Highly sensitive determination of serotonin in human urine by 2D capillary electrophoresis with cyclodextrin separation environment and on-line sample pretreatment



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

An advanced 2D capillary electrophoresis method based on on-line combination of capillary isotachophoresis and capillary zone electrophoresis with cyclodextrin additive in background electrolyte was developed for effective determination of serotonin in human urine. Hydrodynamically closed separation system and large bore capillaries (300-800 mm) were chosen for the possibility to enhance the sample load capacity, and, by that, to decrease limit of detection. Isotachophoresis served for the sample preseparation, defined elimination of sample matrix constituents (sample clean-up), and preconcentration of the analyte. Cyclodextrin separation environment enhanced separation selectivity of capillary zone electrophoresis. In this way, serotonin could be successfully separated from the rest of sample matrix constituents migrating in capillary zone electrophoresis step so that human urine could be directly (i.e. without any external sample preparation) injected into the analyzer. Proposed method was successfully validated, showing favorable parameters of sensitivity (detection limit for serotonin was 2.32 ng.mL^{-1}), linearity (regression coefficient higher than 0.99), precision (repeatability of the migration time and peak area were in the range of 0.02-1.17% and 5.25-7.88%, respectively), and recovery (ranging in the interval of 90.0-93.6%). The developed method was applied for the assay of the human urine samples obtained from healthy volunteers. The determined concentrations of serotonin in such samples were in the range of 12.4-491.2 ng.mL^{-1} that was in good agreement with literature data. This advanced method represents highly effective, reliable, and low cost alternative for the routine determination of serotonin as a biomarker in human urine.

INSTRUMENTATION AND SAMPLE PREPARATION

The ITP–CZE experiments were performed with the use of a modular capillary electrophoresis analyzer EA-102 (Villa Labeco, Spisska Nova Ves, Slovakia), assembled in the column-coupling configuration of the separation units and operated in hydrodynamically (membrane) closed system. The sample was injected by a 30 µL internal sample loop which was a part of the injection valve of the analyzer. The ITP column was provided with an 800 µm I.D. capillary tube made of polytetrafluorethylene (PTFE). Its total length was 90 mm. A contactless conductivity detector was an integral part of the ITP column. The CZE column was provided with a 300 µm I.D. capillary tube made of PTFE of a 160 mm total length. In addition, during the optimization process of BGE composition in the CZE separation step, a 90 mm total length CZE column (with the same characteristics as the 160 mm column) was used. A 0.1% (*w*/*v*) concentration of m-HEC was employed in the separation electrolytes for a dynamic coating of the capillaries to suppress the electroosmotic flow. A photometric detector ECOM (ECOM, Prague, Czech Republic) was connected to an on-column photometric detection cell, integrated in the CZE column, via optical fibers. The detection wavelength was set at 276 nm. To control the separation process and to acquire data from the detectors, ITP Pro software version 1.0 (Villa Labeco, Spisska Nova Ves, Slovakia) was used. The experiments were carried out in a constant current mode at 20 °C (laboratory temperature). The driving currents applied were 300 µA (ITP) and 50 µA (CZE).

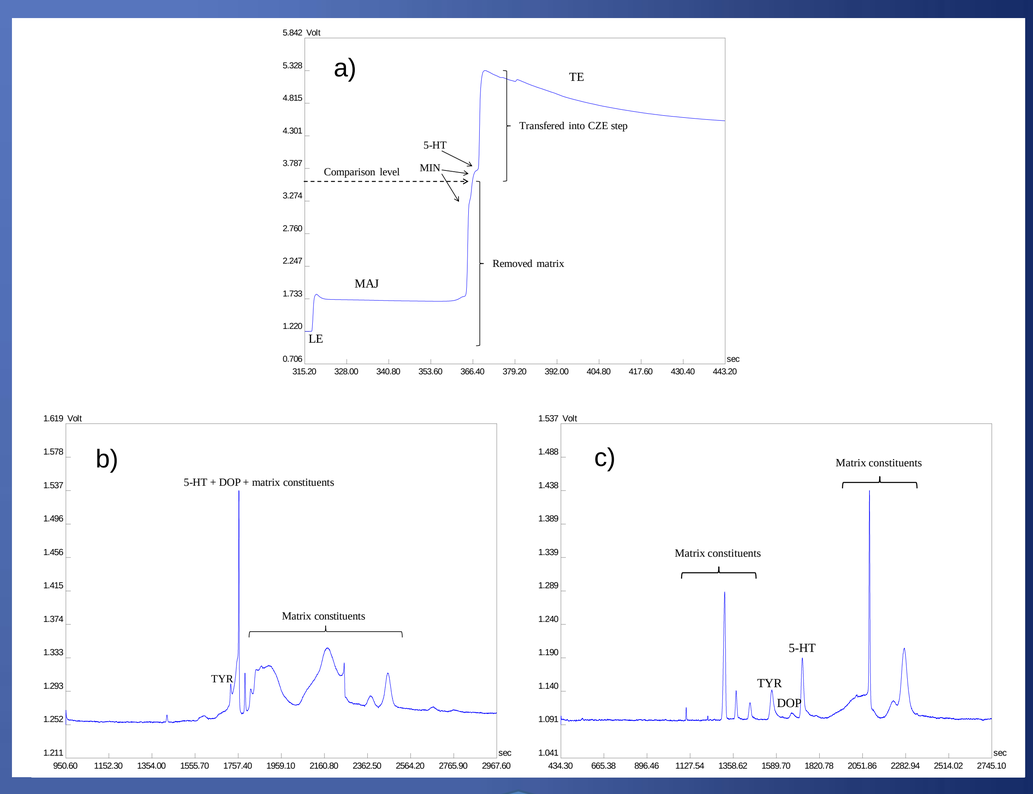
•The stock solution of 5-HT reference substance was prepared by dissolving 10 mg of the powder in 10 mL of demineralized water. Working solutions (calibration solutions and QC samples) of 5-HT were made by a proper dilution of the stock solution with demineralized water or by spiking human urine with the stock solution of 5-HT.

•Urine sample preparation. The first morning urine was taken from healthy adult volunteers. A 10 mL sample was divided into several vials and frozen (−18 °C) immediately after the sampling and kept in the freezer until the use. Prior to the analysis, the sample was melted and equilibrated for 30 min at room temperature. Each sample was centrifuged and 10-times diluted with demineralized water. After the dilution the sample was gently shaken and filtered through disposable membrane filters (0.22 µm pore size, Millipore, Molsheim, France) and immediately injected into the electrophoretic analyzer.

Calibration and selected separation parameters for serotonin in model water and human urine matrices

Performance Parameter	Water	Urine
Slope a	32.94	34.79
SD_a	0.6125	0.5553
Interface b	3.184	66.016
SD_b	0.2636	2.4500
r^2	0.9942	0.9957
Linear range (ng·mL^{-1})	2.07–82.86	4.14–82.86
LOD (ng·mL^{-1}]	1.24	2.32
LLOQ (ng·mL^{-1})	2.07	4.14
N	40,565	29,898
Migration time t_m (min)	27.36	28.73
RSD_{tm} (%)	0.03–0.57	0.02–1.17
RSD_{area} (%)	1.04–4.34	5.25–7.88

Separation efficiency (*N*) was calculated according to the equation $N = 5.545(t_m/w_{1/2})^2$



Isotachophoresis–capillary zone electrophoresis–ultraviolet (ITP–CZE–UV) analysis of model urine samples spiked with three biogenic amines—5-hydroxytryptamine (5-HT), dopamine (DOP), and tyramine (TYR). (**a**) Optimized ITP profile. (**b**) CZE profile without additives influencing separation of the analytes, background electrolyte (BGE): 25 mM ε-aminocaproic acid (EACA) + 50 mM acetic acid (HAc) + 0.1% m-HEC. (**c**) Optimized CZE profile with additives influencing separation. The human urine samples were diluted 10 times. The biogenic amines were present at 50 ng·mL^{-1} concentration levels of their hydrochloride salts (41.43 ng·mL^{-1} of 5-HT, 40.39 ng·mL^{-1} of DOP and 39·50 ng·mL^{-1} of TYR base). LE—leading electrolyte, MAJ—major sample matrix constituents, MIN—minor sample matrix constituents, TE—terminating electrolyte.

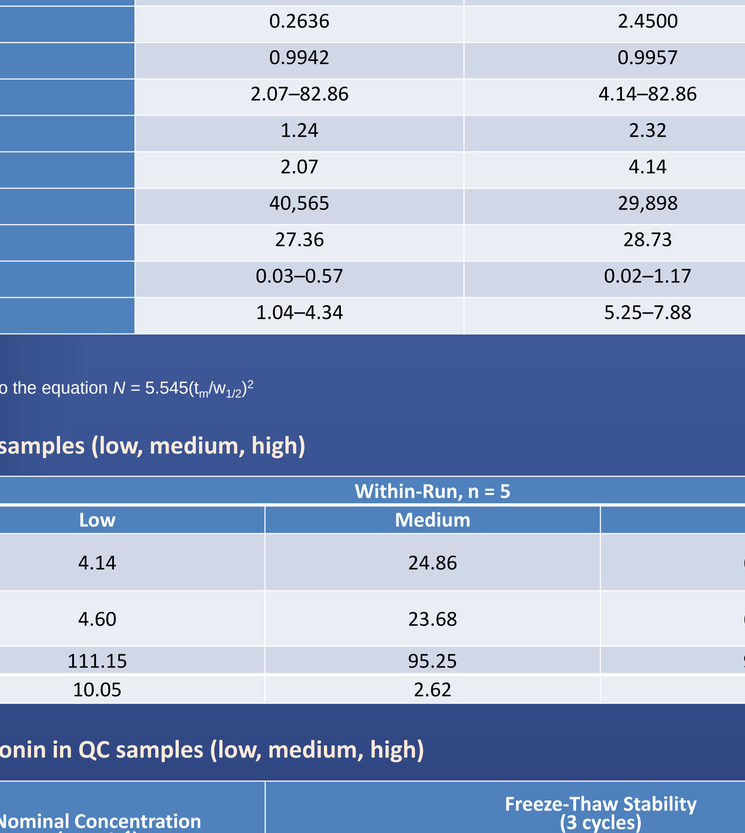
Accuracy and precision data from QC samples (low, medium, high)

Parameter	Within-Run, n = 5			Between-Run, n = 10		
	Low	Medium	High	Low	Medium	High
Nominal concentration (ng·mL^{-1})	4.14	24.86	66.28	4.14	24.86	66.28
Mean found concentration (ng·mL^{-1})	4.60	23.68	62.19	4.62	23.01	59.98
RE (% Nom.)	111.15	95.25	93.83	111.53	92.56	90.49
RSD (%)	10.05	2.62	6.37	12.46	5.69	7.99

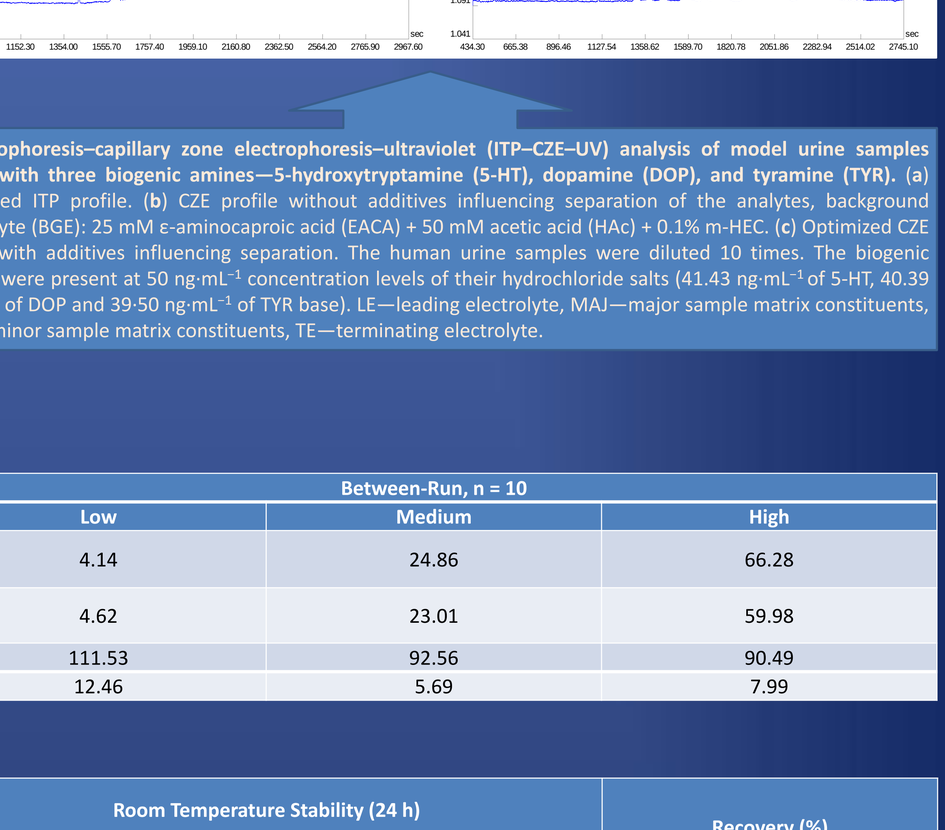
Stability and recovery testing of serotonin in QC samples (low, medium, high)

	Nominal Concentration (ng·mL^{-1})	Freeze-Thaw Stability (3 cycles)		Room Temperature Stability (24 h)		Recovery (%)
		Concentration Found (ng·mL^{-1})	Accuracy (%RE)	Concentration Found (ng·mL^{-1})	Accuracy (%RE)	
Low	4.14	4.46	7.73	4.27	3.14	90.00
Medium	24.86	25.85	3.99	23.79	−4.30	93.05
High	66.28	65.77	−0.76	60.75	−8.34	93.57

Effect of additives (isopropanol and HP–β–CD) on the CZE separation of mixture of three biogenic amines—5-HT, DOP, and TYR in model water matrix using ITP–CZE–UV experimental arrangement. Concentration levels of the injected hydrochloride salts of 5-HT, DOP, and TYR were 100 ng·mL^{-1} for each analyte that represented a 82.86 ng·mL^{-1} concentration of 5-HT, a 80.77 ng·mL^{-1} concentration of DOP, and a 79.00 ng·mL^{-1} concentration of TYR. Tested BGE compositions were following: 25 mM EACA + 50 mM HAc + 0.1% m-HEC (a) -, (b) + 10% isopropanol, (c) + 20% isopropanol, (d) + 30% isopropanol, (e) + 10 mM HP–β–CD, (f) + 10 mM HP–β–CD + 10% isopropanol, (g) + 10 mM HP–β–CD + 15% isopropanol, (h) + 10 mM HP–β–CD + 20% isopropanol. A 90 mm length of the CZE separation capillary was employed.



Electrophoretic profiles of urine samples taken from volunteers for screening of 5-HT concentrations. Concentration levels of 5-HT in urine samples obtained from three healthy volunteers (**a**–**c**) ranged in the interval of 12.36–491.23 ng·mL^{-1}. The human urine samples were diluted 10 times.



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