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Short communication

# Determination of selected drugs in human urine by differential pulse voltammetry technique

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ABSTRACT

A new, simple and selective differential pulse voltammetry (DPV) method for the simultaneous determination of selected drugs in model solutions and spiked human urine samples with prior extraction was developed and validated. The objects of analysis were paracetamol, furosemide, dipyrone, cefazolin and dexamethasone belonging to four different therapeutic groups (antibiotics, analgesic, demulcent and diuretic). Analytical methods for the preparation of urine samples for voltammetric analysis (liquid–liquid extraction — LLE and solid-phase extraction — SPE) were worked out and optimized. Hanging mercury drop electrode (HMDE) and graphite electrode were used as working electrodes. Reference electrode was Ag|AgCl|$KCl_{(sat.)}$, whereas auxiliary electrode — platinum electrode. The optimal conditions for quantitative determination were obtained in a Britton–Robinson (BR) buffer at pH 2.4. Quantification was performed by means of calibration curve and standard addition methods. The calibration curves of analysed drugs are linear within the range of concentration: 6.61–66.10, 6.05–54.42, 6.00–65.00, 4.20–33.58 and 0.51–3.06 μM for paracetamol, furosemide, dipyrone, cefazolin and dexamethasone, respectively. The levels of analysed compounds in human urine can be successfully determined using this developed method with no matrix effect.



## 1. Introduction

It often happens that patients are treated simultaneously with a few drugs representing different groups. Analysis of drugs contents in body fluids is necessary inter alia during following a given drug therapy, in cases of intoxication or suspicion of drug intoxication, drug addiction or in anti-drug control [1]. Used above the therapeutic levels, these drugs can cause a wide variety of adverse effects and their fast analysis could have a significant impact in treatment and recovery of the patients. In view of necessity monitoring of given drugs concentration, particularly in case of simultaneously given of different drugs exists necessity disposing of analytical methods providing simultaneously determination in the shortest time. Therefore a new differential pulse voltammetry (DPV) method for simultaneously determination of selected drugs was elaborated.

Generally for drugs determination distribution techniques i.e. chromatography and electrophoresis are used; rarely spectroscopic and electroanalytical methods. Electroanalytical methods could be used as a comparative methods for others methods in inter-laboratories researches or in certified reference materials (e.g. urine with different drugs) preparation or as a detection methods in high-performance liquid chromatography (HPLC). During sample preparation using standards, authors did not examine the combinations of drugs which are not commonly used in clinical practice or which are not recommended to be used simultaneously.

The subjects of researches in that study were analgesic — paracetamol (PAR) and dipyrone (DPR), demulcent — dexamethasone (DEX), diuretic — furosemide (FUR) and antibiotic — cefazolin (CFZ) drugs. These combinations of cures can be found together in biological samples (urine, plasma, blood). Researches referring above-mentioned drugs by electroanalytical methods include mostly determination drugs placed in table, syrups or model solutions, only small works affect body fluids. Follow from literature data PAR was determined in plasma (individually) [2] and FUR [3], CFZ [4] in urine (individually). For others drugs there are lack of application for body fluids. Literature review referring electrochemical methods of determination researching drugs is given in Table 1.

In the present study, besides basic researches referring to elaboration conditions of determination of nearby above-mentioned drugs, application for urine sample in which researches drugs were let in was also investigated. The procedure was successfully applied to the simultaneous determination of PAR with FUR as well as DPR with CFZ and DEX in spiked human urine samples. The combinations of analysed drugs are in agreement with the ones most often used in the ward and most frequently found together in urine samples. At the best of our knowledge the electrochemical oxidation (PAR and FUR) and reduction (DPR, CFZ and DEX) have not been previously reported.

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**Table 1**
The parameters of samples preparation and determination of selected drugs according to the literature data

| Drug | Method | Working electrode | Reference electrode | Medium | R.D.[a] | Ref. |
|---|---|---|---|---|---|---|
| PAR | Voltammetry | PtE[h] | Ag/AgCl[p] | Phosphate buffer at pH 7.4 | 0–2 mM | [2] |
| | DPV[b] | GCE[i] | SCE[q] | Methanol/0.1 M $HClO_4$ (1/1) | 0–55 μg $mL^{-1}$ | [5] |
| | LSV[c] | CPE[j] | Ag/AgCl[p] | Acetate buffer at pH 4.7 | $3\cdot10^{-6}$–$7.5\cdot10^{-3}$ M | [6] |
| | SWV[d] | CME[k] or GCE[i] | Ag/AgCl[p] | 0.05 M $HClO_4$ | 5–250 μM | [7] |
| | DPV[b] | AuM[l] | Ag/AgCl[p] | Phosphate buffer at pH 7.2 | $2.0\cdot10^{-7}$–$1.5\cdot10^{-3}$ M | [8] |
| FUR | DPV[b] or SWV[d] | GCE[i] | SCE[q] | BR buffer at pH 4.5 and 5.0 | 0–$5.5\cdot10^{-5}$ M | [3] |
| DPR | CV[e] | CPEM[m] | SCE[q] | 0.1 M KCl medium at pH 7.0 | $9.9\cdot10^{-6}$–$2.8\cdot10^{-3}$ M | [9] |
| | DCP[f] | DME[n] | Ag/AgCl[p] | Acetate buffer at pH 3.6 | 0.008–0.06 mg $mL^{-1}$ | [10] |
| DEX | DPP[g] | DME[n] | Ag/AgCl[p] | Acetate buffer at pH 5.0 | 0.008–0.048 mg $mL^{-1}$ | [11] |
| CFZ | DCP[f] | DME[n] | SCE[q] | BR buffer at pH 6 | $8.6\cdot10^{-10}$–$2\cdot10^{-7}$ M | [12] |
| | SWV[d] or CV[e] | HMDE[o] | Ag/AgCl[p] | BR buffer at pH 6 | | |
| | LSV[c] or CV[e] | DME[n] | SCE[q] | 0.1 M acetic/o-phosphoric acid buffer of pH 4.75 | $1.0\cdot10^{-9}$–$1.0\cdot10^{-7}$ M | [13] |
| | LSV[c] or CV[e] | HMDE[o] | Ag/AgCl[p] | BR buffer at pH 4 and acetate buffers (pH 3.6 – 5.0) | – | [4] |

[a]Range of determination, [b]differential pulse voltammetry, [c]linear sweep voltammetry, [d]square wave voltammetry, [e]cyclic voltammetry, [f]direct current polarography, [g]differential pulse polarography, [h]platinum electrode, [i]glassy carbon electrode, [j]carbon paste electrode, [k]nafion®/ruthenium oxide pyrochlore working electrode, [l]gold nanoparticles modified indium tin oxide electrode, [m]carbon paste electrode modified with *N,N′*-ethylenebis(salicylideneiminato)oxovanadium(IV) complex ([VO(Salen)]), [n]dropping mercury electrode, [o]hanging mercury drop electrode, [p]silver/silver chloride electrode, [q]saturated calomel electrode.

The main advantages of the proposed method are related to its rapidity, simplicity and low cost.

## 2. Experimental

### 2.1. Chemicals, reagents and solutions

Dexamethasone {9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one}, furosemide {4-chloro-2-(2-furylmethylamino)-5-sulfamoyl-benzoic acid} and cefazolin sodium salt {sodium 4-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-7-oxo-8-(2-tetrazol-1-ylacetyl)amino-2-thia-6-azabicyclo[4.2.0]oct-4-ene-5-carboxylate} (97–99.9% purity) were purchased from Sigma-Aldrich (Darmstadt, Germany). Paracetamol {*N*-(4-hydroxyphenyl)acetamide} (99.5% purity) was obtained from Fluka BioChemika (Darmstadt, Germany). Dipyrone monohydrate {sodium [(1,5-dimethyl-3-oxo-2-phenyl-pyrazol-4-yl)-methyl-amino]methanesulfonate hydrate} (≥99% purity) was bought from Riedel-de Haën (Seelze, Germany). The structures of the analysed drugs are presented in Fig. 1.

Potassium phosphate dibasic ($K_2HPO_4$), potassium phosphate monobasic ($KH_2PO_4$), sodium phosphate dibasic ($Na_2HPO_4$), *o*-boric acid ($H_3BO_3$), acetic acid ($CH_3COOH$), *o*-phosphoric acid ($H_3PO_4$), hydrochloric acid ($HCl_{aq}$), perchloric acid ($HClO_4$) and sodium hydroxide (NaOH), were purchased from POCH (Gliwice, Poland). Acetonitrile, methanol and ethyl octane for analysis were purchased from POCH (Gliwice, Poland). All reagents and solutions used in this work were of analytical grade.

### 2.2. Human urine samples

Human urine was obtained from six healthy volunteers of different sex and age. Aliquots were centrifuged at 7000 rpm for 5 min at room temperature (22±2 °C) and processed in the manner described in Section 2.9. These urine samples were analysed immediately or they were stored at −18 °C until analysis.

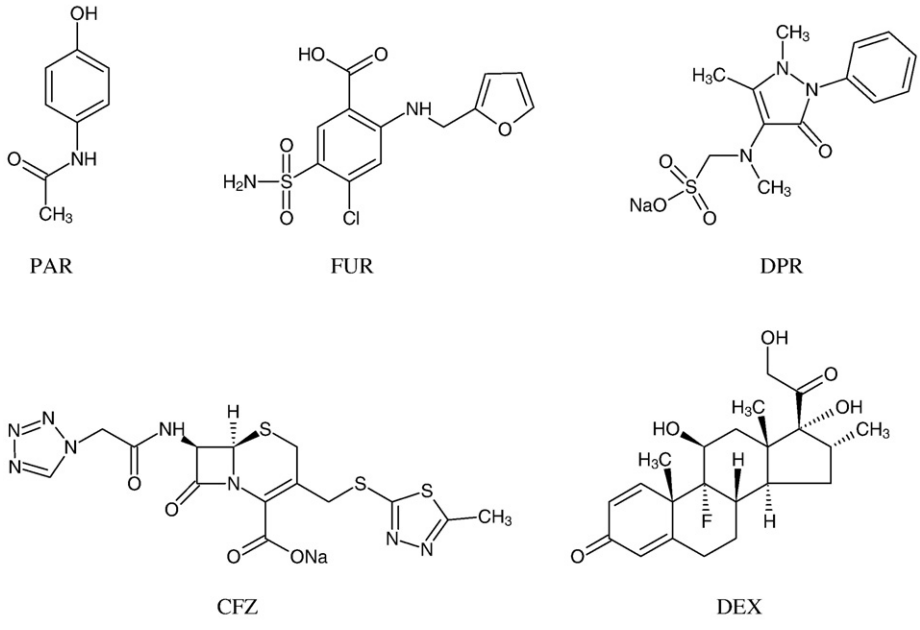


**Fig. 1.** Chemical structures of PAR, FUR, DPR, CFZ and DEX.

### 2.3. Instrumentation

All voltammetric measurements were carried out using computer controlled ECO-TRIBO Polarograph with PolarPro ETP 2.0 software for storing and processing data in combination with a voltammetric mini-microelectrode System UMμE (PolaroSensor) (Prague, Czech Republic). Voltammetric measurements were carried out in a 10 mL glassy electrochemical cell. A three-electrode cell system was completed by means of a hanging mercury drop electrode (HMDE) (area: 0.025 $cm^2$) or graphite electrode working electrodes, $Ag|AgCl|KCl_{(sat.)}$ as a reference electrode and platinum as an auxiliary electrode. All the measurements were automated and controlled through the programming capacity of the apparatus. Argon was used for the removal of dissolved oxygen from the measured solutions. A centrifuge HERMLE Z 323 K (Gosheim, Germany) was used to centrifuge urine samples. Examined urine samples were carried through solid-phase extraction (SPE) on J.T. Baker System with using Bakerbond $NH_2$ (amino, 3 mL volume containing 500 mg of sorbent — Type 7088-03) columns (Deventer, Netherlands). The pH of the buffered solutions was measured by an ELMETRON (Zabrze, Poland), Model CP-401 pH meter using a combined glass electrode.

### 2.4. Buffers and solutions preparation

Stocks Britton–Robinson (BR) buffer solutions (at pH 2.4, 4.5 and 7.0) were prepared by mixing 3.1, 7.5 and 13.1 mL, respectively 200 μM NaOH solution with 25 mL of a mixed acid that contains 40 μM of each of *o*-boric, *o*-phosphoric and acetic acids ($H_3BO_3$–$H_3COOH$–$H_3PO_4$). Phosphate buffer at pH 6.81 was prepared by mixing 25 mL 67 μM $KH_2PO_4$ solutions with 25 mL 67 μM $Na_2HPO_4$ solution.

All chemicals used were of analytical grade and were used without further purification. The pH of the solutions was adjusted by mixing buffer components and was verified before each measurement.

### 2.5. Stock solutions

Separate stock solutions of analysed drugs at concentrations: 6.61, 3.02, 3.00, 2.10 and 2.55 mM for PAR, FUR, DPR, CFZ and DEX, respectively were prepared in 10 mL volumetric flasks by dissolving the appropriate amount of reference substance in a mixture of methanol/water (1/1, v/v). Stock solutions were prepared at the beginning of the study and were stored at 4 °C. Solutions of lower concentrations were prepared by dilution of stock solution with water. All solutions were kept in darkness.

### 2.6. Standard solutions

Individual standard solutions were obtained by diluting stock solutions with BR buffer solutions. Mixed standard solutions containing investigated compounds were prepared by mixing appropriate amount of individual standard solutions and diluting these mixtures with BR buffer solutions. The individual and mixed standard solutions were stable for at least two weeks at 4 °C.

### 2.7. Optimization of parameters for analysed drugs determination

The potentials of reduction and oxidation cited in this article were measured relative to the $Ag|AgCl|KCl_{(sat.)}$ electrode. The graphite working electrode and platinum auxiliary electrode were polished manually to a mirror finish using an alumina (0.3 μm particle sizes) paste and thoroughly rinsed with purified methanol and deionized water between measurements. As supporting electrolyte: BR buffers at pH 2.4, 4.5 and 7.0; BR buffer at pH 2.4 with methanol (1/1, v/v); 100 μM HCl; 100 μM $HClO_4$ and mixture 100 μM $HClO_4$ with methanol (1/1, v/v) were investigated. In the glass electrochemical cell 10 mL of analysed sample were placed. In the aim of removal oxygen, the solution was purged with pure argon for 15 min (and for 30 s before each measurement). The electrochemical accumulation step was carried out at the potential of 0 V for PAR, FUR and −0.3 V for DPR, CFZ and DEX in a stirred solution for 120 s. After equilibrating time of 10 s the voltammetric curve was recorded in the potential range from 0 to +1.3 V for PAR, FUR and from −0.3 to −1.0 V (vs. $Ag|AgCl|KCl_{(sat.)}$) for DPR, CFZ and DEX. The pulse amplitude height of 0.05 V, width of pulse 0.1 s and a scan rate of 0.02 V $s^{-1}$ were used for different pulse voltammetry. After voltammograms samples registration, into electroanalytical cell appropriate amount of individual or mixing standard solutions were added by means of a micropipette. Each measurement was repeated three times using fresh sample solution to ensure reproducibility of the results. All of the electrochemical experiments were carried out at ambient laboratory temperature (22±2 °C). Between experiments, the cell was treated with concentrated nitric (V) acid and then washed with water. PAR, FUR, DPR, CFZ and DEX were then analysed using the optimized voltammetric procedure.

### 2.8. Calibration curves for analysed drug

The calibration curve method was applied to quantitative determination of analysed drugs in model solutions. Calibration curves for PAR, FUR, DPR, CFZ and DEX were prepared separately and for a mixture containing both of these drugs. Working standard solutions, which were used for calibration curves preparation were prepared by adding to 10 mL of BR buffer at pH 2.4 adequately quantity of individual or mixing standard solutions. The calibration curves were measured three times.

### 2.9. Procedure of urine samples preparations

In order to check, the possibility of applying the proposed methods for the determination of PAR, FUR, DPR, CFZ and DEX in spiked human urine samples was also tested. In order to elaboration a procedure of separation examined drugs from urine, drugs model solutions were added to urine samples, which do not contain any of examined drugs. Subsequently analytes were separated by liquid–liquid extraction (LLE) or SPE from urine and determined by DPV technique. The standard addition method was applied for the determination of examined drugs in spiked human urine samples. Urine samples preparation and extraction methods are described below step by step.

#### 2.9.1. Separation of analysed drugs from urine by SPE

Human urine (4 mL) spiked with suitable amounts of PAR and FUR solutions were poured into 15 mL poly(propylene) centrifuge tubes. 4 mL of phosphate buffer at pH 6.81 and 4 mL of acetonitrile were added. The mixture was vortexed for 2 min and centrifuged for 10 min at 2500 rpm. After centrifuging, the clear supernatant was transferred on SPE $NH_2$ (3 mL, 500 mg) column. Earlier this column was conditioned by pulling 3 mL of methanol and 3 mL of water. The column was not allowed to dry. Analytes were eluted by 1 mL of methanol. Samples which were prepared so, were completed by 10 mL of BR buffer at pH 2.4 and were subjected measurements.

Samples preparation procedure for analyses of DPR, CFZ and DEX is the same as in case of determination PAR and FUR, with such difference that to 1 mL of human urine spiked with suitable amounts of DPR, CFZ and DEX 1 mL of phosphate buffer at pH 6.81 and 1 mL of acetonitrile were added.

#### 2.9.2. Separation of par and fur from urine by LLE

In order to separation PAR and FUR from urine single, double and triple extraction by ethyl acetate was performed. To 4 mL of human urine spiked with suitable amounts of PAR and FUR solutions 4 mL of 1 M $KH_2PO_4$ solution were added, so prepared urine was given extraction with ethyl acetate. After single extraction urine with additions of drugs was shaken for 30 min with 8 mL of ethyl acetate.

By double extraction urine was shaken twice for 4 mL of ethyl acetate, and by triple extraction three times for 4 mL of ethyl acetate for 15 min. After shaking both of layers were centrifuged for 5 min at 7000 rpm. After centrifugation organic layers were separated and washed by 8 mL of 100 μM $KH_2PO_4$–$K_2HPO_4$ solution were shaken for 10 min and centrifuged for 5 min at 7000 rpm again. The organic layer was evaporated to dryness under the stream of nitrogen at room temperature. The residues were dissolved in 1 mL of methanol, completed to 10 mL of BR buffer at pH 2.4 and next subjected measurements.

## 3. Results and discussion

### 3.1. Development of DPV method

DPV was used in the voltammetric measurement owing to its good sensitivity and resolving power. It is well known that DPV is suitable for the analysis of the drug mixtures of electrochemically active substances because relatively small difference in peak potentials of the analytes is needed for their simultaneous determination. The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution, and instrumental parameters. We have studied optimization of the proposed procedure and examined conditions which could affect the results. The current was measured and recorded for the sample solution.

As a working electrode for PAR and FUR determination graphite electrode was used, where analysed drugs were oxidated. As a working electrode for DPR, CFZ and DEX determination HMDE was used, where drugs were reduced. Each voltammogram was recorded three times using a new drop of Hg. Peak currents were measured with a graphite electrode at +0.838 and +1.178 V or HMDE at −0.473, −0.681 and −0.906 V (vs. Ag|AgCl|$KCl_{(sat.)}$) for PAR, FUR, DPR, CFZ and DEX, respectively.

BR buffer solutions at different pH values were used as supporting electrolytes. The best supporting electrolyte for determination all of drugs proved BR buffer at pH 2.4 because peaks potentials deriving from each drugs in this buffer let determine PAR and FUR nearby on graphite electrode and DPR, CFZ and DEX on HMDE. When BR buffer at pH 2.4 was used, the peaks of PAR, FUR, DPR, CFZ and DEX were all well defined. The highest difference in peak potentials for the analysed drugs was found in BR buffer at pH 2.4.

Peaks potential of analysed drugs moved into direction of more positive potentials, with the growth of supporting electrolyte acidity. For the lowest value of pH the highest peaks were observed at

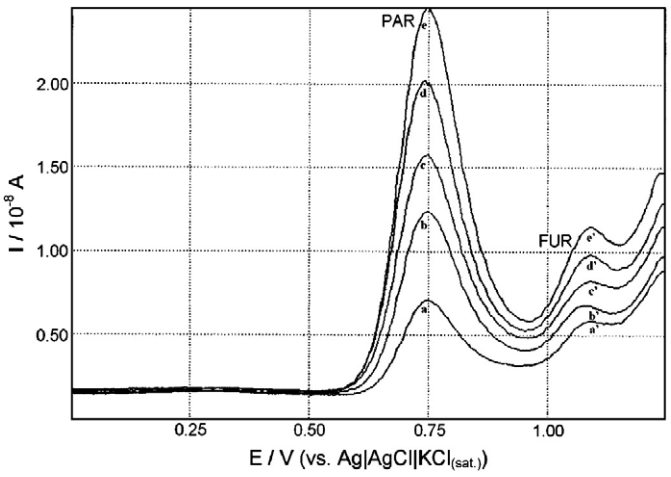


**Fig. 2.** Differential pulse voltammograms obtained for determination of PAR (from a to e: 6.61, 19.85, 33.08, 46.30 and 59.54 μM) and FUR (from a′ to e′: 6.05, 18.14, 30.24, 42.33 and 48.38 μM) in BR buffer at pH 2.4 on graphite electrode. Pulse height 0.05 V, pulse width 0.1 s, scan rate 0.02 V $s^{-1}$, initial potential 0 V, final potential +1.3 V (vs. Ag|AgCl|$KCl_{(sat.)}$).

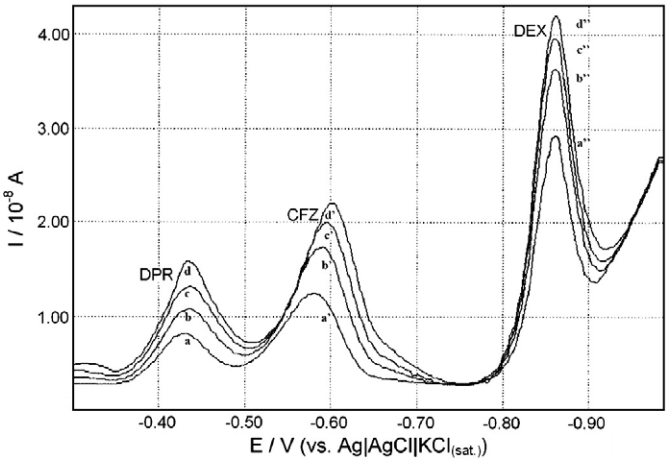


**Fig. 3.** Differential pulse voltammograms obtained for determination of DPR (from a to d: 12.00, 18.00, 30.00 and 42.00 μM), CFZ (from a′ to d′: 4.20, 12.59, 20.99 and 25.18 μM) and DEX (from a″ to d″: 1.27, 2.55, 3.82 and 5.10 μM) in BR buffer at pH 2.4 on HMDE. Pulse height 0.05 V, pulse width 0.1 s, scan rate 0.02 V $s^{-1}$, initial potential −0.3 V, final potential −1.0 V (vs. Ag|AgCl|$KCl_{(sat.)}$).

voltammograms. Representative voltammograms from determination of analysed drugs in BR buffer at pH 2.4 are presented in Figs. 2 and 3.

Validation of analytical procedure determination of analysed drugs was performed. All these determinations were performed in triplicate. Drugs identification was performed according to peaks potential by comparison with standard solutions, and by the standard addition method.

### 3.2. Calibration curves and linearity

For quantitative analysis of selected drugs in human urine samples after extraction procedures standard addition methods were used. The calibration curves were measured in triplicate and evaluated by the least squares linear regression method. Calibration curves and ranges of determinations for analysed drugs in model solutions presented in $y=ax+b$ equation, where "a" is the slope, "b" is the intercept, "y" indicates intensity of current (A) and "x" concentrations (μM) of analysed drugs. Presence of others drugs influenced on quantity of peak current. It may be observed at calibration curves determined for individual drugs and mixture drugs. The calibrations were linear for PAR, FUR, DPR, CFZ and DEX in the concentration ranges of 6.61–66.10, 6.05–54.42, 6.00–65.00, 4.20–33.58 and 0.51–3.06 μM, respectively. The high correlation coefficients ($r^2$) of the all calibration curves were between 0.9974 and 0.9990. The calibration curves show linear response over the whole range of concentration used in the assay procedure. The equations associated with the calibration are summarized in Table 2.

### 3.3. Analytes recoveries

Because other components of the matrix of the human urine samples may interfere with the analysis, potential effects from matrix

**Table 2**
Analytical parameters of calibration curves of all examined compounds in model solutions ($n=6$)

| Drug | Linear range (μM) | $a^a$ | $b^b$ | $r^{2c}$ | LOD[d] (μM) | LOQ[e] (μM) |
|---|---|---|---|---|---|---|
| PAR | 6.61–66.10 | 4.1068 | 4.1852 | 0.9990 | 2.12 | 6.28 |
| FUR | 6.05–54.42 | 1.3343 | 6.2360 | 0.9983 | 2.57 | 5.74 |
| DPR | 6.00–65.00 | 5.8854 | −4.5808 | 0.9974 | 2.37 | 5.85 |
| CFZ | 4.20–33.58 | 2.6884 | 26.631 | 0.9975 | 1.72 | 4.20 |
| DEX | 0.51–3.06 | 53.2981 | 8.7173 | 0.9985 | 0.20 | 0.53 |

[a]Slope, [b]intercept, [c]correlation coefficient, [d]limit of detection, [e]limit of quantification.

**Table 3**
Results of recoveries examination for PAR and FUR from urine samples ($n=3$)

| Method of sample preparation | Concentration added (μM) | Concentration found (μM) | S.D.[a] (μM) | C.V.[b] (%) | Sx[c] (μM) | $L^d$ (μM) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| *PAR* | | | | | | | |
| SPE | 26.46 | 24.81 | 0.63 | 2.54 | 0.36 | 24.81 ± 1.56 | 93.76 |
| Single LLE | 52.92 | 42.53 | 1.04 | 2.44 | 0.60 | 42.53 ± 2.58 | 80.37 |
| Double LLE | 52.92 | 48.29 | 1.22 | 2.53 | 0.70 | 48.29 ± 3.03 | 91.25 |
| Triple LLE | 52.92 | 48.75 | 1.48 | 3.04 | 0.85 | 48.75 ± 3.68 | 92.12 |
| *FUR* | | | | | | | |
| Single LLE | 30.24 | 25.13 | 0.53 | 2.11 | 0.31 | 25.13 ± 1.32 | 83.10 |
| Double LLE | 30.24 | 28.40 | 0.63 | 2.22 | 0.36 | 28.40 ± 1.57 | 93.92 |
| Triple LLE | 30.24 | 28.58 | 0.74 | 2.59 | 0.43 | 28.58 ± 1.84 | 94.51 |

[a]Standard deviation of concentrations found, [b]coefficient of variation of concentrations found, [c]standard deviation of the mean, [d]confidence interval ($\alpha=0.05$, $t=4.303$).

components must be investigated. Recoveries of analysed drugs during their separation from urine were analysed. Extraction experiments were first performed using standard solutions, and then the procedure was checked with human urine samples. The determination of the recovery rates was carried out from spiked human drug-free urine samples. The absolute recovery was calculated for each analyte. Obtained recovery results of spiked human urine samples were given in Tables 3 and 4.

### 3.4. Limits of detection and quantification

The limits of detection (LOD) and limits of quantification (LOQ) for determination of analysed drugs in model solutions were calculated on the peak current using following equations: LOD = 3S.D./$a$ and LOQ = 10S.D./$a$, where "S.D." is the standard deviation of the peak currents and "a" is the slope of the related calibration equation. The LOD and LOQ values are summarized in Table 2. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

### 3.5. Application of the method to urine samples

The developed DPV methods for the selected drugs determination were applied to spiked human urine samples. Conditions of PAR, FUR, DPR, CFZ and DEX determination in urine were elaborated. Methods of urine samples for analysis preparation in order to remove matrix effect were elaborated and optimized. For urine samples containing DPR, CFZ and DEX extraction method — SPE was suggested, which results could suppose, that application of this procedure for analysis of samples coming from patients could be possible. Preparation procedure of urine samples containing DPR, CFZ and DEX by SPE method gave good results and recoveries of these drugs from urine were found as 91.00, 94.19 and 96.08%, respectively.

**Table 4**
Results of recoveries examination for DPR, CFZ and DEX from urine samples by SPE method ($n=3$)

| Drug | Concentration added (μM) | Concentration found (μM) | S.D.[a] (μM) | C.V.[b] (%) | Sx[c] (μM) | $L^d$ (μM) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| DPR | 30.00 | 27.30 | 0.88 | 3.22 | 0.51 | 27.30 ± 2.17 | 91.00 |
| CFZ | 20.99 | 19.77 | 0.54 | 2.73 | 0.31 | 19.77 ± 1.34 | 94.19 |
| DEX | 2.04 | 1.96 | 0.08 | 4.08 | 0.05 | 1.96 ± 0.20 | 96.08 |

[a]Standard deviation of concentrations found, [b]coefficient of variation of concentrations found, [c]standard deviation of the mean, [d]confidence interval ($\alpha=0.05$, $t=4.303$).

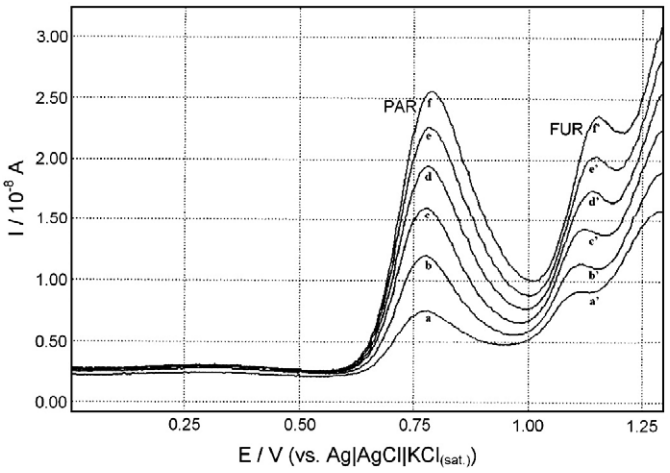


**Fig. 4.** Differential pulse voltammograms obtained for determination of PAR (from a to f: 6.61, 19.85, 33.08, 46.30, 59.54 and 72.77 μM) and FUR (from a′ to f′: 6.05, 18.14, 30.24, 42.33, 48.38 and 54.42 μM) in spiked urine sample after double LLE with ethyl acetate, in mixture of BR buffer at pH 2.4/methanol (9/1, v/v) on graphite electrode. Pulse height 0.05 V, pulse width 0.1 s, scan rate 0.02 V $s^{-1}$, initial potential 0 V, final potential +1.3 V (vs. Ag|AgCl|KCl(sat.)).

For urine samples containing PAR and FUR two methods of analysis sample preparation were proposed. The first method is SPE, which is suitable for preparation of samples containing PAR. The mean recovery of PAR from urine samples by this method was 93.76%. It could not be used for quantitative FUR analysis in view of too high influence of matrix; it could be used for qualitative analysis of this drug.

The second is ethyl acetate extraction method. Though literature [9] recommend single extraction at drugs separation, so that after making measurements, after single, double and triple extraction proved that the most proper is double extraction, which gives greater analytes recovery than single. Triple extraction does not increase meaning analytes recovery, the uncertainty of the result increases, what could be caused greater amount of sample preparation steps, faults are made, which influence on quantity uncertainty of determination. The mean recoveries of PAR and FUR after double ethyl acetate extraction were found as 91.25 and 93.92%, respectively. Receiving results are average of three measurements parallel prepared samples. The results of these

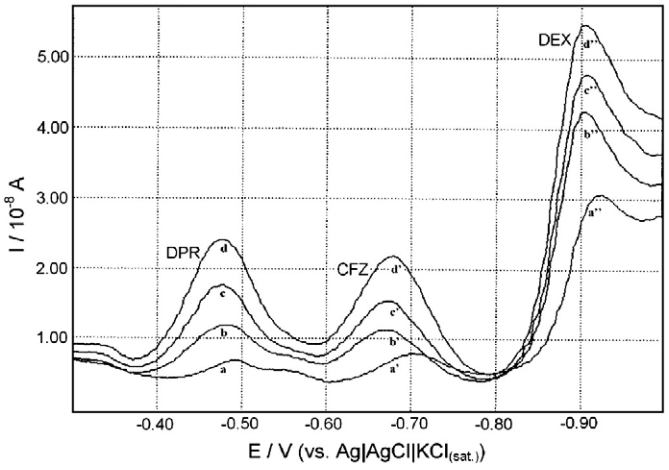


**Fig. 5.** Differential pulse voltammograms obtained for determination of DPR (from a to d: 12.00, 24.00, 42.00 and 54.00 μM), CFZ (from a′ to d′: 2.10, 4.20, 12.59 and 20.99 μM) and DEX (from a″ to d″: 1.27, 2.55, 5.10 and 7.64 μM) in spiked urine sample after SPE, in mixture of BR buffer at pH 2.4/methanol (9/1, v/v) on HMDE. Pulse height 0.05 V, pulse width 0.1 s, scan rate 0.02 V $s^{-1}$, initial potential −0.300 V, final potential −1.0 V (vs. Ag|AgCl|KCl(sat.)).

analyses (recoveries, standard deviations, coefficients of variation, standard deviations of the mean and confidence intervals) are summarized in Tables 3 and 4. The results proved that the proposed method had acceptable recoveries. The data proved the suitability of LLE or SPE procedure for the extraction of investigated compounds from human urine samples. Representative voltammograms of urine samples analysis are presented in Figs. 4 and 5.

### 3.6. Specificity

Sometimes voltammetric techniques can pose difficulties in the analysis of biological fluids, which contain reducing or oxidizing substances. No interfering peaks were observed near the peak potentials of examined drugs in six human urine samples after LLE or SPE (Figs. 4 and 5). The specificity of the method for the analysis of human urine samples was evaluated by the determination of selected drugs in spiked human urine with satisfactory results.

## 4. Conclusion

A new elaborated DPV method and sample preparation procedures let determine PAR with FUR as well as DPR with CFZ and DEX nearby in model solutions, also apply them for urine samples analysis. The analytical method presented in the study may help to understand the underlying processes of with drug excretion when therapeutic goals are not being achieved. It should accent, that analysed drugs are determined simultaneously only by HPLC method [14] and DPV method was applied until now only for determination of some of these drugs individually. This method may be considered as a suitable alternative to the existing chromatographic methods. Finally the methods were not time-consuming and less expensive than the HPLC one.

The proposed methods were successfully applied to the determination these five selected drugs in model solutions and spiked urine samples, and in general, satisfactory results were obtained. A satisfactory separation of all investigated analytes from biological endogenous components in human urine was obtained. Preparation of the sample was easy and the method is not time-consuming and cheap. Therefore this quick and simple analytical procedure is of good applicability and can be introduced to determination of drugs in model solutions and spiked human urine samples.

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