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Analysis of albendazole metabolites by electrospray LC–MS/MS as a probe to elucidate electro-oxidation mechanism of albendazole

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Abstract

The electrochemical oxidation of albendazole was accomplished by controlled potential electrolyses technique. The oxidation was carried out in different pH solutions and yields the same products obtained by in vivo and in vitro metabolism, i.e. albendazole sulfoxide and albendazole sulfone. The identification of albendazole oxidation products was carried out by LC–MS/MS.
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Keywords: Albendazole; Electrochemical oxidation; Metabolites; LC–MS/MS

1. Introduction

The structural diversity of the multitude of modern therapeutic drugs available for the treatment of disease is well documented [1–3]. However, after entry into the body, such therapeutic agents are subjected to metabolism. From the pharmacological point of view it is highly important to investigate interactions between drugs and living organisms and also the pathways for drug metabolism. The majority of metabolic pathway of pharmacologically active substances run just by redox reactions, i.e. electron-transfer processes and understanding the electrochemical behavior is fundamental to predict a pharmacological model for a new drug candidate.

Electrochemical methods are useful tools in these model studies. They can be used for the investigation of electron-transfer reactions and for simulations of biological redox reactions. Although such methods have been widely studied and reviewed [4–8], the subsequent isolation and characterization of the resulting plethora of metabolites still dependent on methods based on comparison with synthesized standards [9–12].

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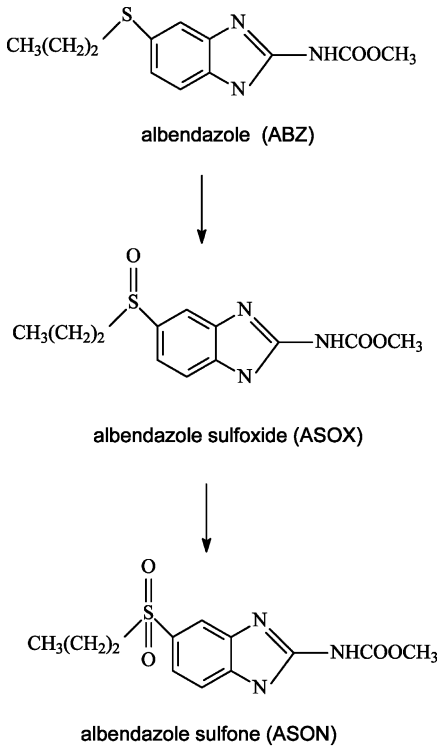
The study of the oxidative electrolyses of albendazole (ABZ) in different electrochemical potential is reported in this paper. ABZ is a drug considered to be effective for the treatment of parenchymal brain neurocysticercosis, an infection of the central nervous system by the larval form of *Taenia solium* [13–15]. ABZ undergoes extensive metabolism by liver microsomal enzymes, and probably in the gastrointestinal tract, to its major active metabolite albendazole sulfoxide (ASOX) which is further metabolized to albendazole sulfone (ASON) (Scheme 1) [16–18].

The interest in the electrochemical oxidation of ABZ results from the reports that mechanistic and product information derived from such studies may provide valuable insights into the biological oxidation of this compound. In fact, some studies involving the use of electrochemical process as a method to quantify ABZ has been recently published [19,20] which was concerning to the formation of ASOX based on the cyclic voltammogram experiments. In these experiments, there is no evidence to the formation of ASON as oxidation product of ASOX. In order to understand the pathway of the electrochemical oxidation of ABZ, some studies have been performed in a wide range of pH by differential pulse voltammetry and by controlled potential electrolysis. The identification of the oxidation ABZ products was based on the use of on-line liquid chromatography–mass spectrometry (LC–MS/MS), which allies the discrimination power of the mass spectrometry with the high separation power of the HPLC [21]. This LC–MS/MS procedure was previously used to analyze ASOX and ASON in plasma samples obtained from patients with neurocysticercosis and treated with ABZ [22].

Account for the observations made on LC–MS/MS experiments a different mechanism to the electrochemical oxidation of albendazole has been proposed here.



Scheme 1.

2. Experimental section

2.1. Reagents and chemicals

The solutions of ABZ dissolved in supporting-electrolyte solution, were prepared with authentic standard kindly supplied by Robert Young & Co. Ltd. (Glasgow, Scotland, UK). Robert Young & Co. also supplied authentic standard of ASOX and ASON.

Methanol used for the mobile phase preparation (EM Science, Gibbstown, NJ, USA) was of HPLC grade. All other reagents were of P.A. grade, supplied by Merck, Darmstadt, Germany (acetic acid), Nuclear (trifluoracetic acid 98% and sodium hydroxide) and Synth (hydrochloric acid). The water was purified with a Milli-Q Plus System (Millipore, Bedford, MA, USA).

2.2. Electrochemical measurement

Physical data were recorded on instrumentation as follow: cyclic and differential pulse voltammetry measurements were carried out with an AUTOLAB® model PGSTAT 30 potentiostat/galvanostat, by using a conventional electrochemical cell containing a 0.78 cm^2 glassy carbon working

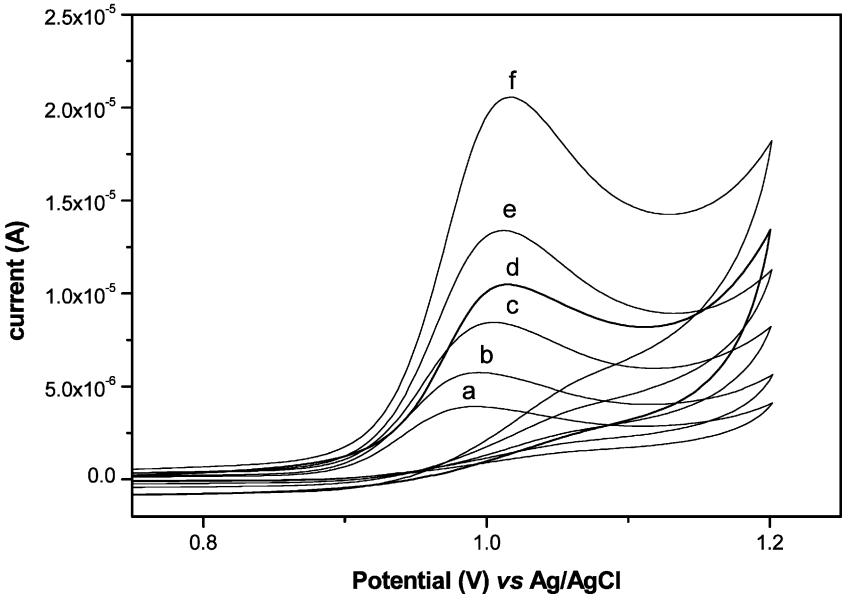


Fig. 1. Cyclic voltamogramms of 1.0 mmol l^{-1} albendazole in 1.0 mol l^{-1} HCl at 25.0 °C. Scan rate 0.01 (a); 0.02 (b); 0.05 (c); 0.10 at 3.0 °C (d); 0.10 (e); 0.20 (f) V s^{-1}.

electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. The controlled potential electrolysis was performed in 0.1 M trifluoroacetate buffer solution pH 1.2 and 3.3, and in 1.0 M HCl. The working electrode was a platinum gauze (1.5 cm diameter, 2 cm long), Ag/AgCl reference electrode and platinum wire auxiliary electrode. The potential was scanned from 0.80 to 1.20 V versus Ag/AgCl during 30 min for each experiment. The electrolysis products were analyzed by LC–MS/MS.

2.3. Equipment and LC–MS/MS conditions

The LC–MS/MS analyses were carried out using a Shimadzu (Kyoto, Japan) HPLC system and a Quatro LC triple-stage quadrupole (Micromass, Manchester, UK), previously described [22]. Separations were carried out at 22 °C on a Lichrospher CN column (125 × 4.6 mm I.D., 5 mm particle size, Merck, Darmstadt, Germany). A CN guard column (4 × 4 mm I.D., Merck) was used to protect the analytical column. The mobile phase consisted of methanol–water (4:6, v/v) acidified with 1% acetic acid, at a flow rate of 1 ml/min. Detection was performed by multi reaction monitoring, MRM of the protonated molecular (precursor) ion ($[M-H]^+$) and their corresponding product ion (266 → 234, 282 → 240 and 298 → 266, for ABZ, ASOX and ASON, respectively).

The analyses were carried out by diluting the electrochemical reaction mixture with the mobile phase (1:5) and injecting 20 μl into the chromatographic system.

3. Results and discussion

The electrochemical method has been proposed as a technique to identify albendazole based on the oxidation of ABZ to ASOX [19,20]. Although two different metabolites (ASOX and ASON) have been proposed due the oxidation of ABZ (Scheme 1) [16] there is no evidence by electrochemistry of the second oxidation of ABZ involved in the formation of ASON. Representative cyclic voltammogram of ABZ in 1.0 M HCl at 25.0 and at 3.0 °C is presented in Fig. 1. On the positive sweep one oxidation peak which is called anodic peak

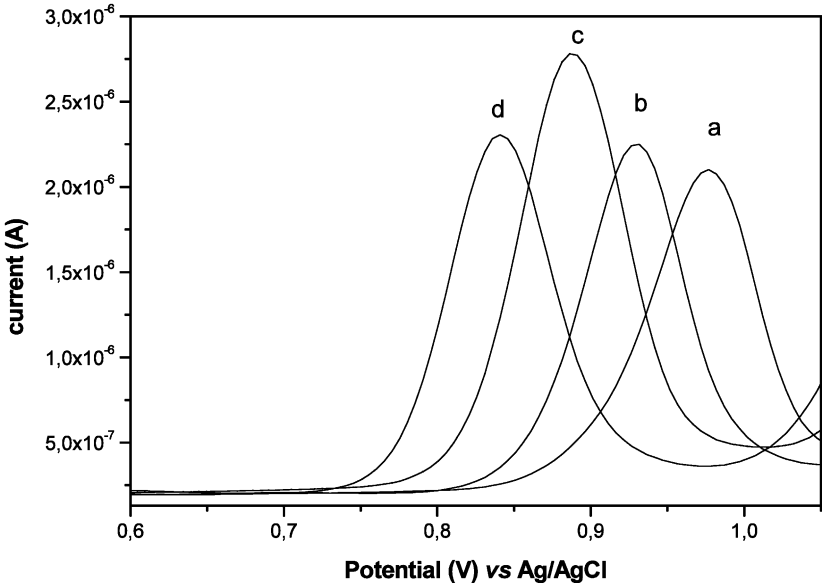


Fig. 2. Pulse differential voltamograms of 1.0 mmol l^{-1} of albendazole at pH 1.06 (a); 2.10 (b); 2.40 (c); 3.30 (d).

potential (E_{pa}) appeared with $E_{pa} = 1.00$ V versus Ag/AgCl at scan rate = 0.10 V s^{-1}.

The oxidation peak potential of ABZ seems to be pH dependent. The differential pulse voltammetry recorded under various pH conditions are presented in Fig. 2. It was also shown that the E_{pa} was linearly dependent on pH (Fig. 3). The peak potential decrease approximately 59 mV per pH-unit in the studied process which is consistent with a electrochemical mechanism involving $2H^+$ and two electrons [23].

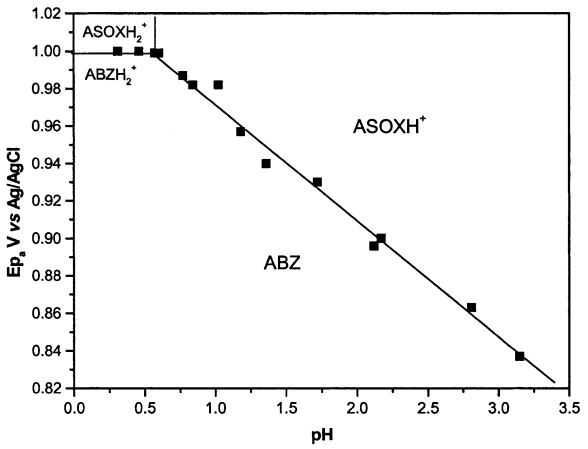


Fig. 3. The E_{anodic}–pH diagram showing the albendazole and albendazole–sulphoxide equilibrium and associated acid–base equilibrium.

The plot potential versus pH defined the thermodynamic stability field of ABZ and its metabolite toward oxidation. The construction of an E–pH diagram has been described as Pourbaix Diagram [23]. Because of the pH-dependence of the E_{pa}, the pK_a value of ASOX could be determined. In the conditions studied we found the value of 0.58 for the pK_a of ASOX, which is a little higher than the pK_a previously described [24] probably due to the difference of ionic strength. The acidity of the solution not only influences the oxidation potential but also has an impact on the pathway of the chemical and electrochemical processes, which take place during the electro-oxidation. Due to the oxidation peak potential dependence on pH we decide to study the electrochemical behavior of albendazole process in pH function.

The electro-oxidation of ABZ in 1.0 M HCl and in trifluoroacetate (TFA) buffer solutions was performed. The identification of the products were done by LC–MS/MS. Therefore, the bulk electrolysis in 0.85–1.20 V versus Ag/AgCl were carried out in TFA buffer solution at pH 3.30. As may be seen, product ASOX is formed in 0.85–

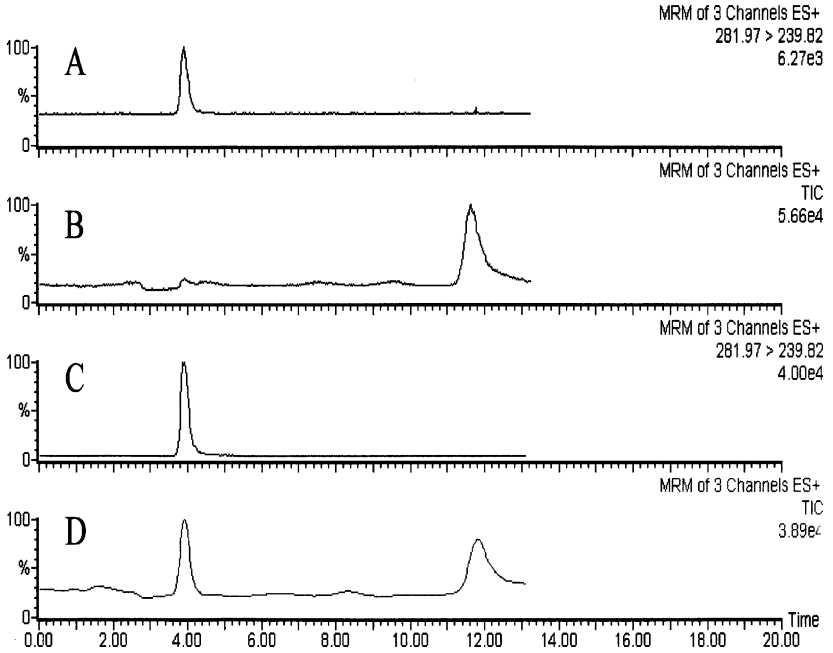


Fig. 4. Ion chromatograms of ASOX (A and C) and total ion chromatograms (B and D) for 30 min ABZ electrolysis mixture in TFA buffer at pH 3.30, acquired by multiple reaction monitoring. Electrolysis carried out at 0.90 (A and B) and 1.20 V (C and D).

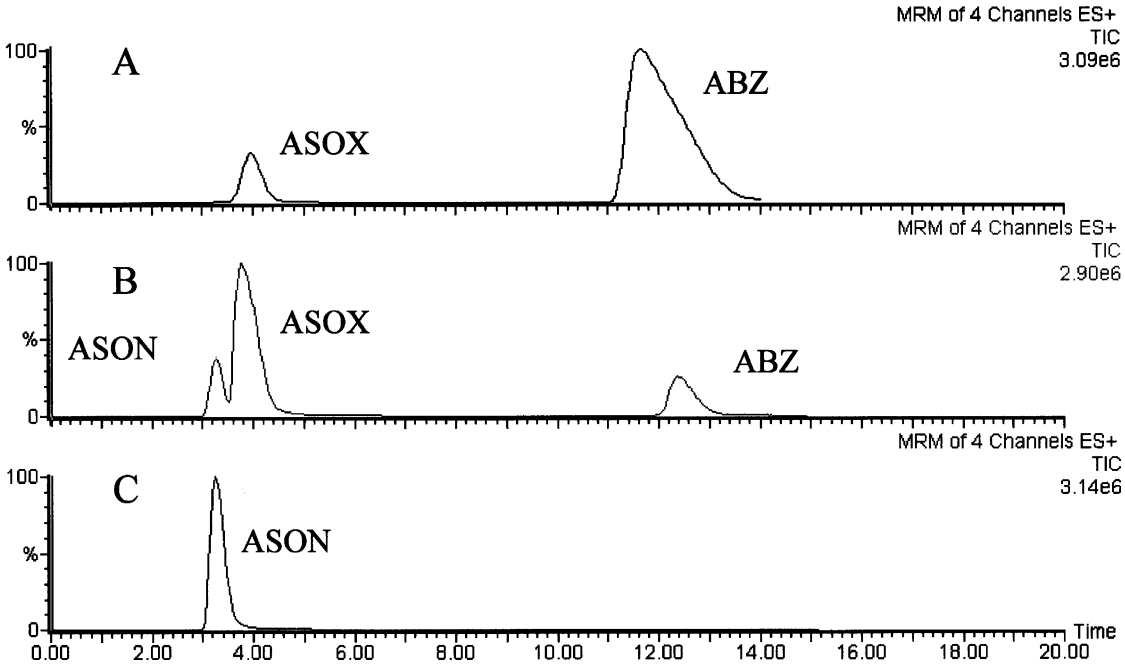


Fig. 5. Total ion chromatograms for 30 min ABZ electrolysis carried out at 0.80 V (A), 1.00 V (B) and 1.10 V (C) in 1.0 mol l^{-1} HCl solution.

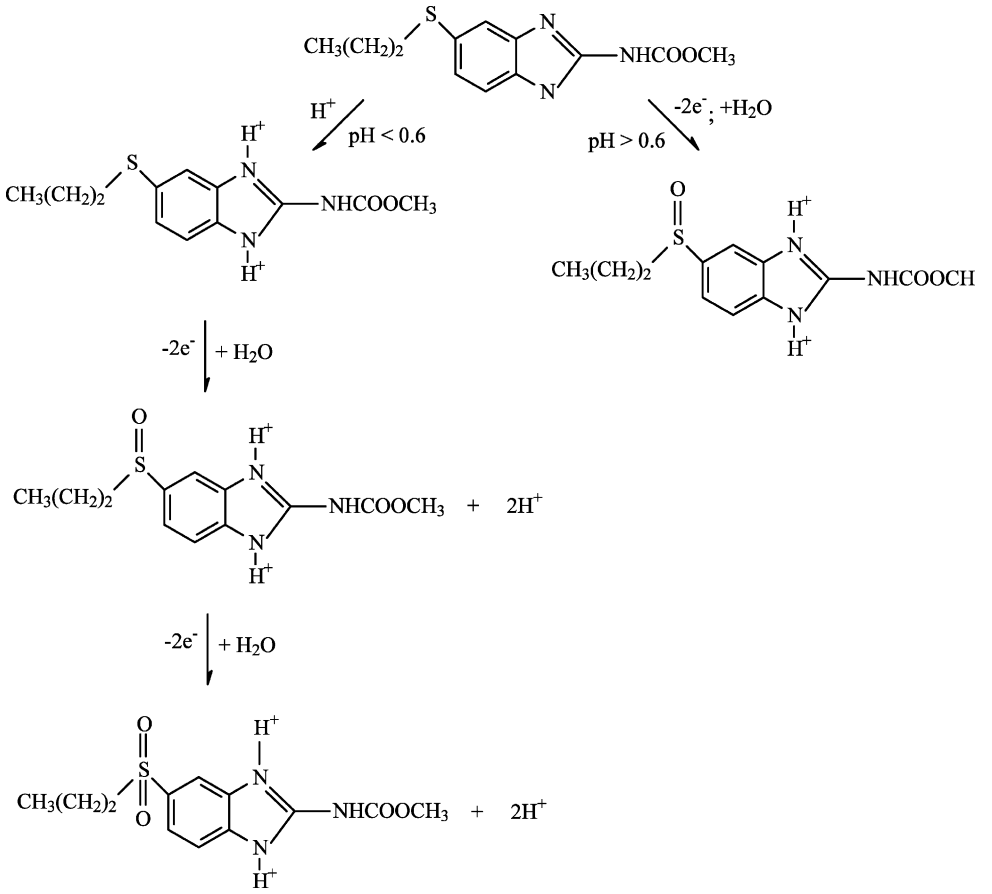
Table 1
The percentages of major constituents found in the electro-oxidation of ABZ

Applied potential (V vs Ag/AgCl)	Electrolyte solution	Product (%)		ABZ (%)
		ASOX	ASON	
0.80	HCl[a]	11.50	0.10	88.40
1.00	HCl[a]	66.20	14.60	19.20
1.10	HCl[a]	0.05	99.80	0.15
0.90	pH 3.30[b]	3.90	–	96.10
1.20	pH 3.30[b]	43.10	–	56.90

[a] 1 mol l^{-1} HCl.
[b] TFA buffer solution.

1.20 V versus Ag/AgCl. Fig. 4 presents the results obtained for 30 min electrolysis using LC–MS/MS in a typical run. The asymmetry observed for the ABZ peak is a result of its large retention time, because the mobile phase composition was optimized to obtain the resolution of ASOX and ASON.

Different electrochemical behavior of ABZ was observed when the electrolysis was carried out in 1.0 M HCl. In that case the electrolysis of



Scheme 2.

albendazole in hydrochloric acidic solution at 0.80, 1.00 and 1.10 V versus Ag/AgCl was performed and the product analyzed by LC–MS/MS (Fig. 5).

The electrolysis carried out at 0.80 V versus Ag/AgCl (Fig. 5A) show preferentially the formation of ASOX. When the electrolysis was carried out at 1.00 V versus Ag/AgCl (Fig. 5B), a mixture of ASOX and ASON was observed although at 1.10 V versus Ag/AgCl the formation of ASON was essentially observed (Fig. 5C). All the results are summarized in Table 1.

The electro-oxidation of albendazole was shown to be an irreversible process greatly influenced by pH conditions. The present studies also revealed the identity of one product after the electrochemical oxidation of ABZ in pH higher than the pK_a of ASOX and two products in 1.0 M HCl, which probably involves a fast chemical step after the electrochemical process. The electrochemical pathway (Scheme 2) was taking based on the LC–MS/MS results of albendazole electrolysis, which is an indubitable way to prove the electrochemical mechanism.

4. Conclusions

The identity of the final products of the electrochemical oxidation allowed confirming the structure of ASOX and ASON as oxidation products of albendazole, which is dependent on oxidation potential. The use of LC–MS/MS and electrochemical techniques described here permit to gain a better understanding into the oxidative mechanism of albendazole and opens a new form for the elucidation of redox reaction products.

Acknowledgements

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