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Determination of 4-Aminobiphenyl and 4-Nitrobiphenyl by HPLC with Electrochemical, UV Spectrophotometric, and Fluorescent Detection

Vít Prchal[*], *Jana Krejčová, Vlastimil Vyskočil, Karolina Pecková, Jan Fischer, Jiří Zima, Jiří Barek*

Charles University in Prague, Faculty of Science, University Research Centre UNCE "Supramolecular Chemistry", Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry, Hlavova 2030/8, 128 43 Prague 2, Czech Republic
[*]E-mail: vit.prchal@natur.cuni.cz

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HPLC systems with electrochemical, UV spectrophotometric, and fluorescent detectors were used to determine genotoxic derivatives of biphenyl – 4-aminobiphenyl (4-ABP) and 4-nitrobiphenyl (4-NBP). As 4-NBP cannot be determined by electrochemical and fluorescent detector directly, reduction of 4-NBP to 4-ABP (making use of a titanium(III)-based reducing agent) was investigated and optimized. UV detection was found to be the least sensitive and fluorescent detection the most sensitive one. Limits of detection (L_Ds) for 4-ABP in the presence of a reducing agent were 6.0×10^{-6} mol L^{-1}, 2.0×10^{-6} mol L^{-1}, and 8.0×10^{-8} mol L^{-1} for UV, electrochemical, and fluorescent detection, respectively. For the determination of 4-NBP after its reduction to 4-ABP, the obtained L_Ds were 8.0×10^{-6} mol L^{-1} (UV), 4.0×10^{-6} mol L^{-1} (electrochemical), and 2.0×10^{-7} mol L^{-1} (fluorescent detection). Moreover, it was also possible to determine 4-NBP directly using HPLC with UV detection, where the L_D was 4.0×10^{-7} mol L^{-1}.

Keywords: High performance liquid chromatography; Electrochemical detection; Platinum tubular detector; UV spectrophotometric detection; Fluorescent detection; Genotoxic derivatives of biphenyl

1. INTRODUCTION

Biphenyl and its derivatives represent a potentially dangerous group of environmental pollutants. Especially 4-nitrobiphenyl (4-NBP) is a well-known carcinogen according to International Agency for Research on Cancer (IARC) and 4-aminobiphenyl (4-ABP) is a common precursor to bladder cancer in humans [1, 2]. This disease was thought to be connected also with 4-NBP; those claims were later dismissed and the relation of this compound to cancer in humans was not confirmed [3],

even though this substance is used for synthesis of 4-ABP. 4-ABP was found in tobacco smoke [4-6], in fumes of cooking oils [7] and it was widely used industrially as a rubber antioxidant and a dye intermediate [8-10]. 4-ABP is also confirmed genotoxic compound [11-13].

For all above stated reasons, there is an increasing demand for analytical methods for the determination of these harmful compounds which are inexpensive, robust and do not require much skill from the operator. The amino and nitro derivatives of biphenyl are easily detectable by various analytical techniques. Many studies were performed using voltammetric techniques, since these derivatives can easily be determined by oxidation of the amino group or reduction of the nitro group, with various traditional and non-traditional electrodes used (such as mercury electrodes [14, 15], glassy carbon electrode [14], boron-doped diamond film electrode [16], and gold electrode [17]).

The most frequently used method for the determination of amino- and nitrobiphenyls is the reverse-phase HPLC with a mixture of methanol/water or acetonitrile/water as a mobile phase [18]. UV spectrophotometry is the most widely used detection technique, even though the limits of detection are relatively high. Better results can be obtained by coupling the HPLC system to an electrochemical detector which is quite simple in construction, robust, inexpensive, and reliable and which also yields good results [18]. Several types of electrochemical detectors can be used depending on the electrode arrangement. For instance, aminobiphenyls were determined using wall-jet, thin-layer, and tubular electrode arrangement with various working electrodes (e.g., boron-doped diamond film electrode [16, 19, 20] or glassy carbon electrode [21]). However, one of the most sensitive methods for the determination of aminobiphenyls nowadays is fluorescence spectrometry [22].

This paper deals with the development of HPLC methods with electrochemical detection (HPLC-ED, amperometric detection with a platinum tubular detector), UV spectrophotometric detection (HPLC-UVD), and fluorescent detection (HPLC-FD) for the determination of 4-ABP and 4-NBP. As 4-NBP cannot be determined by electrochemical and fluorescent detector directly, a method of reduction of 4-NBP to 4-ABP (making use of a titanium(III)-based reducing agent [23]) was also investigated and optimized in this paper.

2. EXPERIMENTAL

4-Aminobiphenyl (4-ABP) and 4-nitrobiphenyl (4-NBP) were obtained from Fluka, Germany (both of 99% purity), and their 1.0×10^{-3} mol L^{-1} stock solutions were prepared by their dissolving in pure methanol (p.a., 99.8%, Lachema Brno, Czech Republic). Lower concentrations were prepared by exact dilution of the stock solutions. Stock solutions were stored in refrigerator. Mobile phase was prepared from pure methanol (for liquid chromatography, LiChrosolv® grade, Merck, Germany) and an aqueous component (77:23, v/v) prepared from deionized water (Milli-Q Plus system, Millipore, USA) containing an acetate buffer and 0.5 mmol L^{-1} EDTA disodium salt dihydrate (Na_2-EDTA, p.a., Lachema Brno, Czech Republic). The acetate buffer was prepared from 0.01 mol L^{-1} sodium acetate trihydrate (p.a., Lachema Brno, Czech Republic) and 0.5 mol L^{-1} acetic acid (p.a., 99.8%, Lachema Brno, Czech Republic). The pH of aqueous component of the mobile phase was adjusted by adding 0.5 mol L^{-1} acetic acid until the desired pH value was reached (the pH was measured using a digital

Conductivity & pH Meter Jenway 4330 (Jenway, UK) with a combined glass electrode). This mobile phase composition was found to be optimal in an earlier study [24].

The HPLC system with electrochemical and UV spectrophotometric detection consisted of a high-pressure pump HPP 5001 (Laboratorní přístroje Praha, Czech Republic) with a 10-μL sample injector (ECOM, Czech Republic) and of a UV-Vis spectrophotometric detector LCD 2082 (ECOM, Czech Republic); electrochemical detection system (coupled in tandem with UV spectrophotometric detection) consisted of a potentiostat ADLC 2 (Laboratorní přístroje Praha, Czech Republic) controlling a tubular platinum working electrode coupled with an Ag/AgCl (1 mol L^{-1} KCl) reference electrode and a platinum auxiliary electrode [25]. Data were processed with the CSW32 software (DataApex, Czech Republic). The HPLC system with fluorescent detection consisted of a high-pressure pump Waters 600E with an autosampler Waters 717 and a Waters 470 Scanning Fluorescence Detector (all three supplied by Waters, USA). Data were processed with the Millennium Session Manager software (version 2.00, Waters, USA). A LiChrospher® 100 RP-18 (5 μm, 125 mm × 4 mm) separation column (Merck, Germany) was used in both HPLC systems. A flow rate of 0.5 mL min^{-1} and an injection volume of 10 μL were always used.

A reducing agent for the reduction of 4-NBP to 4-ABP was titanium(III) trichloride (p.a., 15% aqueous solution, Lachema Brno, Czech Republic). Titanium(III) is widely used in analytical chemistry as a reducing agent. The redox potential of the couple titanium(IV)/titanium(III) can be significantly altered by adding various complexing agents (e.g., trisodium citrate). The reaction must be carried out in an inert atmosphere (e.g., of nitrogen or argon), because titanium(III) is easily oxidized by atmospheric oxygen [24]. Time needed for the reduction of 4-NBP to 4-ABP is several minutes and it can be monitored by the color change of the solution form dark blue to transparent. The reducing agent was prepared as follows: 2.95 g of trisodium citrate dihydrate (p.a., Lachema Brno, Czech Republic) were dissolved in 100 mL of deionized water (c = 0.1 mol L^{-1} of trisodium citrate) and the solution was deaerated for ten minutes by pure nitrogen. Then, 10 mL of a 15% titanium trichloride solution were added and, subsequently, pH was adjusted to desired value by 3 mol L^{-1} hydrochloric acid (p.a., 35%, Lachema Brno, Czech Republic). Finally, zinc amalgam was added to the flask and the solution was again deaerated by nitrogen for ten minutes [23, 24]. After the preparation of this solution, it was left to stabilize for one day; the reaction yields were lower when the reagent was used earlier. This stabilization was accompanied by a slight color change of the solution from deep blue to violet [23, 24]. The reducing agent is then stable up to two weeks. Unless stated otherwise, reduction of 4-NBP was carried out in 10-mL volumetric flasks: 3 mL of the reducing agent were added to 1 mL of methanolic solution of 4-NBP.

3. RESULTS AND DISCUSSION

3.1. Optimization of HPLC with electrochemical and UV spectrophotometric detection

Primary aromatic amines are electrochemically easily oxidized at solid electrodes [26, 27]. Electrochemical oxidation of 4-ABP is shown in Fig. 1. It includes formation of a cation radical followed by formation of dimerization and polymerization products [26, 27]. Thus, 4-ABP can be

detected amperometrically using its anodic oxidation at a platinum tubular electrode [25]. 4-NBP is impossible to be detected at this electrode, so the first step of its determination must be its preliminary reduction to 4-ABP.

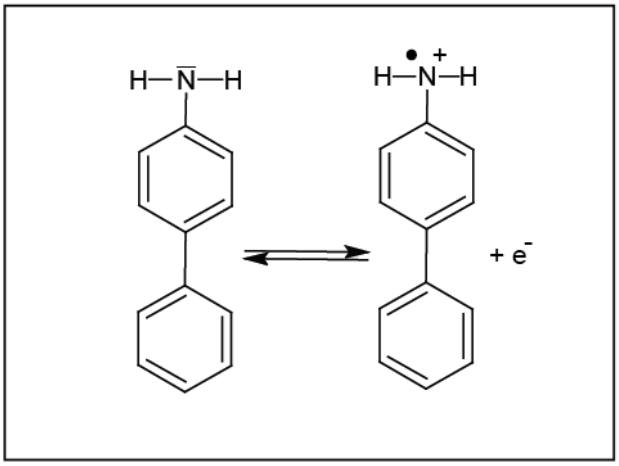

Figure 1. Schematic representation of electrochemical oxidation of 4-ABP on solid electrodes.

The first step of the investigation was to find an optimal detection potential (E_d) to be applied to the working electrode, i.e., a potential at which the highest ratio between the electrochemical signal and the background current is achieved. The optimization procedure consisted of applying potentials from 0 to +1.3 V and recording corresponding HPLC-ED responses (see Fig. 2). 10 µL of 1×10^{-4} mol L^{-1}

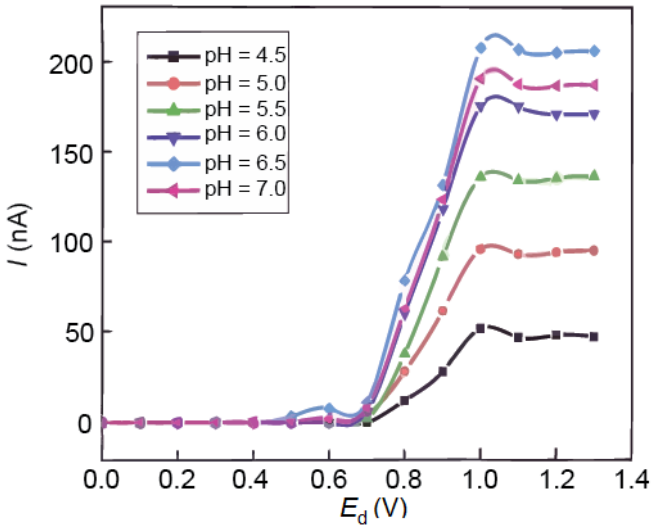

Figure 2. Hydrodynamic voltammograms of 4-ABP measured by HPLC-ED with a platinum tubular detector at different pH of aqueous component of the mobile phase (injected 10 µL of 1×10^{-4} mol L^{-1} 4-ABP methanolic solution; depicted average values for $n = 5$). Mobile phase: methanol : 0.01 mol L^{-1} acetate buffer with 0.5 mmol L^{-1} Na_2-EDTA (77:23, v/v); flow rate 0.5 mL min^{-1}.

4-ABP methanolic solution were injected. The mobile phase was prepared as described in the Experimental part and the pH of its aqueous component was changed from 4.5 to 7.0. It can be seen in Fig. 2 that the highest HPLC-ED responses of 4-ABP were obtained at a detection potential of +1.0 V. Fig. 3A shows the dependence of 4-ABP peak area (measured at this optimal detection potential, E_d = +1.0 V) on pH of aqueous component of the mobile phase. The peak area increased with increasing pH to a threshold value at pH 6.5 and, then, it began to decrease (see Fig. 3A). Therefore, the pH value 6.5 was chosen as the optimal one. In the case of UV spectrophotometric detection, the response of 4-ABP (measured at the optimal detection wavelength, λ_d = 276 nm) did not change significantly with increasing pH of aqueous component of the mobile phase (see Fig. 3B).

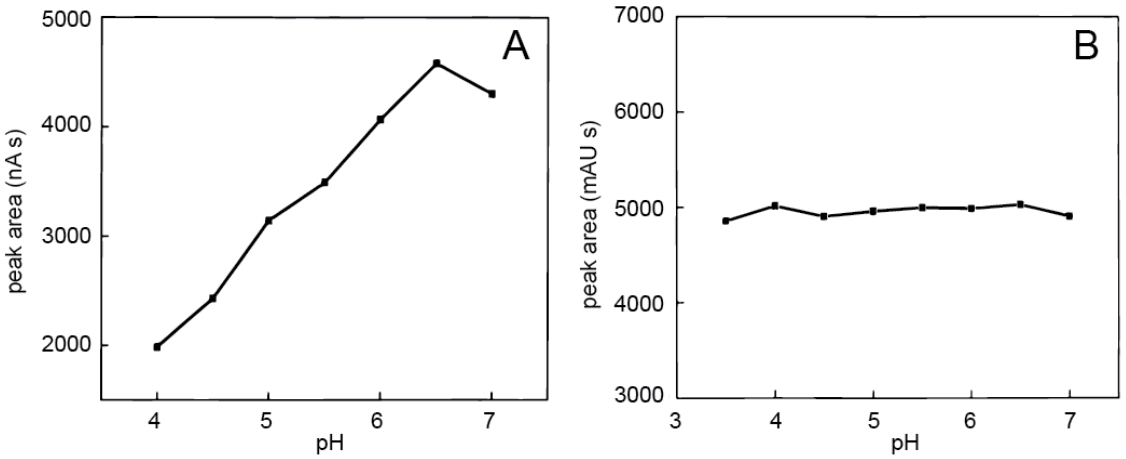

Figure 3. Dependences of 4-ABP peak area on pH of aqueous component of the mobile phase (injected 10 μL of 1×10^{-4} mol L^{-1} 4-ABP methanolic solution; depicted average values for $n = 5$); A) electrochemical detection (E_d = +1.0 V), B) UV spectrophotometric detection (λ_d = 276 nm). Mobile phase: methanol : 0.01 mol L^{-1} acetate buffer with 0.5 mmol L^{-1} Na_2-EDTA (77:23, v/v); flow rate 0.5 mL min^{-1}.

Because of increasing background current at the electrochemical detector, it was necessary to clean up and activate the working electrode of the detector. This procedure was carried out by filling the detector with 0.05 mol L^{-1} sulfuric acid and performing 10 cleaning cycles based on the application of two successive cleaning potentials (+1.4 V and -0.3 V) to the working electrode (each potential was held for five seconds). After the cleaning, the background current of the detector decreased significantly, but the secondary effect was the decrease of the signal of 4-ABP as well. However, when the detector was rinsed (after its aforementioned electrochemical cleaning) with a mixture of methanol and deionized water (77:23, v/v) for several hours, problems with background current and decrease of the 4-ABP signal were eliminated. Thus, this overall procedure was always performed at the end of each measurement day.

The repeatability of HPLC-ED and HPLC-UVD determination of 4-ABP, expressed in term of relative standard deviation (RSD), was estimated from the results of 10 subsequent 10-μL injections of methanolic solution of 4-ABP ($c = 1 \times 10^{-4}$ mol L^{-1}). Table 1 summarizes the repeatability of measurements.

Table 1. Repeatability of HPLC determination of 4-ABP (injected 10 µL of 1×10^{-4} mol L^{-1} 4-ABP methanolic solution; $n = 10$) measured using electrochemical detection (ED, $E_d = +1.0$ V) and UV spectrophotometric detection (UVD, $\lambda_d = 276$ nm). Mobile phase: methanol : 0.01 mol L^{-1} acetate buffer with 0.5 mmol L^{-1} Na_2-EDTA (resulting pH of aqueous component of the mobile phase 6.5) (77:23, v/v); flow rate 0.5 mL min^{-1}.

Peak area		RSD (%)		Peak height		RSD (%)	
ED (nA s)	UVD (mAU s)	ED	UVD	ED (nA)	UVD (mAU)	ED	UVD
4341	5068	2.6	2.1	228.7	264.1	1.9	2.0

3.2. Influence of the composition of the reducing agent on the reduction yield

The yield of reduction of 4-NBP to 4-ABP depends significantly on the resulting pH of the reducing agent [23, 24]. Reduction yields were determined by relative comparison of the peak area of 4-ABP, which was formed after reduction of 4-NBP, with the peak area of authentic 4-ABP measured at the same conditions with reducing agent present in the solution; a value of pH of the reducing agent did not affect the signal of 4-ABP. The highest yield was obtained in the presence of the reducing agent of resulting pH 3.5 (see Table 2). 4-NBP can be quantitatively reduced to 4-ABP with a yield of (92.9 ± 5.8) % and (90.9 ± 6.3) % when using electrochemical detection and UV spectrophotometric detection, respectively (for both $n = 5$).

Table 2. Influence of pH of the reducing agent on the yield of the reduction of 4-NBP to 4-ABP (experimental conditions same as in Table 1).

pH of the reducing agent	Reduction yield (%)	
	ED	UVD
2.0	70.8	73.8
2.5	83.2	84.3
3.0	86.5	87.9
3.5	92.9	90.9
4.0	87.7	85.7
4.5	74.3	79.6
5.0	57.0	50.9

3.3 Determination of 4-ABP and 4-NBP using HPLC with electrochemical and UV spectrophotometric detection

Calibration dependence of 4-ABP in the presence of the reducing agent

Concentration dependences were measured in the concentration range from 2×10^{-6} mol L^{-1} to 1×10^{-4} mol L^{-1} of 4-ABP for HPLC-ED (chromatograms recorded in the concentration order of 10^{-6} mol L^{-1} are shown Fig. 4A) and from 6×10^{-6} mol L^{-1} to 1×10^{-4} mol L^{-1} of 4-ABP for HPLC-UVD (chromatograms recorded in the concentration order of 10^{-5} mol L^{-1} can be seen in Fig. 4B). All solutions of 4-ABP were prepared in one day by diluting the stock solution with methanol. To 1 mL of methanolic solution of 4-ABP, 3 mL of reducing agent of resulting pH 3.5 were added and thoroughly

mixed. The sampled amount of the analyte was thus four times lower due to the dilution effect (when a volume contraction is neglected). All data were processed by linear fitting as the dependence of the peak area on the concentration of 4-ABP. Here and elsewhere in this work, the limit of quantification (L_Q) was calculated with the Adstat 2.0 software (Trilobite, Czech Republic) and the limit of detection (L_D) as the concentration of an analyte which gave a signal three times the background noise. Because of different ways of calculation of the L_Q and L_D, their ratio is not 3.3, which is the case when the same way of calculation is used for both values. Parameters of individual calibration straight lines are summarized in Table 3. The L_D of 4-ABP was lower when using HPLC-ED compared to HPLC-UVD.

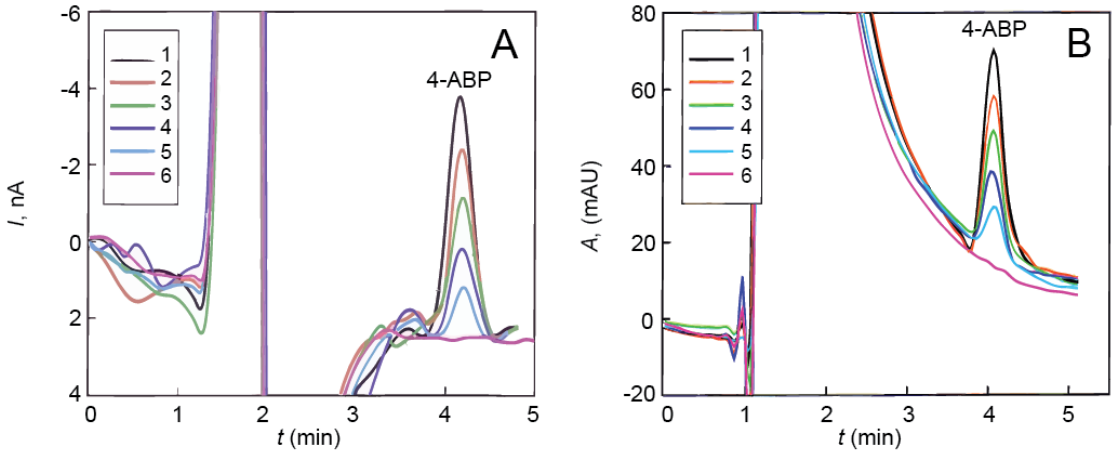

Figure 4. (A) Chromatograms of 4-ABP (injected 10 µL of 4-ABP methanolic solution in the presence of the reducing agent of resulting pH 3.5) measured by HPLC-ED with a platinum tubular detector (E_d = +1.0 V); concentration of the analyte before addition of the reducing agent (mol L^{-1}): 1×10^{-5} (1), 8×10^{-6} (2), 6×10^{-6} (3), 4×10^{-6} (4), 2×10^{-6} (5), and 0 (blank) (6). **(B)** Chromatograms of 4-ABP (injected 10 µL of 4-ABP methanolic solution in the presence of the reducing agent of resulting pH 3.5) measured by HPLC-UVD (λ_d = 276 nm); concentration of the analyte before addition of the reducing agent (mol L^{-1}): 1×10^{-4} (1), 8×10^{-5} (2), 6×10^{-5} (3), 4×10^{-5} (4), 2×10^{-5} (5), and 0 (blank) (6). Mobile phase (for both (A) and (B)): methanol : 0.01 mol L^{-1} acetate buffer with 0.5 mmol L^{-1} Na_2-EDTA (resulting pH of aqueous component of the mobile phase 6.5) (77:23, v/v); flow rate 0.5 mL min^{-1}.

Table 3. Calibration dependences of 4-ABP in the presence of the reducing agent (experimental conditions same as in Fig. 4).

UV spectrophotometric detection of 4-ABP					
Concentration (mol L^{-1})	Slope (AU s mol^{-1} L)	Intercept (AU s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	8.37×10^{3}	1.23×10^{-2}	0.9998	-	-
6×10^{-6} - 1×10^{-5}	8.73×10^{3}	-3.58	0.9987	3.4×10^{-6}	6.0×10^{-6}
Electrochemical detection of 4-ABP					
Concentration (mol L^{-1})	Slope (nA s mol^{-1} L)	Intercept (nA s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	1.00×10^{7}	24.5	0.9993	-	-
2×10^{-6} - 1×10^{-5}	1.17×10^{7}	-3.77	0.9985	4.1×10^{-6}	2.0×10^{-6}

Calibration dependence of 4-NBP after its reduction to 4-ABP

The next step was the measurement of calibration dependences of 4-NBP after its reduction to 4-ABP using HPLC system coupled to electrochemical and UV spectrophotometric detector. Dependences were measured in the concentration range from 4×10^{-6} mol L^{-1} to 1×10^{-4} mol L^{-1} for HPLC-ED and from 8×10^{-6} mol L^{-1} to 1×10^{-4} mol L^{-1} for HPLC-UVD. The procedure for the reduction of 4-NBP to 4-ABP and its optimization are described in the Experimental part and Section 3.2. It was found that 4-NBP can be quantitatively reduced to 4-ABP with (92.9 ± 5.8) % efficiency (determined by HPLC-ED, n = 5) and (90.9 ± 6.3) % efficiency (determined by HPLC-UVD, n = 5). Table 4 shows exact parameters of calibration dependences. The sampled amount of the analyte was four times lower due to the dilution effect occurring during the reducing procedure (see Section 3.2) and the reduction yield also influenced the calibration straight lines. The calibration straight lines were linear in the whole range and their correlation coefficients varied from 0.9981 to 0.9990.

Table 4. Calibration dependences of 4-NBP after its reduction to 4-ABP (experimental conditions as in Fig. 4, but the analyte detected was 4-ABP formed by the reduction of 4-NBP).

UV spectrophotometric detection of 4-NBP after reduction					
Concentration (mol L^{-1})	Slope (AU s mol^{-1} L)	Intercept (AU s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
8×10^{-6} - 1×10^{-4}	7.87×10^{3}	-1.77×10^{-2}	0.9988	1.3×10^{-5}	8.0×10^{-6}
Electrochemical detection of 4-NBP after reduction					
Concentration (mol L^{-1})	Slope (nA s mol^{-1} L)	Intercept (nA s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	9.07×10^{6}	34.3	0.9990	-	-
4×10^{-6} - 1×10^{-5}	9.00×10^{6}	1.99	0.9981	4.4×10^{-6}	4.0×10^{-6}

Calibration dependence of 4-NBP without preceding reduction

4-NBP can also be determined directly using HPLC-UVD at a wavelength of its absorption maximum, i.e., λ_d = 304 nm. Calibration dependences were measured in the concentration range from 4×10^{-7} mol L^{-1} to 1×10^{-4} mol L^{-1}. Parameters of the calibration straight lines obtained are summarized in Table 5. It is obvious that the developed HPLC-UVD method based on the reduction of 4-NBP to 4-ABP (see Table 4) is less sensitive than direct HPLC-UVD determination (see Table 5).

Table 5. Calibration dependence of 4-NBP measured using direct HPLC-UVD (λ_d = 304 nm; the mobile phase and flow rate same as in Fig. 4).

Concentration (mol L^{-1})	Slope (AU s mol^{-1} L)	Intercept (AU s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	4.53×10^{4}	-6.35×10^{-2}	0.9998	-	-
2×10^{-6} - 1×10^{-5}	4.48×10^{4}	-5.05×10^{-3}	0.9989	-	-
4×10^{-7} - 1×10^{-6}	4.10×10^{4}	-2.62×10^{-4}	0.9987	3.1×10^{-7}	4.0×10^{-7}

3.4 Determination of 4-ABP and 4-NBP using HPLC with fluorescent detection

An HPLC-FD technique was used for the determination of 4-ABP and 4-NBP for comparison with the newly developed HPLC-ED and HPLC-UVD methods. It is impossible to determine 4-NBP directly with HPLC-FD [23, 24], so the developed method of reduction of 4-NBP to 4-ABP was used for the formation of detectable 4-ABP. The optimal excitation and emission wavelengths of 4-ABP were 285 nm and 370 nm, respectively [23]. The mobile phase used in HPLC-FD was the same as the optimal one used in both HPLC-ED and HPLC-UVD, but the resulting pH of aqueous component of the mobile phase was 4.5 [23, 24]. The mobile phase was deaerated prior to measurements by pure nitrogen for at least fifteen minutes and, then, it was pumped by multichannel pump with low-pressure gradient; the built-in mixing device was used to mix the components of the mobile phase. Prior to each series of measurements, manual priming was carried out: the air bubbles were removed from the tubing of each mobile phase component (at an open balance valve) using a syringe when the flow rate of the mobile phase of 1 mL min^{-1} was used and, after this procedure, the flow rate was increased to 9 mL min^{-1} to speed up the mixing of mobile phase components. After each series of measurements, whole system was cleaned by a mixture of methanol and deionized water in the same volume ratio as in the mobile phase (i.e., 77:23, v/v) for one hour. In HPLC-FD determinations, a flow rate of 0.5 mL min^{-1} and an injected volume of 10 μL were always used.

The reduction of 4-NBP here was carried in 1-mL vials: 100 μL of methanolic solution of 4-NBP were mixed with 300 μL of reducing agent of resulting pH 3.5 and, then, the vial was filled with 500 μL of pure methanol. Therefore, in following figures and tables, the concentration of 4-NBP (and 4-ABP as well) in pure methanolic solution is considered; the sampled amount of the analyte was nine times lower due to the dilution effect (when a volume contraction is neglected) occurring during the aforementioned reducing procedure and the reduction yield also influenced the calibration straight lines.

Calibration dependence of 4-ABP in the presence of the reducing agent

Concentration dependences of 4-ABP were measured using HPLC-FD in the concentration range from 8×10^{-8} mol L^{-1} to 1×10^{-4} mol L^{-1} and their parameters are summarized in Table 6. Fig. 5 shows HPLC-FD chromatograms of 4-ABP recorded in the lowest concentration range. The L_D obtained when using HPLC-FD was 8×10^{-8} mol L^{-1}, which is more than ten times lower compared to electrochemical and/or UV spectrophotometric detection (see Table 3).

Table 6. Calibration dependence of 4-ABP in the presence of the reducing agent measured using HPLC-FD (experimental conditions same as in Fig. 5).

Concentration (mol L^{-1})	Gain[1]	Slope (AU s mol^{-1} L)	Intercept (AU s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	×10	1.53×10^{8}	-3.86×10^{2}	0.9998	-	-
2×10^{-6} - 1×10^{-5}	×100	1.53×10^{9}	-3.42×10^{2}	0.9985	-	-
8×10^{-8} - 1×10^{-6}	×100	1.42×10^{9}	-3.01	0.9992	1.9×10^{-7}	8.0×10^{-8}

[1] Gain is an amplification of a signal recorded by fluorescent detector, e.g., ×10 means that a signal is amplified ten times.

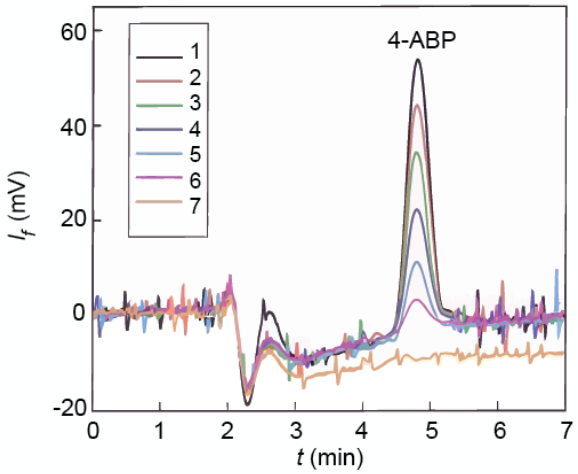

Figure 5. Chromatograms of 4-ABP (injected 10 μL of 4-ABP methanolic solution in the presence of the reducing agent of resulting pH 3.5) measured by HPLC-FD (λ_d = 370 nm); concentration of the analyte before addition of the reducing agent (mol L^{-1}): 1×10^{-6} (1), 8×10^{-7} (2), 6×10^{-7} (3), 4×10^{-7} (4), 2×10^{-7} (5), 1×10^{-7} (6), and 0 (blank) (7). Mobile phase: methanol : 0.01 mol L^{-1} acetate buffer with 0.5 mmol L^{-1} Na_2-EDTA (resulting pH of aqueous component of the mobile phase 4.5) (77:23, v/v); flow rate 0.5 mL min^{-1}.

Calibration dependence of 4-NBP after its reduction to 4-ABP

Concentration dependences of 4-NBP after its reduction to 4-ABP were measured in the concentration range from 2×10^{-7} mol L^{-1} to 1×10^{-4} mol L^{-1} (see Table 7). It was found that 4-NBP can be quantitatively reduced to 4-ABP with an efficiency of (87.9 ± 4.9) % (determined by HPLC-FD, n = 5). It can be seen in Table 7 that the L_D of 4-NBP reached using HPLC-FD is about one order lower than in the case of HPLC-ED and/or HPLC-UVD (see Table 4) and is comparable to the L_D obtained in direct HPLC-UVD determination of 4-NBP (see Table 5).

Table 7. Calibration dependence of 4-NBP after its reduction to 4-ABP measured using HPLC-FD (experimental conditions same as in Fig. 5, but the analyte detected was 4-ABP formed by the reduction of 4-NBP).

Concentration (mol L^{-1})	Gain[1]	Slope (AU s mol^{-1} L)	Intercept (AU s)	R	L_Q (mol L^{-1})	L_D (mol L^{-1})
2×10^{-5} - 1×10^{-4}	×10	1.28×10^{8}	-1.96×10^{2}	0.9997	-	-
2×10^{-6} - 1×10^{-5}	×100	1.19×10^{9}	-2.02×10^{2}	0.9994	-	-
2×10^{-7} - 1×10^{-6}	×100	1.16×10^{9}	-24	0.9982	3.4×10^{-7}	2.0×10^{-7}

[1] Gain is an amplification of a signal recorded by fluorescent detector, e.g., ×10 means that a signal is amplified ten times.

4. CONCLUSIONS

Different methods for determination of electrochemically active derivatives of biphenyl (4-aminobiphenyl (4-ABP) and 4-nitrobiphenyl (4-NBP)) using reverse-phase HPLC systems with electrochemical (ED), UV spectrophotometric (UVD), and fluorescent detectors (FD) were successfully developed. 4-NBP is impossible to be detected by anodic oxidation in an electrochemical detector or by a fluorescent detector. Therefore, optimal conditions for a reduction of 4-NBP to 4-ABP using a citrate complex of titanium(III) were found, as 4-ABP is easily determinable by both techniques.

It was found that the determination of 4-NBP after its reduction to 4-ABP is very time-consuming and, moreover, the limits of detection (L_Ds) reached using HPLC-ED or HPLC-UVD are higher than (and in the case of HPLC-FD comparable to) that one obtained in direct determination of 4-NBP using HPLC-UVD. Generally, HPLC-FD is the most sensitive method used in this work for the determination of both analytes (its L_Ds were considerably lower compared to HPLC-ED and HPLC-UVD). The values obtained are summarized in Table 8.

Table 8. Results summary.

Analyte	L_D (mol L^{-1})	Reduction yield (%)
UV spectrophotometric detection		
4-ABP (in presence of reducing agent)	6.0×10^{-6}	-
4-NBP (after reduction to 4-ABP)	8.0×10^{-6}	90.9
4-NBP (direct determination)	4.0×10^{-7}	-
Electrochemical detection		
4-ABP (in presence of reducing agent)	2.0×10^{-6}	-
4-NBP (after reduction to 4-ABP)	4.0×10^{-6}	92.9
Fluorescent detection		
4-ABP (in presence of reducing agent)	8.0×10^{-8}	-
4-NBP (after reduction to 4-ABP)	2.0×10^{-7}	87.9

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