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Electrochemical determination of oxytetracycline in veterinary drugs

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Abstract

Conductometric, potentiometric and cyclic voltammetric (CV) titration methods are proposed for determination of oxytetracycline (OTC), commonly used in veterinary. The electrochemical titration of OTC hydrochloride with $NH_4Mo_7O_{24}$, $NaVO_3$, NaOH, $AgNO_3$ and $FeCl_3$ as titrants are reported. The proposed methods were found to be highly precise, having a relative standard deviation (R.S.D.) below 1.0%. Proposed electrochemical titrations were successfully applied to the assay of commercial preparations: Tetrox, Tetramutin®OT and Neox, containing the above-mentioned antibiotics. The validity of the methods was tested by the recovery studies of standard addition to pharmaceuticals and results were found to be satisfactory.

Keywords: Oxytetracycline hydrochloride; Conductometry; Potentiometry; Cyclic; Voltammetry; Titrimetric assay

1. Introduction

The structural features of oxytetracycline (OTC) contribute to its high solubility in polar organic solvents and water, which is enhanced at low pH [1]. This compound also forms complexes with calcium and magnesium which have been well characterized [2] (Fig. 1).

Several methods for OTC determination in pure form and in veterinary drugs have been used. British Pharmacopoeia recommends the OTC determination by liquid chromatography method [3]. In recent years the main research interests were focused on such techniques as: HPLC [4–6], TLC [7] and CE [8,9], which are used for trace amounts of OTC determination. However, besides mentioned methods, polarographic [10], amperometric, potentiometric [11–13], spectrophotometric [14–16], spectrofluorimetric [17] and NIR-spectrometric [18] methods were applied for this antibiotic determination.

Potentiometric titration and cyclic voltammetry were also used for antibiotics determination of gentamicin, streptomycin and neomycin with a carbon dioxide gas-sensing electrode [19]. Tetracycline, chlortetracycline and oxytetracycline were determined with copper(II) ions [12] and perchloric acid [13]. The interactions of OTC and TC with Cr(III), Mn(II), Ni(III), Zn(II), Pb(II) ions and UO_2 were studied [20].

Adsorptive stripping voltammetry was adapted for OTC determination at hanging mercury drop electrode in acid medium [21]. Cyclic voltammetry was used in qualitative studies of OTC [22]. Moreover, the separation and quantitative performance parameters for

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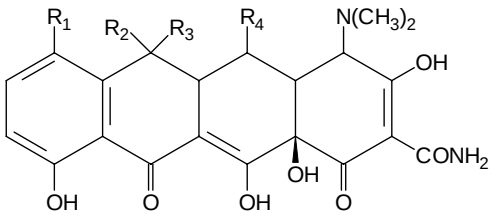

Fig. 1. The chemical structure of the oxytetracycline, R_1–H, R_2–CH_3, R_3–OH, R_4–OH.

OTC were investigated by capillary zone electrophoresis coupled with cyclic voltammetric detection [23].

To the best of our knowledge, there is a lack of publication on OTC determination with $NH_4Mo_7O_{24}$, $NaVO_3$, NaOH and $AgNO_3$. However, these reagents were used in conductometric determination of phenothiazines and hydroxyzine in their pharmaceutical preparations [24,25].

Moreover, in literature we did not find reports on the application of cyclic voltammetric titrations for OTC determination. Therefore, in our studies, above-mentioned titrants were applied for OTC quantitative analysis. One of the advantages of presented methods is that the reagents used in all cases are easily available. Further, electrochemical methods involved simple instrumentation which is cost effective compared with the other instrumental techniques.

The present paper describes conductometric, potentiometric and cyclic voltammetric titrations used in determination of OTC hydrochloride in the pure and dosage forms with the different titrants.

2. Experimental

2.1. Apparatus

Conductivity measurements and potentiometry determinations were carried out with a conductivity meter (CX-742 Elmetron, Poland) and electrode EPS-2ZM (Radelkis, Hungary). Reference electrode Ag/AgCl and platinum electrode as working electrode were employed in potentometric titrations. Cyclic voltammetric measurements were performed using an ECO-Chemie Autolab/PGSTAT 10 with a GPES software package. An electrochemical cell with a conventional three-electrode configuration was used. The Ag/AgCl reference electrode, the platinum auxiliary electrode and the gold working electrode (BAS) were used.

2.2. Chemicals

Hydrochloride OTC in pure form (95%, determined by HPLC method) and veterinary drugs: Tetrox (granules), Tetramutin®OT (powder) and Neox (gel) were supplied by Biowet Ltd. (Poland), whereas $NH_4Mo_7O_{24}$, $NaVO_3$, NaOH, $AgNO_3$ and $FeCl_3$ (all analytical grade), purchased from POCH (Poland) were used as received.

2.3. Sample preparation

OTC hydrochloride and veterinary drugs solutions were prepared in the standard volumetric flask (100 ml) by dissolving the appropriate amount of the drug in bidistilled water. The determinations of OTC (in pure and dosage form) by the cyclic voltammetry and potentiometry with $FeCl_3$ were carried out in phosphate buffer at pH 6.54 (3.52 g KH_2PO_4 and 7.26 g K_2HPO_4 in 1 l water). The concentrations of prepared OTC solutions were similar to those declared in pharmaceutical preparations (1.0×10^{-3} to 7.0×10^{-4} M). In order to minimize the volume correction, titrants concentrations were 2–8 times higher than the studied drug depending on the reaction stoichiometry.

2.4. Procedure

2.4.1. Conductometric titrations

Conductometric titrations were performed with $NH_4Mo_7O_{24}$ (1.67×10^{-3} M), $NaVO_3$ (1.00×10^{-2} M), NaOH (1.00×10^{-2} M) and $AgNO_3$ (5.21×10^{-3} M). An addition of above solutions caused the formation of a precipitates. After the titrant addition (0.1 or 0.2 ml depending on the sample concentration), solutions were stirred for 2 min and left for 2 min to reach the equilibrium, due to formation of a precipitate. Titrations were repeated five times and average values calculated. Considering the volume change, the observed values were corrected by a dilution factor. The end point in conductometric titrations was obtained by extrapolation of the two linear branches of the plot [26].

In order to check the usefulness of the elaborated method in OTC determination, a conductometric titrations of Tetrox, Tetramutin®OT and Neox were carried out. Amounts of these drugs were similar to OTC in pure form. Volumes of 15 ml of the freshly prepared solutions were taken for analysis. The further procedure was the same as for OTC determination in pure form.

2.4.2. Potentiometric determinations

For potentiometric titration, 60 ml of OTC in phosphate buffer (or 30 ml of drug in dosage form) was placed in the beaker and titrated with $FeCl_3$ (1.00×10^{-2} M). After each addition of the titrant (0.1 or 0.2 ml depending on the sample concentration), the procedure was the same as in the conductometric determination.

Titration with $NH_4Mo_7O_{24}$ (2.00×10^{-3} M) was performed at pH 2 (adjusted with 0.5 M HCl). OTC samples containing 20–29 mg of drug (or 30 ml of veterinary drug solution) were dissolved in 60 ml of water. Next step of the procedure was the same as above.

2.4.3. Cyclic voltammetric determination

For CV titration methods, 10 ml of 1×10^{-3} M OTC hydrochloride (in buffer solution, pH 6.54) was used. All measurements were carried out in argon atmosphere, scan rate 50 mV s^{-1}, potential range +1250 to −1250 V. After addition of 0.1 or 0.2 ml $FeCl_3$

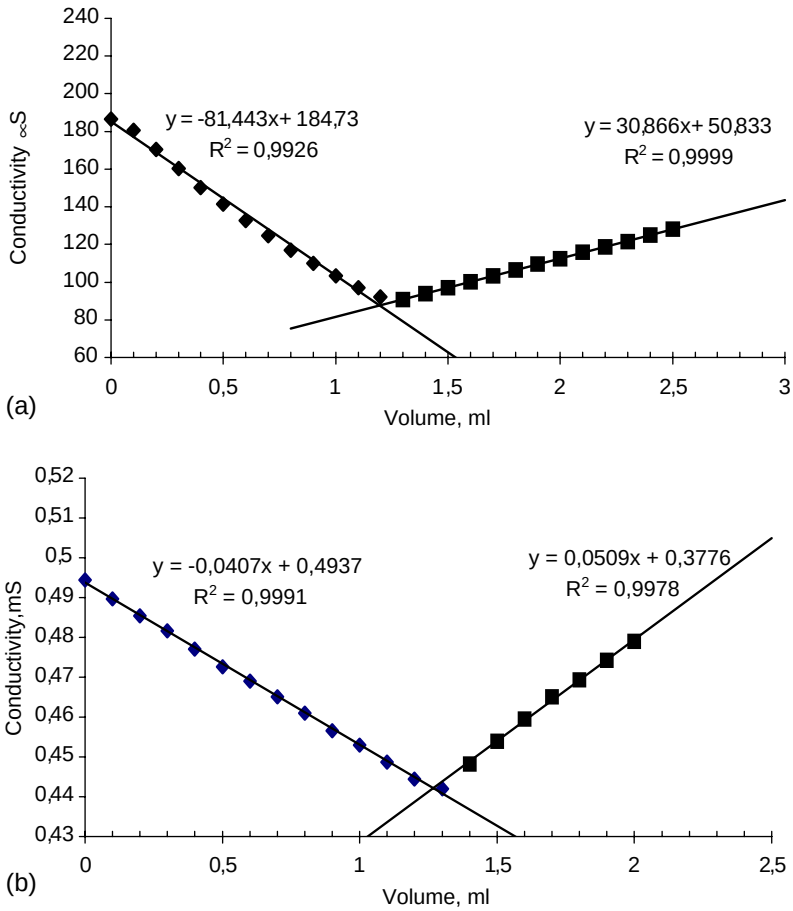

Fig. 2. (a) Conductometric titration curve of oxytetracycline hydrochloride with ammonium molybdate(VI). (b) Conductometric titration curve of Tetramutin®OT with ammonium molybdate(VI).

Table 1
Determination of pure OTC hydrochloride using conductometric titration

Titrants	Mole ratios, OTC:titrant	Amount of OTC taken (mg)	Standard deviation[a] (mg)	Confidence limit[b]	R.S.D. (%)	Recovery (%)
$NH_4Mo_7O_{24}$	6:1	7.10	0.09	7.15 ± 0.11	1.26	100.70
$NaVO_3$	2:1	7.08	0.05	7.13 ± 0.06	0.70	100.71
NaOH	1:1	7.09	0.06	6.89 ± 0.07	0.87	97.18
$AgNO_3$	1:1	7.09	0.07	7.07 ± 0.09	0.99	99.72

[a] $n = 5$.
[b] $P = 0.95$.

(2.00×10^{-3} M), the solution was stirred for 5 min and voltammogram recorded. All measurements were carried out at $T = 293 \pm 1$ K in thermostatic cell.

3. Results and discussion

3.1. Conductometric determinations

The conductometric titration curves of OTC in pure form and in Tetramutin®OT by ammonium molybdate(VI) in the investigated range of concentration are presented as examples in Fig. 2a and b.

The shapes of other titrations curves OTC with $NaVO_3$, NaOH and $AgNO_3$ were similar. Mole ratios for OTC hydrochloride with all titrants (calculated from the curves end point) and results of measurements are listed in Table 1.

The maximum differences between the lowest and highest results (dispersion) in a separate series of determinations were <2%. The mean values obtained by the conductometric method are in good agreement with the nominal value given for the studied drug with the recoveries 97.18–100.71%. The relative standard deviations are less than 2%, what indicates sufficient precision for this type of analyses (Table 1). The results of OTC determination in the studied pharmaceuticals are listed in Table 2.

The applied method was supported by statistical evaluation of the obtained results (Table 2). The repeatability calculated as the relative standard deviation (less than 1%) confirms the high precision of the

Table 2
Results of conductometric determination of OTC in Tetrox, Tetramutin®OT and Neox preparations with ammonium molybdate(VI), sodium vanadate(V), sodium hydroxide and silver nitrate(V)

Veterinary drugs	Titrants	Amount of drugs taken (mg)	Standard deviation[a] (mg)	Confidence limit[b]	R.S.D. (%)	Recovery (%)
Tetrox (granulated)	$NH_4Mo_7O_{24}$	7.09	0.05	7.11 ± 0.06	0.70	100.28
	$NaVO_3$	7.09	0.01	6.68 ± 0.01	0.14	98.44
	NaOH	7.09	0.06	6.87 ± 0.07	0.87	96.90
	$AgNO_3$	7.09	0.01	7.01 ± 0.01	0.14	98.87
Tetramutin®OT (powder)	$NH_4Mo_7O_{24}$	7.09	0.04	7.10 ± 0.05	0.56	100.14
	$NaVO_3$	7.09	0.01	6.92 ± 0.01	0.14	97.60
	NaOH	7.09	0.04	7.02 ± 0.05	0.57	99.01
	$AgNO_3$	7.09	0.02	6.90 ± 0.02	0.29	97.60
Neox (gel)	$NH_4Mo_7O_{24}$	4.28	0.01	4.20 ± 0.01	0.24	98.25
	$NaVO_3$	4.28	0.02	4.14 ± 0.02	0.48	96.84
	NaOH	4.28	0.03	4.28 ± 0.04	0.70	100.12
	$AgNO_3$	4.28	0.01	4.13 ± 0.01	0.24	96.61

[a] $n = 5$.
[b] $P = 0.95$.

measurements. Recovery values ranging from 96.61 to 100.28% in veterinary drug solutions were found satisfactory [27].

3.2. Potentiometric determinations

The measured potential ranges from +335 to +695 mV for $FeCl_3$, and +265 to +285 mV for $(NH_4)_6Mo_7O_{24}$ titration. The end point of titration was determined with the first derivatives for all titrations. The potentiometric plot for the titration of OTC in pure form with $FeCl_3$ and $(NH_4)_6Mo_7O_{24}$ are presented in Fig. 3a and b.

The tested method is based on the fact that molybdate(VI) ions in an acidic medium created with OTC hydrochloride ion-pairs formation like phenothiazines, probably [25,28]. In the case of iron(III) ions, a complex with OTC was formed [20,28].

Mole ratios calculated from potentiometric curves for OTC with $NH_4Mo_7O_{24}$ and $FeCl_3$ are listed in Table 3. The recoveries results were 99.13 and 101.69% with R.S.D. values 0.39 and 0.87%, respectively. The method was applied next for determination of the studied drugs in dosage forms (Table 4).

The validity of potentiometric titration was tested by recovery studies of OTC in pharmaceuticals and

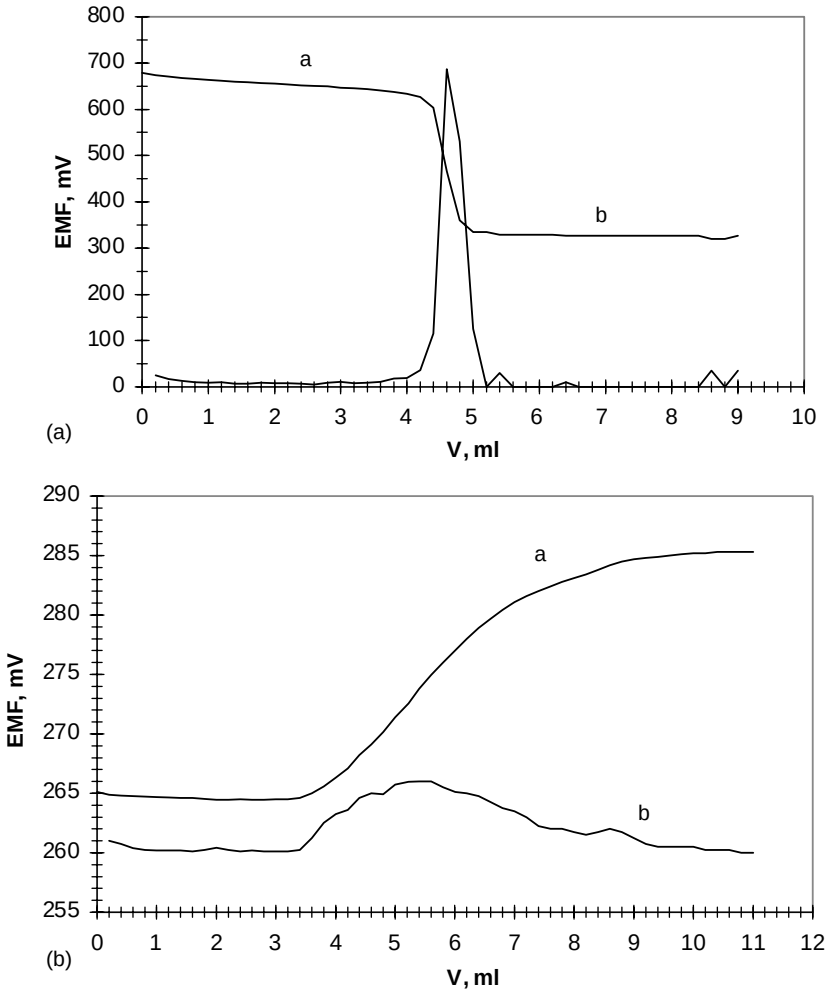

Fig. 3. (a) Potentiometric titration curve (marked a) and first derivative (marked b) of OTC hydrochloride in pure form with iron(III) chloride. (b) Potentiometric titration curve (marked a) and first derivative (marked b) of OTC hydrochloride in pure form with ammonium molybdate(VI).

Table 3
Results of potentiometric determination of OTC hydrochloride in pure form with ammonium molybdate(VI) and iron(III) chloride

Titrants	Mole ratios, OTC:titrant	Amount of OTC taken (mg)	Standard deviation[a] (mg)	Confidence limit[b]	R.S.D. (%)	Recovery (%)
$NH_4Mo_7O_{24}$	6:1	28.33	0.11	28.08 ± 0.14	0.39	99.13
$FeCl_3$	2:1	28.37	0.25	28.85 ± 0.31	0.87	101.69

[a] $n = 5$.
[b] $P = 0.95$.

Table 4
Results of potentiometric determination of OTC hydrochloride in veterinary drugs with ammonium molybdate(VI) and iron(III) chloride

Veterinary drugs	Titrants	Amount of drug taken (mg)	Standard deviation[a] (mg)	Confidence limit[b]	R.S.D. (%)	Recovery (%)
Tetrox (granulated)	$NH_4Mo_7O_{24}$	14.16	0.15	13.89 ± 0.19	1.08	98.09
	$FeCl_3$	14.16	0.10	14.02 ± 0.12	0.71	99.01
Tetramutin®OT (powder)	$NH_4Mo_7O_{24}$	14.16	0.12	14.34 ± 0.15	0.83	101.27
	$FeCl_3$	14.16	0.09	13.92 ± 0.11	0.65	98.31
Neox (gel)	$NH_4Mo_7O_{24}$	8.55	0.04	8.35 ± 0.09	0.48	97.66
	$FeCl_3$	9.41	0.08	9.39 ± 0.10	0.85	99.79

[a] $n = 5$.
[b] $P = 0.95$.

range 97.66–101.27% can be found satisfactory [27]. Relative standard deviations (R.S.D.) of this method were below 1.08%, what makes the method highly precise. The results in Table 4 indicate that the OTC hydrochloride in veterinary drugs in the range from 8.00 to 14.50 mg can be determined by the proposed method.

3.3. Cyclic voltammetric-titration method

Redox and complexing properties of OTC hydrochloride was (like in potentiometric titration) applied in quantitative drug determination employing cyclic voltammetry [13,20].

An example of voltammogram for such titration is presented in Fig. 4a. In order to plot the titration curve of OTC with $FeCl_3$, cathodic Fe^{3+} reduction at potential of −0.75 V was used (Fig. 4b). The end point for titration was determined in the same way as in the case of conductometric titration.

The reagent's mole ratios were calculated from curves end point and listed in Table 5. In order to examine suitability of this method to determine veterinary drugs, a CV titration for Tetrox, Tetramutin®OT and Neox preparations was carried out. Amount of studied drugs solution were the same as for OTC in pure form. In the case of pharmaceuticals: Tetramutin®OT and Neox besides the active substance (OTC hydrochloride) also contained supporting substances or others antibiotics (tiamuline, neomycine), the obtained results revealed that this method is not selective.

Table 5
Determination of OTC hydrochloride in pure form and veterinary drug with iron(III) chloride

Compound	Amount of OTC taken (mg)	Standard deviation[a] (mg)	Confidence limit[b]	R.S.D. (%)	Recovery (%)
OTC in pure form with $FeCl_3$ (2:1)	4.72	0.01	4.56 ± 0.01	0.23	96.61
In veterinary drug Tetrox	4.72	0.04	4.71 ± 0.05	0.85	99.79

[a] $n = 5$.
[b] $P = 0.95$.

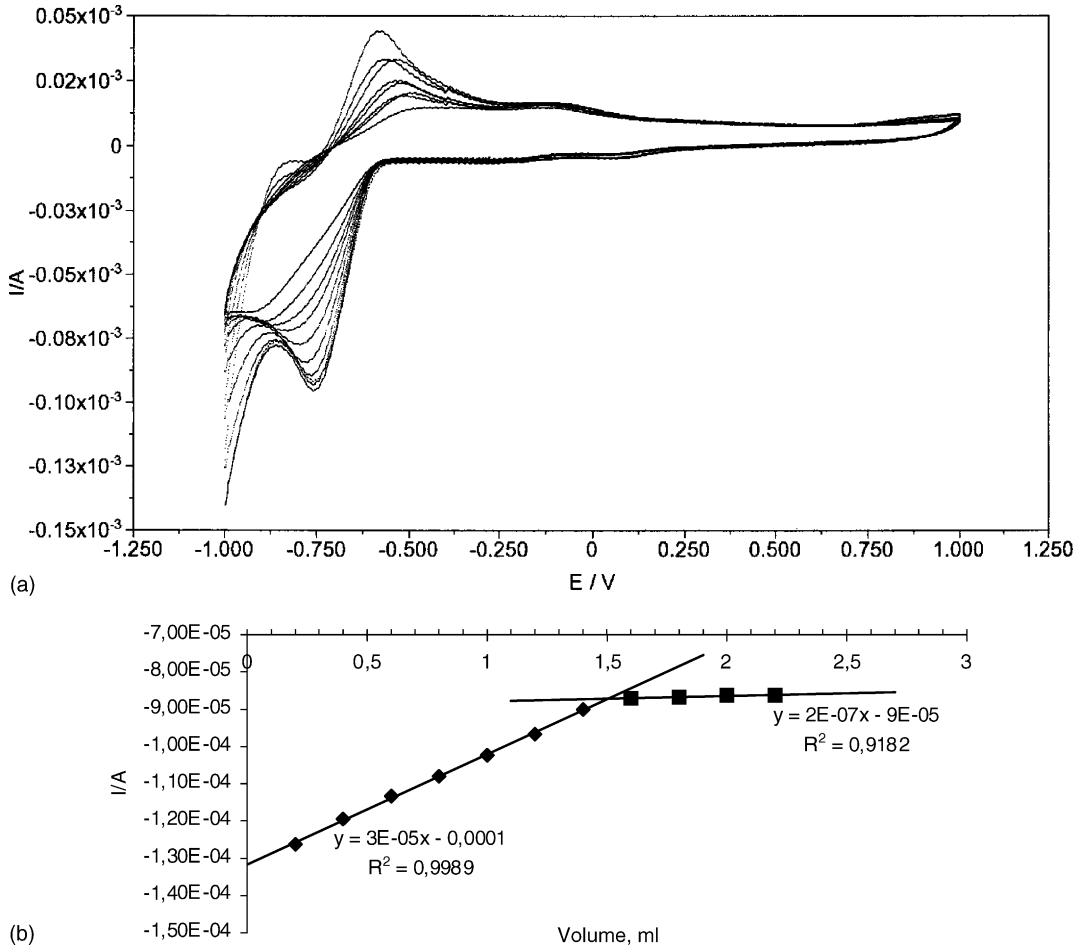

Fig. 4. (a) Cyclic voltammogram of OTC hydrochloride in pure form with iron(III) chloride. (b) Cyclic voltammogram titration curve of Tetrox with iron(III) chloride.

In order to fully characterize this method, the statistical evaluation was made. The recoveries (96.01–99.79%) agree well enough with the nominal contents. The elaborated method is precise for the determination of OTC hydrochloride in veterinary drugs because of the R.S.D. values are less than ±1% [27]. Unfortunately, this method revealed low selectivity and is not suitable for determination of OTC in a complex pharmaceuticals.

4. Conclusion

Potentiometry and conductometry can be applied for the determination of OTC hydrochloride in pure and dosage forms. These titrations have been studied due to their simplicity, precision, accuracy and therefore, they can be recommended for laboratory practice. The conductometric and potentiometric determination of OTC are faster and less expensive than Polish Pharmacopoeia V or British Pharmacopoeia recommended methods. The cyclic voltammetry can be used for determination of OTC hydrochloride in pure form and in pharmaceutical preparations, but this method is not specific for composed drugs.

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