Pharmacy Department[1], University Hospital Carl Gustav Carus, Technical University, Dresden; Department of Pharmaceutical Chemistry[2], University of Jena, Germany

Electrochemical behavior of the antifungal agents itraconazole, posaconazole and ketoconazole at a glassy carbon electrode

H. KNOTH[1], G. K. E. SCRIBA[2], B. BUETTNER[1]

Received November 3, 2014, accepted January 9, 2015

Holger Knoth, Pharmacy Department, University Hospital Carl Gustav Carus TU Dresden, Fetscherstr. 74, 01307 Dresden, Germany
holger.knoth@uniklinikum-dresden.de

Pharmazie 70: 374–378 (2015) *doi: 10.1691/ph.2015.4174*

The electrochemical behavior of the azole antifungal agents itraconazole, posaconazole and ketoconazole has been investigated at a glassy carbon working electrode using cyclic voltammetry. All measurements were carried out in a supporting electrolyte solution consisting of a 1:1 (v/v) mixture of 0.1 mol L^{-1} sodium phosphate buffers and acetonitrile at various substance concentrations and pH values. An amperometric cell with a three electrode system consisting of a working electrode, a palladium reference electrode and a platinum disk as the auxiliary electrode was used in all experiments. All azoles showed a similar electrochemical behavior involving two reactions. An irreversible oxidation occurred at potentials of about 0.5 V. A reduction peak was detected at potentials between −0.28 V and −0.14 V with an associated oxidation peak, which was observed in consecutive repeated measurements at potentials between −0.03 and 0.28 V. The reduction and corresponding oxidation can be regarded as a quasi-reversible process. The proposed reaction mechanisms are an irreversible oxidation of the piperazine moiety at higher potentials as well as a reduction at lower potentials of the carbonyl group of the triazolone moiety in the case of itraconazole and posaconazole or a reduction of the methoxy group of ketoconazole.

1. Introduction

Azole antifungals are important antifungal drugs for the therapy and prophylaxis of systemic and topic mycoses. One of the first substances of this group introduced into therapy was the imidazole derivative ketoconazole (Fig. 1). Due to severe side effects upon oral administration, the drug is nowadays used only for the topical treatment of mycoses (Mutschler et al. 2008). Further development led to the triazole antifungal drugs including itraconazole and posaconazole (Fig. 1). These compounds can be administered orally or intravenously and are used for the prophylaxis and treatment of systemic mycoses (Mutschler et al. 2008).

Several analytical methods using high performance liquid chromatography (HPLC) with UV or mass spectrometric detection have been reported for the analysis of the triazole antifungals (Reddy et al. 2011; Xiong et al. 2013; Jourdil et al. 2013; Cendejas-Bueno 2013; Zhang et al. 2013; Xu et al. 2013; Vujanovic et al. 2013). However, the electrochemical behavior of the compounds has not been studied in detail despite their widespread use. A differential pulse polarographic investigation of itraconazole indicated electrochemical activity of the compound with a reduction peak within the pH range 5.5 to 9.0 (Knoth et al. 2012). The peak current was pH dependent and decreased at higher pH values. Electrochemical oxidation or reduction of posaconazole has not been reported. In contrast, the electrochemical behavior of the older drug ketoconazole has been widely studied (Knoth et al. 2013; Shamsipur and Farhadi (2000); Peng et al. 2001; Arranz et al. 2003; Dantas et al. 2010). The compound displayed two electrochemical reactions, an irreversible oxidation at higher positive potentials and a reversible oxidation at lower potentials. Due to structural similarities it can be expected that the compounds show similar electrochemical behavior. Therefore, the aim of the present study was the inves-

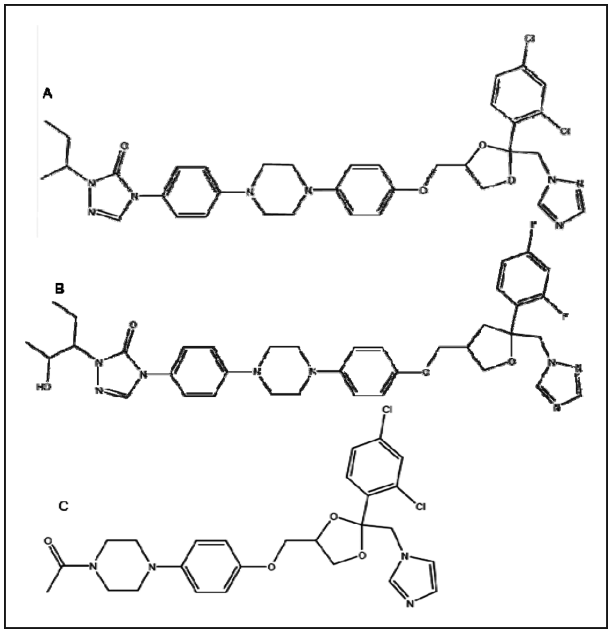

Fig. 1: Chemical structures of itraconazole (A), posaconazole (B) and ketoconazole (C).

tigation of the electrochemical reaction of the azole antifungals itraconazole and posaconazole in comparison to ketoconazole using cyclic voltammetry at a glassy carbon.

2. Investigations, results and discussion

2.1. Electrochemical reactions

The three azole antifungals itraconazole, ketoconazole and posaconazole were investigated using cyclic voltammetry at a glassy carbon electrode at pH 3.0, 5.0 and 7.0. A potential range between −0.7 and 1.2 V was selected. All three compounds exhibited comparable electrochemical reactions characterized by an oxidation *ox1* at potentials of about 0.5 V, followed by a reduction *red2* with an associated oxidation *ox2* within a potential range between −0.28 and 0.28 V.

As an example of the first oxidation *ox1*, the voltammograms of the compounds at pH 7.0 at different anodic and cathodic sweep rates are shown in Fig. 2.

Each voltammogram was obtained from a separate experiment with purging of the electrochemical cell between measurements. In the anodic run, the peak *ox1* in the potential range between 0.4 and 0.6 V was identified. Because no significant associated peak was observed in the cathodic run this reaction appears to be irreversible. The intensity of the reaction was pH-dependent. The peak currents $i_{p[ox1]}/nA$ and potentials $E_{p[ox1]}/V$ (*versus* the palladium electrode) at the pH values studied are summarized in Table 1.

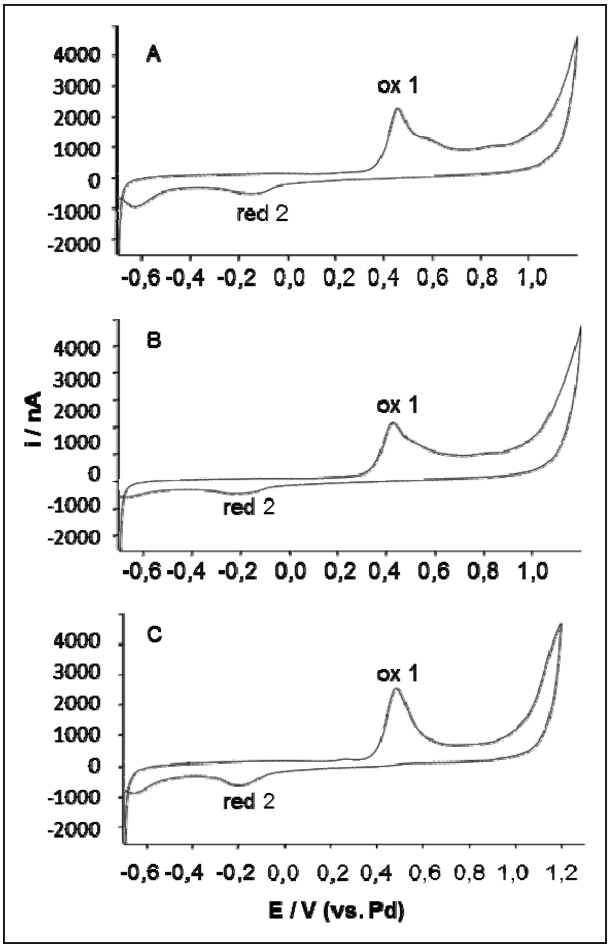

Fig. 2: Cyclic voltammograms of itraconazole 100 µg mL^{-1} (A), posaconazole 100 µg mL^{-1} (B) and ketoconazole 100 µg mL^{-1} (C) at a glassy carbon working electrode at pH 7.0 with scan rate 10 mV s^{-1}.

Table 1: Peak potentials $E_{p[ox1]}$ and peak currents $i_{p[ox1]}$ of itraconazole, posaconazole and ketoconazole for peak *ox*1 at variable pH values 3.0, 5.0 and 7.0 calculated from cyclovoltammograms with scan rate 50 mV s^{-1} (1st scan)

pH value	$Ep_{[ox1]}$ / V (vs. Pd)	$ip_{[ox1]}$ / nA
Itraconazole		
3.0	0,536	3707
5.0	0,536	4815
7.0	0,480	5209
Posaconazole		
3.0	0,543	3452
5.0	0,514	4185
7.0	0,444	4565
Ketoconazole		
3.0	0,536	1759
5.0	0,536	2794
7.0	0,522	4433

The highest current of the irreversible oxidation *ox1* was determined at pH 7.0 and decreased towards pH 3.0. Peak currents were linear over a concentration range of 20–100 µg mL^{-1}. Coefficients of determination were 0.9923 (itraconazole), 0.9984 (posaconazole) and 0.9929 (ketoconazole) with residuals randomly distributed around the linear line. In principle, the oxidative reaction may be used for quantitative determinations although this was not further evaluated in the present study.

The second electrochemical reaction is illustrated by the voltammograms displayed in Fig. 3, which were obtained at pH 3.0 in consecutive measurements without intermediate purging.

The curves of the first, third and fifth consecutive run are shown. In the initial run, a peak *red2* was identified at potentials between −0.14 V and −0.28 V besides the peak of *ox1* around 0.5 V. In the following runs, an oxidation peak *ox2* could be observed at about 0.2 V during the anodic sweep. Because the peak currents of red2 and ox2 increased with repeated measurements, it can be concluded, that both reactions are associated. The currents $i_{p[ox2]}/nA$ and $i_{p[red2]}/nA$ as well as the potentials $E_{p[ox2]}/V$ and $E_{p[red2]}/V$ (both versus the palladium electrode) of the fifth consecutive measurement are summarized in Table 2. The fifth run was selected because it gave the strongest response in the present study. In subsequent consecutive measurements no further increase of currents was observed. The peak currents $ip_{[ox2]}$ and $i_{p[red2]}$ increased at lower pH values and approximately doubled at pH 3.0 as compared to pH 7.0 (Table 2). In addition, the peak potentials showed a shift to less positive values at higher pH values, which fulfills a general rule for a reversible charge exchange (Henze 2001).

The electrochemical behavior of ketoconazole in the cyclic voltammetric study is in accordance with earlier electrochemical investigations of the compound. Shamsipur and Farhadi 2000 detected an irreversible anodic peak at 1.32 V as well as a reversible reaction with a cathodic peak at 0.79 V and an associated anodic peak at 0.87 V. Compared to our data the respective reactions occurred at higher potential which may be explained by the use of a palladium reference in the present study compared to an Ag/AgCl reference electrode described by Shamsipur and Farhadi (2000). This will shift the reaction potentials to lower values.

The present work indicated that itraconazole and posaconazole show an electrochemical behavior comparable to that of ketoconazole. Based on the structural similarity of these three azole antifungals presumably similar functional groups are involved into electrochemical reactions.

Table 2: Peak potentials E_p, peak currents i_p, potential separation ΔE_p and ratio of peak currents $i_{p[ox]}/i_{p[red]}$ of itraconazole, ketoconazole and posaconazole for peaks *ox*2 and *red*2 at variable pH values 3.0, 5.0 and 7.0 calculated from cyclovoltammograms with scan rate 250 mV/s (5th consecutive scan)

pH value	$E_{p[ox2]}$ / V (vs. Pd)	$E_{p[red2]}$ / V (vs. Pd)	$\Delta E_{p[ox2-red2]}$	$i_{p[ox2]}$ / nA	$i_{p[red2]}$ / nA	$i_{p[ox2]}/i_{p[red2]}$
Itraconazole						
3.0	0,280	−0,140	0,420	3058	−2965	1,03
5.0	0,150	−0,210	0,360	2222	−2193	1,01
7.0	0,035	−0,210	0,245	1686	−1770	0,95
Posaconazole						
3.0	0,280	−0,140	0,420	2734	−2762	0,99
5.0	0,070	−0,244	0,315	2235	−2471	0,90
7.0	−0,030	−0,280	0,25	1050	−957	1,10
Ketoconazole						
3.0	0,280	−0,140	0,42	7119	−7283	0,98
5.0	0,245	−0,245	0,49	4898	−4534	1,08
7.0	0,140	−0,280	0,42	2666	−2801	0,95

2.2. Reversibility of the reactions

The reversibility of electrochemical reactions can be derived from repeated measurements of the same solutions. As shown in Fig. 3, the current of *ox1* significantly decreased from the first to the consecutive scans. This is characteristic for an irreversible reaction because fewer reacting molecules are available in the vicinity of the electrode in the case of an irreversible reaction. This results in a weaker current for each consecutive run. In contrast, the second reaction including the reduction step *red2* and *ox2* displayed increasing currents in repeated measurements (Fig. 3). As an indication of the reversibility of these reactions, the ratio of peak currents $i_{p[ox2]}$ and $i_{p[red2]}$, the change of $i_{p[red2]}$ with increasing scan rates v and the potential difference ΔE_p have to be taken into account (Henze 2001). The ratio $i_{p[ox2]}/i_{p[red2]}$ of the fifth consecutive measurement at pH 3.0 at a scan rate 250 mV s^{-1} is reported in Table 2.

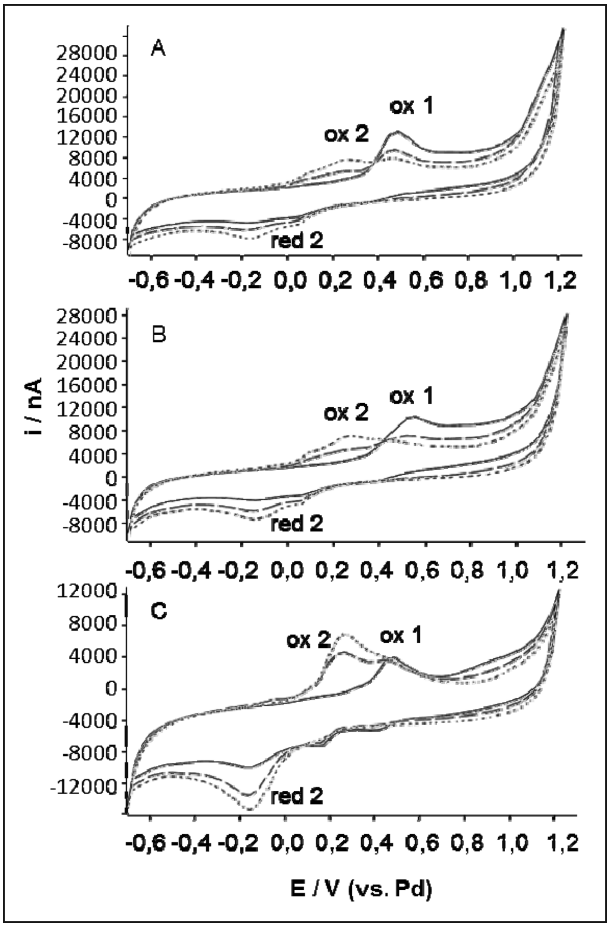

Fig. 3: Cyclic voltammograms of itraconazole 100 µg mL^{-1} (A), posaconazole 100 µg mL^{-1} (B) and ketoconazole 100 µg mL^{-1} (C) at a glassy carbon working electrode at pH 3.0 with scan rate 250 mV s^{-1}; 1st (solid line), 3rd (broken line) and 5th (dotted line) consecutive measurements.

The value varies between 0.91 and 1.10. This meets the first criterion for a reversible reaction, i.e., a $i_{p[ox2]}/i_{p[red2]}$ ratio of 1 (Henze 2001). The second condition is the proportionality between the peak current $i_{p[red2]}$ and the square root of the scan rate, $\sqrt{v}$. This requirement is fulfilled only at scan rates between 10 (square root $\sqrt{v}$ 3,16) and 100 mV s^{-1} (square root $\sqrt{v}$ 10). At higher scan rates v (square root $\sqrt{v} > 10$) a deviation from the linear behavior is observed. It may be concluded, that at the highest scan rate the reaction is controlled not only by the charge-transfer reaction, but also by the diffusion of analyte. The third condition according to Henze 2001 is a ΔE_p close to 0.057 V. This is not fulfilled for the studied azole compounds as $\Delta E_p > 0.25$ V was observed in all cases (Table 2). Therefore, it may be concluded that the observed reduction *red2* and the associated oxidation *ox2* undergo a quasi-reversible charge exchange (Henze 2001).

2.3. Reaction mechanisms

As described above, comparable electrochemical behavior for the three azole antifungals has been observed. Therefore, similar electrochemical reactions can be assumed. Regarding the irreversible oxidation ox1, the most likely mechanism is the loss of an electron in the piperazine ring as reported for other piperazine-containing compound (Kauffmann et al. 1987; Arranz et al. 1997; Özkan et al. 2003; Popa et al. 2013). This oxidation yielded lower currents with decreasing pH-values. In view of the pK_a-values of 3.7 for itraconazole (AB-Kommentar 2009), 3.6 for posaconazole (Courtney et al. 2003) and 2.94 for ketoconazole (Skiba et al. 2000) as well as the pH dependence, the deprotonated piperazine moiety may be regarded as the electroactive form. Another hint for this mechanism is the shift to higher reaction potentials. This indicates that the electroactive deprotonated piperazine must be formed by proton dissociation first as described for other bases (Özkan et al. 2003; Popa et al. 2013).

In the case of ketoconazole, the reversible reactions *red2* and *ox2* might be reactions of the imidazole ring. However, it has been reported that imidazole cannot be reduced in aqueous solutions which have been applied in the present study (Pereira

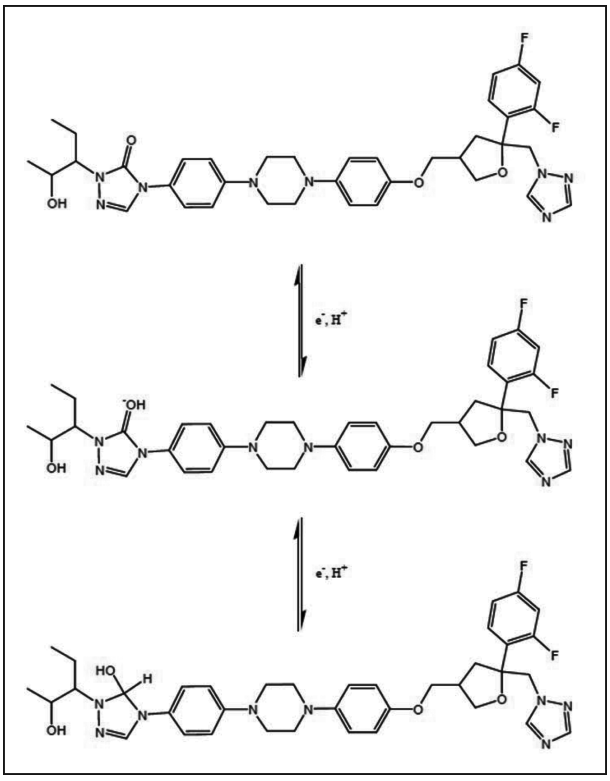

Fig. 4: Proposed mechanism for the reduction step *red2* and associated oxidation step *ox2* illustrated for the triazolone moiety of posaconazole.

et al. 2001). Therefore, a reversible reduction and subsequent oxidation of the methoxy group of ketoconazole has been proposed as mechanism of this reaction (Shamsipur and Farhadi 2000). In contrast to the imidazole ring, the triazole ring can be reduced in aqueous solutions so that this reaction might be envisioned for itraconazole and posaconazole. However, this reaction seems unlikely considering the respective potentials at which *red2* and *ox2* were observed. Reduction of the triazole ring has been reported at potentials lower than -1.5 V (Lokesh et al 2010). Therefore, the most likely mechanism appears to be the reversible reduction of the carbonyl group of the triazolone moiety of the compounds with an uptake of two electrons and two protons as illustrated in Fig. 4. This mechanism has been previously postulated for itraconazole in a polarographic study (Knoth et al. 2012).

2.4. Conclusions

Comparable electrochemical behavior of the antifungal agents itraconazole, posaconazole and ketoconazole at a glassy carbon electrode has been observed by cyclic voltammetry. Irreversible oxidation occurred at potentials of about 0.5 V while a reversible reduction with an associated oxidation was noted in the potential range between -0.28 and 0.28 V. A loss of an electron at the piperazine moiety is the assumed mechanism of the irreversible process. The proposed mechanism for the second reaction is a reversible reduction of the carbonyl group of the triazolone moiety in the case of itraconazole and posaconazole. For ketoconazole a reduction of the methoxy group can be assumed. Both reactions displayed pH-dependence in the pH range 3 – 7. While the irreversible oxidation displayed linearity of peak currents in the concentration range of 20–100 $\mu g\,mL^{-1}$, no linear relationship could be found for the reversible reaction. Thus, the irreversible oxidation might be utilized for quantitative determination of the drugs.

3. Experimental

3.1. Chemicals

Ketoconazole was obtained from Caelo (Hilden, Germany), itraconazole from Inresa (Bartenheim, Germany) and posaconazole from Schering-Plough (Hérouville-Saint-Clair, France). Acetonitrile was purchased from VWR (Darmstadt, Germany), methanol, sodium dihydrogen phosphate monohydrate and sodium hydroxide from Merck (Darmstadt, Germany) and phosphoric acid 85 wt. % from Sigma-Aldrich (Munich, Germany). All reagents were of analytical or HPLC grade and used as received. Stock solutions of the drugs were prepared in methanol at a concentration of 1 mg mL^{-1} and stored in a refrigerator.

3.2. Equipment

All cyclic voltammetry measurements were carried out using an 896 IC amperometric detector (Metrohm, Filderstadt, Germany) controlled by a computer using MagicNet version 2.3 software. An amperometric cell with a three electrode system consisting of a glassy carbon working electrode (disc diameter 3 mm, Metrohm, Filderstadt, Germany), a palladium reference electrode and a platinum disk as the auxiliary electrode was used. To run the electrode and deaerate the sample solutions, nitrogen gas with a reagent grade of 5.0 was obtained from Messer (Sulzbach/Germany). The pH of electrolyte solutions was adjusted using a SevenEasy pH-Meter (Mettler-Toledo, Gießen, Germany). Phosphate buffer and sodium hydroxide solution were prepared with water purified by a UO 400 - 1950 System (BWT Pharma & Biotech, Mondsee, Germany) and distillated in a Dewadest System (DEWA, Goslar, Germany).

3.3. Working procedures

The electrolyte support solution consisted of equal volumes of 0.1 mol L^{-1} phosphate buffer and acetonitrile. Prior to each scan, the amperometric cell was purged for 600 s with the appropriate support solution. All measurements were performed at a temperature of $35 \pm 0.05\,^{\circ}C$. Cyclic voltammograms were measured in the potential range between -0.8 and 1.2 V at various scan rates v. The stock solutions of the drugs were diluted to working concentrations of $20-100\,\mu g\,mL^{-1}$. For the determination of the pH-dependence, phosphate buffers were adjusted to pH 3.0, 5.0 and 7.0 using 0.1 mol L^{-1} sodium hydroxide solution or 0.1 mol L^{-1} phosphoric acid, respectively. For the determination of the reversibility of the reactions, each test solution was measured five times consecutively without intermediate purging of the electrochemical cell.

References

Arranz A, Betono SF, Moreda JM, Cid A, Arranz JF (1997) Cathodic stripping voltammetric determination of doxazosin in urine and pharmaceutical tablets using carbon paste electrodes. Analyst 122: 849–854.

Arranz P, Arranz A, Moreda JM, Cid A, Arranz JF (2003) Stripping voltammetric and polarographic techniques for the determination of antifungal ketoconazole on the mercury electrode. J Pharm Biomed Anal 33: 589–596.

Arzneibuch-Kommentar (2009) - Wissenschaftliche Erläuterungen zum Arzneibuch, Wissenschaftliche Verlagsgesellschaft, Govi-Verlag, Stuttgart, Eschborn, Gesamtwerk einschließlich 33. Aktualisierungslieferung, Itraconazol.

Cendejas-Bueno E, Cuenca-Estrella M, Gomez-Lopez A (2013) A simple, sensitive HPLC-PDA method for the quantification of itraconazole and hydroxy itraconazole in human serum: a reference laboratory experience. Diagn Microbiol Infect Dis 76: 314–320.

Courtney R, Wexler D, Radwanski E, Lim J, Laughlin M (2004) Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. Br J Clin Pharmacol 57: 218–222.

Dantas AN, Souza DD, Lima JE, Lima-Neto P, Correia AN (2010) Voltammetric determination of ketoconazole using a polished silver solid amalgam electrode. Electrochim Acta 55: 9083–9089.

Henze G (2001) Polarographie und Voltammetrie - Grundlagen und analytische Praxis, 2 Methoden, Springer, Heidelberg, pp. 28–38.

Jourdil JF, Tonini J, Stanke-Labesque F (2013) Simultaneous quantitation of azole antifungals, antibiotics, imatinib, and raltegravir in human plasma by two-dimensional high-performance liquid chromatography-tandem mass spectrometry, J Chromatogr B 919: 1–9.

Kauffmann JM, Vire JC, Patriarche GJ, Nunez-Vergara LJ, Squella JA (1987) Voltammetric oxidation of trazodone. Electrochim Acta 32: 1159–1162.

Knoth H, Petry T, Gärtner P (2012) Differential pulse polarographic investigation of the antifungal drugs itraconazole, ketoconazole, fluconazole

and voriconazole using a dropping mercury electrode, Pharmazie 67: 987–990.

Lokesh SV, Satpati AK, Sherigara BS (2010) Electrochemical behavior of 1,2,4-triazole and benzotriazole at glassy carbon electrode in acidic media. Open Electrochem J 2: 15–21.

Mutschler E, Geisslinger G, Kroemer HK, Ruth P, Schaefer-Korting M (2008) Mutschler Arzneimittelwirkungen - Lehrbuch der Pharmakologie und Toxikologie, 11.4.1 Antimykotisch wirksame Azolderivate, Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp. 857.

Özkan SA, Uslu B, Zuman P (2003) Electrochemical oxidation of sildenafil citrate (Viagra) on carbon electrodes. Anal Chim Acta 501: 227–233.

Peng TZ, Cheng Q, Yang CF (2001) Adsorptive behavior and electrochemical determination of the anti-fungal agent ketoconazole. Fresen J Anal Chem 370: 1082–1086.

Pereira FC, Stradiotto NR, Zanoni MVB (2001) Electrochemical reduction and cathodic stripping voltammetric determination of clotrimazole. J Brazil Chem Soc 12: 202–2007.

Popa OM, Diculescu VC (2013) Electrochemical and spectrophotometric characterisation of proteinkinase inhibitor and anticancer drug danusertib. Electrochim Acta 112: 486–492.

Reddy TM, Tama CI, Hayes RN (2011) A dried blood spots technique based LC–MS/MS method for the analysis of posaconazole in human whole blood samples. J Chromatogr B 879: 3626–3638.

Shamsipur M, Farhadi K (2000) Electrochemical behavior and determination of ketoconazole from pharmaceutical preparations. Electroanalysis 12: 429–433.

Skiba M, Skiba-Lahiani M, Marchais H, Duclos R, Arnaud P (2000) Stability assessment of ketoconazole in aqueous formulations. Int J Pharm 198: 1–6.

Vujanović IK, Malenović A, Jovanović M, Rakić T, Stojanović BJ, Ivanović D (2013) Chemometrically assissted optimization and validation of RP-HPLC method for the analysis of itraconazole and its impurities. Acta Pharm 63 (2013) 159173.

Xiong X, Zhai S, Duan J (2013) Validation of a fast and reliable liquid chromatography-tandem mass spectrometry (LC-MS/MS) with atmospheric pressure chemical ionization method for simultaneous quantitation of voriconazole, itraconazole and its active metabolite hydroxyitraconazole in human plasma. Clin Chem Lab Med 51: 339–346.

Xu HR, Chu NN, Chen WL, Wang ZD, Li XN (2013) Liquid chromatography-mass spectrometry method for the quantification of posaconazole in human plasma: application to pharmacokinetics following single-dose administration in the fasted state and with a high-fat meal. Pharmazie 68: 173–177.

Zhang M, Moore GA, Barclay ML, Begg EJ (2013) A simple high-performance liquid chromatography method for simultaneous determination of three triazole antifungals in human plasma. Antimicrob Agents Chemother 57: 484–489.