out in clinical laboratories for diagnostic purpose as well as therapy optimization.

INSTRUMENTATION

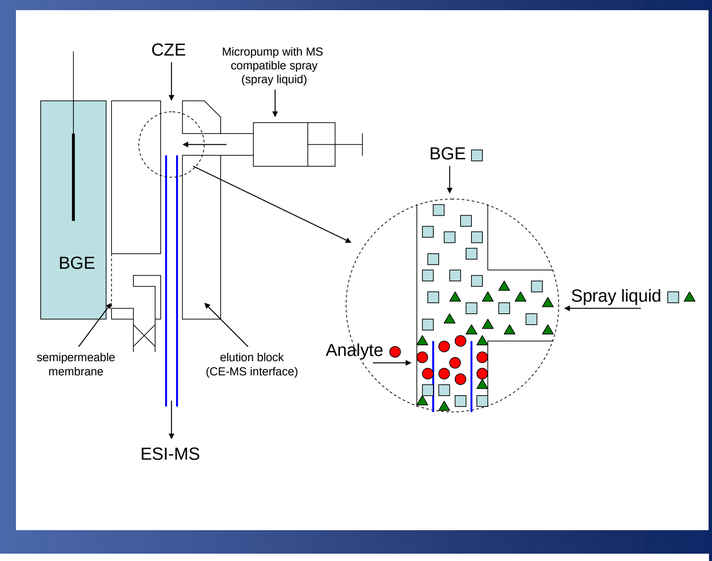
A modified modular capillary electrophoresis analyzer EA-102 (Villa Labeco, Spišská Nová Ves, Slovakia), assembled in the column-coupling configuration of the separation unit, was used in this work for perfomning the ITP-CZE runs. The analytical protocol was controlled by Win ACES software, ver. 1.4 (Faculty of Natural Sciences Comenius University, Bratislava, Slovensko). An ITP column was provided with an 800 µm I.D. polytetrafluoroethylene (PTFE) capillary tube of a 90 mm total length and a contactless conductivity detector. A CZE column was the same as the ITP one except for a 300 µm I.D. and a 160 mm total length

The separating electrolytes in the capillaries were replaced by the fresh ones between each run. ITP and CZE experiments were carried out in the cationic regime of the separation (i.e. cathodic movement of the analytes) and the samples were injected by Hamilton syringe via a rubber septum into the injection block. The experiments were performed in constant current mode at 20°C. The driving currents applied were 300 µA (ITP) and 40 µA (CZE).

Detection was performed on a triple quadrupole mass spectrometer (QqQ) Agilent 6410 Series Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA), operated with an ESI interface in the positive ionization mode. The control of the MS system and data aquisition were performed using Mass Hunter Work Station B.03.01 (Agilent Technologies).

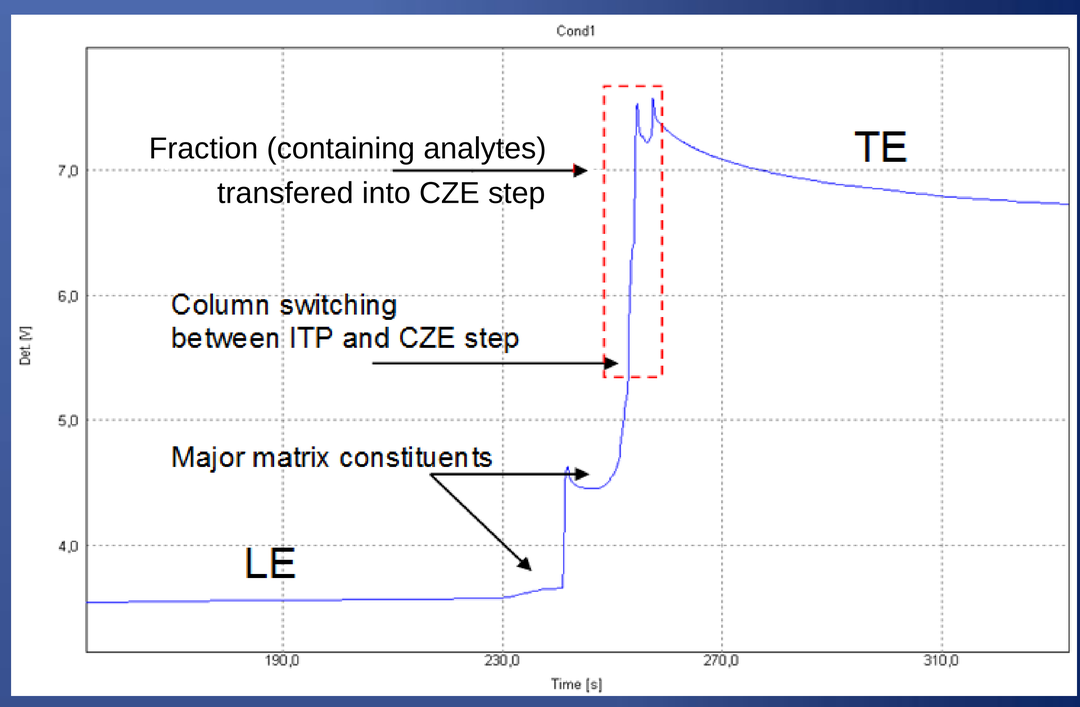
CE-MS coupling was carried out using an elution block with a short capillary transfer line (transport/electrospray capillary). The transfer line (fused silica capillary with a 75 µm I.D.) served also as the ESI tip of the sheathless electrospray interface. The transfer of separated zones from CE towards the mass spectrometer was performed using a spray liquid. The spray liquid was delivered to the elution block by a syringe pump (KDS 100, KD Scientific, Holliston, MA, USA). Nitrogen was used as a nebulizing as well as drying gas. The capillary voltage of 5000 V was set in the MS detector, and dwell time for each ion was 200 ms. The quantification of the analytes (VAR, PHM, PHE) was achieved using the multiple reaction monitoring (MRM) mode.

Scheme of the elution block (i.e. CE-ESI interface) showing a position of the transport/electrospray capillary inside it. The detail shows the arrangement of electrolyte systems in the CZE-ESI combination of the ITP-CZE-ESI-QqQ techniqe.



Optimized analytical conditions of ITP-CZE-ESI-QqQ method for varenicline (VAR), pheniramine (PHM) and phenylephrine (PHE).

Parameter	ITP-CZE	Parameter	ESI-QqQ
ITP stage		**Spray liquid**	
LE	10 mM NH_4Ac + 20 mM HAc (pH 4.5)	Composition	MeOH:water:HAc
TE	10 mM HAc (pH 3.1)	Concentration (%, v/v)	50:49.9:0.1
Current (µA)	300	Flow rate (µL/min)	2
CZE stage		**Nebulizer pressure (psi)**	15
BGE	10 mM Hac (pH 3.1)	**Drying gas**	N_2
Current (µA)	40	Temperature (°C)	300
		Flow rate (L/min)	5
		QqQ stage	
		Capillary voltage (kV)	5
		Fragmentor (V)	160 (VAR), 100 (PHM, PHE)
		Collision energy (eV)	18 (VAR), 10 (PHM), 8 (PHE)

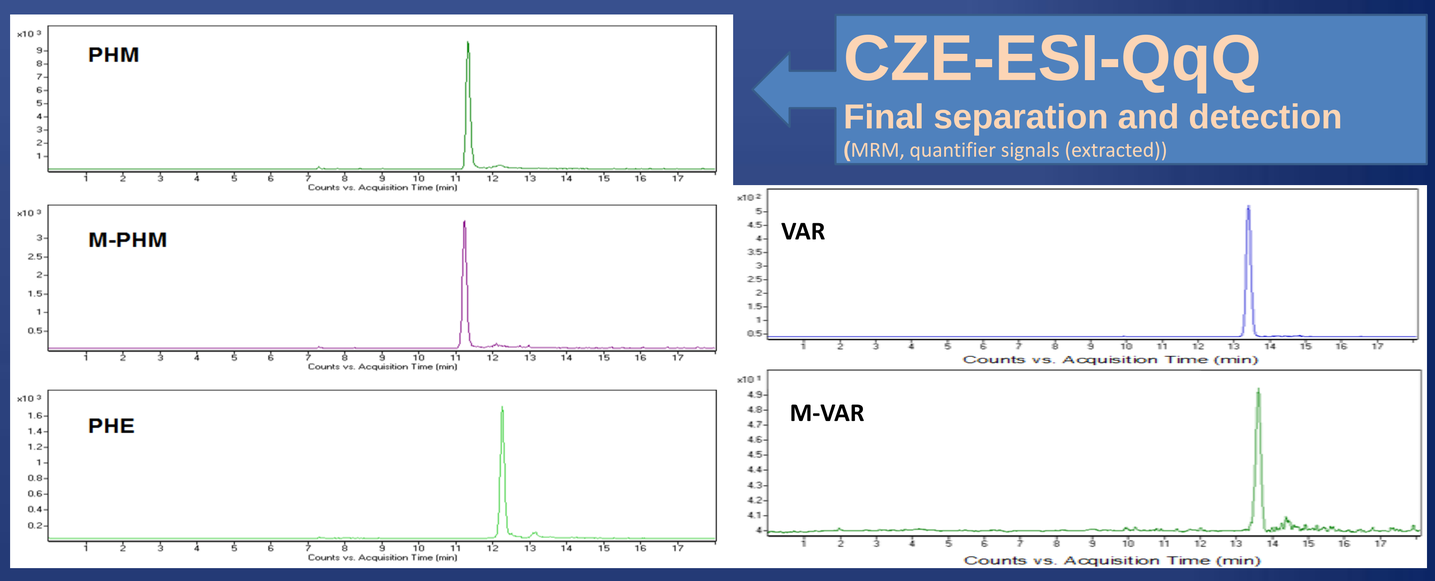


ITP
On-line sample treatment

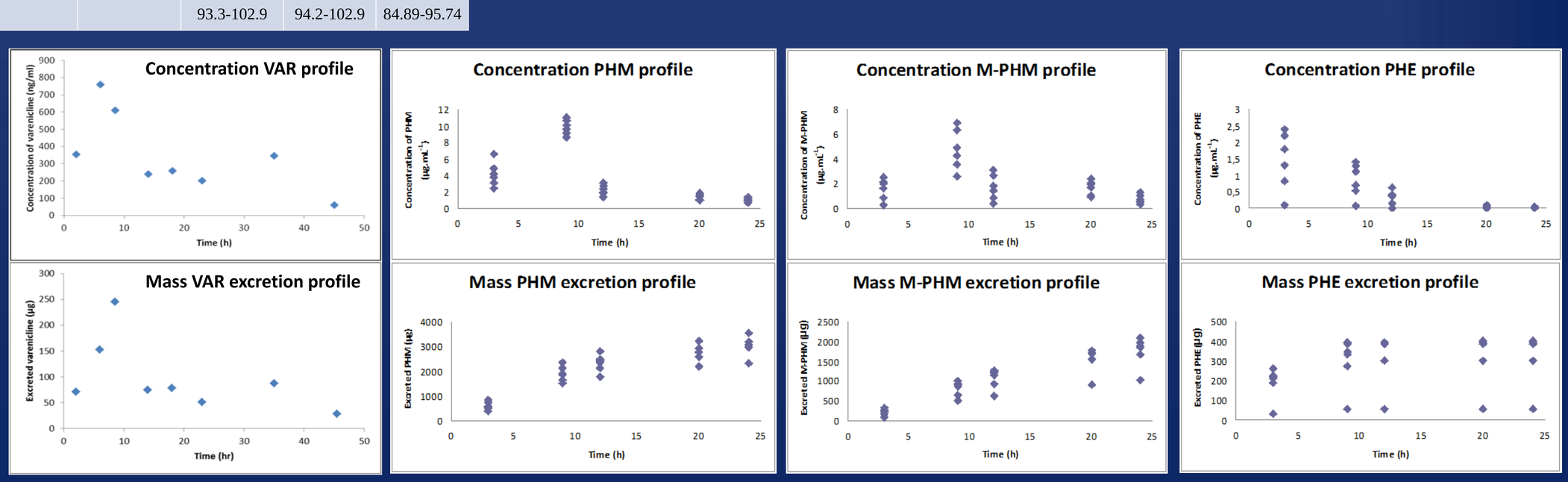
Performance parameters of ITP-CZE-ESI-QqQ method for varenicline (VAR), pheniramine (PHM) and phenylephrine (PHE).

Parameter	Water			Urine		
	PHM	PHE	VAR	PHM	PHE	VAR
t_m [min]	11.315	12.347	11.42	11.062	11.911	12.36
RSD_{tm} [%], n = 6	0.77	0.63	0.89	1.46	1.15	1.57
RSD_{area} [%], n = 6a	3.71	5.61	6.29	7.76	3.51	5.37
a [counts]	98.85	2.9721	-7.574	57.37	– 14.92	7.961
b [counts.ng^{-1}.mL]	1262.6	346.58	286.0	851.43	470.23	210.4
r^2	0.9973	0.9979	0.998	0.9995	0.9993	0.995
LOD [pg.mL^{-1}]	57.1	181.3	44.12	126.0	129.6	51.72
LOQ [pg.mL^{-1}]	190.3	604.4	144.2	421.5	431.8	154.6
N	53800	46700	18 600	28500	40600	33 980
H [µm]	3.01	3.62	49.60	5.75	3.99	26.98
R	4.84			3.40		
Recovery [%], n = 3				93.3-102.9	94.2-102.9	84.89-95.74

CZE-ESI-QqQ
Final separation and detection
(MRM, quantifier signals (extracted))



Time profiles of excreted drugs and their metabolites (varenicline VAR, pheniramine PHM, N-desmethyl pheniramine M-PHM, phenylephrine PHE) in human urine after administration of one dose of comercial pharmaceutical preparation (VAR in Champix®, PHM and PHE in Theraflu®) to healthy volunteers.



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