CROATICA CHEMICA ACTA
CCACAA **79** (2) 253–259 (2006)

ISSN-0011-1643
CCA-3089
Original Scientific Paper

Differential Pulse Adsorptive Stripping Voltammetric Determination of Benzocaine and Butacaine with Nafion Modified Glassy Carbon Electrode

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RECEIVED AUGUST 31, 2005; REVISED DECEMBER 5, 2005; ACCEPTED DECEMBER 16, 2005

A sensitive, simple and accurate differential pulse adsorptive stripping voltammetric (DPAdSV) method for determination of benzocaine (BZE) and butacaine (BTE) using a nafion modified glassy carbon electrode (NMGCE) is described. NMGCE exhibited significantly increased sensitivity and selectivity for BZE and BTE compared to the bare glassy carbon electrode (GCE). Factors affecting peak currents, such as accumulation potential, accumulation time, presence of surfactants, buffer pH, *etc.* for the determination of BZE and BTE, were studied using DPAdSV. The modified electrode was less susceptible to peak depression than the bare electrode in the presence of surfactants and ascorbic acid. Peak currents showed a linear response in the concentration range 3.2×10^{-9} to 4.5×10^{-7} mol dm^{-3} for BZE and 4.2×10^{-9} to 3.6×10^{-7} mol dm^{-3} for BTE, with the respective limits of detection (LOD) of 2.4×10^{-9} mol dm^{-3} and 3.6×10^{-9} mol dm^{-3} at NMGCE. The method has been successfully applied for the determination of BZE and BTE in a spiked human urine sample. Repeatability and reproducibility of the proposed method were also studied.

Keywords
• benzocaine
• butacaine
• urine samples
• differential pulse adsorptive stripping voltammetry
• nafion modified glassy carbon electrode

INTRODUCTION

Benzocaine (4-aminobenzoic ethyl ester) and butacaine (3-(dibutyl amine)-1-propanol-4-aminobenzoate) are widely used as local and topical anesthetics (Figures 1a and 1b). BZE sensitivity produces classic allergic contact dermatitis reactions. Sometimes it may be seen as a flare or spread of an existing treated rash.

Various methods such as coulometry,[1] potentiometric titrations,[2] colorimetry,[3,4] spectrophotometry,[5–10] and HPLC[11–16] have been reported for the determination of local anesthetics. However, although the sensitivity and detection limits of the methods have been improved, these are rather time consuming methods and the analyses involve a large number of complicated steps. Therefore, a simple and easy technique was urgently required for the detection of BZE and BTE. In contrast, electrochemical methods are rapid, inexpensive and directly applicable to many pharmaceutical preparations. Some local anesthetics have been investigated electrochemically.[17–19] The voltammetric properties and determination of local anesthetics benzocaine, cinchocaine, lidocaine and procaine and the antithusic codeine[20,21] have been reported.

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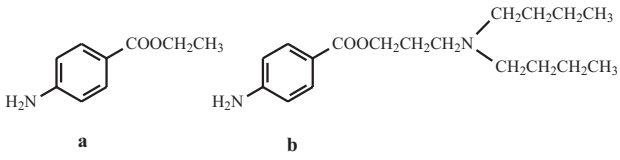

Figure 1. a) Structure of BZE; b) Structure of BTE.

Chemically Modified Electrodes (CMEs) have drawn much attention in the field of electrochemistry due to their easy fabrication, excellent sensitivity, fast response, good selectivity and low cost.

Chemically modified electrodes were introduced in 1973 by Lane and Hubbard, who studied the chemisorption of electroactive allyl compounds on a Pt electrode.[22] Nafion has been widely used as a modifier for analytical purposes because of its chemical and electrochemical inertness, as well as its high stability in aqueous solution.[23] Nafion, having a hydrophilic charged sulphonate group in the polymer structure, selectively enables preconcentrating positively charged molecules through electrostatic interaction.[24] The nafion film has the advantage of rapid and reproducible preparation of electrode as well as of low background currents. Li *et al.* determined the trace procaine hydrochloride by differential pulse adsorptive stripping voltammetry with a NMGCE.[25] Z. Hu *et al.* developed voltammetric stripping determination of lead using 1–(2-pyridylazo)-2-naphthol (PAN) nafion modified spectroscopic graphite electrodes.[26] The main aim of the present paper is to develop a simple, sensitive, selective and low-cost method for the determination of BZE and BTE by DPAdSV with a NMGCE. The method has been successfully applied for the determination of BZE and BTE in spiked human urine samples.

EXPERIMENTAL

Apparatus

The differential pulse adsorptive stripping voltammetric experiment was performed using a Metrohm 757 VA Computrace (Herisau, Switzerland) controlled by running electrochemical analysis software; the output was a Hewlett packed plotter. pH measurements were carried out with a Metrohm 632 pH meter. The electrolytic cell consisted of a stirrer and a three-electrode system incorporating the laboratory made nafion modified glassy carbon electrode or bare glassy carbon electrode as a working electrode, an Ag/AgCl (3 mol dm^{-3} KCl) as a reference electrode and a platinum wire as an auxiliary electrode.

Reagents and Solutions

Compounds BZE and BTE were purchased from Sigma (USA) and were used as received. Nafion perfluorinated polymer 5 % solution was purchased from Aldrich. Paraffin oil was purchased from Fluka and surfactants Triton X-100 and SDS were used as received from Aldrich. Stock solutions of BZE and BTE (1×10^{-3} mol dm^{-3}) were prepared by dissolving appropriate amounts of electroactive species in ethanol. Standard stock solutions were protected from light throughout the experimental procedure. Supporting electrolyte, Britton Robinson buffer solution, was prepared with 0.04 mol dm^{-3} acetic acid, 0.04 mol dm^{-3} boric acid and 0.04 mol dm^{-3} orthophosphoric acid and mixed with 0.2 mol dm^{-3} NaOH solution to obtain the desired pH values. All chemicals and solvents used were of analytical grade. Triple distilled water was used throughout. Desired concentrations of solutions were prepared daily from the stock solutions.

Modification of Electrode

Before surface modification of the glassy carbon electrode, it was polished to a mirror finish with 0.3 μm alumina powder and then sonicated in a water bath. Then, the electrode was rinsed with triple distilled water and alcohol, dried and used for electrode modification. The NMGCE was prepared by dropping 5 μL of nafion suspension onto the surface of the electrode, which was air dried for 20 min using a domestic hair dryer. A uniform film was formed over the entire surface. The modified electrode was washed with deionized water and then scanned between 0 and +1.4 V in BR buffer of pH 2.0 solution before being used for analysis.

Process of Analysis

A standard solution (1.0×10^{-3} mol dm^{-3}) of BZE and BTE was diluted to an adequate concentration and used immediately before measurement. The modified electrode was immersed in the voltammetric cell containing 10 mL of supporting electrolyte (BR buffer) and a suitable amount of BZE or BTE for a certain period of time at the desired accumulation potential. During the deposition time, the solution was stirred at 2000 rpm. The stirring was then stopped and the solution was allowed to stand for 30 s, after which a positive sweep was carried out from 0 to +1.4 V. All the measurements were made at 21±1 °C.

RESULTS AND DISCUSSION

Differential Pulse Adsorptive Stripping Voltammetry

Oxidation of BZE and BTE by DPAdSV at GCE and NMGCE is displayed in Figures 2 and 3, which show the response of 2×10^{-7} mol dm^{-3} BZE and BTE in BR buffer of pH 2.0. Each of the two compounds yielded one peak, which was observed due to the oxidation of amine group in BZE[21] and BTE compounds, respectively. In Figures 2 and 3, 'a' is the peak obtained at bare GCE with the peak potentials of +1.18 V and +1.28 V, 'b' is the peak obtained at NMGCE with the peak potentials of +1.03 V and +1.17 V for BZE and BTE respectively. The oxidation peak for BZE and BTE at bare GCE was less intense with a broader peak whereas at NMGCE a well defined and sharper oxidation peak was observed. This in-

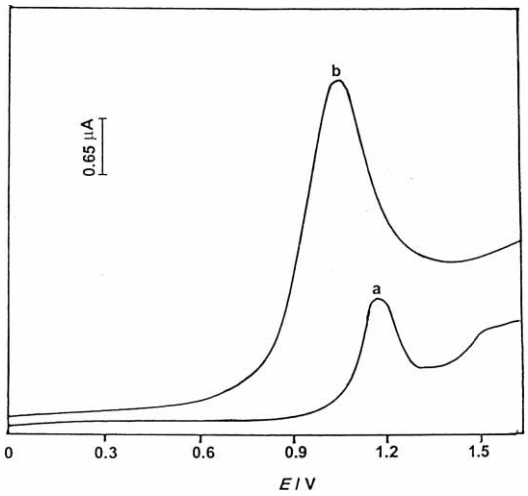

Figure 2. DPAdSV of BZE of 2×10^{-7} mol dm^{-3} a) bare GCE; b) NMGCE in BR buffer of pH 2.0, pulse amplitude 50 mV, stirring rate 2000 rpm, accumulation time 3.5 min, accumulation potential – 0.2 V, scan rate 10 mVs^{-1}.

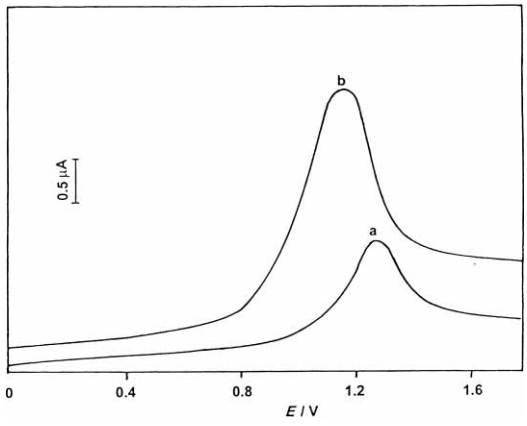

Figure 3. DPAdSV of BTE of 2×10^{-7} mol dm^{-3} a) bare GCE; b) NMGCE in BR buffer of pH 2.0, pulse amplitude 50 mV, stirring rate 2000 rpm, accumulation time 3.5 min, accumulation potential – 0.2 V, scan rate 10 mVs^{-1}.

dicates that remarkable improvement could be achieved in the voltammetric determination of BZE and BTE by combining the effect of nafion film on glassy carbon electrode. Firstly, the peak currents at NMGCE are nearly two and half times greater than peak currents at bare GCE. Secondly, the electrochemical oxidation of BZE and BTE at NMGCE was more facile due to the shift in peak potentials to less positive values compared to those obtained at bare GCE. The enhancement of peak currents at NMGCE is due to the preconcentration of BZE and BTE into the nafion film.[27] It is evident that application of nafion film not only enhances the preconcentration of BZE and BTE on the electrode surface, but it also has catalytic properties.[28,29] The shift of oxidation potential of BZE and BTE to lower values at NMGCE with a concomitant increase in the peak currents reflects a faster electron transfer reaction; the faster electron transfer leads to sharper and better defined peaks.

Effect of pH

The effect of pH on the peak currents of BZE and BTE of concentration 2×10^{-7} mol dm^{-3} at NMGCE was investigated. It was observed that peak currents increase as the pH increases from 1.0 to 2.0 and decreases with a further increase in pH beyond 2.0. This observation reveals the importance of the protonation effect of buffer on the peak currents. At a higher pH, the BZE and BTE molecules are not sufficiently protonated to form cations and thus cannot be effectively preconcentrated into the nafion film, wereas at lower pH values, the nafion film attracts more proton ions and holds them inside the polymer matrix, thereby reducing the BZE and BTE preconcentration time. Hence the pH 2.0 of the supporting electrolyte was selected in all subsequent experiments in order to achieve maximum sensitivity. The peak potentials of BZE and BTE get shifted towards lower positive values as the solution becomes more basic. This behavior indicates that the reaction of BZE and BTE is associated with the proton transfer process. The effect of pH on the peak current response of BZE and BTE at NMGCE is shown in Figure 4.

Effect of Supporting Electrolytes

Supporting electrolytes such as Britton-Robinson (BR), KCl, acetate, phosphate and borate were investigated for the determination of BZE and BTE. Acetate buffer, however, shows similar voltammograms for BZE and BTE to those in BR, but with less sensitivity. The remaining buffers have shown poor sensitivity for the determination of BZE and BTE. The most suitable supporting electrolyte for the voltammetric study of BZE and BTE is probably the BR buffer. 0.04 mol dm^{-3} concentration of the buffer was selected to obtain an adequate buffering capacity.

Effect of Accumulation Potential

The effect of accumulation potential on the stripping peak response of BZE and BTE (Figure 5) was studied by varying the potential from +0.4 to –0.4 V with an accu-

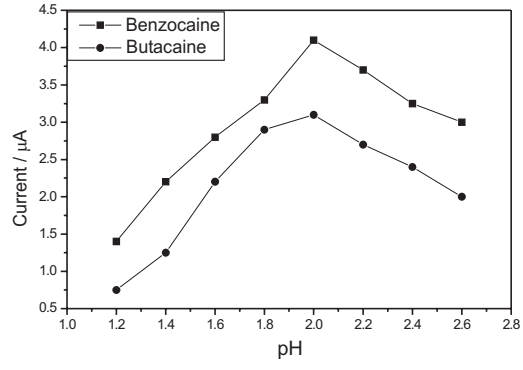

Figure 4. Effect of pH on the peak currents for 2×10^{-7} mol dm^{-3} BZE and BTE at a NMGCE.

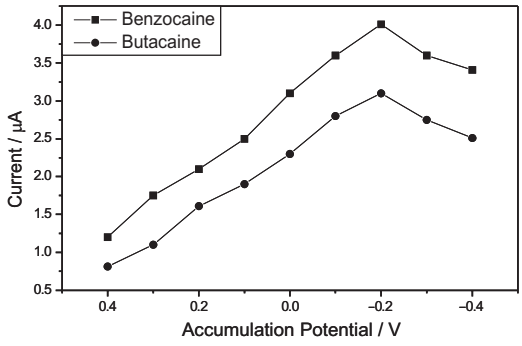

Figure 5. Effect of accumulation potential on the peak currents for 2×10^{-7} mol dm^{-3} BZE and BTE at a NMGCE.

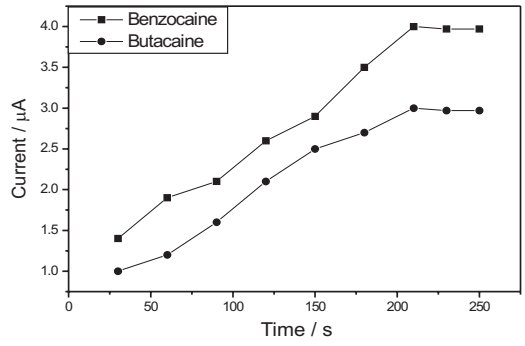

Figure 6. Effect of preconcentration time on peak currents of 2×10^{-7} mol dm^{-3} BZE and BTE at a NMGCE.

mulation time of 210 s. It was observed that the peak currents increased as the accumulation potential shifted in the direction from +0.4 to –0.4 V. Amine groups in the BZE and BTE molecules are protonated in acidic solutions. Protonated BZE and BTE have a positive charge; the more negative the accumulation potential, the higher is the stripping peak current. When the accumulation potential was increased above –0.2 V, there was a decrease in the peak current. Hence the potential of –0.2 V was selected for further studies.

Effect of Preconcentration Time

The dependence of peak currents on the preconcentration time was studied with solutions of various concentrations of BZE and BTE at NMGCE. At a higher concentration of BZE and BTE, 2×10^{-7} mol dm^{-3}, the peak currents increase and start to level off in about 3.5 min. At a lower concentration of BZE and BTE, 6×10^{-8} mol dm^{-3}, it took 5.5 min to reach maximum peak currents. Apparently, the rate of BZE and BTE uptake is dependent on concentration; the higher is the concentration, the adsorption of BZE and BTE on the NMGCE is more effective. The peak currents are nearly two and a half times greater at NMGCE in comparison with bare GCE; better preconcentration at NMGCE is due to the ion-exchange property of nafion film. Figure 6 shows the effect of preconcentration time on peak currents.

Effect of Nafion Layer Thickness

Figure 7 shows the effect of nafion quantity on the glassy carbon electrode. The dependence of the peak current on the volume of nafion added onto the glassy carbon electrode surface was examined over the range from 0 to 7 μL. The electrode response increases rapidly with the increase of nafion concentration. It was observed that the peak currents were maximal when the concentration was 5 μL of nafion solution. The response of the electrode declines when the concentration is higher than 5 μL. Hence the 5 μL concentration of nafion solution was selected for modification of the electrode surface.

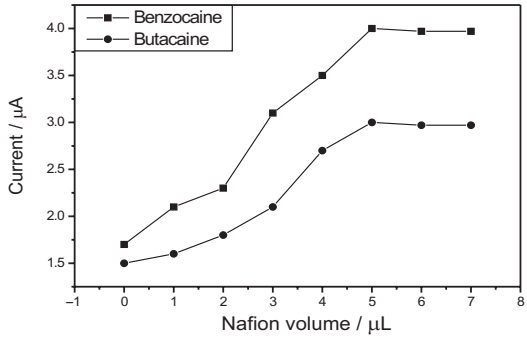

Figure 7. Effect of volume of nafion on the electrode surface.

Effect of Surfactants and Interferents

The presence of surface active substances has a pronounced effect on peak currents; this happens by adsorption of the surfactants on the surface of the electrode, causing depression of the peak currents.[30,31] Thus, the effects of nonionic surfactant Triton X-100 and anionic surfactant SDS on the differential pulse adsorptive stripping voltammetric signals of BZE and BTE at NMGCE and bare GCE were investigated in this study.

The surfactants of different concentrations in 1 x 10^{-6} mol dm^{-3} are spiked into the BZE or BTE solution of concentration 2×10^{-5} mol dm^{-3}. At bare GCE, the peak currents response resulted in higher depression compared to NMGCE, where 95 % of the original peak current response was observed. From these results, it was seen that modified electrode is less susceptible to the peak currents than bare electrode in the presence of surfactants. The NMGCE is more resistant than bare GCE due to the function of the nafion film membrane coated on the glassy carbon electrode, which prevents organic interferents (Triton X-100, SDS) from reaching the interface at which the deposition takes place.

Also, the interference of ascorbic acid (AA) typical anionic species was examined because AA is always present in biological fluids. AA of concentration 2×10^{-4} mol dm^{-3} was added to BZE and BTE of concentration 2×10^{-6} mol dm^{-3}. It was seen that at bare GCE, the inter-

TABLE I. Determination of BZE and BTE in spiked human urine sample by DPASV at NMGCE

Sample	Amount added / μg L^{-1}	Average[a] amount found / μg L^{-1}	Recovery percentage	±SD	% RSD
BZE	1.0	0.97	97.33	0.015	1.56
	2.5	2.40	96.13	0.025	1.04
	5.0	4.84	96.86	0.081	1.68
	7.5	7.38	98.44	0.076	1.03
BTE	1.0	0.98	98.13	0.010	1.04
	2.5	2.44	97.73	0.037	1.54
	5.0	4.86	97.33	0.057	1.18
	7.5	7.31	97.55	0.076	1.04

[a] Each value is an average of five determinations.

TABLE II. Regression equations and statistical parameters of voltammetric determination of BZE and BTE by DPV

Parameters	BZE		BTE	
	NMGCE	bare GCE	NMGCE	bare GCE
Calibration curve equation / 1×10^{-6} mol dm^{-3}	y (A) = 0.0424 + 1.76×10^{7}	y (A) = 0.0743 + 6.65×10^{6}	y (A) = 0.0227 + 1.49×10^{7}	y (A) = 0.104 + 7.06×10^{6}
Correlation coefficient	0.9977	0.9980	0.9983	0.9977
Linear range concentration / mol dm^{-3}	3.2×10^{-9} to 4.5×10^{-7}	2.0×10^{-8} to 6.4×10^{-7}	4.2×10^{-9} to 3.6×10^{-7}	2.6×10^{-8} to 5.6×10^{-7}
L.O.D. / mol dm^{-3}	2.4×10^{-9}	1.2×10^{-8}	3.6×10^{-9}	1.8×10^{-8}
L.O.Q. / mol dm^{-3}	7.97×10^{-9}	3.9×10^{-8}	1.2×10^{-8}	6.0×10^{-8}
Repeatability of peak currents (% R.S.D.)	3.34	3.54	3.28	3.60
Repeatability of peak potentials (% R.S.D.)	0.93	0.83	0.96	0.80
Reproducibility of peak currents (% R.S.D.)	3.62	3.73	3.45	3.75
Reproducibility of peak potentials (% R.S.D)	0.99	0.90	0.98	0.97
Number of assays	15	15	15	15

ference with AA was significantly higher. On the other hand, by employing NMGCE, the interference from AA was effectively eliminated due to the cation-exchange feature of nafion film. Nafion film coated electrode eliminates the interference of anions.[32]

Determination of BZE and BTE in Spiked Human Urine

An aliquot of original urine was collected and was added to the volumetric cell containing BR buffer of pH 2.0 (9:1. BR buffer:urine). The voltammograms were recorded for the blank urine sample, then a certain concentration of BZE or BTE was added into the volumetric cell and DPASV was recorded. The concentration of BZE and BTE was calculated with reference to the calibration plot. The average recovery of BZE and BTE in urine was found to be 97.19 and 97.68 %, respectively. This confirms the good selectivity of the method. Results obtained for determination of BZE and BTE in the urine sample are presented in Table I.

Calibration Plots and Detection Limits

Under the optimized conditions of pH 2.0 of BR buffers, the accumulation potential of –0.2 V and pulse amplitude of 50 mV, scan rate of 10 mVs^{-1}, the peak currents of BZE and BTE were found to be proportional to their concentrations over the range from 3.2×10^{-9} to 4.5×10^{-7} mol dm^{-3} and 4.2×10^{-9} to 3.6×10^{-7} mol dm^{-3} at NMGCE, respectively.

The precision of the proposed method was evaluated by repeating five experiments on the same day and in the same standard solution (repeatability), and then after some days by preparing different standard solutions, different analysts and different electrodes of the same compositions (reproducibility) and repeating the experiments five times.[33] To study these experiments, 2×10^{-7} mol dm^{-3} concentrations were used. The results are given in Table II. LOD and LOQ were calculated using the equations LOD = 3 S.D. m^{-1}, LOQ = 10 S.D. m^{-1}, where S.D. is the standard deviation of the peak currents and 'm' is the slope of the calibration curves.[34]

CONCLUSIONS

The above results indicate that the NMGCE significantly increases the sensitivity and selectivity compared to bare GCE for the determination of BZE and BTE. DPAdSV at a NMGCE has been shown to be a suitable method for the determination of trace amounts of BZE and BTE in a spiked human urine sample. The NMGCE is less susceptible to the peak depression than the bare GCE in the presence of surfactants Triton X-100 and SDS; also, improved resistance to interference from AA at NMGCE indicates that the modification of the electrode surface by nafion film increases the electrode resistance to the surface-active substances.

Acknowledgment. – The authors gratefully acknowledge financial support from the University Grants Commission (UGC), New Delhi.

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SAŽETAK

Određivanje benzokaina i butakaina diferencijalnom pulsnom voltametrijom s adsorptivnom akumulacijom na elektrodi od staklastog grafita modificiranoj nafionom

Tukiakula Madhusudana Reddy, Kodigutta Balaji i Srinivasulu Reddy Jayarama Reddy

Opisana je osjetljiva, jednostavna i točna metoda za određivanje benzokaina i butakaina korištenjem diferencijalne pulsne voltametrije s adsorptivnom akumulacijom analita na elektrodi od staklastog grafita modificiranoj nafionom. Modifikacijom elektrode povećava se njena osjetljivost i selektivnost za navedene spojeve. Istraženi su faktori koji utječu na voltametrijski odziv, kao što su potencijal i trajanje akumulacije, prisutnost površinski aktivnih tvari, pH puferske otopine, itd. Modifikacijom elektrode smanjuje se utjecaj površinski aktivnih tvari i askorbinske kiseline na vršnu struju voltametrijskog odziva analiziranih spojeva. Vršna struja je linearna funkcija koncentracija analita u rasponu od $3{,}2 \times 10^{-9}$ mol dm^{-3} do $4{,}5 \times 10^{-7}$ mol dm^{-3} za benzokain, odnosno od $4{,}2 \times 10^{-9}$ mol dm^{-3} do $3{,}6 \times 10^{-7}$ mol dm^{-3} za butakain, a granice detekcije su $2{,}4 \times 10^{-9}$ mol dm^{-3} za prvi i $3{,}6 \times 10^{-9}$ mol dm^{-3} za drugi spoj. Metoda je uspješno primijenjena za određivanje benzokaina i butakaina u obogaćenom uzorku ljudskog urina. Istražena je ponovljivost rezultata predloženog postupka.