DETERMINATION OF 2,6–DIMETHYLANILINE AND O–TOLUIDINE IMPURITIES IN PREPARATIONS FOR LOCAL ANAESTHESIA BY THE HPLC METHOD WITH AMPEROMETRIC DETECTION

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Abstract: 2,6–Dimethylaniline (2,6–DMA) and o–toluidine (o–TLD) together with their decomposition products are potential technological impurities of Pharmaceuticals used for local anaesthesia, in which lidocaine and prilocaine appear as active substances. Pharmacopoeial analytical methods for the determination of these impurities are little sensitive (from about 1 μg ml^{-1}) and accurate and provide results which are difficult to interpret. Taking the above into account, a sensitive and specific amperometric method has been developed, which enables, after separation with the use of HPLC, an accurate determination of the content of 2,6–DMA and o–TLD in various pharmaceutical preparations. The determinations were performed at a glassy carbon electrode at a potential of +0.85 V. The limit of detection for both 2,6–DMA and o–TLD was 0.8 ng ml^{-1}. On the other hand, the limit of quantitation, considering a signal to noise ratio, was 1.5 ng ml^{-1}. The developed method allows to determine low concentrations of the impurities in question, which are hardly 1/120000 of the main substance. Preparation and determination of samples is carried out in a relatively short time, thus the method can be applied to routine investigations. Statistical evaluation of the obtained results shows that the accuracy and precision of the elaborated HPLC–ED method is good.

Keywords: 2,6–dimethylaniline, o–toluidine, determination, HPLC, amperometric detection.

There are many preparations for local anaesthesia on the pharmaceutical market, in which lidocaine or prilocaine can occur as active substances. At present, lidocaine is applied as a valuable agent suitable for all kinds of local anaesthesia (surface, infiltration, medullary and conducting) in surgery, gynaecology and dentistry. In the human organism, lidocaine, under the influence of macrosomatic enzymes of liver, undergoes hydrolytic decomposition to 2,6–dimethylaniline which also demonstrates anaesthetic activity, however it is significantly more toxic than the parent compound.

In respect of pharmacology, prilocaine is similar to lidocaine, however, its action is longer. From among amide agents applied in anaesthetic practice, prilocaine is least toxic. During biotransformation prilocaine decomposes to o–toluidine, which may oxidize hemoglobin to methemoglobin. Prilocaine is applied in all types of anaesthesia. Recently, because of the discovered risk of the occurrence of methemoglobinemia, particularly in babies, its application has been limited (1,2). 2,6–Dimethylaniline may also cause methemoglobinemia, however in a somewhat less degree than o–toluidine. In studies on animals, it was found an

additional cancerogenic activity of both these compounds on bladder (3) and nassal fosse (4).

2,6–Dimethylaniline and o–toluidine can be potential technological impurities of medicinal products because they are used as substrates in the synthesis of pharmaceuticals. In addition, they can also be decomposition products during storage of drugs containing lidocaine or prilocaine. Studies for the presence of 2,6–DMA impurity in lidocaine are recomended by pharmacopoeias of FPV, Ph. Eur. 2000, DAB 10 and BP 1998 and by numerous standards of the producers. The permissible level of 2,6–DMA in lidocaine–substance and of o–TLD in prilocaine is 100 ppm (Ph. Eur. 2000). The British Pharmacopoeia 1998 is forcasting an impurity of 2,6–DMA amounting to 400 ppm (calculated for the acitve substance) in preparations containing lidocaine.

On the other hand, in injections containing prilocaine a maximum impurity with o–TLD may-be as high as 10000 ppm. In determination experiments, the recommended reactions include binding of the substance studied with dimethyl amino-benzaldehyde (Ph. Eur. 2000, BP 1998) or diazation and binding with thymol (FPV) followed by a comparison of the obtained colour with the

standard. Looking for the presence of o–toluidine can be performed by thin layer chromatography method (Ph. Eur. 2000) and by colorimetric method (BP 1998).

Pharmacopoeial analytical methods for the determination of these impurities are little accurate and provide results difficult to interpret. For this reason, elaboration of a sensitive and specific method was desirable. This method should enable accurate and explicit evaluation of 2,6–DMA and o–toluidine impurities in various pharmaceutical preparations applied for local anaesthesia.

In the literature reporting determination of these compounds, the most frequent techniques include high performance liquid chromatography (HPLC) (5–9) and gas chromatography (GC) (10–15) combined with various detection methods such as: UV (5–7,9,16) mass spectrometry (10,11,14,17), electrochémistry (8) or eventually with the application of a detector for electron capture (15). For isolation and determination of toluidine and its derivatives capillary electrophoresis has also been used (18).

The application of electrochemical detection to the determination of low concentrations of 2,6–DMA or o–TLD is based on their anodic oxidation reaction. Unsubstituted aromatic amines, N–alkyl and N,N–dialkyl follow these reactions in the potential range 0.4–1.0 V against the normal hydrogen electrode. As an example of utilization of these properties the determination of p–aminophenol in preparations containing paracetamol maybe quoted (19,20). Anodic oxidation of aromatic amines is a complex process and can lead to a series of various products depending on their structure and conditions of electrolysis (21). In Figure 1 schematically are shown oxidation reactions of aniline derivatives.

In this study, the chosen working electrode was a glassy carbon electrode because mercury electrodes undergo anodic oxidation already at a potential of +0.4 V versus the saturated calomel electrode (SCE) and the surface of platinum or gold electrodes covers with a layer of oxides. Due to these processes, during polarization of the electrodes great residual currents occur and reduction of oxides as well as adsorption and desorption of various compounds take place. On the other hand, the glassy carbon electrode is characterized by good mechanical properties, great reaction rate of charge transfer and low background current. Thanks to these qualities this electrode is suitable for repeatable and reproducible determinations. In addition, this electrode makes it possible studying of processes occurring in a broad range of potentials, depending on pH of the investigated solution:
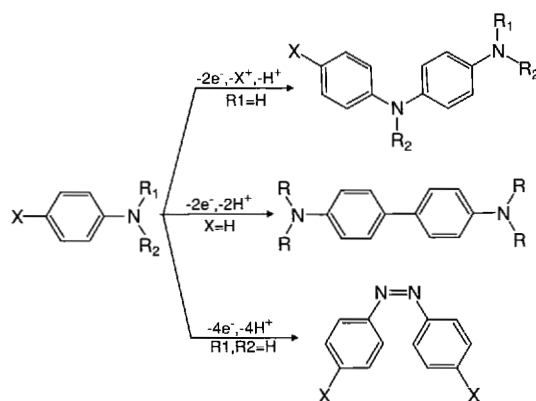

Figure 1. Oxidation reactions of aniline derivatives.

from –1.6 V to +1.0 V for basic solutions, from –0.2 V to +1.5 V for acidic solutions and for buffered solutions at pH 4.5 from –0.8 V to +1.2 V (vs. SCE).

EXPERIMENTAL

Reagents

Acetic acid 100% – AppliChem, anhydrous sodium acetate – Merck, acetonitrile – Labscan, lithium chloride – BDH. All these reagents were of purity suitable for AAS and HPLC. Redistilled water was additionally purified in the Nanopure Deionization System (Barnstead).

Mobile phase: acetate buffer (0.01 mol l^{-1} solution of sodium acetate at pH 4.8 adjusted by addition of 100% acetic acid): acetonitrile (4:6) with an addition of lithium chloride (0.5 mol l^{-1} in mobile phase).

Standard solution of 2,6–DMA was freshly prepared before each series of measurements by accurately weighing about 10 mg of 2,6–DMA and dissolving it in the mobile phase in a 10 ml volumetric flask. From this solution successive dilutions were then prepared of concentrations from 1 to 450 ng ml^{-1}.

Standard solution of o–TLD was freshly prepared before each series of measurements by accurately weighing about 10 mg of o–TLD and dissolving it in the mobile phase in a 10 ml volumetric flask. From this solution successive dilutions were then prepared of concentrations from 1 to 230 ng ml^{-1}.

Studied materials

Calgel (GlaxoWellcome), a gel with 0.33% of lidocaine hydrochloride; Emla 5% (Astra), a cream with 25 mg of lidocaine and 25 mg of prilocaine in 1 g of the cream; Lignocain 2% (Braun), injections; Cathejell (Montavit), a gel with 2% of lidocaine

hydrochloride; Plidocain 100 mg/10 ml (Pliva-
-Krakow), injections; Plidocain 200 mg/10 ml (Pli-
va-Krakow), injections; Plidocain 2% (Pliva-Kra-
kow), injections; Tytanoreina (Jonhson & John-
son), a cream with 2% of lidocaine; Lignocainum
5% „grave" (WZF Polfa), injections; Lidocain
10% (EGIS), a spray; Lignocainum 1% (WZF
Polfa), injections; Lignocainum 2% (WZF Polfa),
injections; Lidocaine hydrochloride monohydrate
(AstraZeneca); Prilocaine hydrochloride (Astra);
2,6-dimethylaniline hydrochloride (Astra); o-tolui-
dine hydrochloride (AstraZeneca).

Apparatus

A μ Autolab voltameter (Eco Chemie, Utrecht),
controlled by a computer with an accompanying
software GPES (General Purpose Electrochemical
System, Version 4.8); a liquid chromatograph, type
LC–10AP (Shimadzu); a steel column Luna, 100
mm long and 4.6 mm in diameter with a column
packing C18 of particle size 5 μm (Phenomenex);
an electrochemical detector Intro (Antec, Leyden)
with a glassy carbon electrode (working electrode),
a silver–silver chloride electrode (Ag/AgCl – refe-
rence electrode) and an auxiliary electrode; effec-
tive volume of the flow cell was 0.5 μl and a 20 μl
loop were used throughout.

Preparations of samples and determination of 2,6–DMA and o–TLD in pharmaceuticals

From the studied preparations in the form of
injection samples corresponding to 10 mg of lido-

caine were measured out into 10 ml volumetric
flasks and made up to volume with the mobile
phase. An aliquot of preparation Emla – 5% cream,
corresponding to 20 mg of lidocaine and prilocaine,
was weighed to a 100 ml volumetric flask, dissol-
ved in the mobile phase and made up to the mark
with the same solution. An aliquot of preparation
Cathejell–gel, corresponding to 20 mg of lidocaine,
was weighed to a 10 ml volumetric flask and made
up to volume with the mobile phase. An aliquot of
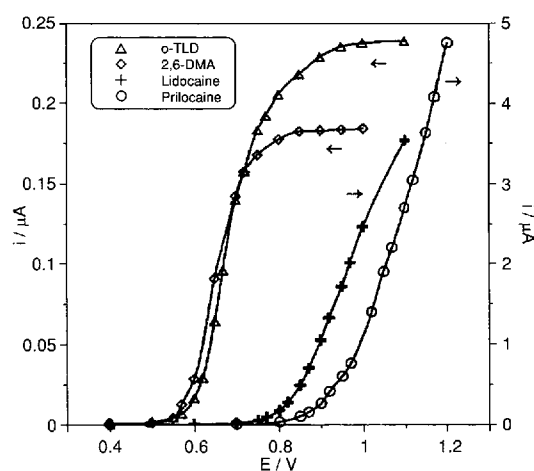preparations Tytanoreina– cream, corresponding to

Figure 3. Dependence of the peak current intensities of o–TLD, 2,6–DMA, lidocaine and prilocaine on the electrode potential in the mobile phase: 0.01 mol l⁻¹ acetate buffer pH 4.8: acetonitrile (4:6); flow rate 1.5 ml min⁻¹; amperometric detection.
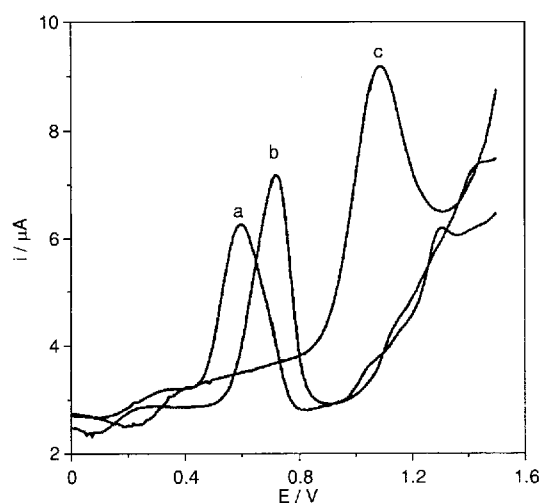

Figure 2. Voltammograms recorded in 0.01 mol l⁻¹ acetate buffer pH 4.8 from a solution containing 20 μg ml⁻¹ of 2,6–dimet-hylaniline (a), 20 μg l⁻¹ of o–toluidine (b) and 170 μg ml⁻¹ of lidocaine (c).
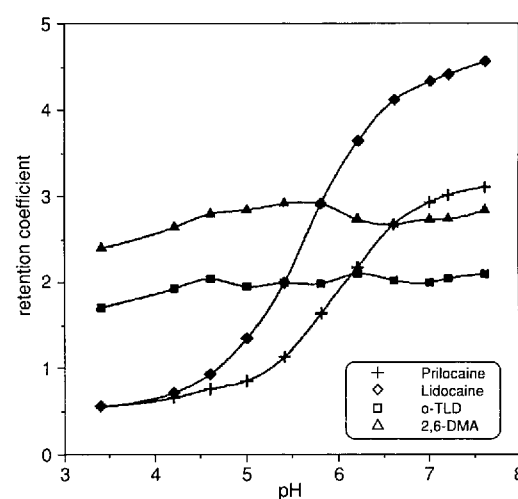

Figure 4. Relation between the retention coefficient k, of prilocai-ne and lidocaine (100 μg ml⁻¹ each), and o–toluidine and 2,6–di-methylaniline (100 ng ml⁻¹ each) and the pH of 0.01 mol l⁻¹ acetate buffer in the mobile phase with acetonitrile (4:6); flow rate 1.5 ml min⁻¹; amperometric detection; electrode potential +0.85 V.

Table 1. Statistical evaluation of the determination results of o–toluidine in model solutions by HPLC–ED

Conc. of studied solution [ng · ml⁻¹]	Concetration found [ng · ml⁻¹]		Standard deviation	RSD [%]	Confidence interval of arithmetic mean $\bar{x} \pm t \cdot s$ [ng · ml⁻¹]
84.07	92.27 91.95 91.39	90.70 91.07 90.24	0.76	0.83	91.27 ± 0.80
84.52	86.60 87.06 85.90	86.00 85.31	0.67	0.78	86.17 ± 0.83
8.41	8.61 8.50 8.68	9.03 9.41 8.94	0.34	3.79	8.86 ± 0.35
8.45	8.21 8.59 8.08	8.85 8.47	0.31	3.63	8.44 ± 0.38
1.68	1.63 1.38 1.75	1.70 1.53	0.15	9.16	1.60 ± 0.18
1.69	1.84 1.73 2.20	1.51 1.73	0.25	14.11	1.80 ± 0.31

Table 2. Statistical evaluation of the determination results of 2,6–dimethylaniline in model solutions by HPLC–ED.

Conc. of studied solution [ng · ml⁻¹]	Concetration found [ng · ml⁻¹]		Standard deviation	RSD [%]	Confidence interval of arithmetic mean $\bar{x} \pm t \cdot s$ [ng · ml⁻¹]
86.97	88.89 89.33 88.39	88.28 87.33	0.75	0.85	88.44 ± 0.93
86.2	86.33 87.22 86.66	86.94 85.43	0.69	0.80	86.51 ± 0.85
8.7	9.44 9.32 9.04	9.15 9.02	0.18	1.97	9.19 ± 0.22
7.99	7.80 7.79 8.12	7.94 7.81 8.01	0.13	1.70	7.91 ± 0.14
1.72	1.43 1.67 1.66	1.62 1.60	0.10	5.98	1.59 ± 0.12
1.60	1.09 1.19	1.16 1.08	0.05	4.60	1.13 ± 0.09

20 mg of lidocaine, was weighed to a 50 ml volumetric flask, 1 ml of 0.1 mol l⁻¹ hydrochloric acid and 20 ml of water was added, the mixture was shaken untill complete dissolution. Then, the solution was made up to the mark with water and filtered through a hard filter paper. 20 μl of the prepared solution was passed through the chroma-tographic column and chromatograms were recorded during 150 s with the use of the amperometric detector, the potential of the glassy carbon electrode being +0.85 V vs. the Ag/AgCl reference electrode. The current intensity of the o–TLD peak was measured for the retention time of 73 s, and that for the 2,6–DMA peak was 93 s.

Table 3. Statistical evaluation of the determination results of o–TLD impurities in preparations Emla–cream and of 2,6–DMA in preparation Lignocainum–injections obtained by the methods of standard curve and standard addition using the HPLC–ED technique.

Preparation	Content [μg · ml⁻¹]		Standard deviation	RSD [%]	Confidence interval of arithmetic mean $\bar{x} \pm t \cdot s$ [μg · ml⁻¹]
o–TLD w EMLA – – 5% cream	26.05 25.69 25.17	25.00 24.84 24.52	0.56	2.24	25.21 ± 0.59[a]
	30.81 29.33 28.16	28.83 26.16 27.36	1.61	5.67	28.44 ± 1.69[b]
2,6–DMA w Lignocainum 5% „grave" – injections	1.18 1.18 1.17	1.19 1.16 1.16	0.01	1.03	1.17 ± 0.01[a]
	0.98 0.98 1.05	1.15 1.09 1.02	0.07	6.37	1.05 ± 0.07[b]

a – standard calibration curve
b – standard addition technique

Table 4. Determination results of impurities in the selected preparations by the HPLC–ED method.

Preparation	Impurity content recounted to active substance [ppm]	Excess of active substance in relation to that determined
Calgel (GlaxoWellcome), gel containing 0.33% of lidocaine hydrochloride	below detection limit (2,6–DMA)	–
Cathejell (Montavit), gel with 2% of lidocaine hydrochloride	below detection limit (2,6–DMA)	–
Emla 5% (Astra), cream containing 25 mg of lidocaine and 25 mg of prilocaine in 1 g of cream	1000 (o–TLD)	1000
Titanoreina (Johnson&Johnson), cream with 2% of lidocaine	below detection limit (2,6–DMA)	–
Lignocainum 1% (WZF Polfa), injections	8 (2,6–DMA)	120 000
Lignocainum 2% (WZF Polfa), injections	9 (2,6–DMA)	110000
Lignocain 2% (Braun), injections	10 (2,6–DMA)	100 000
Lidocain 10% (EGIS), spray	below detection limit (2,6–DMA)	–
Lignocainum 5% „grave" (WZF Polfa), injections	20 (2,6–DMA)	50 000
Plidocain 100 mg/10 ml (Pliva, Kraków), injections	25 (2,6–DMA)	40 000
Plidocain 200 mg/10 ml (Pliva, Kraków), injections	9 (2,6–DMA)	110 000
Plidocain 2% (Pliva, Kraków), injections	11 (2,6–DMA)	90000

RESULTS DISCUSSION

By using the cyclic voltammetry and differential voltammetry methods, the electrode behaviour of 2,6–dimethylaniline, o–toluidine, lidocaine and prilocaine at the glassy carbon electrode has been studied. The measurements have shown that in 0.1 mol l⁻¹ acetate buffer at pH 5.0, 2,6–DMA under-
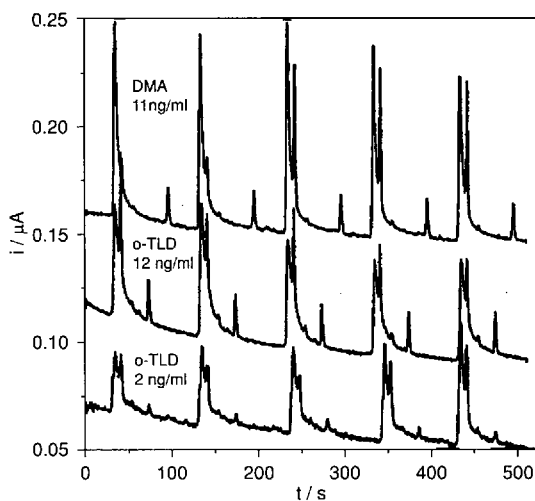
Figure 5. Chromatograms recorded for a multiple injection of the studied solutions; mobile phase:
0.01 mol l⁻¹ acetate buffer pH 4.8: acetonitrile (4:6); flow rate 1.5 ml min⁻¹; amperometric detection; electrode potential +0.85 V.
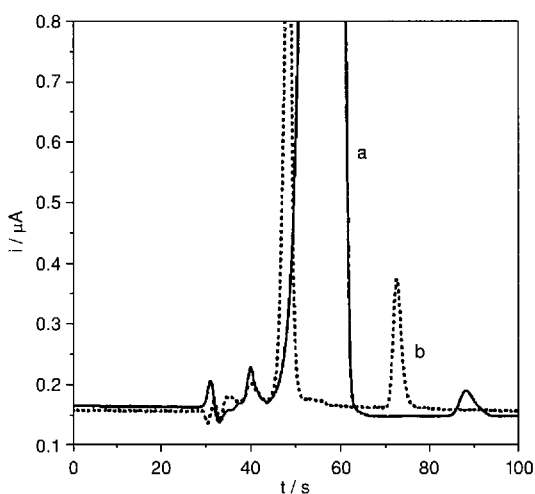

Figure 6. Chromatograms recorded from solutions containing preparation Lignocainum–injections (a) and preparation Emla–cream (b); mobile phase: 0.01 mol l⁻¹ acetate buffer pH 4.8: acetonitrile (4:6); flow rate 1.5 ml min⁻¹; amperometric detection; electrode potential +0.85 V.

goes anodic oxidation at a potential of +0.66 V, and o–TLD at +0.69 V vs. the Ag/AgCl electrode. On the other hand, lidocaine and prilocaine undergo anodic oxidation at a potential which is near to the upper limit of available measuring potentials for the glassy carbon electrode (Figure 2).

The HPLC method with amperometric detection was used to study the dependence of peak current intensities in the range from 0.4 to 1.2 V (Figure 3) and the retention coefficient k, on pH

of the mobile phase for 2,6–DMA, o–TLD, lidocaine and prilocaine (Figure 4). Based on the obtained results as the optimal potential 0.85 V and the optimal value of pH 4.8 have been selected.

Using the developed method, curves were obtained of the dependence of current intensities on the concentration of 2,6–DMA and o–TLD. The obtained curves have a rectilinear character in the whole analyzed range of concentrations: for 2,6–DMA from 1 to 40 ng ml⁻¹ (y=0.8645x+0.3431, r=0.9997) and from 40 to 450 ng ml⁻¹ (y=0.8348x+1.399, r=0.9998); for o–TLD from 1 to 15 ng ml⁻¹ (y=1.8085x+0.8857, r=0.9985) and from 15 to 230 ng ml⁻¹ (y=1.6369x+5.7727, r=0.9990). The detection limit for both 2,6–DMA and o–TLD is equal to 0.8 ng ml⁻¹, however, the quantitation limit, considering the signal to noise ratio, is equal to 1.5 ng ml⁻¹. The determination was carried out with 2,6–DMA and o–TLD solutions prepared from three independent weighed portion of standards from which dilute solutions containing 2, 10 and 100 ng ml⁻¹ of these compounds were obtained (Tables 1 and 2). Reproducibility of the recorded peaks from the standard solutions are shown in Figure 5. The content of impurities in all the preparations chosen for this study was determined by the method of standard curve (Table 4), however, in Emla–5% cream and Lignocainum 5% „grave" also by the method of standard addition (Table 3). Examples of the chromatograms recorded from the solutions containing preparations Emla cream and Lignocainum injections are presented in Figure 6.

The proposed HPLC–ED method allows to determine low concentrations of the impurities under question, which are amounting hardly to 1/120000 of the main substance in the various forms of pharmaceutical preparations containing different additional substances (Table 4). The preparation of samples and determination is performed in a relatively short time. Thanks to this advantage, the method can be applied to routine investigations. Pharmacopoeial methods do not give such possibilities by means of which significantly higher concentrations of 2,6–DMA and o–TLD, of the range 1 µg ml⁻¹, can be detected. The statistical evaluation of the obtained results presented in Tables 1 –3 indicates that the accuracy and precision of the developed HPLC–ED method are good.

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