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RECENT ADVANCES IN DOPING ANALYSIS (12)

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Perspectives of capillary electrophoresis using dynamic capillary coating for routine screening procedures in antidoping analysis

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INTRODUCTION

The new list of doping substances and methods led to a deep reorganization of the internal screening procedures of our laboratory, in order to allow both the detection of the newly added substances/classes of substances, and to satisfy the Minimum Required Performance Limits (MRPL) fixed by the World Antidoping Agency (WADA) for all the searched compounds. This new regulatory situation, considered in combination to the need of a satisfying cost/benefit ratio for all the analytical methods of the laboratory, suggested us to evaluate the usefulness of new screening techniques, and primarily among them of capillary electrophoresis (CE), already widely diffused for the analysis of illicit drugs (1-3).

This work presents a CE method, specifically designed for the detection, in biological matrices, of beta-blockers, phenolalkylamines and other compounds banned in sport, based on the use of fused-silica uncoated capillaries and of a Ceofix kit system. The latter consists of two buffers, which produce a dynamic coating of the capillary surface, resulting in a uniform electro-osmotic flow (EOF) and in a parallel decrease of the interaction of the solutes with the capillary wall (3-5). The method is selective and reproducible, with a limit of detection of 50-500ng/ml, and a good linearity in the range 100-1000 ng/ml for most of the compounds here considered, matching the WADA MRPL for all beta blockers and phenolalkylamines, and reaching an even lower limit of detection for most of the considered beta agonists.

EXPERIMENTAL SECTION

Apparatus and methods

All CE experiments were performed using a P/ACE system MDQ (Beckman Coulter), with a built-in UV diode-array detector, interfaced to a personal computer. The applied

voltage was 22 kV for all the separations and temperature control (20°C) was used in all experiments. Detection was accomplished on-column by monitoring UV absorbance at 195 nm and 210 nm. Separations were performed using an uncoated silica capillary 60 cm × 75 μm I.D. Hydrostatic injection was applied for 3-5 s at 5 psi, followed by a 3 s flush with buffer.

The procedure for the CE run was the following: firstly, a buffer (initiator) containing polycations is injected to form a positively charged layer on the capillary surface; a second solution (accelerator) of polyanions is then introduced, to adsorb the positively charged layer thus forming a highly negatively charged layer, which is insensitive to pH changes, resulting in a strong and costant EOF. Before each run the capillary was rinsed with 0.1M NaOH, 1min with CElixir initiator solution and 2min with accelerator solution at controlled pH (2.5 for beta agonists and 6.2 for beta blockers/phenolalkylamines) at 20psi, and the separation voltage was 22 kV (normal polarity, ramp 0.5 s). In order to improve the migration time and the peak-shape reproducibilities, after each run the system was programmed for the following successive operations: 5 min rinsing with 0,1 M NaOH followed by 5 min with water and 5 min with running buffer.

Sample preparation

Beta-blockers and phenolalkilamine

To 5ml urine, 0.5 ml of acetate buffer (pH 5.2), 50μl of β-glucuronidase/arylsulfatase and 50μl ISTD (bambuterol) were added and incubated for three hours at 50°C. After hydrolysis 2 ml of carbonate buffer (pH 9) and 50μl of NaOH 2M were added to alkalinize the hydrolyzed solution. Extraction was carried out by 10 ml of *tert.*-butanol:diethylether 1:9 for 20 minutes; after centrifugation the organic phase layer was transferred and evaporated to dryness. The residue was re-dissolved in 50μl of deionized water and injected.

Beta-agonists

To 5ml urine, 0.5 ml of phosphate buffer (pH 7.4), 50μl of β-glucuronidase and 50μl ISTD (bisoprolol) were added and incubated for one hours at 50°C. The hydrolyzed urine was applied to a SPE column preconditioned with methanol (3 ml) and water (3 ml). The column was rinsed with water (1ml), 10% methanol in water (1 ml), and hexane (1ml), dried with nitrogen (3 min), and the retentate eluted with ethyl acetate (3 ml). The organic phase was evaporated to dryness, re-dissolved in 50μl of deionized water and injected.

RESULTS AND DISCUSSION

Good linearity (R^2 0.9876-0.9997) was obtained for beta-blockers, beta-agonists and phenolalkylamines within the 100-1000 ng/ml urine concentration range. The back-calculated concentrations for the standards showed acceptable deviation from the nominal values, the limits of detection (LOD) of the method was in the range 50-500ng/ml in urine (depending on the specific class of substances) with a signal to noise ratio 3:1. To assess the accuracy and precision of the CE assays, triplicate quality controls samples of beta-blockers, phenolalkylamines and beta-agonists in human urine at three different concentrations (300ng/ml; 500ng/ml; 1000ng/ml) were prepared and analysed: the results show good accuracy (≤ 15%) and precision (≤ 4%) for all quality controls. Figures 2-5 show representative electropherograms of standards of beta-blockers, phenolalkylamines and beta-agonists in human urine.

In principle, this technique is simpler, faster and less expensive than GC/MS analysis: (i) the derivatization step is not necessary; (ii) the run time for each assay is 20min shorter than the corresponding GC/MS technique (10 minutes instead of 32); and (iii) the cost of a complete CE system does not exceed 50000 €.

The same analytical approach can also be followed for the detection of all chemical structures that can be easily charged: preliminary results showed that this technique is also suitable for the screening analysis of diuretics and of other polar substances excreted either conjugated or unconjugated in the urine; while the analysis of less hydrophilic compounds (e.g. steroids or lipophilic drugs like modafinil) is more effectively carried out by other electrophoretic techniques, like MECK (micellar electrokinetic chromatography).

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Table 1

Substance	Linearity			Sensitivity (ng/ml)		Repeatability	
	R^2	Slope	Intercept	LOD	LOQ	RMT	CV%
Acebutolol	0.9971	0.0011	0.02	100	200	0.99	2.50
Alprenolol	0.9960	0.0040	0.05	200	300	0.89	1.35
Atenolol	0.9965	0.0050	0.03	100	250	0.93	3.90
Bamethane	0.9987	0.0044	-0.02	400	400	0.89	4.50
Betaxolol	0.9999	0.0038	0	100	200	0.98	1.34
Bupranolol	0.9997	0.0030	0	200	250	0.90	1.22
Carteolol	0.9987	0.0014	-0.01	400	400	0.94	2.54
Carvedilol	0.9876	0.0023	-0.05	300	350	0.94	2.67
Celiprolol	0.998	0.0015	0.01	300	350	1.02	2.45
Etilephrine	0.9969	0.0049	0.1	300	400	0.85	1.25
Dimetofrine	0.993	0.0110	0.0035	500	500	0.89	1.89
Pholedrine	0.9955	0.0058	0.05	100	200	0.81	1.67
Isosuprine	0.9976	0.0012	-0.05	500	500	0.97	0.93
Labetalol	0.9998	0.0005	0.03	400	400	1.04	4.98
Levobunolol	0.9985	0.0012	0	100	200	0.94	4.56
Moprolol	0.9986	0.0042	-0.02	300	350	0.94	2.78
Nadolol	0.9968	0.0035	0.07	200	300	0.87	3.65
Oxprenolol	0.9984	0.0021	0.04	100	200	0.97	1.38
Pindolol	0.9959	0.0026	0.05	100	200	0.95	3.80
Synephrine	0.9992	0.0065	0.01	300	300	0.90	0.87
Sotalol	0.9967	0.0033	-0.06	200	300	0.94	1.74
Timolol	0.9878	0.0006	0.01	100	200	0.88	1.90
Clenbuterol	0.9978	0.0026	0.002	50	100	0.89	1.35
Fenoterol	0.9967	0.0024	0.04	50	100	0.93	3.90
Procaterol	0.9998	0.0060	-0.001	50	100	0.89	4.50
Salbutamol	0.9976	0.0015	0.01	50	100	0.98	1.34
Salmeterol	0.9989	0.0056	0.04	100	200	0.90	1.22
Terbutaline	0.9976	0.0029	0.005	50	100	0.94	2.54
Tolbuterol	0.9997	0.0045	0.01	50	100	0.94	2.67

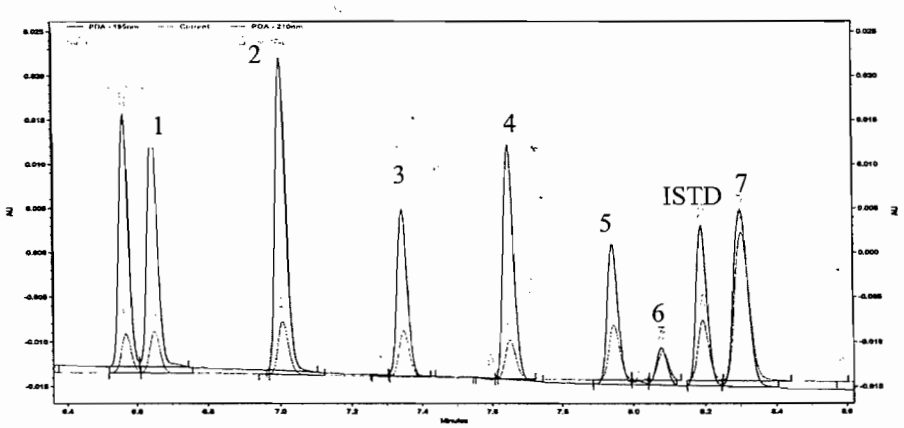

Figure 1. 1: pholedrine; 2: etilephrine; 3: alprenolol; 4: atenolol; 5: nadolol; 6: acebutolol; 7: celiprolol

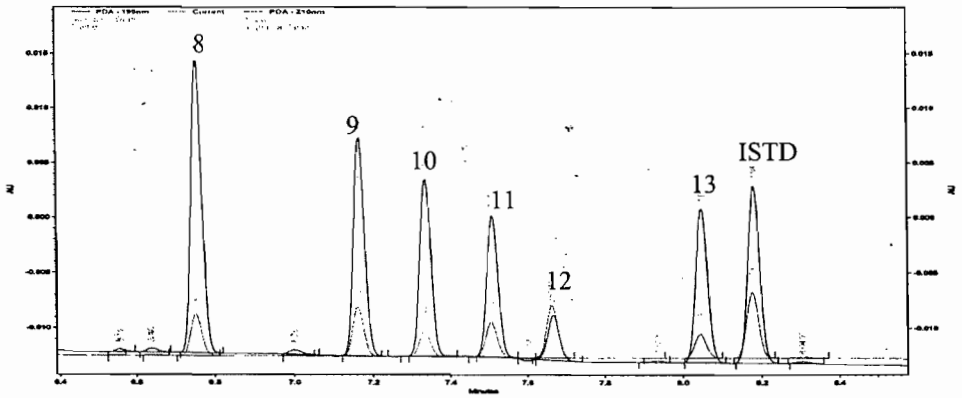

Figure 2. 8: synephrine; 9: moprolol; 10: bamethane; 11: sotalol; 12: levobunolol; 13: betaxolol.

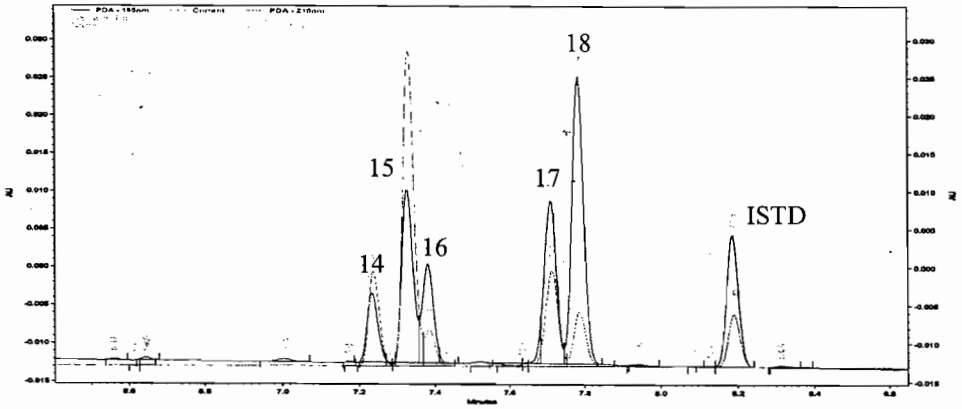

Figure 3. 14: pindolol; 15: dimetofrine; 16: oxprenolol; 17: carvedilol; 18: isosuprine.

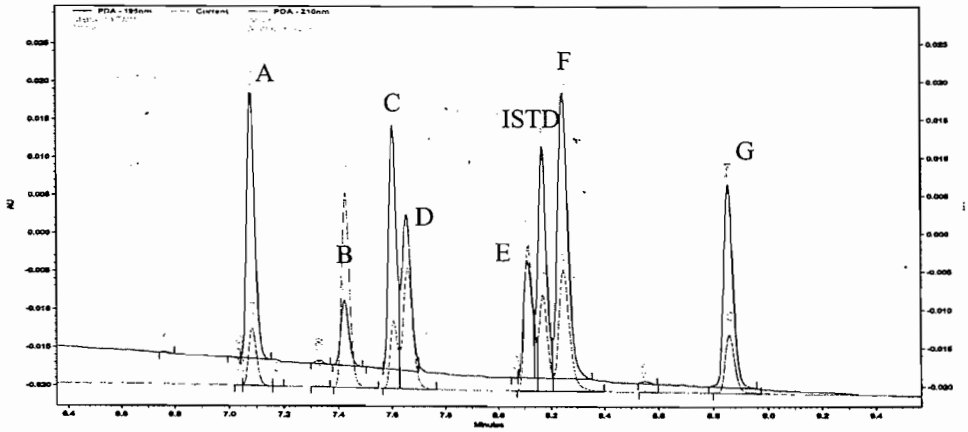

Figure 4. A: tulobuterol; B: clenbuterol; C: salbutamol; D: terbutaline; E: fenoterol; F: procaterol; G: salmeterol.