Monica Mazzarino[1]
Xavier de la Torre[1]
Franco Mazzei[2]
Francesco Botrè[1, 3]

[1]Laboratorio Antidoping, Federazione Medico Sportiva Italiana, Rome, Italy
[2]Dipartimento di Chimica e Tecnologie del Farmaco Università "La Sapienza", Rome, Italy
[3]Dipartimento per le Tecnologie, le Risorse e lo Sviluppo, Università "La Sapienza", Rome, Italy

Original Paper

Rapid screening of beta-adrenergic agents and related compounds in human urine for anti-doping purpose using capillary electrophoresis with dynamic coating

This paper presents a capillary electrophoresis method, developed for the detection, in human urine, of beta-adrenergic agents and phenolalkylamines. The electrophoretic separation is achieved in less than 10 min and is based on the use of CEofix kit, for the dynamic capillary coating. The effects of accelerator buffer pH and separation voltage were investigated. The optimum buffer pH was found to be 2.5 for beta2-agonists and 6.2 for beta-blockers and phenoalkylamines with a separation voltage of 15 kV. Urine samples spiked with the compounds here studied were treated according to the standard procedure (SPE and evaporation to dryness) and analyzed by CE interfaced with an UV diode-array, set at 195 and 210 nm. The quantitative validation results, obtained analyzing samples at three different concentrations, show a good precision of peak areas that do not exceed 5% for intra-day assays and 10% for inter-day assays. Good linearity ($r^2 > 0.995$) was obtained within the 50 – 500 ng/mL concentration range. The qualitative validation data show a relative migration times (MTs) variation lower than 1%. The analytes were clearly distinguishable in urine, with LOD and LOQ in the range of 10 – 80 and 40 – 100 ng/mL, respectively.

Keywords: Anti-doping analysis / Beta-adrenergic / Capillary electrophoresis / Dynamic capillary coating

Received: June 15, 2009; revised: August 2, 2009; accepted: August 3, 2009

DOI 10.1002/jssc.200900450

1 Introduction

Beta-adrenergic agents cover a wide range of chemical products and can be classified according to their chemical structure and/or their effect (agonist/antagonist) on the adrenergic receptor. Beta-blockers (or antagonists) can be divided into two groups: (i) one consists of a phenolic ring structure carrying an oxypropanolamine side chain that terminates either in a isopropyl or tertiary butyl group; (ii) the other consists of a substituted phenylethanolamine nucleus and is considered to be the less potent group of drugs [1] (see Fig. 1 for chemical structures). Beta2-agonists are mainly phenylethanolamines, variously substituted on their aryl moiety and terminal amino group [1] (see Fig. 1 for chemical structures).

Correspondence: Dr. Francesco Botrè, Scientific Director, Antidoping Laboratory of Rome, Largo Giulio Onesti 1, 00197 Rome RM, Italy
Email: francesco.botre@uniroma1.it
Fax: +39-06-8078971

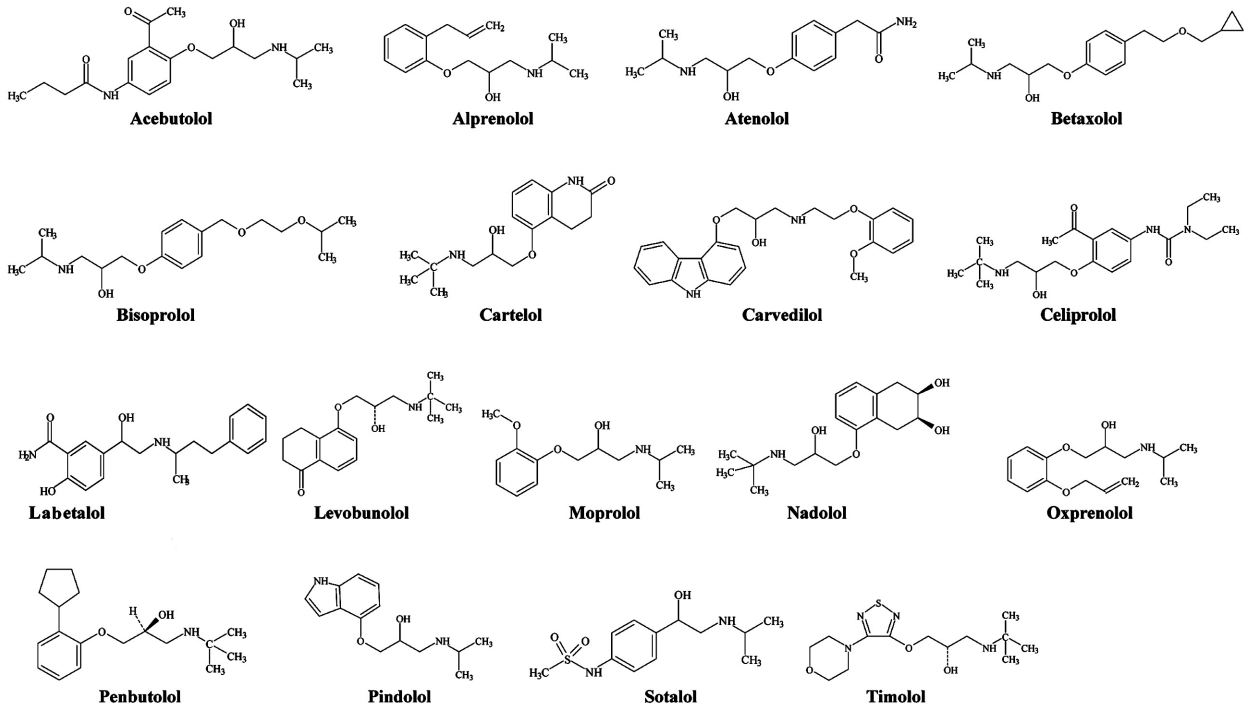
Abbreviations: GC/MS, gas chromatography/mass spectrometry; **ISTD,** internal standard; **MRPL,** minimum required performance level; **MT,** migration time; **WADA,** World Anti-doping Agency

The metabolism of these compounds depends directly on their physiochemical properties as shown by Bourne [2]. The more lipophilic substances are extensively metabolized to produce more water-soluble derivatives by oxidation, hydroxylation, or dealkylation following occasional conjugation to glucuronides or sulfates [2].

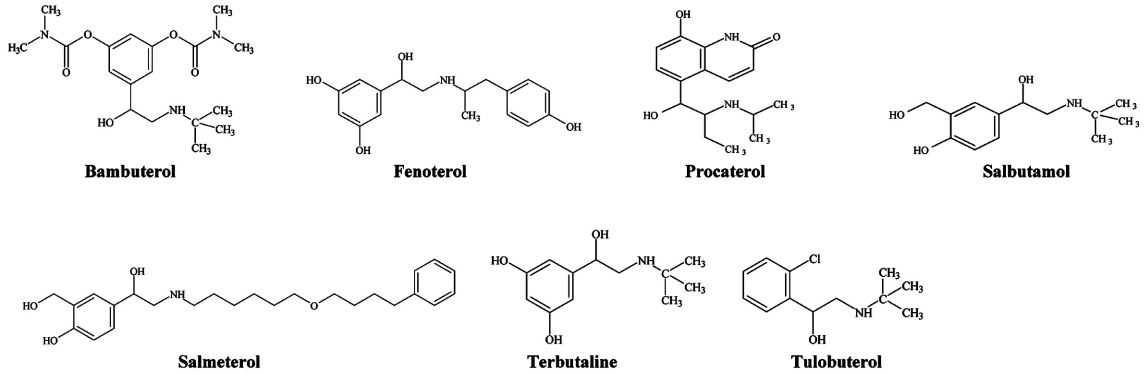
These drugs are included in the list of prohibited substances, published every year by the World Anti-Doping Agency (WADA) [3]. More specifically, beta-blockers are banned only for sports where the reduction of anxiety and control of hand tremor are important for a better sportive performance, whereas beta2-agonists are always banned as stimulants and at higher doses as anabolic agents.

Different kinds of techniques have been developed for monitoring these compounds in urine, such as enzyme immunoassay (EIA), RIA [4 – 6], gas chromatographic/mass spectrometric techniques (GC/MS) [7 – 12], and LC interfaced with different detectors such as UV, electrochemical, or MS [13 – 17]. However, GC/MS analysis for the compounds considered in this study requires a time-consuming derivatization step. In addition to the above, the instability of beta-blockers and phenolalkylamines *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) [7 – 9] and/or *N*-

a) Beta-blockers



b) Beta2-agonists



c) Phenolalkylamines

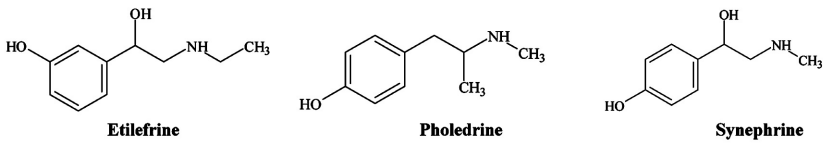


Figure 1. Chemical structures of the compounds here considered: (a) beta-blockers; (b) beta2-agonists; (c) phenoalkylamines.

methyl-*N*-bis-trifluoroacetamide (MBTFA) [10] derivatives does not permit reproducible quali-quantitative results.

CE can be an alternative efficient technique which is characterized by high separation efficiency and selectivity, high sample throughput, small sample consumption, robustness, and simple instrumentation [17 – 21]. Moreover, all CE modes employ the same instrumentation (samples can be easily analyzed subsequently as differencing separation mechanisms). At the present time, CE is a very promising alternative technique to HPLC and GC for clinical and forensic analysis and was utilized for separation of complex mixtures of target analytes (*e.g.*, abused drugs, diagnostic markers, *etc*.) from matrix without multistep sample preparation [22 – 26]. Moreover, the possibility to use wall-coated capillaries to mask the silanol groups on the capillary surface and therefore eliminate or modify the electro-osmotic flow (EOF) and reduce the interaction of the basic solutes with the capillary wall, permits an increase of migration time (MT) and peak area integration reproducibility. In this context a dynamic coating system has been developed by Analis SA, Belgium, and commercialized under the name CElixir or CEofix. This system consists of two buffers: a buffer containing polycations is injected first to form a positively charged layer on the capillary surface; the second buffer consisting of polyanions is then introduced. The polyanions adsorb to the positively charged layer and form a highly negatively charged layer, which is sensitive to pH changes, resulting in a strong and constant EOF, and consequently in a more reproducible peak timing.

The aim of this work was to develop a simple and rapid CE method for the screening analysis of basic drug such as, for example beta-adrenergic agents in human urine using the CEofix kit for the dynamic capillary coating. The developed method was applied to real urine samples and the results obtained have been then compared with those obtained by the reference methods, *i.e.*, the screening method – accredited according to the ISO17025 – presently followed for the analysis of beta-adrenergic agents, and phenolalkylamines by the WADA-accredited anti-doping laboratory of Rome [27, 28]. Although the method has been specifically designed and evaluated in view of its potential application for anti-doping analyses, it can be effective also in other areas of analytical toxicology.

2 Materials and methods

2.1 Chemicals and reagents

Acebutolol, acetic acid, alprenol, ammonium acetate, atenolol, bamethane (used as internal standard), bambuterol, betaxol, bisoprolol, carteolol, carvedilol, celiprolol, clenbuterol, dichloromethane, etilefrine, fenoterol, hydrochloric acid, isopropanol, labetalol, levobunolol, moprolol, nadolol, oxprenolol, penbutolol, pholedrine, pindolol, procaterol, salbutamol, synephrine, sotalol, terbutaline, timolol, tulobuterol were supplied by Sigma – Aldrich (Milano, Italy). Salmeterol was purchased from NMIA (National Measurement Institute of Australia, Pymble, Australia).

The Ceofix kit system, developed by Analis SA, Belgium, consists of two buffers: one buffer (initiator) containing a polycation and a second buffer (accelerator) consisting of a polyanion and of the background electrolyte (BGE), was purchased from Beckman Coulter (Torino, Italy).

All chemicals (ethanol, methanol, hexane, and ethyl acetate) were from Carlo Erba (Milano, Italy).

Stock solutions of the various beta-blockers, phenolalkylamines, and beta2-agonists were made up in methanol and stored in screwed cap vials at −20°C.

2.2 Sample preparation

To 3 mL of urine, 50 μL of the internal standard (ISTD: bamethane final concentration 300 ng/mL) were added. The urine sample was then applied to MCX OASIS (Waters, Milano, Italy) or STRATA XC (Phenomenex, Bologna, Italy) solid phase extraction (SPE) column (3 mL, phase: 60 mg) preconditioned with 2 mL of water and 2 mL of methanol. Once the sample was applied, the column was rinsed with 1 mL of methanol (10%) in acetate buffer (0.1 M, pH 4). The retentate was then eluted with 3 mL of dichloromethane/isopropanol (80:20) mixture. The organic phase was evaporated to dryness and dissolved in 10 μL of deionized water (to improve the solubility of the compounds containing amine groups and insoluble in water a little amount of HCl was added) for the CE analysis (injection condition: 1 psi for 10 s).

2.3 CE/UV procedure

All CE experiments were performed using a P/ACE system MDQ (Beckman Coulter, Torino Italy), with a built-in UV diode-array detector. A fused silica uncoated capillary (L: 50 cm; id: 75 μm; 60 cm effective length) and a CEofix kit were used for the separation.

CE conditions were set as follows: applied voltage 15 kV (80 μA) (normal polarity), capillary temperature 20°C. Signals at 195 and 210 nm were recorded for the quali-quantitative analysis.

The capillary was conditioned before each run for 2 min with NaOH 0.1 M, then it was first coated with the CEofix buffer containing a polycation (initiator: pH 2.5), then with the CEofix running buffer containing polyanions and the BGE (accelerator: different buffers were tested: 75 mM phosphate buffer, pH 2.5; 50 mM phosphate buffer, pH 6.2; and 150 mM borate buffer, pH 9.2). Sample was injected (the injection condition suggested

is 0.5 psi for 5 s, in this study we tested different injection conditions: 0.3, 0.5, and 1 psi for 5 and 10 s) followed by a water plug injection (0.1 psi for 10 s). Then voltage was applied (different separation voltage were tested; 15, 20, and 25 kV) between two vials containing the accelerator buffer. After separation, this dynamic coating is cleared by a rinse with NaOH and distilled water, 1 min each, and the capillary is ready for the next analysis.

The values of the urinary concentration of the compounds considered here were calculated by the peak areas of the detected signals relative to the ISTD bamethane.

2.4 Validation parameters

Experiments were performed, in urine, to determine all the parameters (namely: LOD, LOQ, linearity, selectivity, precision, accuracy, robustness, and recovery) necessary to validate a quali-quantitative screening method according to ISO 17025.

2.4.1 Limit of detection (LOD)

The LOD defined as the lowest concentration can be measured at a S/N $\geq$ 3, thus indicating the S/N of the least intense wavelength selected for the detection of each target compound. Ten different blank urine samples spiked with the internal standard (ISTD: bamethane final concentration 300 ng/mL) only and ten different blank samples fortified with different concentrations of the target compounds and with the ISTD were prepared and analyzed, according to the established protocol providing the data necessary to estimate the LOD.

2.4.2 Limit of quantification (LOQ)

The LOQ of an analytical method is the lowest measured concentration level that can be determined in a reproducible way. Ten different blank samples fortified with different concentration of the target compounds were prepared and analyzed, according to the established protocol providing the data necessary to estimate the LOQ.

2.4.3 Linearity

The calibration curve was obtained fortifying different aliquots of 3 mL of urine, with all the compounds studied here, at 10 equidistant concentration levels (50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ng/mL) prepared in triplicate. Averages of the triplicate were used to construct the calibration curve. The area ratios between each compound and internal standard (ISTD: bamethane final concentration: 300 ng/mL) were plotted *versus* the concentrations.

2.4.4 Selectivity

The selectivity of the method was tested analyzing 20 different urine samples prepared and analyzed using the method described above, in order to probe for interfering peaks in the electropherogram at the expected relative MTs of all the substances considered here. No significant interferences were found at the expected relative MTs of the analytes of interest, thus excluding the risk of false-positive results. In addition to this, the described techniques showed no carryover at the concentrations tested.

2.4.5 Precision

Precision can be divided in repeatability and reproducibility. Precision is calculated from the determination of a number ($n > 6$) of analyses of an identical sample.

a) Repeatability

The repeatability is defined as the similarity between successive measurements obtained under identical circumstances (same operator, same method, same equipment, and same time of analysis). Therefore, at least six negative samples were spiked with compounds of interest at three different levels (80, 200, and 500 ng/mL).

b) Reproducibility

The reproducibility is the level of correspondence between results obtained with the same analytical method but analyzed by different analysts and spread times. Therefore, at least six negative samples spiked at three different levels (80, 200, and 500 ng/mL) as for repeatability were analyzed by different persons.

2.4.6 Accuracy

The accuracy was defined as the difference between the calculated amount and the specified amount for the selected compound and expressed as a percentage. The accuracy was tested at three levels (80, 200, and 500 ng/mL).

2.4.7 Robustness

The robustness of the methods was demonstrated by analyzing the same reference sample (an originally negative urine spiked with all the analytes considered here) once a week for 7 weeks, randomly changing the operators involved in the instrumental analysis and in the preparation of the urine samples.

2.4.8 Recovery

The recovery of all compounds tested from urine by SPE extraction was determined at the minimum required performance level (MRPL) set by the WADA. Ten blank urine samples were fortified with all compounds studied before sample preparation, while another set of ten blank urines were extracted according to the described protocol followed by the same addition of all compounds studied into the organic layer before evaporation. To both the sets of samples, the ISTD was added into the

Table 1. Quali-quantitative validation results

Compound	r^2	LOQ (ng/mL)	RMT (MTc/MTistd)	LOD (ng/mL)	MRPL (ng/mL)
Beta-blockers					
Acebutolol	0.9971	80	0.99	50	500
Alprenolol	0.9960	80	0.89	50	500
Atenolol	0.9965	80	0.93	50	500
Betaxolol	0.9999	80	0.98	50	500
Bisoprolol	0.9930	80	0.89	50	500
Carteolol	0.9987	70	0.94	40	500
Carvedilol	0.9876	80	0.94	50	500
Celiprolol	0.9980	70	1.02	40	500
Labetolol	0.9998	70	1.04	40	500
Levobunolol	0.9985	70	0.94	40	500
Moprolol	0.9986	90	0.94	60	500
Nadolol	0.9968	70	0.87	40	500
Oxprenolol	0.9984	90	0.97	60	500
Penbutolol	0.9976	70	0.97	40	500
Pindolol	0.9959	100	0.95	80	500
Sotalol	0.9968	70	0.94	40	500
Timolol	0.9878	70	0.88	40	500
Beta2-agonists					
Bambuterol	0.9967	60	0.94	20	100
Fenoterol	0.9998	60	0.93	30	100
Procaterol	0.9976	50	0.89	20	100
Salbutamol	0.9989	50	0.98	20	100
Salmeterol	0.9989	50	0.90	20	100
Terbutaline	0.9976	50	0.94	20	100
Tulobuterol	0.9997	70	0.94	40	100
Phenolalkylamines					
Etilefrine	0.9969	90	0.85	60	500
Pholedrine	0.9955	90	0.81	60	500
Synephrine	0.9992	90	0.90	60	500

organic layer before evaporation. Recovery was then calculated by comparison of mean peak area ratios of analytes and ISTD of samples fortified prior to and after SPE extraction.

3 Results

The experimental data demonstrate that it is possible, using CE/UV, to carry out a rapid quali-quantitative screening in human urine sample of 24 beta-adrenergic agents (17 beta-blockers and 7 beta2-agonists) and 3 phenolalkylamines. Buffer pH (pHs tested: 2.5, 6.2, and 9.2), separation voltage (separation voltages tested: 15, 20, and 25 kV) and injection conditions (injection conditions tested: 0.3, 0.5, and 1 psi for a time of 5 and 10 s) were optimized to achieve an increase of separation efficiency, repeatability, and sensitivity (data not shown). The optimum buffer pH was found to be 2.5 for beta2-agonists and 6.2 for beta-blockers and phenoalkylamines with a separation voltage of 15 kV (normal polarity) and an injection pressure of 1 psi for a time of 10 s.

Good linearity ($r^2 > 0.995$) (see Table 1 for the r^2 results) was obtained in urine samples within the 50 – 500 ng/mL concentration range. To assess the accuracy, intra-day and inter-day precision of the method presented here, quality control samples (at least six for each concentration) spiked with the compounds studied at three different concentrations (80, 200, and 500 ng/mL) were prepared and analyzed under identical circumstances or/and using different analysts and spread times: the results obtained for the lowest quality control samples show a good precision of peak areas (RSD) not exceeding 5% for intra-day assays and 10% for inter-day assays, whereas the results obtained for the two high quality control samples show an excellent peak area precision (RSD) not exceeding 3% for intra-day assays and 8% for inter-day assays. The deviation of the mean measured concentration from the theoretical concentration for all compounds considered in this study was below the acceptable threshold of 15% for all the three levels tested. The repeatability of relative MTs is very satisfactory (RSD <1%). No significant interferences were found at the expected MTs of the analytes of interest, thus excluding the possibility of false positive results. Carryover signal was not detected in blank urine samples that were injected in a sequence after the analysis of the fortified urine samples at the highest concentration. The

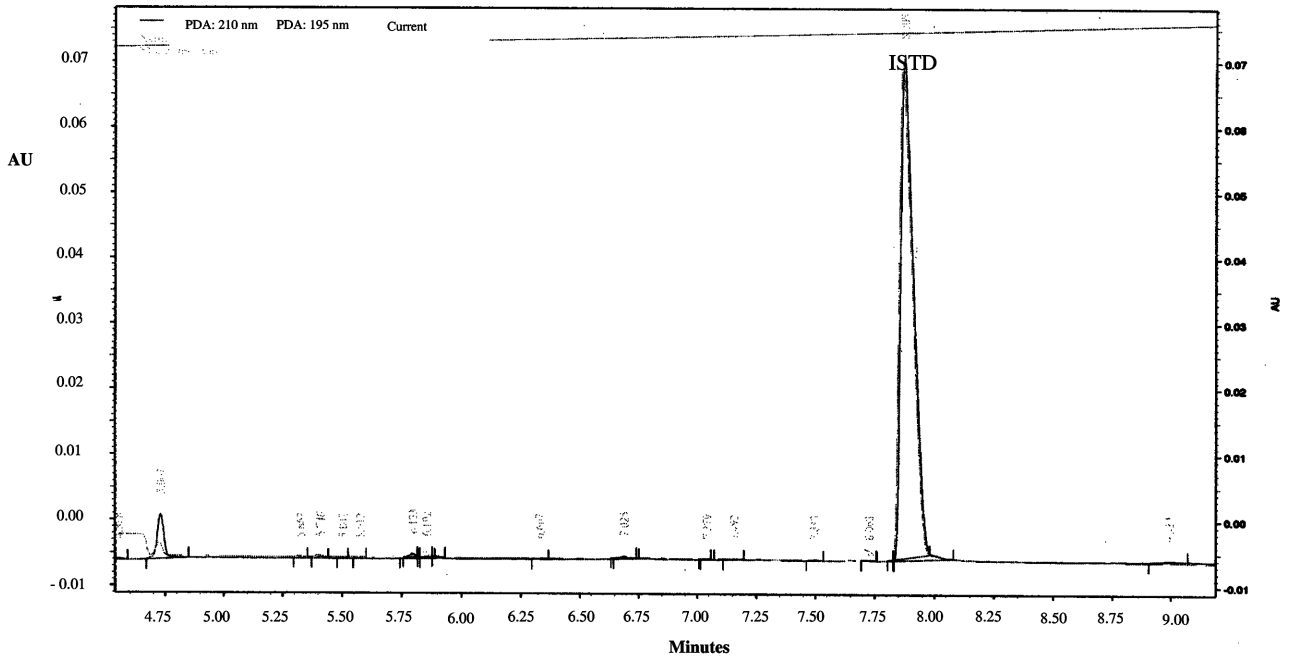
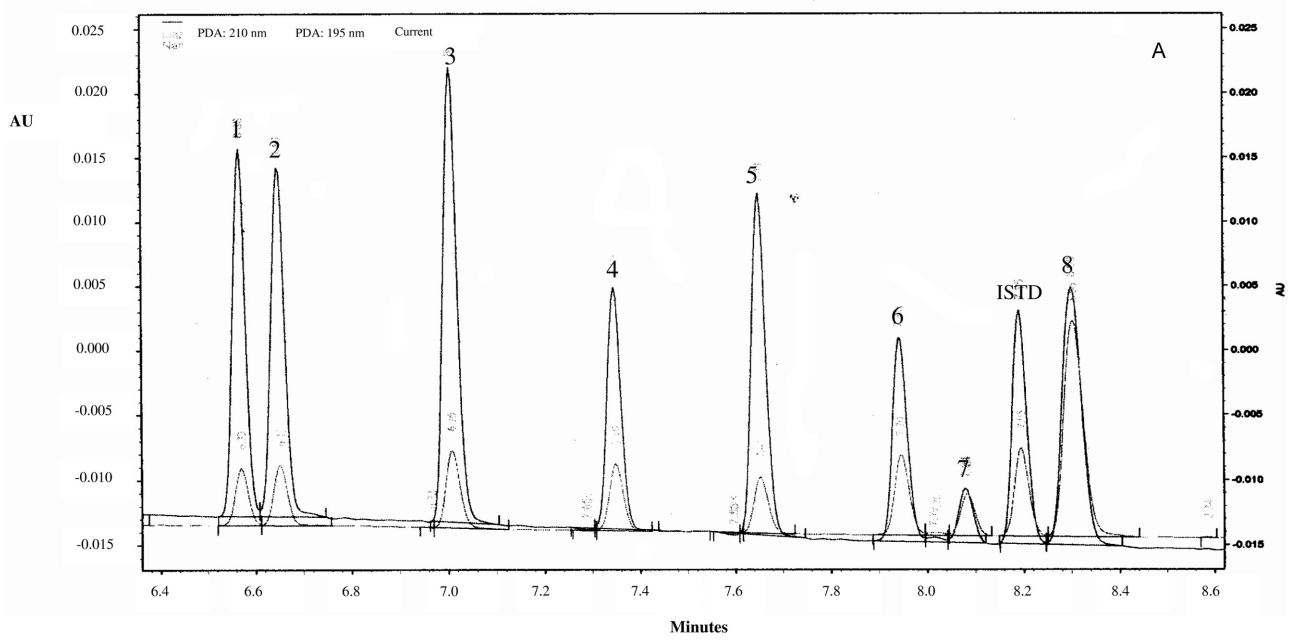


Figure 2. Electropherogram of blank urine. Signals at 195 and 210 nm.

LOQ were in the range of 40 – 100 ng/mL, whereas the LOD were in the range of 10 – 80 ng/mL, thus both significantly lower than the MRPLs for laboratories set by the WADA (see Table 1) [29]. Particularly, the requirements for the accredited laboratories include a MRPL of 500 ng/mL for the whole class of beta-blockers and phenoalkylamines and of 100 ng/mL for the beta2-agonists (with a cut off value set at 1000 ng/mL for salbutamol). At the end with both tested SPE columns (MCX OASIS and STRATA XC) a clean extract was obtained with a recovery higher than 70% for all compounds considered in this study.

The suitability of the developed method for routine analysis was checked by analyzing real samples found, during the normal laboratories routine carried out with the reference screening method, to be positive for the presence of one or more substances considered here.

More specifically, Figs. 2 – 4 show representative electropherograms of a blank urine and of a sample containing 17 beta-blockers and 2 phenolalkylamines (Figs. 3A – C), to a final concentration of 300 ng/mL, and a sample containing all beta2-agonists considered in this study (Fig. 4), to a final concentration of 300 ng/mL, confirming



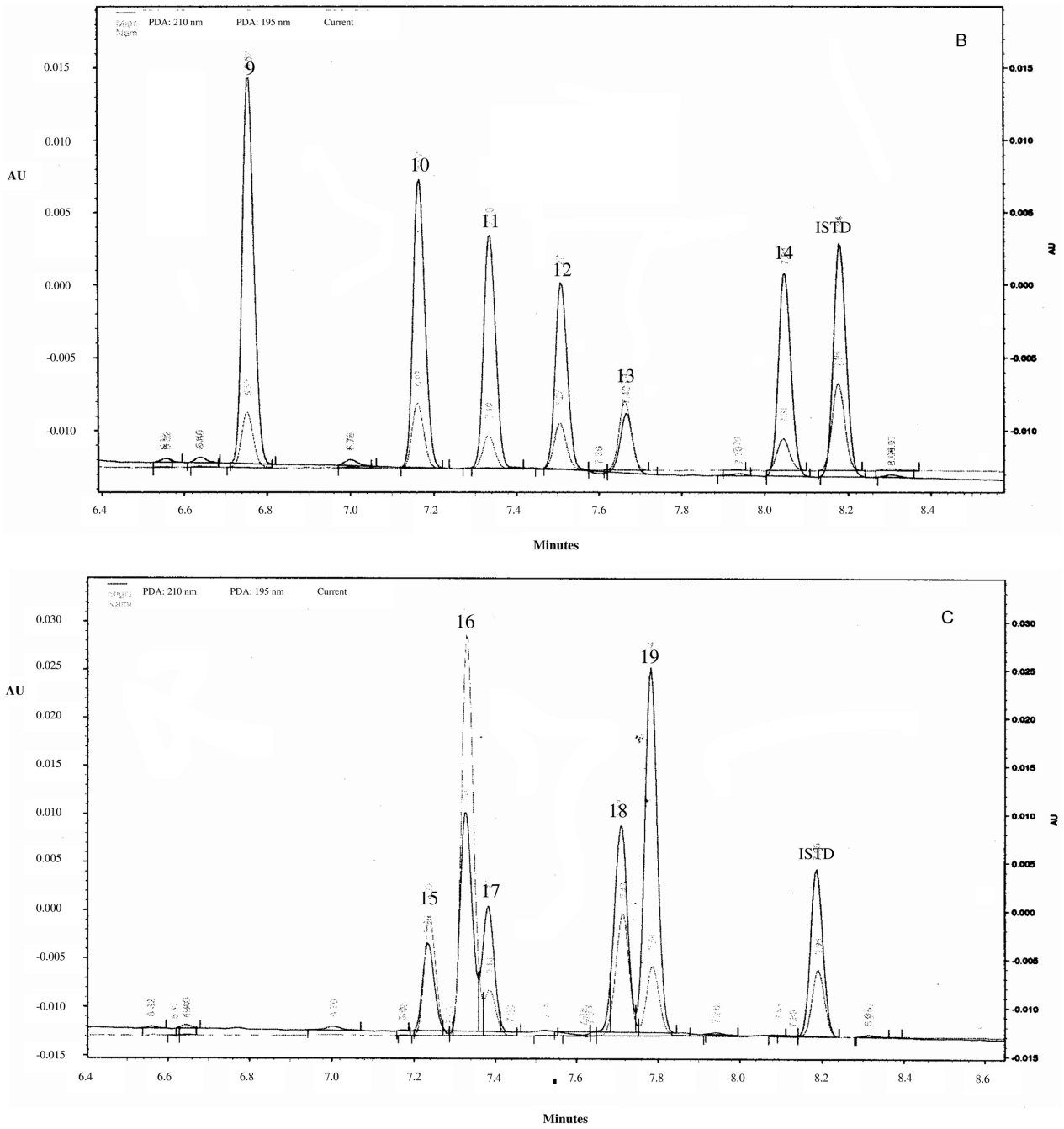


Figure 3. (A) Electropherogram of spiked urine. Peak identity: 1, synephrine; 2, etilefrine; 3, alprenolol; 4, atenolol; 5, nadolol; 6, acebutolol; 7, celiprolol; 8, timolol. Signals at 195 and 210 nm. (B) Electropherogram of spiked urine. Peak identity: 9, bisoprolol; 10, moprolol; 11, propranolol; 12, sotalol; 13, levobunolol; 14, betaxolol. Signals at 195 and 210 nm. (C) Electropherogram of spiked urine. Peak identity: 15, pindolol; 16, carteolol; 17, oxprenolol; 18, carvedilol; 19, penbutolol. Signals at 195 and 210 nm.

that in less then 10 min all analytes considered in this study were clearly distinguishable from matrix interferences.

4 Discussion

Analytical techniques based on CE can be used to complement GC and HPLC methods because of their high efficiency, accuracy, very high resolution, and tolerance to biological matrices. Moreover, this technique is more efficient, simpler, faster, and less expensive than GC/MS or LC/MS analysis, especially in term of solvents and supplies consumption.

The poor sensitivity, due to low short optical pathlength, of CE with on-column UV detection can be

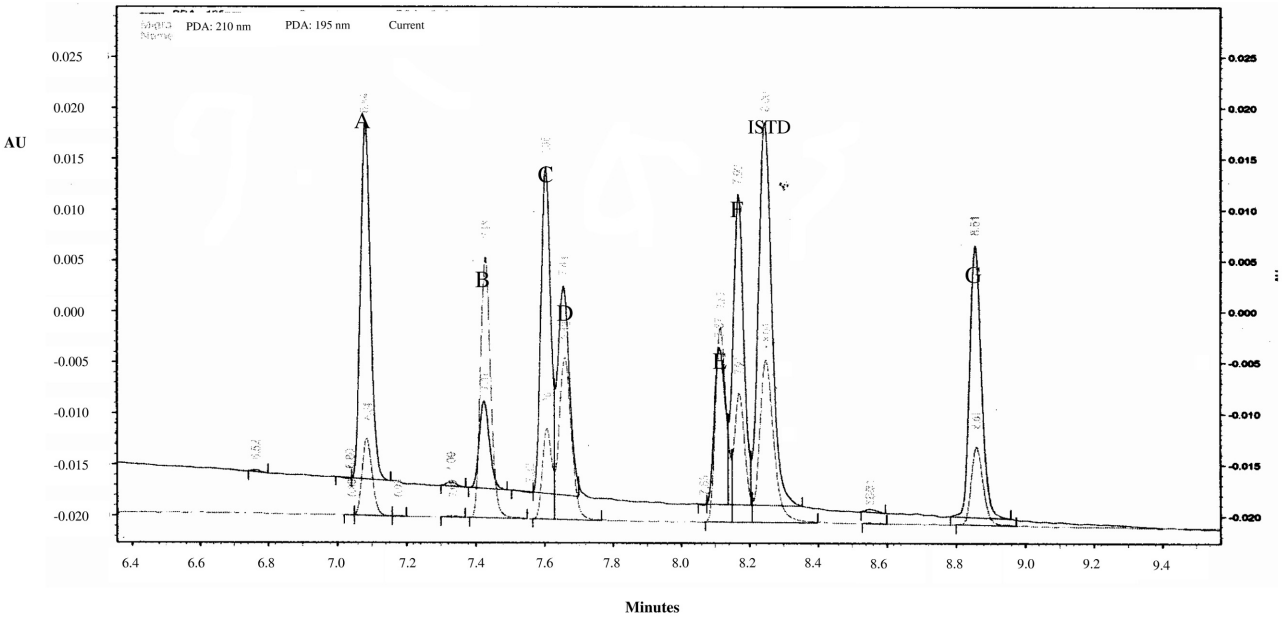


Figure 4. Electropherogram of spiked urine. Peak identity: A tulobuterol; B bambuterol; C salbutamol; D terbutaline; E fenoterol; F procaterol; G salmeterol. Signals at 195 nm and 210 nm.

improved using different online or/and offline concentration methods developed in previous researches [30 – 36]. Previous approaches in the forensic field were applied to the screening of some drugs of abuse, diuretics, steroids, and beta-blockers, using both CE and micellar electrokinetic chromatography (MEKC) [37 – 41]. Here we have developed a simple and rapid capillary electrophoretic method for screening analysis of beta-adrenergic agents and other basic compounds included in the official list of doping substances and methods, using a CEofix kit for the dynamic capillary coating. This patented double coating stabilizes the electroendosmosis (EEO) improving migration-time reproducibility and peak shape of basic drug. On the basis of our experiments a good MT and area integration reproducibility were obtained for all compounds tested; moreover, production of peak tailing was not observed. Other observations accomplished with the results obtained were reported: (i) Although the CE technique is characterized by high separation efficiency, in our study it was not possible to separate all compounds also at the lowest separation voltage (15 kV) tested. Nevertheless the possibility to clearly distinguish all analytes from matrix interferences is sufficient for a screening method. (ii) It is possible to set up a unique screening method, using the same accelerator buffer pH even if the optimum pH was found to be 2.5 for beta2-agonists and 6.2 for beta-blockers and phenoalkylamines. (iii) Thanks to the very low sample volume injected, it is possible to increase the method sensitivity using different online or/and offline concentration methods, and/or using different injection conditions. In this study we tested different pressures and times obtaining the best sensitivity at pressure of 1 psi for a time of 10 s. (iv) The LODs for most of the target compounds considered in the present study are significantly lower than MRPL for laboratories established by WADA (see Table 1) ensuring that the detection of a prohibited substance is often possible days after administration.

In conclusion a highly sensitive method with good reproducibility based on dynamic capillary coating for the detection of beta-adrenergic agents and phenolalkylamines in urine samples was developed in the presented work. Our approach is suitable as screening method on the occasion of "in and/or out competition" sport antidoping control tests, matching, the requirements of the WADA for the accredited anti-doping laboratories.

Additional experiments are currently in progress to verify the suitability of the proposed method for the detection of other drugs that can easily be charged.

This work has been supported in part by a Research Grant of the Italian Department of Health ("Ministero della Salute, Commissione per la vigilanza sul doping e sulla tutela sanitaria delle attività sportive").

The authors declared no conflict of interest.

5 References

[1] Weiner, N., Drugs that inhibit adrenergic nerves and block adrenergic receptors, in: *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Eds.: Goodman Gilman, A., Goodman, L. S., Rall, T. W., Murad, F.), 7th ed., Macmillan Publishing Company, New York 1985, pp 192 – 203.

[2] Bourne, G. R., *Prog. Drug Metab.* 1981, *6*, 77 – 80.

[3] *The World Anti-Doping Code, The 2009 Prohibited List International Standard*, World Anti-Doping Agency, Montreal, Canada 2009. www.wada-ama.org.

[4] Howells, L., Sauer, M., Sayer, R., Clark, D., *Anal. Chim. Acta* 1993, *275*, 275 – 278.

[5] Ploum, M. E., Haasnoot, W., Paulussen, R. J. A. *et al.*, *J. Chromatogr.* 1991, *564*, 413 – 427.

[6] Pickett, R. J. H., Sauer, M. J., *Anal. Chim. Acta* 1993, *275*, 269 – 273.

[7] Donike, M., Derenbach, J., *Fresenius Z. Anal. Chem.* 1976, *279 (2)*, 128 – 135.

[8] Donike, M., Gola, R., Jaenicke, L., *J. Chromatogr.* 1977, *134*, 385 – 390.

[9] Hemmersbach, P., de la Torre, R., *J. Chromatogr.* 1996, *687*, 221 – 225.

[10] Contrade, M. P., Le Bizec, B., Monteau, F., Siliart, B., Andre, F., *Anal. Chim. Acta* 1993, *275*, 253 – 268.

[11] Polettini, A., Ricossa, M. C., Groppi, A., Montagna, M., *J. Chromatogr.* 1991, *564*, 529 – 535.

[12] Amendola, L., Molaioni, F., Botrè, F., *J. Pharm. Biomed. Anal.* 2000, *23*, 211 – 221.

[13] Botterblom, M. H. A., Feenestra, M. G. P., Erdtsieck-Ernste, E. B. H. W., *J. Chromatogr.* 1993, *613*, 121 – 126.

[14] Hamann, J. A., Johnson, K., Jeter, D. T., *J. Chromatogr. Sci.* 1985, *23*, 34 – 36.

[15] Haasnoot, W., Ploum, M. E., Paulussen, R. J. A., Schilt, R., Huf, F. A., *J. Chromatogr.* 1990, *519*, 323 – 335.

[16] Leloux, M. S., Dost, F., *Chromatographia* 1991, *32*, 429 – 435.

[17] Chen, F. T. A., Evangelista, R. A., *Clin. Chem.* 1994, *40*, 1819 – 1822.

[18] Boone, C. M., Waterval, J. C. M., Lingeman, H., Ensing, K., Underberg, W. J. M., *J. Pharm. Biomed. Anal.* 1999, *20*, 831 – 863.

[19] Caslavska, J., Allemann, D., Thormann, W., *J. Chromatogr. A* 1999, *838*, 197 – 211.

[20] Ševcík, J., Lemr, K., Smysl, B., Jirovsky, D., Hradil, P., *J. Liq. Chromatogr. Relat. Technol.* 1998, *21*, 2473 – 2784.

[21] Petersen, J. R., Okorodudu, A. O., Mohammad, A., Payne, D. A., *Clin. Chim. Acta* 2003, *330*, 1 – 30.

[22] Tagliaro, F., Bortolotti, F., *Electrophoresis* 2006, *27*, 231 – 243.

[23] Brettell, T. A., Butler, J. M., Saferstein, R., *Anal. Chem.* 2005, *77*, 3839 – 3860.

[24] Thormann, W., Lurie, I. S., McCord, B., Marti, U., Cenni, B., Malik, N., *Electrophoresis* 2001, *22*, 4216 – 4243.

[25] Weinberger, R., Lurie, I. S., *Anal. Chem.* 1991, *63*, 823 – 827.

[26] Hilhorst, M. J., Somsen, G. W., de Jong, J., *Electrophoresis* 2001, *22*, 2542 – 2564.

[27] Botrè, F., Borrelli, R., Colamonici, C., Garribba, F., Mazzarino, M., Rossi, F., in: *Recent Advances in Doping Analysis* (Eds.: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U.), Vol. 13, Sportverlag Strauß, Köln, Germany 2005, pp. 11 – 20.

[28] Mazzarino, M., de la Torre, X., Botrè, F., *Anal. Bioanal. Chem.* 2008, *392*, 681 – 698.

[29] *WADA Technical Document TD2007MRPL, Minimum required performance levels for detection of prohibited substances*, World Anti-Doping Agency, Montreal, Canada 2009. www.wada-ama.org.

[30] Quirino, J. P., Terabe, S., *J. Chromatogr. A* 1997, *781*, 119 – 128.

[31] Quirino, J. P., Terabe, S., *J. Chromatogr. A* 1997, *791*, 255 – 267.

[32] Quirino, J. P., Terabe, S., *Anal. Chem.* 1998, *70*, 149 – 157.

[33] Quirino, J. P., Terabe, S., *J. Chromatogr. A* 1998, *798*, 251 – 257.

[34] Quirino, J. P., Terabe, S., *Anal. Chem.* 1998, *70*, 1893 – 1901.

[35] Quirino, J. P., Otsuka, K., Terabe, S., *J. Chromatogr. B* 1998, *714*, 29 – 38.

[36] Quirino, J. P., Terabe, S., *Science* 1998, *282*, 465 – 468.

[37] Thormann, W., Aebi, Y., Lanz, M., Caslavska, J., *Forensic Sci. Int.* 1998, *92*, 157 – 183.

[38] Tagliaro, F., Turrina, S., Pisi, P., Smith, F. P., Marigo, M., *J. Chromatogr. B* 1998, *713*, 27 – 49.

[39] Anastos, N., Barnett, N. W., Lewis, S. W., *Talanta* 2005, *67*, 269 – 279.

[40] Boone, C. M., Douma, J. W., Franke, J. P., de Zeeuw, R. A., Ensing, K., *Forensic. Sci. Int.* 2001, *121*, 89 – 96.

[41] Maguregui, M. I., Barroso, M. B., Alonso, R. M., Jimenez, R. M., in *Recent Advances in Doping Analysis* (Eds.: Schänzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U.), Vol. 5, Sport & Buch Staub, Köln Germany, 1997, pp. 303 – 304.

