ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 247–255

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Simultaneous determination of amphetamine and one of its metabolites by HPLC with electrochemical detection

Natale Alfredo Santagati *, Giuseppina Ferrara, Agostino Marrazzo, Giuseppe Ronsisvalle

Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Catania, Viale A. Doria, 6-95125 Catania Italy

Received 25 March 2001; received in revised form 24 April 2002; accepted 27 April 2002

Abstract

A high-performance liquid chromatographic method coupled with electrochemical detector was developed for the separation and quantitation of amphetamine and one of its metabolites, the 4-hydroxynorephedrine. The pre-column derivatisation of these compounds was carried out with 2,5-dihydroxybenzaldehyde as electroactive labelling reagent, in presence of Borohydride Exchange Resin. The new synthetic method developed was fast, clean and high yielding. The analysis was performed in isocratic mode on a reversed phase column 5 μm Hypersil ODS RP-18, 15 cm, using as a mobile phase methanol-NaH_2PO_4 buffer (50 mM, pH 5.5)(30:70 v/v) containing trietylamine (0.5% v/v) and the products were detected by a porous graphite electrode set at an oxidation potential of +0.6 V. The linearity of response was examined for each derivatised compound and was analysed using solutions in the range 10–40 nmol/ml. The correlation coefficients of the linear regression of the standard curves were greater than 0.99. The method developed in this study was sensitive and very selective. Because of the specificity for primary phenylethylamines, it could be applicable for the assay of other related substances in toxicology and drugs abuse.

Keywords: HPLC; Electrochemical detection; Amphetamine; Metabolite; 4-Hydroxynorephedrine; Derivatization; 2,5-Dihydroxybenzaldehyde

1. Introduction

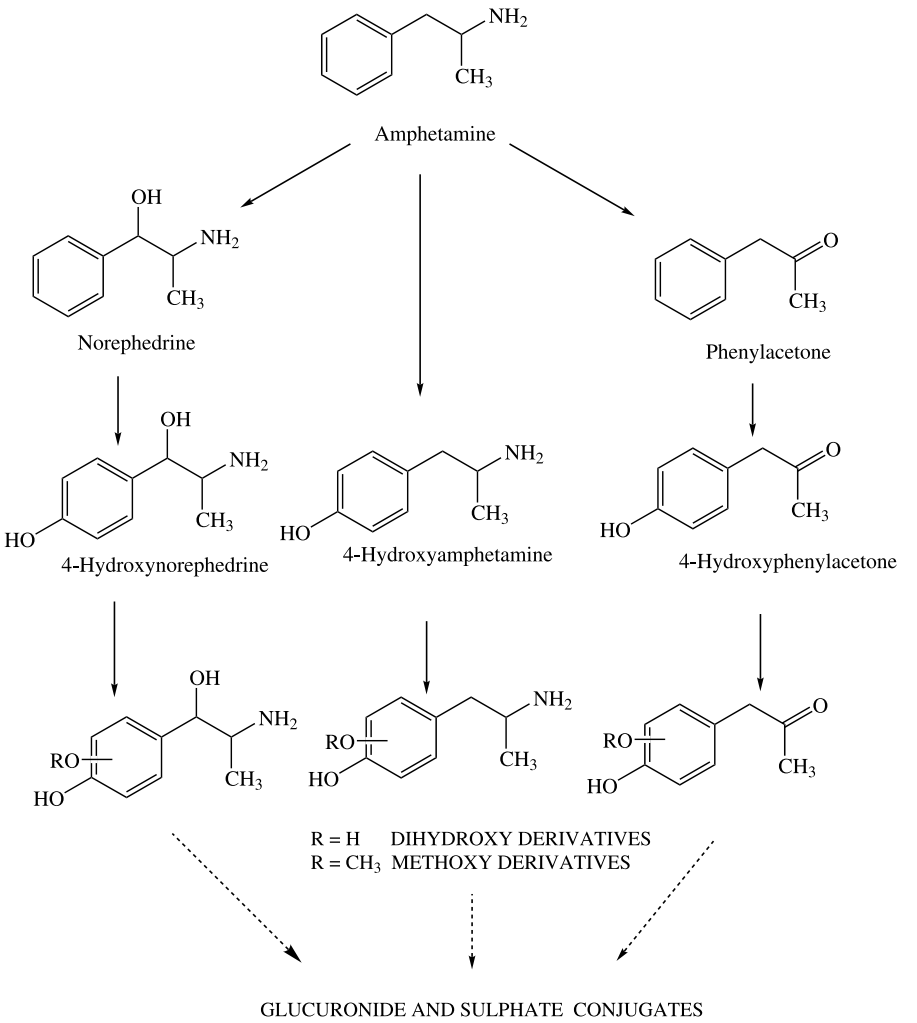
Amphetamine (AMP) and other related phenylethylamines are drugs of abuse as well as doping agents in many sports. In the last years, these substances have been extensively used among teenagers because of their psychoactive properties [1]. The ability of stimulants with an essential phenylethylamine structure cause neurotoxicity, particularly during chronic administration, was well established in experimental models [2]. Phenylethylamine-induced neurotoxicity was primarily characterized by degeneration of dopaminergic nerve terminal fields within the nucleus accumbens, olfactory area and frontal cortex. In addition, substantial destruction of

* Corresponding author. Tel.: +39-095-58-0526; fax: +39-095-22-2339
E-mail address: santagat@unict.it (N.A. Santagati).

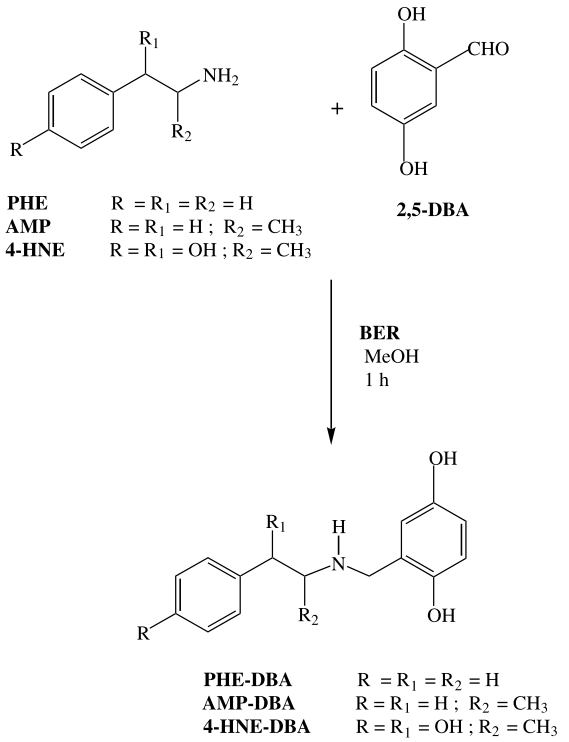
0731-7085/02/$ - see front matter
PII: S0731-7085(02)00330-8

serotonergic terminals in the hippocampus, cerebral cortex, amygdala and striatum was associated with administration of high dose of phenylethylamine stimulants [3]. Recent evidence indicates that production of phenylethylamine stimulant-induced dopaminergic neurotoxicity requires interaction with the glutamatergic neurotrasmission [4].

The main metabolic pathways of AMP [5] is shown in Scheme 1. The compounds formed in the first phase of metabolism of AMP are produced from (i) oxidative deamination; (ii) one and two-fold ring hydroxylation, followed by methylation of one of the hydroxy group; (iii) *N*-demethylation. In the subsequent phase they are mainly excreted as glucoronide and/or sulphate conjugates. The hydroxy-derivatives metabolites of AMP are the principal compounds excreted in urine that can be detected for 7/8 days after ingestion, whereas the parent compounds are only detectable for about 2/3 days. Some of these metabolites may have activity that contribute to the effects seen after AMP administration, in particular 4-hydroxyamphetamine (4-HNE) acts as a false neurotransmitter.



Scheme 1. Main metabolic pathways of amphetamine.



Scheme 2. Derivatization reaction of AMP, 4-HNE and PHE with 2,5-DBA to give the electroactive amines AMP-DBA, 4-HNE-DBA and PHE-DBA (IS).

Many analytical methodologies used in toxicological studies and forensic science have been described and the great number of publications on AMP analysis published in the last 5 years indicates that it was necessary to improve the methods of analysis [5–7]. However, only few chromatography methods were described for the simultaneous analysis of AMP and 4-HNE [8,9].

Gas chromatographic (GC) methods are the most widely used for AMP and related substances analysis in biological samples [10–21] and are traditionally recommended, especially if coupled to mass spectrometric (GC/MS) detection. Recently, capillary electrophoresis (CE) methods were also developed [22–24].

In high performance liquid chromatography (HPLC) several methods have been described for the determination of AMP and derivatives but HPLC procedures without derivatisation reactions have not been widely applied to the analysis of phenylethylamines because they show low UV absorbances and very little natural fluorescence [25–27]. In addition, primary and secondary amines often show poor chromatographic performance which could be improved by derivatisation. To improve both chromatographic behaviour or detectability of the AMP and derivatives, a great number of procedures involving precolumn or postcolumn derivatization using different reagents were developed [28–36].

HPLC with electrochemical detection represents a very sensitive method, providing enhanced selectivity as a result of the limited number of substances which could undergo redox reactions under certain conditions [37–39].

The aim of this study was the development of a sensitive and selective method for the simultaneous determination of AMP and one of its metabolites (4-HNE) by HPLC with electrochemical coulometric detection (HPLC-ECD).

A new derivatization method using 2,5-dihydroxybenzaldehyde (2,5-DBA) as an electrochemical probe, selected for the very low oxidation potential was developed. The 2,5-DBA was rapidly aminated with the primary amines AMP, 4-HNE and phenylethylamine (PHE), using borohydride exchange resin (BER) as a chemoselective reducing agent to give the electroactive secondary amines AMP-DBA, 4-HNE-DBA and PHE-DBA, the latter working as an internal standard (IS), respectively. The procedure is shown in Scheme 2.

In order to optimise the detection of the electroactive derivatives several parameters such as oxidation potential, pH and ionic strength of mobile phase were examined.

2. Experimental

2.1. Apparatus

The HPLC apparatus comprised two Model 510 pumps, a Model 712 WISP auto-injector and a Model 490E absorbance detector (Waters Assoc., Milford, MA, USA) set at 276 nm and 0.05 absorbance units full scale. The UV detector was connected in series with the electrochemical detec-

tor (Model 5100A Coulochem; ESA, Bedford, MA, USA) which consists of a control module and an analytical cell (Model 5010) containing two on-line porous graphite coulometric electrodes.

The analysis was performed in the oxidative mode. The ECD sensitivity range and the response time were set at 100 nA and 10 s, respectively. Signals from the detectors were converted to chromatographic traces and integrated by an APC IV computer system (NEC, Boxborough, MA, USA) using MAXIMA 820 software (Waters Assoc.).

^{1}H NMR spectra and ^{13}C NMR spectra were recorded on Varian Unit Inova (200 MHz) instrument for DMSO solutions with tetramethylsilane (TMS) as IS. Chemical shifts are expressed in ppm (δ). Molecular weights of the obtained products were determined by MS (EI at 70 eV) on a Kratos 25 RF spectrometer. Elementary analyses for C, H, N were obtained on a Carlo Erba 1106 analyser (Milan, Italy) and they agree with theoretical values within $\pm$ 0.4%. UV absorption spectra were recorded on a Uvikon 860 (Kontron, Zurich, Switzerland) spectrometer in MeOH solution. Analytical thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel plates.

2.2. Chemicals

2,5-DBA, PHE and BER were purchased from Sigma-Aldrich (Milano, Italy). AMP and 4-HNE were obtained from Salars S.p.A. (Como, Italy). HPLC-grade methanol and water were purchased from Carlo Erba (Milano). Methanol anhydrous used for synthesis was stored on 4A activated molecular sieves. Other chemicals used were of reagent grade or better.

2.3. Derivatization procedure

The derivatization was achieved by adding a suitable amount of methanolic solution of 2,5-DBA to a methanolic solution of the individual phenylethylamine. The molar ratio between the derivatizing agent and amine was fixed at 1:1.

2.4. Synthesis of electroactive compounds

2.4.1. 2-[(phenylethylamino)methyl]benzene-1,4-diol oxalate (PHE-DBA)

1 ml of 2,5-DBA solution in methanol (0.57 M, 0.0795 g, 0.57 mmol), 1 ml of 2-phenylethylamine hydrochloride solution in methanol (0,57 M, 0.0907 g, 0.57 mmol) and $NaHCO_3$ (0.4836 g, 0.57 mmol) were placed in a 15 ml flask, which is fitted with rubber-capped side arm connected to a mercury bubbler. The mixture was stirred at room temperature for 10 min and then 2 ml of triethylamine hydrochloride solution in methanol (0.57 M, 0.1585 g, 1.1515 mmol) and BER (0.1919 g, 0.57 mmol) were added. The reaction was checked by TLC using $CHCl_3$–Cicloexane–EtOH–NH_4OH (5:4:1:0.05 v/v/v) as an eluent. After 1 h, the acidic solution was neutralised with $NaHCO_3$ (0.9673 g, 1.1515 mmol) and the resin was removed by filtration. The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and washed with a solution of $NaHCO_3$ (4%, w/w). The mixture was dried over anhydrous Na_2SO_4 and evaporated in vacuum. The final product was stored and analysed as oxalic salt.

Yellow solid 0.1639 g, 84% yield; m.p. 168–170 °C; $\lambda_{max} = 300$ nm; ^{1}H NMR (DMSO-d_6) δ : 8.10 (br s, 4H), 7.33–7.22 (m, 6H), 6.77–6.67 (m, 2H), 4.05 (s, 2H), 3.11(t, 2H, $J = 7.1$ Hz), 2.86 (t, 2H, $J = 7.1$ Hz); ^{13}C NMR (DMSO-d_6) δ : 173.52, 169.31, 150.74, 150.62, 136.61, 128.34, 127.03, 127.90, 118.43, 116.84, 114.87, 47.77, 42.44, 31.34; MS: m/z 243 $[M]^+$; Anal. $C_{15}H_{17}NO_2 \cdot H_2C_2O_4 \cdot 0.5H_2O$ (C, H, N).

2.4.2. 2-{[(1-methyl-2-phenylethyl]amino} methyl)benzene-1,4-diol oxalate (AMP-DBA)

Bright orange solid 0.1502 g, yield 80%; m.p. 148–150 °C; $\lambda_{max} = 299$ nm; ^{1}H NMR (DMSO-d_6) δ: 7.38–7.20 (m, 6H), 6.80–6.68 (m, 2H), 5.01 (br, s, 4H), 4.09 (s, 2H), 3.44–3.22 (m, 2H), 2.70–2.58 (m, 1H), 1.14–1.09 (d, 3H, $J = 6.4$ Hz);

^{13}C NMR (DMSO-d_6) δ : 172.44, 168.53, 150.40, 148.42, 137.81, 131.02, 127.04, 125.66, 118.05, 115.33, 114.25, 47.35, 39.87, 38.95, 37.23, 19.74; MS: m/z 257 $[M]^+$; Anal. $C_{16}H_{19}NO_2$. $0{,}5H_2C_2O_4.1{,}5H_2O$ (C, H, N).

2.4.3. 2-({[2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}methyl)benzene-1,4-diol oxalate (4-HNE-DBA)

Yellow solid 0.1807 g, yield 90%; m.p. 215–216 °C; $\lambda_{max} = 276$ nm; ^{1}H NMR (DMSO-d_6) δ: 8.24 (s, 1H), 7.16–6.63 (m, 6H), 4.46 (d, 1H, $J = 6$ Hz), 3.56–3.33 (m, 3H), 1.16 (d, 3H, $J = 6.2$ Hz); ^{13}C NMR (DMSO-d_6) δ : 171.65, 167.12, 155.33, 149.73, 148.13, 126.35, 125.23, 117.11, 116.01, 114.68, 113.24, 112.05, 63.66, 55.51, 38.13, 11.83; MS m/z 289 $[M]^+$; Anal. ($C_{16}H_{19}NO_4.0{,}5H_2C_2O_4.1H_2O$) C, H, N.

2.5. Chromatography

Separations were performed on a 5 μm Hypersil ODS RP-18 column (15 cm × 4.6 mm) (Alltech, Deerfield, IL, USA) fitted with a guard column (5 μm Hypersyl ODS RP-18) and eluted isocratically with methanol–sodium hydrogen phosphate buffer (50 mM, pH 5.5) (30:70 v/v) containing trietylamine (0.5% v/v) (TEA). The mobile phase was filtered through GS-type filters (0.45 μm, Millipore, Bedford, MA, USA) and on-line degassed with a Model ERC-3311 solvent degaser (Erma, Tokyo, Japan). Chromatography was performed at room temperature (22 °C) and at a flow-rate of 1.0 ml/min.

2.6. Standard solutions

Standard solutions of AMP-DBA, 4-HNE-DBA and IS in the concentration range 10–40 nmol/ml were prepared diluting known amounts of methanol stock solution. All solutions were stored in the dark at 4 °C.

3. Results and discussion

3.1. Derivatization procedure

Scheme 2 shows the procedure of derivatization using 2,5-DBA as a derivatizing agent. This aromatic aldehyde was selected for its easy reactivity and its lower oxidation potential compared with other isomers such as 3,4-dihydroxybenzaldeide [40]. The electrochemical probe was reductively aminated by reaction with the primary aromatic amines AMP, 4-HNE and PHE to give the corresponding secondary amines using BER as a chemoselective reducing agent [41]. The most commonly used hydride reducing agent is cyanoborohydride, but in this case the reaction time results very long and the reaction is not suitable for derivatization because it needs a large amount of amine. In addition, the syntheses that use solid supported reagents are attractive because the reactions are very rapid (very often they involve only one step), clean, high yielding and the workup involves simple filtrations and evaporation of the solvent [42].

3.2. Optimisation of derivatization procedure

The electroactive derivatives of the amine standards were obtained in one step. Experiments were performed to establish the optimum derivatization time. For this purpose, samples of each reaction were taken at appropriate time intervals (15 min), diluted with methanol and immediately analysed by HPLC-UV. The reactions were completed after 60 min. Fig. 1 shows the trend of reactions.

3.3. Optimisation of electrochemical detection

In this study several parameters were examined in order to optimise the ECD detection of AMP-DBA, 4-HNE-DBA and IS. The electrochemical properties of derivatised compounds were studied using their hydrodynamic voltammograms (Fig. 2). The data analysis showed that increasing the applied potential from $+0.2$ to $+0.8$ V, the detector response was enhanced for all the electroactive compounds. Under the chromatographic conditions previously reported, the electrochemical derivatives responded at ECD oxidation potentials higher than $+0.2$ V. At potential $+0.6$ V

no further increases in peak area was recorded. Particularly, the high response of the electroactive probe 2,5-DBA, recorded at +0.2 V, was observed. The 4-HNE-DBA showed a good response at +0.6 V, while the other compounds were detectable at lower potential. The observed data indicate that the optimum potential is +0.6 V.

The ECD performance was moreover markedly influenced by the ionic strength of mobile phase and the best condition was 50 mM phosphate buffer because no significant improvement in detector response was achieved by further increasing of phosphate concentration. A good robustness was recorded for the pH of mobile phase because no significant variation in the chromatograms was recorded in the range 5–7.5.

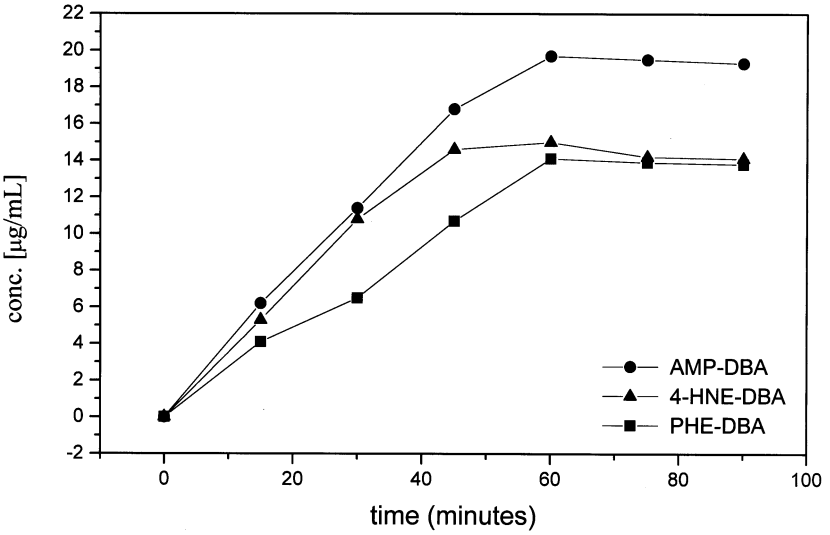


Fig. 1. Curve of concentration of derivatised compounds AMP-DBA, 4-HNE-DBA and PHE-DBA (IS) vs. time of reaction.

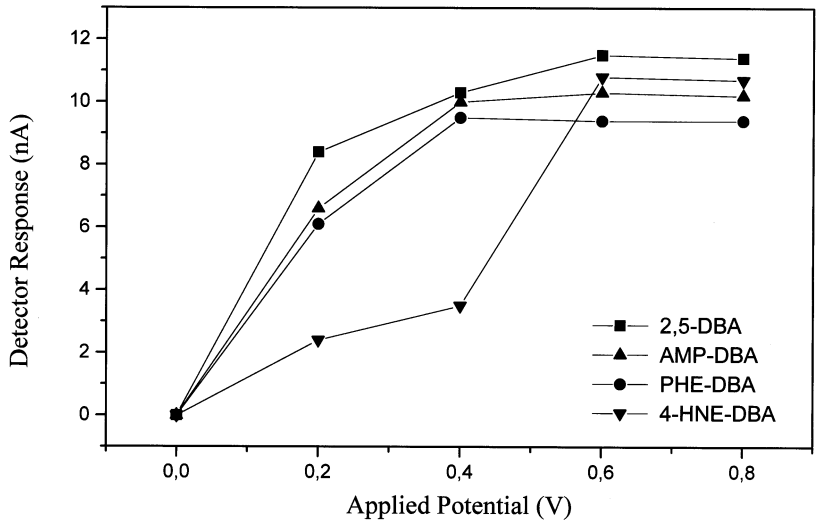


Fig. 2. Hydrodynamic voltammograms of electroactive 2,5-DBA, AMP-DBA, PHE-DBA (IS) and 4-HNE-DBA.

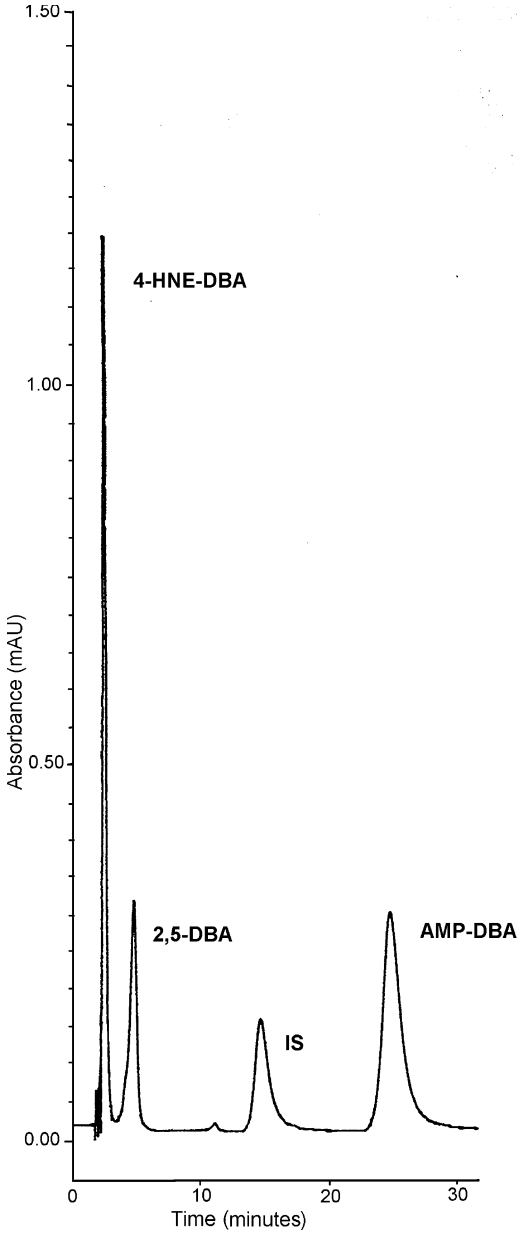


Fig. 3. Typical HPLC-UV chromatogram of standard mixture of electroactive compounds: 4-HNE-DBA (2.8 min), 2,5-DBA (4.8 min), IS (14.8 min) and AMP-DBA (24.8 min).

3.4. Chromatographic separation

Fig. 3 shows a representative HPLC chromatogram with UV detection at 276 nm of AMP-DBA, 4-HNE-DBA and IS at concentrations of 2.2, 1.9 and 2.3 μmol/ml, respectively. Figure 4 shows the chromatogram of the same standards compounds with ECD detection at concentrations of 19.4 nmol/ml for AMP-DBA, 17.2 nmol/ml for 4-HNE-DBA and 20.5 nmol/ml for IS. In order to obtain an optimal chromatographic separation, different mobile phases and columns were evaluated. The simultaneous elution of all electroactive derivatives would be possible in a shorter time under gradient conditions, but this procedure is not suitable for ECD detection.

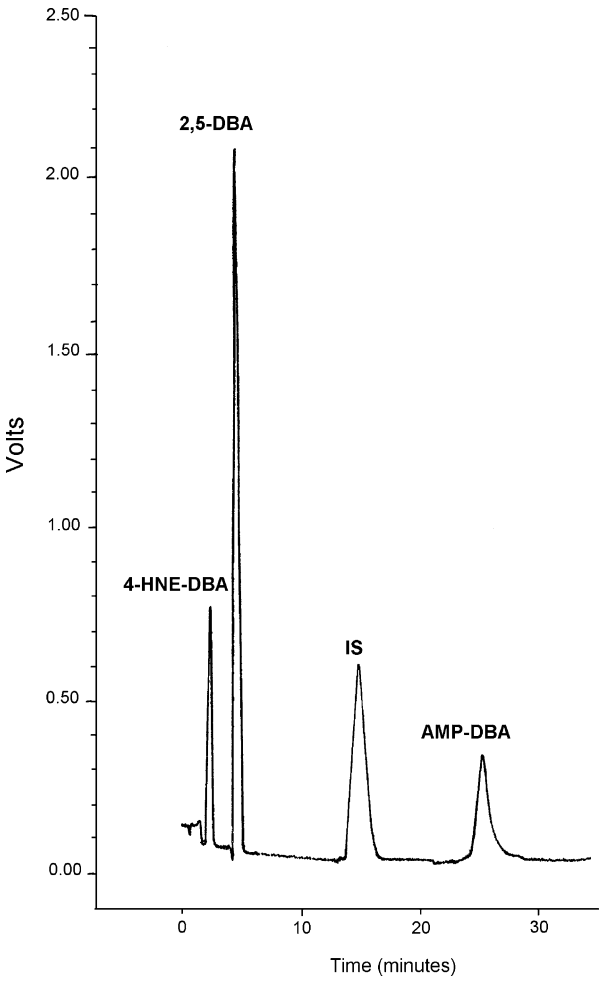


Fig. 4. Typical HPLC-ECD chromatogram of standard mixture of electroactive compounds: 4-HNE-DBA (2.8 min), 2,5-DBA (4.8 min), IS (15.0 min) and AMP-DBA (25.4 min).

Chromatographic separation was carried out in about 25 min under isocratic condition on a 5 μm Hypersil ODS RP-18, 15 cm reversed phase column and eluted with methanol–sodium hydrogen phosphate buffer (50 mM, pH 5.5) (30:70 v/v) containing TEA (0.5% v/v) as a competing base because it improves chromatographic performance of standards electroactive compounds.

3.5. Linearity and detection limit

The linearity of response was examined for each derivatised compound, analysing five solutions in the range 10–40 nmol/ml. The correlation coefficients of the linear regression of the standard curves were greater than 0.99. Detection limits (LOD) were determined, from five runs, using progressively lower concentrations of the electroactive compounds for a signal/noise ratio of 3:1 ($S/N = 3$) with an injected volume of 10 μl. The limit of detection were less than 50 ng/ml for each compound and the limits of quantitation (LOQ) were comprised in the range 0.3–0.6 μg/ml.

4. Conclusions

The HPLC-ECD method described in this study was applied for the determination of AMP and its metabolite 4-HNE. The selective and easy derivatization of AMP, 4-HNE and PHE by the electroactive labelling 2,5-DBA yields stable and highly sensitive electroactive secondary amines that can undergo specific redox reaction under the condition reported. The HPLC-ECD method developed is a versatile technique because other primary phenylethylamines of forensic interest can be determinated with this system.

Moreover, the described method is applicable for all cases where it is possible to determine selectively small amount of aromatic primary amines through the conversion of the compounds in detectable electroactive derivatives, without complicated steps of purification.

Acknowledgements

This work was supported by 'Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST)', Rome, Italy.

References

[1] C. Giround, M. Augsburger, F. Sadeghipour, E. Varesio, J.L. Veuthey, L. Rivier, Schweiz. Rundsch. Med. Prax. 86 (13) (1997) 510–523.
[2] L.S. Seiden, G.A. Ricaurte, in: H.Y. Meltzer (Ed.), Psychopharmacology, The Third Generation of Progress, Raven Press, New York, 1987, pp. 359–366.
[3] R.W. Rockhold, in: E. Jucker (Ed.), Progress in Drug Research, vol. 50, Birkhäuser Verlag, Basel, Switzerland, 1998, pp. 155–192.
[4] P.K. Sonsalla, Drug Alcohol Depend. 37 (1995) 101–105.
[5] T. Kraemer, H.H. Maurer, J. Chromatogr. B 713 (1998) 163–187.
[6] A. Brettell, R. Saferstein, Anal. Chem. 69 (1997) 123R–143R.
[7] T.A. Brettell, K. Inman, N. Rudin, R. Saferstein, Anal. Chem. 71 (1999) 235R–255R.
[8] B.M. Farrel, T.M. Jefferies, J. Chromatogr. Biomed. Appl. 272 (1983) 111–128.
[9] J. Sevcik, K. Lemr, B. Smysl, D. Jirovsky, P. Hradil, J. Liq. Chromatogr. Rel. Technol. 21 (1988) 2473–2484.
[10] W. Weinmann, M. Renz, S. Vogt, S. Pollak, Int. J. Leg. Med. 113 (4) (2000) 229–235.
[11] S. Cheung, H. Nolte, S.V. Otton, R.F. Tendale, P.H. Wu, E.M. Sellers, J. Chromatogr. B 690 (1–2) (1997) 77–87.
[12] M. Sato, T. Mitsui, J. Pharm. Biomed. Anal. 16 (1) (1997) 139–145.
[13] G.W. Kunsman, R. Jones, B. Levine, M.L. Smith, J. Anal. Toxicol. 22 (4) (1998) 310–313.
[14] K. McCambly, R.C. Kelly, T. Johnson, J.E. Johnson, W.C. Brown, J. Anal. Toxicol. 21 (6) (1997) 438–444.
[15] J.T. Cody, S. Valtier, J. Anal. Toxicol. 22 (4) (1998) 299–309.
[16] P. Kintz, J. Anal. Toxicol. 21 (7) (1997) 570–575.
[17] J.F. Van Bocxlaer, W.E. Lambert, L. Thienpont, A.P. DeLeenheer, J. Anal. Toxicol. 21 (1) (1997) 5–11.
[18] C. Battu, P. Parquet, A. Fauconnet, E. Lacassie, G. Lachatre, J. Chromatogr. Sci. 36 (1) (1998) 1–7.
[19] J. Rohrich, G. Kauert, Forensic Sci. Int. 84 (1–3) (1997) 179–188.
[20] Y. Nakahara, R. Kikura, M. Yasuhara, T. Mukai, Forensic Sci. Int. 84 (1–3) (1997) 157–164.
[21] K. Hara, S. Kashimura, Y. Hieda, M. Kageura, J. Anal. Toxicol. 21 (1) (1997) 54–58.
[22] A. Ramseier, C. Siethoff, J. Caslavska, W. Thormann, Electrophoresis 21 (2) (2000) 380–387.

[23] A. Ramseier, F. Von Heeren, W. Thormann, Electrophoresis 19 (16–17) (1998) 2967–2975.
[24] X. Paez, P. Rada, S. Tucci, N. Rodriguez, L. Hernandez, J. Chromatogr. A 735 (1–2) (1996) 263–269.
[25] R. Backer, D. Tautmann, J. Forensic Sci. 42 (1) (1997) 157–161.
[26] O. Al-Dirbashi, J. Qvarnstrom, K. Irgum, K.L. Nakashima, J. Chromatogr. B Biomed. Appl. 712 (1–2) (1998) 105–112.
[27] B.S. Foster, D.D. Gilbert, A. Hutchaleelaha, M. Mayersohn, J. Anal. Toxicol. 22 (4) (1998) 265–269.
[28] O.Y. Al-Dirbashi, N. Kuroda, M. Wada, M. Takashi, K. Nakashima, Biomed. Chromatogr. 14 (5) (2000) 293–300.
[29] D. Talwar, I.D. Watson, M.J. Stewart, J. Chromatogr. B Biomed. Sci. Appl. 735 (2) (1999) 229–241.
[30] R. Herraez-Hernandez, P. Campins-Falco, L.A. Tortajada-Genaro, Analyst 123 (10) (1998) 2131–2137.
[31] R. Herraez-Hernandez, P. Campins-Falco, A. Sevillano-Cabeza, J. Chromatogr. Sci. 35 (4) (1997) 169–175.
[32] R. Herraez-Hernandez, P. Campins-Falco, A. Sevillano-Cabeza, J. Chromatogr. B Biomed. Sci. Appl. 679 (1–2) (1996) 69–78.
[33] R. Herraez-Hernandez, P. Campins-Falco, A. Sevillano-Cabeza, Anal. Chem. 68 (5) (1996) 734–739.
[34] P. Campins-Falco, A. Sevillano-Cabeza, C. Molins-Legua, M. Kohlmann, J. Chromatogr. B Biomed. Appl. 687 (1) (1996) 239–246.
[35] T.K. Wang, M.S. Fuh, J. Chromatogr. B Biomed. Appl. 686 (2) (1996) 285–290.
[36] C. Molins-Legua, P. Campins-Falco, A. Sevillano-Cabeza, J. Chromatogr. B Biomed. Appl. 672 (1) (1995) 81–88.
[37] E. Bousquet, N.A. Santagati, S. Tirendi, J. Liq. Chromatogr. Rel. Technol. 20 (5) (1997) 757–770.
[38] E. Bousquet, V. Cavrini, R. Gatti, A. Spadaro, J. Liq. Chromatogr. Rel. Technol. 21 (18) (1998) 2873–2886.
[39] E. Bousquet, R. Acquaviva, A. Spadaro, A. Vanella, J. Liq. Chromatogr. Rel. Technol. 22 (19) (1999) 2957–2974.
[40] K. Ueno, T. Umeda, J. Chromatogr. 585 (1991) 225–231.
[41] N.M. Yoon, E.G. Kim, H.S. Son, Choi, J. Synth. Commun. 23 (11) (1993) 1595–1599.
[42] D.H. Drewry, D.M. Coe, S. Poom, Med. Res. Rev. 19 (1999) 97–148.