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Determination of local anaesthetics and their impurities in pharmaceutical preparations using HPLC method with amperometric detection

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Abstract

A method for the determination of local anaesthetics and their impurities – 2,6-dimethylaniline and *o*-toluidine – by high-performance liquid chromatographic method with amperometric detection has been developed. The analysis was performed in an isocratic mode on a reversed phase Luna column 5 μm C-18 (100 mm × 4.6 mm). A mobile phase [0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55)] was selected for the separation and determination of studied anaesthetics and their impurities. Chromatograms were recorded for 500 s by means of an amperometric detector at a potential of +1.0 V of the glassy carbon electrode versus the reference electrode Ag/AgCl. The proposed liquid chromatographic method was successfully applied to the analysis of commercially available pharmaceutical preparations. The limit of the detection for 2,6-dimethylaniline and *o*-toluidine was 0.8 ng ml^{-1}. The limit of qantitation, considering a signal to noise ratio was 1.5 ng ml^{-1}. The method developed in this study is sensitive and selective and can be applied to routine studies of pharmaceuticals in the form of cream and injection.

Keywords: Local anaesthetics; 2,6-Dimethylaniline; *o*-Toluidine; HPLC method with amperometric detection

1. Introduction

There are many preparations for local anaesthesia on the pharmaceutical market, in which lidocaine bupivacaine, mepivacaine, prilocaine or ropivacaine can occur as active substances. They are amide type substances and are widely used to alleviate pain associated with medical procedures in surgery, gynaecology and dentistry. Local anaesthetics drugs are suitable for all kinds of local anaesthesia (surface, infiltration, modularly and conducting). The main impurities, which may be present in preparations containing lidocaine buvpivacaine, mepivacaine, prilocaine or ropivacaine are 2,6-dimethylaniline (2,6-DMA) and *o*-toluidine (*o*-TLD). These impurities may be formed during the synthesis of local anaesthetics or during the storage of preparations.

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 appear as decomposition products during storage of drugs containing lidocaine or prilocaine. 2,6-Dimethylaniline also demonstrates anaesthetic activity, but it is significantly more toxic than the parent compound. Among amide agents used in anaesthetic practice, prilocaine is the least toxic. During biotransformation prilocaine undergoes decomposition to *o*-toluidine, which may then oxidize hemoglobin to methemoglobin. 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 methemoglobine-

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mia, but to a somewhat less degree than *o*-toluidine. In studies on animals, an additional cancerigenic activity of both these compounds on bladder [3] and nasal cavity was found [4].

Quantification of 2,6-DMA impurities in lidocaine, bupivacaine or mepivacaine are described by pharmacopoeias of FP VI, Ph. Eur. 4, DAB 10 and BP 2003 and by numerous specifications of manufacturers. In determination experiments, the recommended reactions include binding of 2,6-DMA with dimethyl aminobenzaldehyde (Ph. Eur. 4, BP 2003, FP VI) or diazation and binding with thymol (FP VI) followed by a comparison of the obtained colour with a standard. Determination of *o*-toluidine can be performed using the high-performance (BP 2003) and thin layer chromatographic methods (Ph. Eur. 4).

The permissible level of 2,6-DMA and of *o*-TLD in substances is 100 ppm (Ph. Eur. 4). The British Pharmacopoeia 2003 allows for lidocaine and bupivacaine injections and gels not more than 400 ppm of 2,6-DMA, while for prilocaine preparations the content of *o*-TLD is limited to 1% (calculated for the active substance). On the other hand, in injections containing prilocaine a maximum content of the *o*-TLD impurity may be as high as 10,000 ppm. Pharmacopoeial analytical methods for the determination of the 2,6-DMA and *o*-TLD impurities are not sufficiently sensitive and accurate, so often provide ambiguous results. This has motivated us to develop a sensitive and specific high-performance liquid chromatographic method with amperometric detection (HPLC-ED) for the quantification of 2,6-DMA, *o*-TLD and local anaesthetics in various pharmaceutical preparations.

Techniques most often recommended in the literature for the determination of these compounds are high-performance liquid chromatography (HPLC) [5–8] and gas chromatography (GC) [11–17] coupled with various detection methods such as: UV [5,6,8,14,15], mass spectrometry [9,10,12,16], electrochemistry [7] or possibly with the application of a detector for electron capture [13]. For isolation and determination of toluidine and its derivatives capillary electrophoresis has also been used [17].

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 undergo these reactions in the potential range +0.4 to +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 may be quoted [18,19]. Anodic oxidation of aromatic amines is a complex process and can lead to various products depending on their structure and conditions of electrolysis [20].

In part one of this study [21] we investigated the electrode behaviour of 2,6-dimethylaniline, *o*-toluidine, lidocaine and prilocaine using the cyclic and differential pulse voltammetric methods in various non-buffered and buffered solutions at the glassy carbon electrode. We also performed preliminary investigations of these substances by HPLC with amperometric detection. The measurements have shown that in 0.1 mol l^{-1} acetate buffer at pH 5.0, 2,6-DMA and *o*-TLD undergo anodic oxidation at the potentials of +0.66 and +0.69 V, respectively, against the Ag/AgCl electrode. On the other hand, lidocaine and prilocaine undergo anodic oxidation at a potential which is close to the upper limit of available measuring potentials for the glassy carbon electrode.

2. Experimental

2.1. Apparatus and conditions

The following apparatus were used throughout this work: a μAutolab voltammeter (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 and a degasser DGU-14A (Shimadzu), an electrochemical detector Flexell of active volume 0.5 μl with a glassy carbon electrode (working electrode), a silver-silver chloride electrode (Ag/AgCl, reference electrode), a steel wire (auxiliary electrode), an SSI Pulse Damper (Antec, Leyden). Effective volume of the flow cell was 0.5 μl and a 20 μl loop was used throughout.

The analysis was performed in an isocratic mode on a Luna 5 μm C-18 analytical column (100 mm × 4.6 mm, i.d., Phenomenex, Torrance, C.A). The mobile phase [Tris buffer (0.01 mol l^{-1} Tris solution adjusted to pH 7.9 with hydrochloric acid):acetonitrile (45:55)] was filtered through filters (0.45 μm, Nylon) and dedegassed with a Sonifier 250/450 (Branson Ultrasonics Corporation, USA) before use. The column was maintained at 30 °C in a column block heater. A flow rate was set at 1.5 ml min^{-1} and sample injections were typically 20 μl.

Oxygen was removed from the system by passing argon for 15 min.

2.2. Materials studied

Emla 5% (Astra), a cream containing 25 mg of lidocaine and 25 mg of prilocaine in 1 g of the cream; Plidocain 20 mg/1 ml (Pliva, Kraków), injections; Scandonest 3% (Septodont), containing 30 mg of mepivacaine hydrochloride in 1 ml; Marcaine spinal 0.5% (AstraZeneca) heavy, containing 20 mg of bupivacaine hydrochloride in 1 ml; lidocaine hydrochloride monohydrate, Working Standard (AstraZeneca); prilocaine hydrochloride, Working Standard (Astra); bupivacaine hydrochloride monohydrate, Working Standard (AstraZeneca); mepivacaine hydrochloride, Working Standard (Astra); ropivacaine hydrochloride monohydrate, Working Standard (Astra); 2,6-dimethylaniline hydrochloride, Working Standard (Astra); *o*-toluidine hydrochloride, Working Standard (AstraZeneca).

2.3. Reagents

Hydrochloric acid from J.T. Baker, Tris (tris-(hydroxymethyl)-aminomethan) from AppliChem, ace-

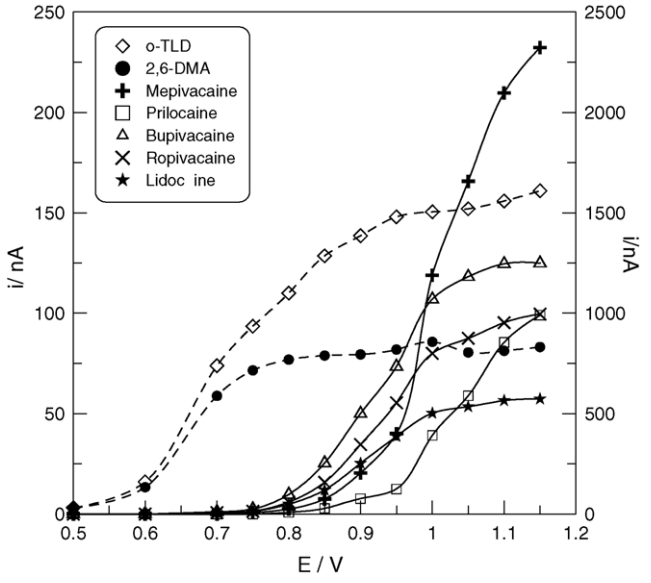

Fig. 1. Dependence of the anodic peak current intensities on the electrode potential; mobile phase: 0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55); flow rate 1.5 ml min^{-1}; amperometric detection.

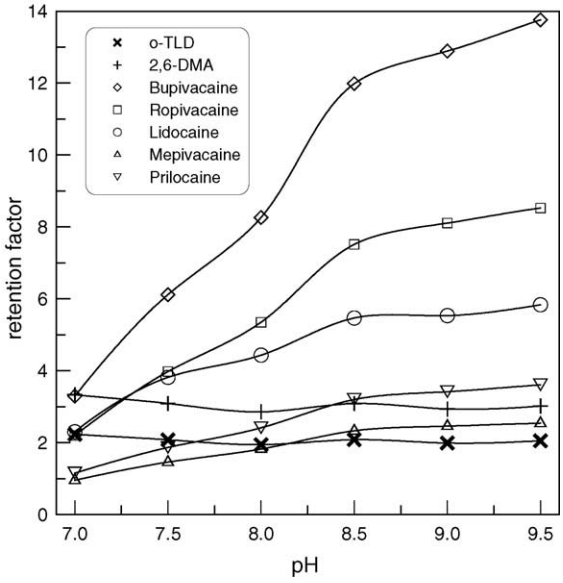

Fig. 2. Relation between the retention factor *k* and the pH of the buffer in the mobile phase; flow rate 1.5 ml min^{-1}; amperometric detection; electrode potential +1.0 V.

tonitrile from Labscan—all of them of purity suitable for AAS and HPLC; doubly distilled water additionally purified in the Nanopure Deionization System (Barnstead) were used throughout.

2.3.1. Mobile phase

Tris buffer—0.01 mol l^{-1} Tris solution adjusted to pH 7.9 with hydrochloric acid:acetonitrile (45:55).

2.3.2. Standard solution

Standard solutions of lidocaine, prilocaine, mepivcaine, ropivacaine and bupivacaine were daily prepared by dissolving about 10 mg of one of the working standard in the mobile phase in a 10 ml volumetric flask. Thus prepared solutions were diluted with mobile phase to the concentration of 10 μg ml^{-1}.

Standard solutions of 2,6-DMA and *o*-TLD were daily prepared in a 10 ml volumetric flask by dissolving about 10 mg of 2,6-DMA or *o*-TLD in the mobile phase. From

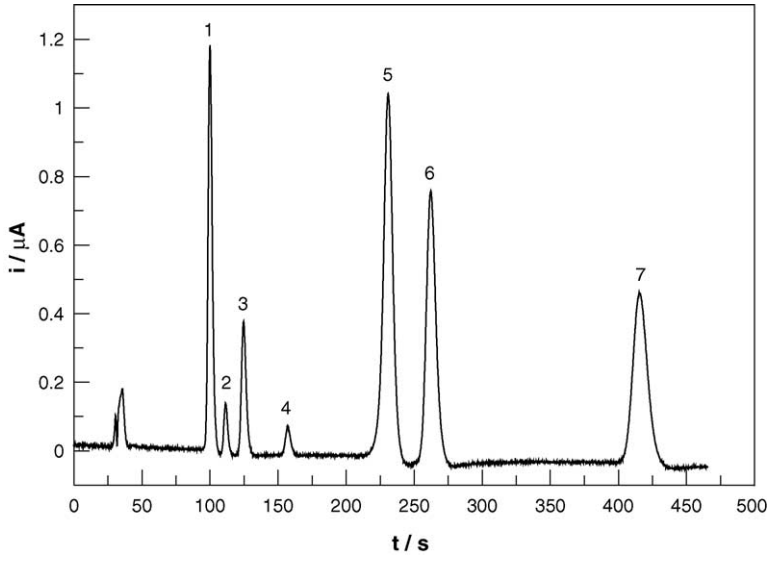

Fig. 3. Chromatogram recorded from solutions containing mixture of 10 μg ml^{-1} of mepivacaine (1), prilocaine (3), lidocaine (5), ropivacaine (6), bupivacaine (7) and 100 ng ml^{-1} of *o*-TLD (2) or 2,6-DMA (4); mobile phase: 0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55); flow rate 1.5 ml min^{-1}; amperometric detection; electrode potential +1.0 V.

Table 1
Quantitative parameters of 2,6-dimethylaniline and *o*-toluidine determination by HPLC-ED method

Parameter	2,6-Dimethylaniline	*o*-Toluidine
Concentration range (ng ml^{-1})	(1) 1–40	(1) 1–15
	(2) 40–450	(2) 15–230
$y = ax + b$	(1) $0.8645x + 0.3431$	(1) $1.8085x + 0.8857$
	(2) $0.8348x + 1.3990$	(2) $1.6369x + 5.7727$
Correlation coefficient	(1) 0.9997	(1) 0.9985
	(2) 0.9998	(2) 0.9990
Standard error of the slope	(1) 0.002	(1) 0.002
	(2) 0.001	(2) 0.002
Standard error of the intercept	(1) 0.002	(1) 0.002
	(2) 0.001	(2) 0.002
Detection limit (ng ml^{-1})	0.8	0.8
Quantitation limit (ng ml^{-1})	1.5	1.5
Within-day R.S.D. (%)	(a) 0.80	(a) 0.78
	(b) 1.70	(b) 3.63
	(c) 4.60	(c) 9.16
Between-day R.S.D. (%)	(a) 1.12	(a) 1.08
	(b) 2.36	(b) 4.71
	(c) 5.98	(c) 14.11
Precision of determination mean $\pm tS/\sqrt{N}$, $N = 6$ (ng ml^{-1})	86.51 ± 0.85	86.17 ± 0.83
	7.91 ± 0.14	8.44 ± 0.38
	1.59 ± 0.12	1.60 ± 0.18

Concentration: (a) 85.0 ng ml^{-1}; (b) 8.5 ng ml^{-1}; (c) 1.6 ng ml^{-1}.

these solutions successive dilutions were then prepared of concentrations from 1 to 450 ng ml^{-1} for 2,6-DMA or from 1 to 230 ng ml^{-1} for *o*-TLD.

All solutions were stored in the dark when not in use.

2.4. Preparations of samples and determination of lidocaine, prilocaine, mepivcaine, ropivacaine and bupivacaine in pharmaceutical formulations

From the studied preparations in the form of injections, samples corresponding to 10 mg of active substance were measured out into 10 ml volumetric flasks and made up to volume with the mobile phase. Thus prepared solutions were diluted to the concentration of 10 μg ml^{-1}. An accurate weight of Emla—5% cream, equivalent to 5 mg of prilocaine, was transferred into a 100 ml volumetric flask, dissolved in the mobile phase and made up to the mark with the same solution. Thus obtained solution was diluted to the concentration of 10 μg ml^{-1}. Then 20 μl portions of the prepared solutions were introduced into the column and the chromatograms

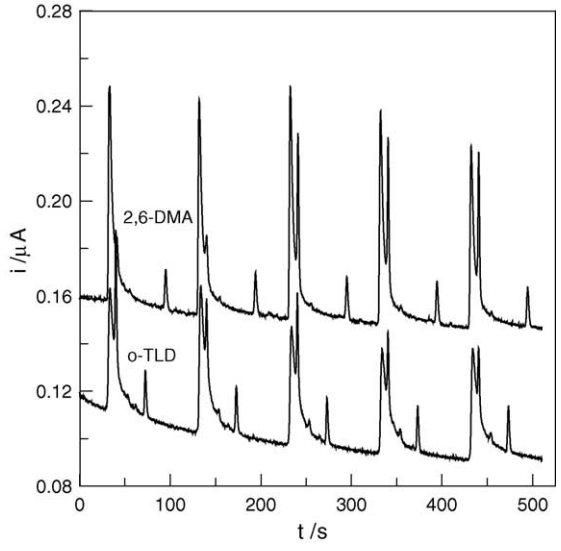

Fig. 4. Chromatograms recorded for a multiple injection of the studied solutions containing 10 μg ml^{-1} of 2,6-DMA or *o*-TLD; mobile phase: 0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55); flow rate 1.5 ml min^{-1}; amperometric detection; electrode potential +1.0 V.

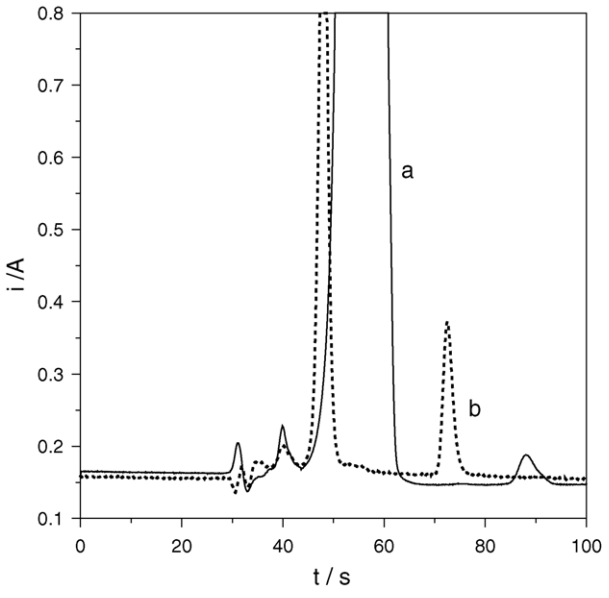

Fig. 5. Chromatogram recorded from solutions containing preparation lignocainum injections (a) and Emla cream (b); mobile phase: 0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55); flow rate 1.5 ml min^{-1}; amperometric detection; electrode potential +1.0 V.

Table 2
Statistical estimation of the results of local anaesthetics determination in pharmaceutical formulations by the HPLC-ED method ($n = 7$)

Preparation (active substance)	Content of active substance	S.D. of arithmetic mean	R.S.D. (%)	$V_x \pm t \cdot S_x$, confidence level 95% (mg ml^{-1})
Plidocain 20 mg ml^{-1} (lidocaine hydrochloride)	20.40 20.22 20.33 20.27 20.24 20.55 20.27	0.116	0.57	20.32 ± 0.10
Marcaine 0.5% (bupivacaine hydrochloride)	5.06 5.01 5.02 5.13 5.11 5.06 5.10	0.046	0.90	5.07 ± 0.04
Scandonest 3% (mepivacaine hydrochloride)	31.66 32.03 32.40 31.77 31.10 32.29 32.25	0.456	1.43	31.93 ± 0.42
Emla 5% (prilocaine 2.5%)	24.45 24.21 24.24 23.56 23.35 24.38 24.50	0.456	1.89	24.10 ± 0.42

were recorded for 500 s using an amperometric detector at a potential of +1.0 V of the glassy carbon electrode versus the reference electrode Ag/AgCl. The current intensity was measured for the retention times of about 100, 111, 124, 156, 230, 262 and 415 s for *o*-TLD, mepivcaine, prilocaine, 2,6-DMA, lidocaine, ropivacaine and bupivacaine, respectively.

3. Results and discussion

In order to obtain optimal chromatographic separation, different mobile phases and columns were evaluated. The resolution and separation of all electroactive substances would be more sufficient with the use of a gradient system, but this procedure is not suitable for amperometric detection. In this study several parameters were examined in order to optimize amperometric detection of the studied anaesthetics.

The HPLC method with amperometric detection was used to measure the dependence of peak current intensities on the potential in the range from +0.5 to +1.5 V (Fig. 1). The same method was used to study the relation between the retention factor *k*, and the pH of the mobile phase (Fig. 2). It was found that a 0.01 mol l^{-1} Tris buffer of pH 7.9:acetonitrile (45:55) was the best for the separation of all studied local anaesthetics and their impurities from each other or from pharmaceutical excipients present in pharmaceutical preparations (Fig. 3). The +1.0 V potential has been chosen as optimal for this study (Fig. 2). Using the proposed method, the dependence of the current intensity on the concentration of 2,6-DMA and *o*-TLD was studied. Thus obtained calibration curves were linear in the following ranges 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 detectability limit for both 2,6-DMA and *o*-TLD is equal to 0.8 ng m^{-1}. However, considering the signal to noise ratio, the determinability limit is equal to 1.5 ng ml^{-1}. The determination was carried out with the model 2,6-DMA and *o*-TLD solutions prepared on the basis of three independent samples, from which dilute solutions containing 2, 10 and 100 ng ml^{-1} of these compounds were obtained (Table 1). Reproducibility of the recorded peaks from the model solutions is shown in Fig. 4. The content of impurities in all the preparations chosen for this study was determined by the standard curve method,

while the content of local anaesthetics in pharmaceutical formulations studied was assayed by the standard addition method (Fig. 5).

Although some aromatic compounds are detected easily at solid electrodes using a constant applied potential, this is not so in the case for local anaesthetics. Intermediate oxidation products of these substances can adsorb on the surface of solid electrodes. Unfortunately, a serious consequence of adsorption is the fouling of the electrode by accumulation of reaction products, resulting in the gradual loss of electrode response. The problem of electrode fouling can be solved by application of the pulsed potential waveforms. The waveform consist of three distinct potential steps to achieve the sequential anodic detection. At the detection potential (E_{det} = +1.0 V), applied during a period t_{det} = 0.4 s, the components to be analysed are oxidized. Following the detection process, the electrode surface is reductively cleaned by application of the subsequent negative potential (E_{red} = −0.55 V) during a period t_{red} = 0.2 s. Than the electrode surface is additionally cleaned by the application of a large positive potential (E_{oxd} = +1.3 V) over a period of t_{oxd} = 0.07 s. Following anodic dissolution of the surface film, new analytes can already adsorb at the clean electrode surface prior to the next cycle of waveform (Fig. 5).

The proposed HPLC-ED method allowed us to determine low concentrations of the studied impurities, which constituted hardly a 1/120,000 of the main substance in the various forms of pharmaceutical preparations. The preparation and analysis of the samples was performed in a relatively short time. Owing to this advantage, the method can be applied to routine investigations. Present pharmacopoeial methods do not give such possibilities and are suitable for significantly higher concentrations of 2,6-DMA and *o*-TLD (ca. 1 $\mu g\,ml^{-1}$).

The statistical estimation of the results of the local anaesthetics determination (Table 2) has shown that the developed HPLC-ED method operates with a good accuracy and high precision and can be applied to routine analysis of pharmaceutical preparations in the form of cream and injection.

4. Conclusions

The HPLC-ED procedure has been developed to provide a very sensitive, quantitative assay of local anaesthetics in cream or injection. This method allows one to determine also low concentrations of their impurities, amounting hardly to 1/120,000 of the main substance in the various forms of pharmaceutical preparations. The preparation of samples and their analysis is performed in a relatively short time. Owing to this advantage, the method can be applied to routine analysis of pharmaceutical preparations.

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