

Amperometric and Spectrophotometric Detection of Aminobiphenyls and Aminonaphthalenes in HPLC

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Abstract: A new method for the determination of selected aminobiphenyls (ABs) and aminonaphthalenes (ANs), proved or suspected carcinogens and mutagens, was developed using reversed-phase HPLC coupled to an amperometric platinum tubular detector and a spectrophotometric detector. Using mobile phase consisting of acetonitrile and phosphate buffer pH 2.5 (40:60, v/v), separation of 2- and 4-aminobiphenyl and 1- and 2-aminonaphthalene was achieved within 10 min and limits of determination between $3 \cdot 10^{-8} \text{ mol L}^{-1}$ and $8 \cdot 10^{-8} \text{ mol L}^{-1}$ were obtained for amperometric detection. In comparison with spectrophotometric detection, it is superior in both sensitivity and selectivity. The developed method was applied on the analysis of spiked samples of urine. Solid phase extraction was used for a preliminary separation and preconcentration of the tested analytes, leading to the decrease of limits of determination to the values between $7.2 \cdot 10^{-9} \text{ mol L}^{-1}$ and $2.7 \cdot 10^{-8} \text{ mol L}^{-1}$.

Keywords: Aminobiphenyls; Aminonaphthalenes; Platinum tubular electrode; Urine; Solid phase extraction; HPLC; Amperometric detection; Spectrophotometric detection.

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Introduction

Aminobiphenyls (ABs) and aminonaphthalenes (ANs) are, as well as other amino-derivatives of polycyclic aromatic hydrocarbons, proved or suspected carcinogens and mutagens [1, 2]. Among them, 4-aminobiphenyl (4-AB) and 2-aminonaphthalene (2-AN) are particularly dangerous, belonging to the Group 1 (the so-called "proven human carcinogens") according to the IARC classification [3].

The occurrence of ABs and ANs in the environment is associated with their utilization in chemical industry, where they serve as a raw material or intermediates in manufacturing of pesticides, dyestuffs, polymers and other industrial chemicals [4]. The manufactured products might contain residues of these compounds and the contamination of the environment by the production waste is possible [5-7]. Besides, they are also produced during the combustion of organic compounds, particularly of fossil fuels, and during smoking [8]. ANs can serve as markers to the exposition to nitronaphthalenes, another group of hazardous substances, which transform to amino derivatives during metabolizing [9,10]. The impact of these compounds on human health leads to the increased effort in development of analytical methods for their determination.

Among them, chromatographic methods dominate mainly due to the occurrence of ABs and ANs in complicated matrices. UV-VIS spectrophotometric detection is common in HPLC [11, 12], fluorescence detection is also used [13]. Gas chromatography employs flame-ionization [14] or mass spectrometric detector [15, 16]. The presence of easily oxidizable amino group offers the possibility to use electrochemical methods, based both on voltammetric techniques [17, 18] and on electrochemical detection in combination with HPLC [6,19-21]. Several indicator electrodes were used, including glassy carbon electrodes [6], carbon paste electrodes [17, 21], and boron-doped diamond film electrodes [18, 20].

Various settings for the construction of tubular detector were applied in the past, using platinum, gold or graphite as the electrode material [22-25]. The arrangement employed in this work and introduced by Cvačka et al. [26] consists of platinum tube with applied voltage inserted in the outlet of the Teflon tubing. In spite of its relatively high geometric volume, its effective volume is low, as only a small part near the end of the tube is employed in electrooxidation of the analytes. Together with a simple and robust construction, the detector exhibits advantageous sensitivity.

This work deals with the development of a method for the determination of selected ABs and ANs (namely 2-aminobiphenyl (2-AB), 4-aminobiphenyl (4-AB), 1-amino-

naphthalene (1-AN), and 2-aminonaphthalene (2-AN)) using reversed-phase HPLC coupled to spectrophotometric and amperometric platinum tubular detector. Practical applicability of the method was tested on the determination of spiked samples of urine after the preconcentration and preliminary separation of the analytes by solid phase extraction.

Experimental

Chemicals and Reagents

The $1 \cdot 10^{-4}$ mol L⁻¹ stock solutions of 2-AB, 4-AB (both Sigma-Aldrich, 97%), 1-AN (Sigma-Aldrich, 98%), and 2-AN (Sigma-Aldrich, 95%) were prepared by dissolution of exact mass of each compound in deionized water (Millipore Q-plus System, Millipore, USA) and kept in the dark at laboratory temperature. Acetonitrile (for HPLC, Merck, Germany) was used as the organic part of the mobile phase. The aqueous part of the mobile phase was phosphate buffer consisting of 0.01 mol L⁻¹ disodium hydrogen phosphate (p.a., Lachema, Czech Republic), whose pH was adjusted by the addition of concentrated phosphoric acid (p.a., Lach-Ner, Czech Republic). Other used chemicals were: methanol (p.a., Merck, Czech Republic), diethyl ether (p.a., Lachema, Czech Republic), ethyl acetate, sodium hydroxide, and concentrated sulphuric acid (all p.a., Lach-Ner, Czech Republic).

Apparatus and Other Instrumentation

HPLC system consisted of a high-pressure pump HPP 5001 (Laboratorní přístroje Praha, Czech Republic), precolumn LiChroCART[®] PAH 4-4 (Merck, Germany), column LiChrospher[®] 100 RP-18, 5 μm, 125×4 mm (Merck, Germany), UV spectrophotometric detector LCD 2083 (ECOM, Czech Republic) and amperometric detector ADLC 2 (Laboratorní přístroje Praha, Czech Republic). UV spectrophotometric detector (detection wavelength of 290 nm) and amperometric detector were connected in series. The system was controlled by software CSW 32 Chromatography station (DataApex, Czech Republic) working under Windows 98 (Microsoft, USA). The injected volume was 10 μL and the flow rate F_M was 1 mL min⁻¹. All measurements were carried out at laboratory temperature. The mobile phase was degassed by 10min sonication using PSO 2000A Ultrasonic Compact Cleaner (Powersonic, USA).

The amperometric detection was realized in a three-electrode arrangement with platinum tubular indicator electrode (see Fig. 1). The indicator electrode was made of a 15 mm piece of platinum tubing (300 μm o.d., 150 μm i.d., geometric area ca 7.07 mm^2 , geometric volume 265 nL) fixed in Teflon tubes (1/16" o.d., 0.010" i.d.) on both sides. The free end of shorter Teflon tube was cut flat with the end of platinum tube, the other end served for the connection to the chromatographic system [26]. The electrode was immersed in an overflow vessel together with Ag/AgCl (3 mol L^{-1} KCl) reference electrode (ETP CZ-R00408), and Pt-auxiliary electrode (both Elektrochemické detektory, Turnov, Czech Rep.).

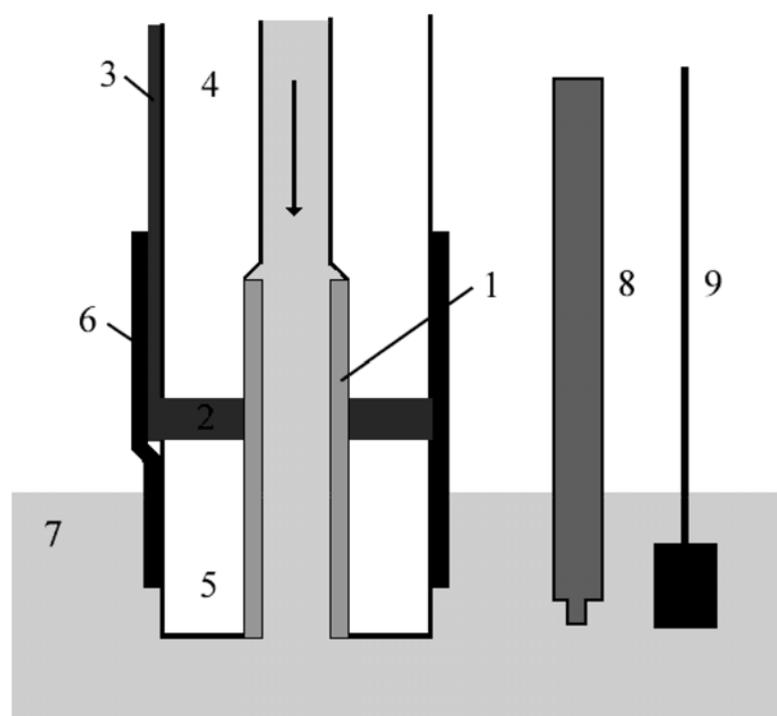


Figure 1. Scheme of electrochemical platinum tubular detector: 1 – platinum tube; 2 – tin solder; 3 – electrical contact; 4 and 5 – Teflon tubes; 6 – shrinkable tube; 7 – electrolyte solution in overflow vessel (mobile phase); 8 – reference electrode; 9 – counter electrode.

The tubular electrode was activated once a day in $5 \cdot 10^{-2} \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ by switching 10 times between the potential -0.3 V and $+1.4 \text{ V}$, applying the potentials always for 10 s.

For solid phase extraction (SPE), LiChrolut[®] EN 200 mg/3 mL polypropylene tubes (Merck, Germany) and vacuum manifold were used.

Procedures

Solid phase extraction was performed as follows: the solid phase was conditioned on a vacuum manifold with 3 mL of ethyl acetate followed by 3 mL of methanol and 3 mL of deionized water, which was allowed to pass through the cartridge without the use of vacuum. Hereafter, 100 mL of spiked sample of urine was loaded on the column at the flow rate of 1 mL min^{-1} . Prior to sample application, the urine sample pH was adjusted to pH 9.0 with 5 mol L^{-1} sodium hydroxide. Following the sample application, the cartridges were washed with 3 mL of deionized water and dried under the vacuum for 1 min. Elution of adsorbed aminobiphenyls and aminonaphthalenes was carried out with 6 mL of acetonitrile, which was allowed to pass through the column without the use of vacuum. Finally, the vacuum was applied for 1 min to remove the residual acetonitrile from the cartridge. $10 \mu\text{L}$ of the solution after the extraction was injected into the HPLC system for the analysis.

The calibration dependences were processed using linear regression method. The limits of determination were calculated as the concentration of the analytes corresponding to the signal height ten times the background noise ($S/N = 10$).

Results and Discussion

Optimization of Separation

For the successful separation of 2-AB, 4-AB, 1-AN, and 2-AN, it was necessary to find appropriate separation conditions. The mobile phase pH has significant influence on the retention in the reverse phase system because the amino group is protonized in the acidic media, which causes weaker retention of the studied analytes on column. Optimization of the pH of the phosphate buffer was carried out in the range from pH 2.5 to pH 7.0 with the mobile phase consisting of acetonitrile and phosphate buffer of given pH (50:50; v/v). Aminobiphenyls were separated from the mixture in all selected pH values, but aminonaphthalenes were not separated when using mobile phase at higher pH values than 4.0 as both ANs are unprotonized and thus strongly retained at the column. At lower pH values than 4.0, progressive protonization depending on the pK_a values of analytes (3.82, 4.22, 3.92, and 4.15 for 2-AB, 4-AB, 1-AN, and 2-AN) causes weaker and differing retention of studied analytes. The pH 2.5 of phosphate buffer was set as optimal for the following measurements.

Polarity of the mobile phase also has an important influence on the retention. It is usually regulated by the concentration of organic modifier such as acetonitrile. Optimization of the acetonitrile content was carried out in the mobile phase consisting of acetonitrile and phosphate buffer pH 2.5 in ratio from 60:40 (v/v) to 30:70 (v/v); the ratio 40:60 (v/v) was selected as a compromise between long analysis time and low resolution. Chromatograms obtained under the optimum conditions, i.e. mobile phase consisting of acetonitrile and 0.01 mol L^{-1} phosphate buffer pH 2.5 (40:60; v/v), using both, amperometric and spectrometric detection modes are shown in Fig. 2.

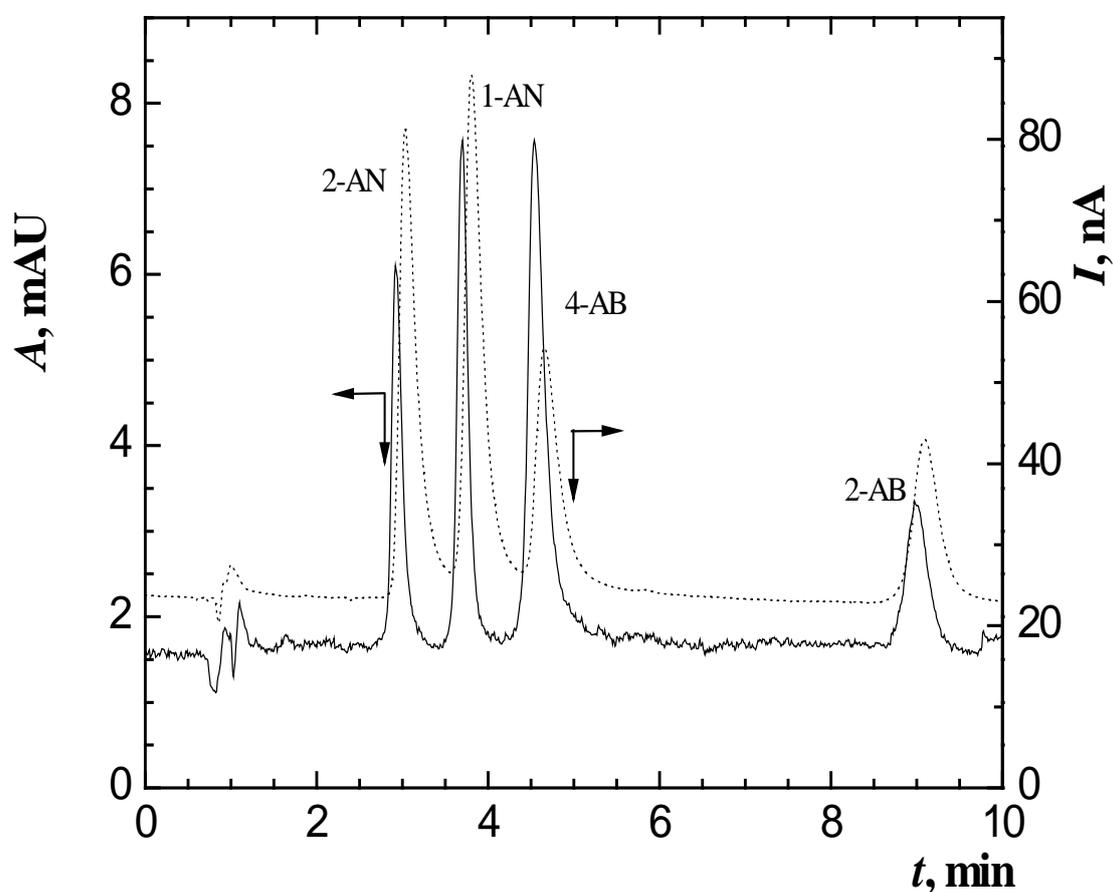


Figure 2. Chromatograms of 2-AB, 4-AB, 1-AN and 2-AN ($c = 1 \cdot 10^{-5} \text{ mol L}^{-1}$ of each) obtained under the optimum conditions by spectrophotometric (solid line, $\lambda_{\text{det}} = 290 \text{ nm}$) and amperometric (dotted line, $E_{\text{det}} = +1.0 \text{ V}$) detection. Column LiChrospher[®] 100 RP-18, $5 \mu\text{m}$, $125 \times 4 \text{ mm}$, mobile phase acetonitrile and phosphate buffer pH 2.5 (40:60; v/v), injection volume $10 \mu\text{L}$, $F_{\text{M}} = 1 \text{ mL min}^{-1}$.

Optimization of Electrochemical Detection

As the next step, optimization of the potential imposed on the indicator electrode was carried out. It was selected on the basis of hydrodynamic voltammograms obtained under the optimum separation conditions. The potential on the indicator electrode was set in the range from +0.5 V to +1.3 V. The hydrodynamic voltammograms of all analytes and background current are shown in Fig. 3. Detection potential of +1.0 V was set as optimum, based on maximum ratio of the peak height and the background current. Chromatogram of analytes measured under the optimum detection conditions is shown in dotted line in Fig. 2. Increased peak width for amperometric detection compared to the spectrophotometric one is caused by the serial connection of both detectors.

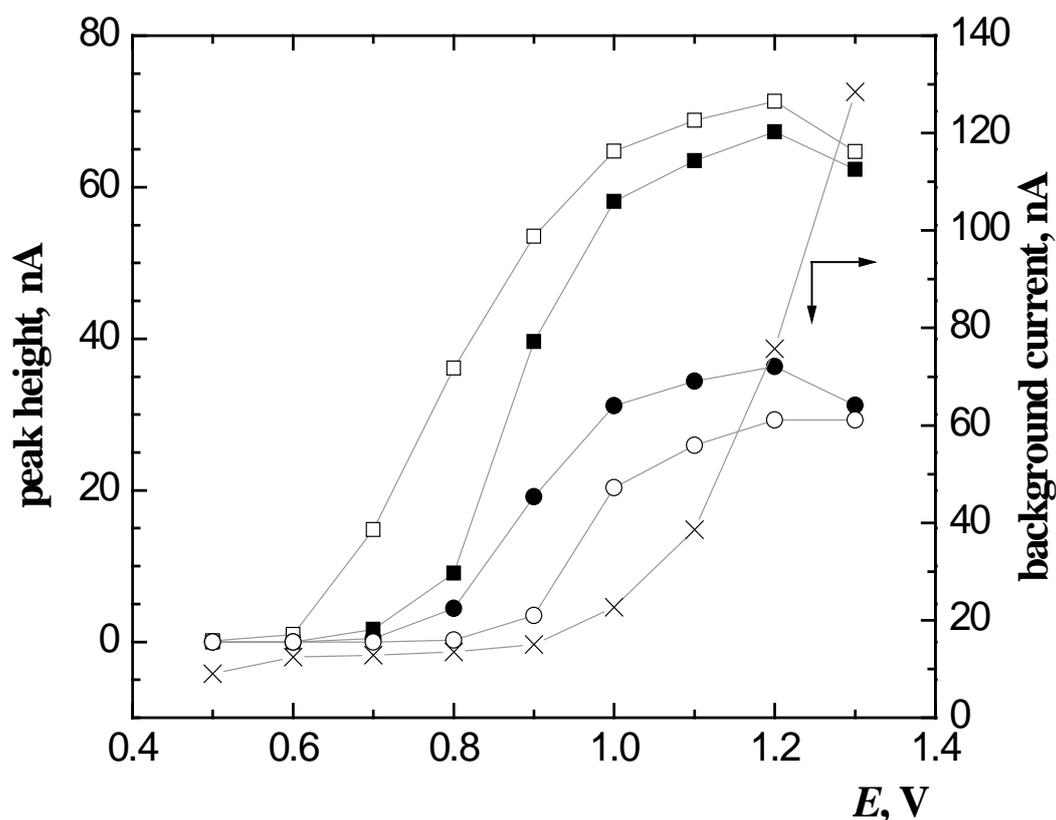


Figure 3. Hydrodynamic voltammograms of 2-AB (\circ), 4-AB (\bullet), 1-AN (\square), 2-AN (\blacksquare), and the background current (\times). Injected $10 \mu\text{L}$ of the mixture of analytes, $c = 1 \cdot 10^{-5} \text{ mol L}^{-1}$. For other conditions see Fig. 2.

Concentration Dependences

Under the optimized conditions of the separation and detection, the concentration dependences were measured. Selected parameters of the dependences evaluated of the peak heights are shown in Table 1. For spectrophotometric detection, the calibration dependences were measured in the concentration range from $4 \cdot 10^{-7}$ mol L⁻¹ to $1 \cdot 10^{-5}$ mol L⁻¹. For amperometric detection, the calibration dependences were measured in the concentration range from $8 \cdot 10^{-8}$ mol L⁻¹ to $1 \cdot 10^{-5}$ mol L⁻¹. Concentration dependences are linear up to the concentration $1 \cdot 10^{-5}$ mol L⁻¹ for spectrophotometric detection and up to the concentration $8 \cdot 10^{-6}$ mol L⁻¹ for amperometric detection. Limits of determination reach values between $8.5 \cdot 10^{-7}$ mol L⁻¹ and $3.1 \cdot 10^{-6}$ mol L⁻¹ for spectrophotometric detection and between $2.6 \cdot 10^{-8}$ mol L⁻¹ and $8.9 \cdot 10^{-8}$ mol L⁻¹ for amperometric detection, which means, that the electrochemical detection is more than one order of magnitude more sensitive than the spectrophotometric detection.

Table 1. Parameters of calibrations dependences evaluated from peak heights for the determination of 2-AB, 4-AB, 1-AN and 2-AN using HPLC with spectrophotometric and amperometric detection. For conditions see Fig. 2.

spectrophotometric detection, $\lambda_{\text{det}} = 290$ nm					
analyte	linear dynamic range, mol L ⁻¹	slope, mAU mol ⁻¹ L	intercept, mAU	correlation coefficient	limit of determination, mol L ⁻¹
2-AB	$1 \cdot 10^{-6} - 1 \cdot 10^{-5}$	$1.97 \cdot 10^5$	0.05	0.9961	$3.1 \cdot 10^{-6}$
4-AB	$4 \cdot 10^{-7} - 1 \cdot 10^{-5}$	$7.15 \cdot 10^5$	0.02	0.9981	$8.5 \cdot 10^{-7}$
1-AN	$4 \cdot 10^{-7} - 1 \cdot 10^{-5}$	$6.11 \cdot 10^5$	-0.03	0.9998	$1.0 \cdot 10^{-6}$
2-AN	$4 \cdot 10^{-7} - 1 \cdot 10^{-5}$	$4.76 \cdot 10^5$	-0.01	0.9998	$1.3 \cdot 10^{-6}$
amperometric detection, $E_{\text{det}} = +1.0$ V					
analyte	linear dynamic range, mol L ⁻¹	slope, nA mol ⁻¹ L	intercept, nA	correlation coefficient	limit of determination, mol L ⁻¹
2-AB	$1 \cdot 10^{-7} - 8 \cdot 10^{-6}$	$1.85 \cdot 10^6$	0.3	0.9988	$8.9 \cdot 10^{-8}$
4-AB	$8 \cdot 10^{-8} - 8 \cdot 10^{-6}$	$3.04 \cdot 10^6$	0.1	0.9996	$5.4 \cdot 10^{-8}$
1-AN	$8 \cdot 10^{-8} - 8 \cdot 10^{-6}$	$6.32 \cdot 10^6$	-0.4	0.9996	$2.6 \cdot 10^{-8}$
2-AN	$8 \cdot 10^{-8} - 8 \cdot 10^{-6}$	$5.41 \cdot 10^6$	-0.1	0.9998	$3.0 \cdot 10^{-8}$

Urine Sample Extraction

The developed HPLC-ED method was further used for the determination of 2-AB, 4-AB, 1-AN and 2-AN in human urine. For their preliminary separation from the matrix and preconcentration, solid-phase extraction was used. We took over the procedure recommended by the supplier for column activation and sample preparation for anilines [27] and optimized the procedure for elution with acetonitrile, using 100 mL of urine spiked with aminobiphenyls and aminonaphthalenes as model sample. Elution of adsorbed analytes was carried out with 6 mL of acetonitrile, thus the preconcentration factor expecting 100% extraction recovery was 17. The extraction efficiency was found to be 97 % for 2-AB, 73 % for 4-AB, 86 % for 1-AN and 71 % for 2-AN for the analyte concentration of $1 \cdot 10^{-6} \text{ mol L}^{-1}$.

After the optimization of extraction conditions, calibration dependences were measured. The selected parameters of the dependences evaluated from peak heights are shown in Table 2. For spectrophotometric detection, the calibration dependences were measured in the concentration range from $1 \cdot 10^{-7} \text{ mol L}^{-1}$ to $1 \cdot 10^{-6} \text{ mol L}^{-1}$. For amperometric detection, the calibration dependences were measured in the concentration range from $7.5 \cdot 10^{-9} \text{ mol L}^{-1}$ to $1 \cdot 10^{-6} \text{ mol L}^{-1}$. They are linear for both detectors. Limits of determination lie between $3.9 \cdot 10^{-8} \text{ mol L}^{-1}$ and $1.7 \cdot 10^{-7} \text{ mol L}^{-1}$ for spectrophotometric and between $7.2 \cdot 10^{-9} \text{ mol L}^{-1}$ and $2.7 \cdot 10^{-8} \text{ mol L}^{-1}$ for amperometric detection. A broad peak caused by the presence of weakly retained compounds in eluate and practically coeluting with the system peak — i.e., the signal characterizing the dead time of the column — complicated the measurement, but this undesirable effect was lower in amperometric detection due to its higher selectivity.

Table 2. Limits of determination of 2-AB, 4-AB, 1-AN, and 2-AN evaluated from peak heights for their determination in urine using HPLC with spectrophotometric and amperometric detection.

analyte	limit of determination, mol L^{-1}	
	spectrophotometric detection	amperometric detection
2-AB	$1.7 \cdot 10^{-7}$	$2.7 \cdot 10^{-8}$
4-AB	$3.9 \cdot 10^{-8}$	$1.7 \cdot 10^{-8}$
1-AN	$5.5 \cdot 10^{-8}$	$7.2 \cdot 10^{-9}$
2-AN	$6.3 \cdot 10^{-8}$	$9.1 \cdot 10^{-9}$

Conclusions

A method for the determination of 2-AB, 4-AB, 1-AN, and 2-AN was developed using HPLC with amperometric detection on platinum tubular electrode and spectrophotometric detection. The conditions for the determination are: LiChrospher[®] 100 RP-18, 5 μm , 125 \times 4 mm column, mobile phase consisting of acetonitrile and 0.01 mol L⁻¹ phosphate buffer pH 2.5 (40:60, v/v), and detection potential +1.0 V or wavelength of 290 nm.

Using amperometric detection, concentration dependences are linear in the range from 8 \cdot 10⁻⁸ mol L⁻¹ to 8 \cdot 10⁻⁶ mol L⁻¹ and limits of determination reach values between 2.6 \cdot 10⁻⁸ mol L⁻¹ and 8.9 \cdot 10⁻⁸ mol L⁻¹, which is over one order of magnitude higher sensitivity than using spectrophotometric detection.

The method was applied on spiked samples of human urine after preliminary separation and preconcentration of 2-AB, 4-AB, 1-AN, and 2-AN using LiChrolut[®] EN sorbent. Limits of determination in the range from 7.2 \cdot 10⁻⁹ mol L⁻¹ to 2.7 \cdot 10⁻⁸ mol L⁻¹ were obtained. Amperometric detection exceeds spectrophotometric detection both in sensitivity and selectivity, which confirms the qualities of the indicator electrode.

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