Scientific paper

The Possibility of Simultaneous Voltammetric Determination of Desloratadine and 3-Hydroxydesloratadine

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Abstract

The electrochemical behaviour of desloratadine (DLOR) and its derivative 3-hydroxydesloratadine (3OH-DLOR) was investigated by direct current (DCP) polarography, cyclic (CV), differential pulse (DPV) and square–wave (SWV) voltammetry in Britton-Robinson (BR) buffer solutions (pH 4–11). Both compounds are reduced at mercury electrode in irreversible two electron reduction of the C=N bond of the pyridine ring in their molecules. The difference in their electrochemical behaviour was investigated, and the most pronounced distinction is observed at pH > 9, as a consequence of the deprotonation of the phenolic moiety in 3OH-DLOR molecule, yielding significant change in their reduction potentials ($E_{p\ DLOR}$ = –1.48 V, and $E_{p\ 3OH\text{-}DLOR}$ = –1.6 V). The observed results correlate with calculated LUMO energy levels and Hammet substituent constants (σ).

Based on the difference in the reduction potential for DLOR and 3OH-DLOR, conditions for simultaneous determination these two molecules in alkaline medium were established. The best selectivity was achieved using SWV method at pH 10. The linearity of the calibration graphs were achieved in the concentration range from 1.5×10^{-6} M – 1×10^{-5} M for DLOR and 7.5×10^{-6} M – 5×10^{-5} M for 3OH-DLOR with detection limits of 2.29×10^{-7} M and 2.08×10^{-6} M, and determination limits of 7.64×10^{-7} M and 6.94×10^{-6} M, for DLOR and 3OH-DLOR, respectively. The method was checked in human plasma sample. Good response was obtained with LOD and LOQ values of 4.63×10^{-7} M and 1.54×10^{-6} M, for DLOR and 2.39×10^{-6} M and 7.97×10^{-6} M, 3OH-DLOR, respectively.

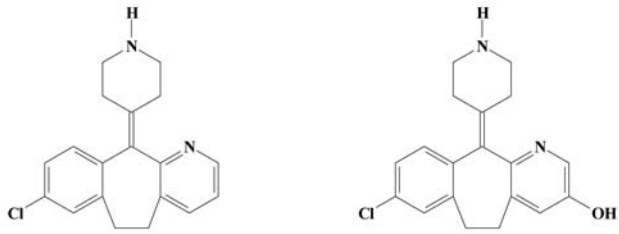
Keywords: Desloratadine, 3-hydroxydesloratadine, simultaneous determination, voltammetry, buffer, plasma.

1. Introduction

Desloratadine (DLOR)[1] is the active metabolite of loratadine, the most extensively used competitive histamine H1-receptor antagonist.

Several papers concerning the analytical approach for DLOR determination in plasma and pharmaceutical formulations are mentioned in literature[2–7]. Most of these papers deal with simultaneous determination of loratadine and DLOR preferably using HPLC, capillary electrophoresis and mass spectroscopy.

This antihistamine is metabolized to 3-hydroxydesloratadine (3OH-DLOR), which retains biological acti-



Scheme 1. DLOR and 3OH-DLOR.

vity. Recently a bioanalytical method for determination DLOR and its metabolyte 3OH-DLOR using ultrahigh pressure liquid chromatography in conjunction with mix mode solid phase extraction is published[8].

No literature data dealing with electrochemical behaviour or voltammetric methods for DLOR or 3OH-DLOR determination were found.

This paper deals with electrochemical study of those two compounds with the aim of finding the difference in their electrochemical behaviour which can be used in purpose of their monitoring and determination simultaneously.

2. Experimental

2. 1. Reagents and Solutions

DLOR, 5H-benzo[5,6]cyclohepta [1,2-b]pyridine, 8-chloro- 6,11-dihydro-11- (4-piperidinylidene), is produced by Sigma-Aldrich (Munich, Germany), and kindly donated by Agency of Drugs and Medical Devices, Belgrade, Serbia. 3OH-DLOR, 5H-benzo[5,6]cyclohepta[1,2-b]pyridin-3-ol, 8-chloro-6,11-dihydro-11-(4-piperidinylidene), was produced by TRC – Toronto Research Chemicals Inc., and all other chemicals were of analytical grade quality. Stock solutions of 1×10^{-3} M DLOR and 3OH-DLOR were prepared in methanol and stored in freezer. More diluted solutions were prepared daily from stock solutions.

Britton-Robinson buffer solution, used as supporting electrolyte was prepared in the usual way[9].

Plasma sample was kindly provided by Institute of Biochemistry, Faculty of Pharmacy, University of Belgrade.

2. 2. Instrumentation

Polarographic analyzer PAR 174A connected with three-electrode cell (DME, SCE and Pt) was used. Dropping time of 1 s, scan rate 2 mV/s, and the mercury column height of 80 cm were used for direct polarographic measurements.

The voltammetric measurements were performed with an Amel 433-A computerized polarographic analyzer. Three-electrode system was employed: hanging mercury drop electrode (HMDE), Ag/AgCl reference electrode and a Pt-auxiliary electrode.

CV mode was applied with the scan rates from 5 to 100 mVs^{-1}. DPV was performed with scan rate 50 mVs^{-1}, pulse repetition 100 ms, pulse amplitude –100 mV, pulse width 20 mV and 4 ms sampling time, while SW mode used the following parameters: pulse amplitude 50 mV, frequency of 125 Hz , pulse increment 4 mV and sampling time 1 ms. All techniques used the mercury drop size of 10 arb.un., and when needed, the stirring speed of 300 r.p.m. was applied.

A SCALTEC SBC 31 balance, Radiometer pH meter, PHM 220, with combined pH electrode Radiometer GK2401B with appropriate standard buffer solutions, and centrifuge Tehtnica (Železniki) LC – 320 were used.

2. 3. Procedures

2. 3. 1. Procedure for Polarographic and Voltammetric pH – Investigations

In electrochemical cell 13.5 ml (for DCP) or 19.8 ml (for CV and DPV experiments) of BR buffer of different pHs was transferred, de-aerated for 10 minutes with nitrogen of high purity and 1.5 ml (for DCP) or 0.2 ml (for CV and DPV) of DLOR or 3OH-DLOR stock solution was added to make its final concentration of 1×10^{-4} M (DCP) or 1×10^{-5} M (CV, DPV).

The solution was purged for another 3min and the current -voltage curves were recorded.

2. 3. 2. Procedure for Square Wave Voltammetric Measurements

An aliquot of 15 ml of Britton Robinson's buffer pH 10 was introduced into electrochemical cell and de-aerated with pure nitrogen for 10 min. If adsorptive stripping was performed, the selected accumulation potential was applied to a mercury drop, for a selected accumulation period, while the solution was stirred at 300 rpm, if not, the fresh mercury drop was established. The stirring was then stopped, and after the 10 s rest period, a square–wave voltammogram was scanned in the negative direction over the range from –1.0 V to – 1.8 V vs. Ag/AgCl. After the background voltammogram had been recorded, the aliquot of the DLOR, 3OH-DLOR or their mixture was introduced into cell and the voltammogram was then recorded at a new mercury drop.

Calibration graphs were constructed using data from these measurements and evaluated by the least-squares linear regression method. Measurements were performed at room temperature.

2. 3. 3. Procedure for DLOR and 3OH-DLOR Determination in Human Plasma

For determination of DLOR and 3OH-DLOR, 0.4 ml of concentrated human plasma was taken, 4 ml of concentrated methanol was added to precipitate the proteins and centrifuged 25 min at 3900 rpm. The clear supernatant (2 ml), was filtrated through the membrane filter porosity of 2 μm and this solution was used for further investigations.

This plasma solution was mixed with BR buffer solution, pH 10, and spiked with different volumes of DLOR/3OH-DLOR (1 : 5) mixture. The procedure for square wave voltammetric measurements was the same as in buffer solution (Section 2.3.2.).

3. Results and Discussion

The electrode reduction process of DLOR, and 3OH-DLOR, has been examined in the pH range 4.0–11.0 using DC, CV and DPV.

3. 1. Influence of pH of the Supporting Electrolyte

The preliminary information about the behaviour of these molecules in buffered solutions was obtained from polarographic curves. Both DLOR and 3OH-DLOR showed one reduction wave at negative potentials. Those polarographic waves are not very well defined in acid medium, due to negative reduction potential which is only slightly more positive than the reduction of H^+ ions from the supporting electrolyte used, but with increasing of pH the waves are better shaped. Polarographic reduction wave of 3OH-DLOR is shifted to even more negative values compared to DLOR.

The effect of the pH on the polarographic halfwave potential $E_{1/2}$, for DLOR and 3OH-DLOR is presented in Figure 1. The shape and the position of the waves are pH dependent, indicating that protonation of the reactive part of the molecule is involved in the electrode reaction mechanism[10].

Both molecules showed the negative shift of the halfwave potential with increasing pH. In the case of DLOR the slope of $E_{1/2}$ vs. pH dependence is 0.059 V/pH, being constant in whole pH range. At pH < 9, 3OH-DLOR showed slope of the $E_{1/2}$ vs. pH dependence ~0.051 V/pH, which is almost the same as for DLOR, but with further increasing of pH (pH > 9), the peak is drastically shifted to much more negative values, $\Delta E_{1/2}/\Delta pH = 0.280$ V/pH.

3. 2. Electrode Reaction Pathway

Since these molecules possess the same electrochemical active centre as antihistaminic drug loratadine, the polarographic wave of DLOR and 3OH-DLOR can be attributed to the irreversible two electron reduction of the C=N bond of the pyridine ring in their molecules[11].

The unsaturated C=N bond of pyridine derivatives are well known to be reduced on mercury electrodes[12]. It has been early observed that the reduction occurs preferably on the protonated form and involves, under optimum conditions, transfer of two electrons, yielding saturated bond.

According to the changes of reduction potential with pH shown in Figure 1, it can be concluded that the reduction of C=N bond of pyridine ring at pH ≤ 9 is proceeding through the same mechanism for both investigated molecules DLOR and 3OH-DLOR, consuming two protons and two electrons.

$$> C = N - + 2H^+ + 2e^- \rightarrow > CH - NH - \qquad (1)$$

3. 3. Nature of the Electrode Process

Voltammograms of these molecules in BR buffer solutions pH ≥ 4 exhibit a single cathodic peak (Fig. 2a.) at

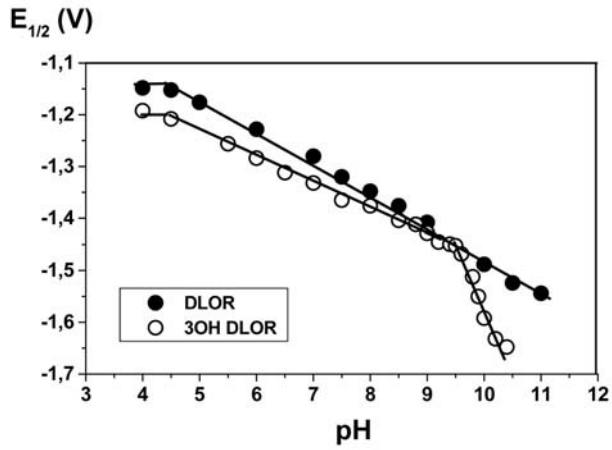


Figure 1. The effect of pH on halfwave potential for 1×10^{-4} M DLOR (●) and 3OH-DLOR (○) in BR buffer solutions.

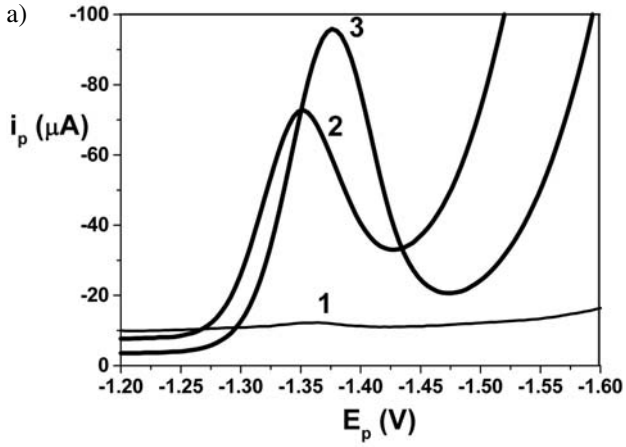


Figure 2a. The representative DP voltammograms of 1×10^{-5} M DLOR (2) and 1×10^{-5} M 3OH-DLOR (3) in BR buffer solutions (1) of pH 8 ($\nu = 50$ mVs^{-1}, pulse amplitude –100 mV, drop size 10 arb.un.).

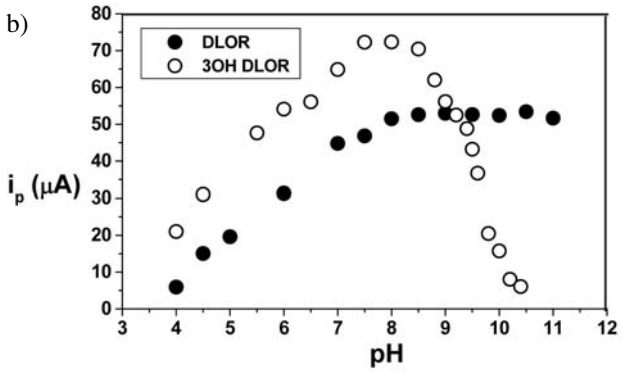


Figure 2b. The effect of pH on peak current for 1×10^{-5} M DLOR (●) and 3OH-DLOR (○) in BR buffer solutions ($\nu = 50$ mVs^{-1}, pulse amplitude –100 mV, drop size 10 arb.un.).

negative potentials. Both molecules exhibit well shaped peaks especially in alkaline solutions, but 3OH-DLOR, which is reduced at more negative potential, showed more symmetrical peak with higher peak current.

The peak current of both molecules increases with the pH increasing up to 8. At pH > 8, peak current for DLOR remaines almost constant, while the current of 3OH-DLOR reaches its maximum, and then very suddenly decreases. (Fig.2b.).

Since no anodic peak was observed in whole pH range, the irreversibility of both DLOR and 3OH-DLOR reduction process, was confirmed.

The nature of the reduction process was studied by following the effect of the scan rate on the peak current showing that the electroreduction of 3OH-DLOR is much more influenced by the adsorption comparing to DLOR. Figure 3 shows linear $\mathbf{i_p}$ **vs.** $\boldsymbol{\nu}$ dependence obtained for 3OH-DLOR at pH 8 and 10 which is characteristic for adsorption controlled processes. Slope obtained at pH 8 is six times higher then at pH 10. On the other hand the same relationship obtained for DLOR gave nonlinear curves.

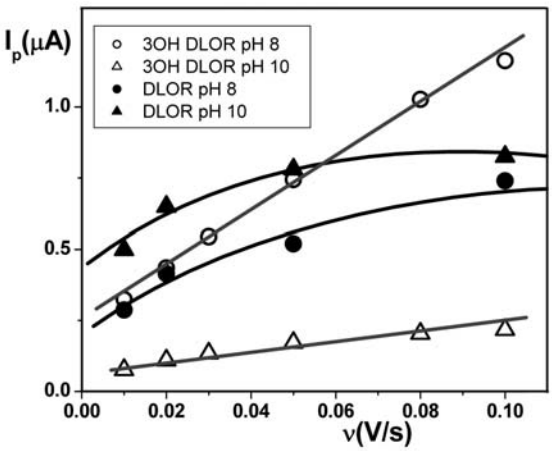


Figure 3. Effect of the scan rate on the CV peak current for 1×10^{-5} M DLOR (●, ▲) and 3OH-DLOR (○, △) in BR buffer solutions pH 8 (●,○) and 10 (▲, △).

The appearance of the "broad" maximum for 3OH-DLOR and its higher currents (Fig. 2b), also suggests that this molecule is strongly adsorbed at mercury surface regarded to DLOR.

3. 4. Simultaneous Determination of DLOR and 3OH-DLOR

Based on the results obtained from the pH dependences presented in Figure 1, it was supposed that DLOR and 3OH-DLOR can be detected from the same solution in alkaline medium at pH ~10. In order to establish the best conditions for their simultaneous detection the composition of the mixture was examined. The best composition in which both compounds showed good current signal response was found to be DLOR **:** 3OH-DLOR = 1 **:** 5. The obtained results are presented in the Figure 4. In BR buffer pH 10, DLOR gave a well defined peak at –1.48V, and 3OH-DLOR at –1.61 V (Fig. 4 a, b). DP voltammogram of their mixture is presented at Fig. 4c–1.

In order to achieve better resolution and higher sensitivity of determination of DLOR and 3OH-DLOR in the mixture, the square-wave voltammetry was applied (Figure 4c –2). As shown, two well separated peaks better resolved from the background electrolyte, with higher peak currents compared to DP voltammograms are obtained. Several experimental parameters were examined in order to establish the best conditions for simultaneous determination of DLOR and 3OH-DLOR from their mixture. The frequency of 125 Hz, pulse amplitude of 50 mV, scan increment of 4 mV, and mercury drop size of 10 arb. un. were chosen.

Having in mind the adsorption characteristics of the investigated compounds, the cathodic adsorptive stripping SW-voltammetry was applied. The AdSSW voltammograms of DLOR : 3OH-DLOR mixture were recorded in BR buffer pH 10, at accumulation potentials in the range from –0.9 V to –1.6V, followed by preconcentration of 10–30 s. It was evident that signal obtained for AdSSWV was much higher after an accumulation step, but the peaks overlapped forming one peak at $E_p \approx -1.6$V (Fig. 4d–1). According to peak potential, this peak is probably caused by the 3OH-DLOR accumulation at the electrode surface that is in agreement with its pronounced adsorption characteristics. Unfortunately, this makes adsorptive stripping technique inapplicable for determination of the components of this particular mixture.

3. 5. Analytical Parameters

Calibration curves were constructed for DLOR and 3OH-DLOR in the mixture at pH 10 under the optimal conditions of the proposed SW voltammetric method. Concentration range and regression equations are presented in Table I. The limits of detection (LOD) and quantification (LOQ) were calculated using the relation $3S_a/b$ and $10S_a/b$ for LOD and LOQ, respectively, where S_a is the standard deviation of the intercept and b is the slope of the calibration curve.

The precision of the SWV method was checked for DLOR : 3OH-DLOR mixture of concentrations 1.60 μM : 8.00 μM, and 2.00 μM : 10.00 μM, at pH 10. Five determinations were performed in both cases, and the RSD were less then 3.8% (Table II).

The robustness[13] of the method proposed was examined by evaluating the influence of small variations of some most important operational parameters such as pH, wave amplitude, wave increment, pulse period, drop size, and stirring speed, on the recovery of the DLOR : 3OH DLOR concentration of 2×10^{-6} M : 1×10^{-5} M. Differen-

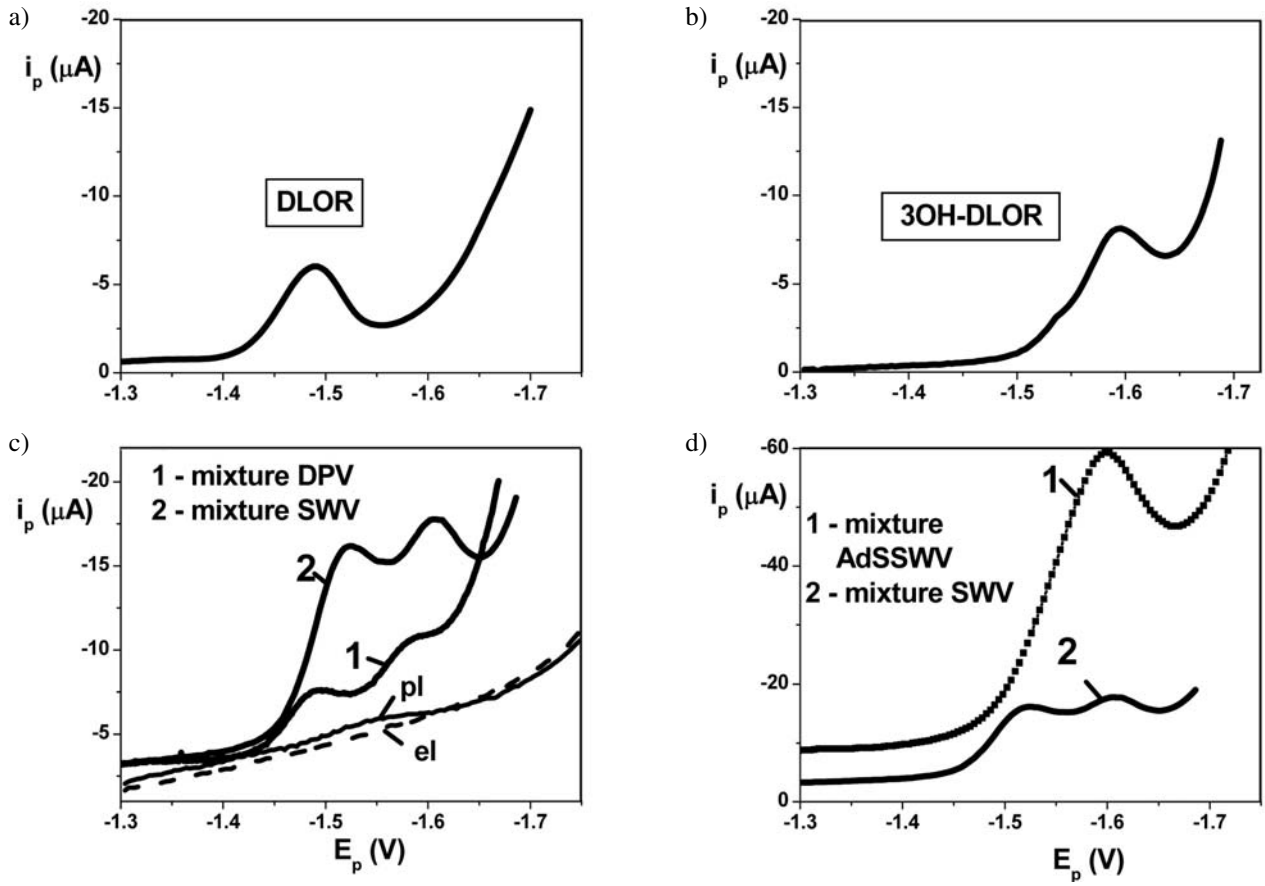


Figure 4. (a) DP voltammogram of 2×10^{-6} M DLOR, pH 10 (b) DP voltammogram of 1×10^{-5} M 3OH-DLOR, pH 10 (c) SW voltammograms of (el) BR buffer pH 10, (pl) plasma sample in BR buffer pH 10 DP (1), and SW (2) voltammogram of the mixture of 2×10^{-6} M DLOR and 1×10^{-5} M 3OH-DLOR in BR buffer solutions pH 10 (d) AdSSW (1) and SW (2) voltammogram of the mixture of 2×10^{-6} M DLOR and 1×10^{-5} M 3OH-DLOR in BR buffer solutions pH 10 (DPV: scan speed 50 mVs^{-1}, pulse amplitude –100 mV, pulse width 20 ms, sampling time 4 ms, drop size 10 arb.un, and stirring speed of 300 rpm.). (SWV: frequency of 125 Hz, pulse amplitude of 50 mV, scan increment of 4 mV, drop size 10 arb.un.). (AdSSWV: E_{acc} = –1500 mV, t_{acc} = 10 s , other parameters as already given for SWV).

ce between the recovery obtained under the chosen experimental conditions and the recovery obtained within the studied range of variation of the operational parameters was between 2 and 4%. The most pronounced influence was observed with the change of pH, but since this variable did not significantly effect the determination, the proposed procedure can be considered robust.

The possibility of simultaneous detection and determination of both compounds was checked in real human plasma since 3OH-DLOR is an active metabolite of DLOR.

The main problem for determination of both compounds in plasma compared to the simple buffer solution is the presence of proteins, which makes the ratio of signal to noise unacceptable. After the precipitation of proteins and filtration of the supernatant through the membrane filter, the background currents were minimized (Figure 4c curves denoted as **el** and **pl**) and determination of both compounds was possible. Therefore the protein free human plasma was spiked with DLOR : 3OH-DLOR = 1 : 5 mixture and the results are presented in Table I. The limits of detection (LOD) and quantification (LOQ) of investigated

Table 1. Statistical parameters for SWV, simultaneous determination of DLOR and 3OH-DLOR in BR buffer, pH 10, and plasma samples.

Drug	Linear concentration range (M)	Medium	Regression equation y (μA), x (M)	S_a	S_b	R	SD (μA)	LOD (M)	LOQ (M)
DLOR	$1.50 \times 10^{-6} – 1.00 \times 10^{-5}$	BR	$y = –4.60 + 6.02 \times 10^6 x$	0.46	2.9×10^5	0.994	0.378	2.29×10^{-7}	7.64×10^{-7}
		*Plasma	$y = –6.55 + 5.12 \times 10^6 x$	0.79	3.8×10^5	0.994	0.277	4.63×10^{-7}	1.54×10^{-6}
3OH-DLOR	$7.50 \times 10^{-6} – 5.00 \times 10^{-5}$	BR	$y = –0.56 + 3.46 \times 10^5 x$	0.24	3.3×10^4	0.991	0.169	2.08×10^{-6}	6.94×10^{-6}
		*Plasma	$y = –1.72 + 2.51 \times 10^5 x$	0.20	1.8×10^4	0.993	0.098	2.39×10^{-6}	7.97×10^{-6}

S_a Standard deviation of the intercept. S_b Standard deviation of the slope. *Protein free plasma samples, pH 10 (pH adjusted with BR buffer)

mixture in plasma are somewhat higher compared to buffer solutions, indicating that plasma medium is also suitable for simultaneous detection and determination of DLOR and 3OH-DLOR. The presence of endogenous factors does not interfere with the main voltammetric response.

Table 2. Results obtained for simultaneous DLOR and 3OH-DLOR determination

	Added (μM)	Found (μM)	Recovery (%)	RSD (%) (n = 5)
DLOR	1.60	1.57	98.12	2.7
	2.00	1.96	98.00	2.9
3OH-	8.00	7.89	98.62	2.1
DLOR	10.00	10.41	104.10	3.8

3. 6. The sequence of the Reduction

According to the results presented, it is supposed that pathway of DLOR and 3OH-DLOR reduction is the same up to pH 8; i.e. the unsaturated C = N bond of pyridine ring is reduced on mercury electrodes preferably of the protonated form, and involves transfer of two electrons, yielding saturated bond. In more alkaline solutions, as the rate of protonation decreases with increasing pH, above a certain pH that is several pH units higher than would correspond to the pKa, no further current increase is expected. In the case of DLOR, at pH > 8 curve representing reduction current vs. pH is almost constant due to the rate of the re-establishment of the acid-base equilibrium on N-pyridine (pKa_{calc} 4.55[14]) in the vicinity of the electrode surface.

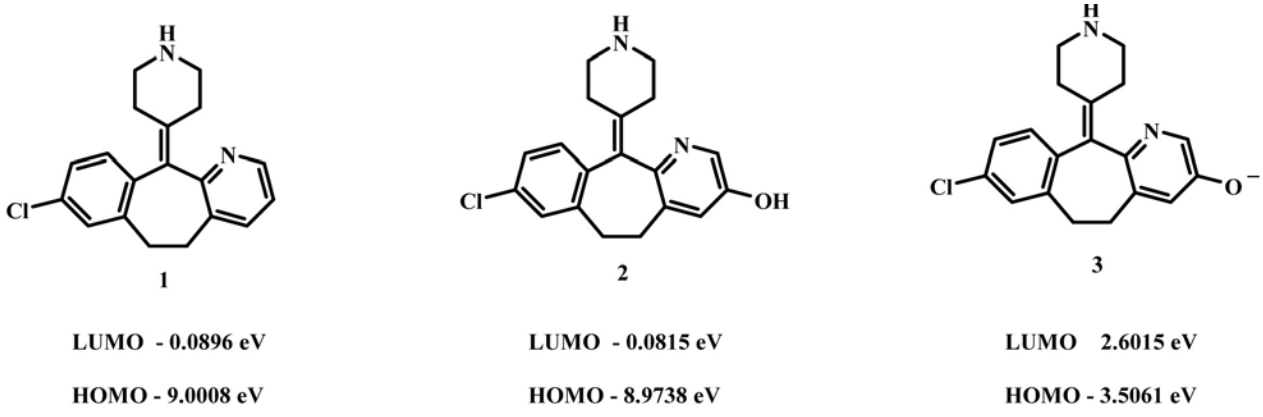
On the other hand at pH > 8 for 3OH-DLOR two deprotonation processes occurred; besides N-pyridine, deprotonation of the phenolic moiety in the position 3 (pKa_{calc} 8.8[14]) is also taking place. This provokes the sudden decrease of the peak current at higher pH values, pH > 9, caused by the repulsion forces between formed anion and negatively charged mercury surface making the reduction process more difficult.

In order to confirm the reduction sequence of investigated compounds, the theoretical approach was made through the calculation of the corresponding LUMO energy levels and Hammet substituent constants.

The observed results, that the reduction potential for 3OH-DLOR (Scheme 2 – compound 2) is more negative than for DLOR (Scheme 2 – compound 1), correlate with calculated LUMO energy levels (1_{LUMO} –0.0896 eV; 2_{LUMO} –0.0815 eV)[15] which suggest that the reduction of compound 1 is more feasible due to lower LUMO energy[16–18]. Under acidic conditions both N-atoms (N-piperidine pKa_{calc} 9.79; N-pyridine pKa_{calc} 4.55)[14] are protonated and this consequently lowers the LUMO energy level. Figure 1 shows significant decrease of reduction potential for compound 2 at pH > 9. The result, as already said, can be attributed to deprotonation of the phenolic moiety (pKa_{calc} 8.8) and therefore the effect of the ionized substituent on the LUMO energy level (Scheme 2 – compound 3). Correlation between the LUMO energy and the Hammet substituent constant (σ) is well established in the literature[19–22]. Generally, more negative σ value of the substituent results in higher LUMO energy level shifting the reduction potential towards more negative values. This, as expected, was supported by our results, going from compound 1(σ_H 0) and 2 (σ_{OH} –0.38) to compound 3 (σ_O– –0.81) the LUMO energy levels increase (LUMO: –0.0896eV, –0.0815eV, 2.6015eV respectively) which is reflected in the reduction potential.

4. Conclusions

The investigated compound DLOR and its derivative 3OH-DLOR, both undergo the irreversible two electron reduction of the C = N bond of the pyridine ring. The reduction of both compounds is the same up to pH 9. In more alkaline medium, due to the deprotonation of the phenolic moiety in 3OH-DLOR molecule, its reduction process happens at more negative potential compared to DLOR. Calculated values of LUMO energy levels and the



Scheme 2. LUMO energy levels and Hammet substituent constants

Hammet substituent constants confirmed the sequence of the reduction of the investigated compounds, already experimentally established.

The observed difference of the peak potentials enables simultaneous determination of these two drugs in alkaline medium, at pH 10. The best selectivity and sensitivity was achieved using SW voltammetric method. The method was checked in real human plasma. Good response was obtained after protein precipitation in plasma, according to LOD and LOQ values. The more sensitive detection could be achieved after the preconcentration of plasma sample either by liquid or solid phase extraction.

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6. References

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Povzetek

Z DC polarografijo, ciklično voltametrijo, diferencialno pulzno in square wave voltametrijo smo študirali elektrokemijske lastnosti desloratadina (DLOR) in njegovega derivata 3-hydroksidesloratadina (3OH-DLOR) v Britton-Robinsonovem (BR) pufru (pH 4–11). Obe spojini se na živosrebrni elektrodi ireveribilno reducirata, pri čemer poteče dvoelektronska redukcija C = N vezi piridinskega obroča. Največje razlike smo ugotovili v raztopinah s pH > 9, ki so posledica deprotonacije fenolnih skupin v molekuli 3OH-DLOR, kar povzroča znatne spremembe njihovih redukcijskih potencialov ($E_{p\ DLOR}$ = –1.48 V, and $E_{p\ 3OH\text{-}DLOR}$ = –1.6 V). Dobljeni rezultati se ujemajo z izračunanimi LUMO energijskimi nivoji in Hammet-ovimi substitucijskimi konstantami (σ).

Na osnovi razlik v redukcijskih potencialih za DLOR in 3OH-DLOR, smo določili pogoje za simultano določanje teh spojin v alkalnih pogojih. Najboljšo selektivnost smo dosegli z uporabo SWV pri pH 10. Pri teh pogojih je linearnost umeritvenih krivulj v območju od 1.5×10^{-6} M – 1×10^{-5} M za DLOR in 7.5×10^{-6} M – 5×10^{-5} M za 3OH-DLOR. Doseženi meji zaznave sta bili 2.29×10^{-7} M za DLOR in 2.08×10^{-6} M za 3OH-DLOR, meji določljivosti pa 7.64×10^{-7} M za DLOR in 6.94×10^{-6} M za 3OH-DLOR. Metodo smo uporabili za določevanje omenjenih substance v humani plazmi, pri čemer sta bili doseženi meji zaznave 4.63×10^{-7} M za DLOR in 2.39×10^{-6} M za 3OH-DLOR ter meji določitve 1.54×10^{-6} M za DLOR in 7.97×10^{-6} M za 2OH-DLOR.