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Joint determination of todralazine and acetazolamide in human serum by differential pulse polarography

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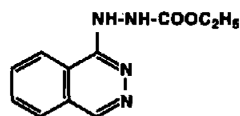
Abstract: Differential pulse polarography (DPP) is proposed as a direct method for the quantitation of todralazine and acetazolamide in human serum. The method was applied to the determination of these drugs in human serum, after a liquid–liquid extraction process. This extraction process together with the use of the standard additions method is essential for the elimination of the matrix effect. The proposed method enables detection limits of 0.107 μg ml^{-1} for acetazolamide and 0.111 μg ml^{-1} for todralazine to be achieved at reduction potentials of −0.59 and −0.86 V, respectively, using Britton–Robinson buffer (pH 1.65) as the supporting electrolyte.

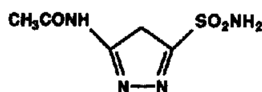
Keywords: *Differential pulse polarography; todralazine; acetazolamide.*

Introduction

Todralazine is a vasodilator with an extensive clinical application in the treatment of hypertension. Acetazolamide is a carbonic anhydrase inhibitor that has a diuretic and natriuretic action.



TODRALAZINE



ACETAZOLAMIDE

Todralazine can provoke toxicity problems that affect 70% of treated patients. If doses are high (500–1000 mg daily) serious side-effects are produced in 15% of cases. These side-effects disappear when doses lower than 200 mg daily are administered [1].

Sometimes todralazine produces renal effects with retention of sodium and water. This problem can be solved by the administration of acetazolamide [2] which increases the elimination of water and sodium. However, when both drugs are administered, an exhaustive control must be carried out since these drugs can produce synergism which may give rise to hypotensive reactions.

Several techniques have been used to determine these drugs in biological fluids. Ishii and Deguchi [3] proposed a spectrofluorimetric technique for the determination in plasma after derivatization of the todralazine. Todralazine hydrochloride has also been determined using a selective chloride electrode or by indirect potentiometric titration with a silver electrode [4]. Kracmar *et al.* [5] employed UV spectrophotometry for the determination of this substance; the detection limits are in the range 0.4–5.0 μg ml^{-1}.

Methods of determining acetazolamide in biological fluids include measurement of carbonic anhydrase inhibition [6], visible spectrophotometry [7], gas chromatography [8] and liquid chromatography [9]. The detection limits are in the range 0.5–1.0 μg ml^{-1}.

These molecules have been investigated by electrochemical techniques, the emphasis being placed on reduction at the mercury electrode. Functional groups such as C=N— give rise to reduction waves that have been used in the determination of the drugs in formulations and biological fluids. In previous work [10] todralazine has been studied by adsorptive stripping voltammetry (AdSV) with a detection limit of 0.86 ng ml^{-1}; acetazolamide has been determined by FIA with amperometric detection [11] for concentrations

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of about 10 μg ml^{-1}. It has been shown that acetazolamide is not adsorbed on the surface electrode; therefore adsorptive stripping techniques cannot be used in the joint determination of both sustances.

In the present paper, different pulse polarography (DPP) is proposed as a direct and sensitive method for the assay of todralazine and acetazolamide. The method has been applied to the determination of these sustances in human serum.

Experimental

Apparatus

A multifunctional electroanalytical system consisting of a Metrohn E-506 Polarecord, an E-612 scanner, a VA-663 unit stand and an XY Linseis LY-1800 register were used for the cyclic voltammetric studies. A Metrohm VA-646 processor coupled to a VA-647 unit stand was also used. Both incorporated a Metrohm Multimode 6.1246.020 as the working electrode, a Ag/AgCl reference electrode and a platinum auxiliary electrode. A Metrohm E-605 pH-meter was used to measure the pH. An 8000 rpm Selecta Centronic centrifuge and a Thermolyne vortex mixer were used for the separation of serum proteins.

Reagent

Stock solutions (1×10^{-3} M) of pure todralazine and acetazolamide (Sigma) were prepared by dissolving the compounds in deionized water. A Britton–Robinson buffer solution containing 0.04 M of each acid component was used as the supporting electrolyte. All reagents used were of analytical grade. The human serum samples were pools from five subjects.

Preparation of the samples

Four millilitres of ethanol was added to 2 ml of serum as a deproteinizer. The mixture was shaken in a vortex mixer for 2 min and centrifuged at 8000 rpm for 5 min. An aliquot of the solution (2 ml) was diluted to 20 ml with Britton–Robinson buffer (pH 1.65), transferred to the polarographic cell and then deaerated for 8 min with a stream of oxygen-free nitrogen. The polarograms were recorded under the optimum instrumental conditions and the peak current was measured from the base-line of each polarogram.

Results and Discussion

The cyclic voltammograms of a solution of todralazine and acetazolamide show the reversibility of the electrochemical process which is used for the identification of the peaks of both drugs.

The presence of azometine reducible groups (C═N—) on the hanging mercury drop electrode (HMDE) can be used for the electroanalytical determination of todralazine and acetazolamide. The reduction occurs generally in the potential range of −0.4 V [12] to −1.1 V [13].

In the cyclic voltammograms of acetazolamide solutions at pH 1.65 two peaks were observed but only the peak that appeared at −560 mV could be measured (Fig. 1a). On the other hand, in the study of todralazine (Fig. 1b) two peaks appeared but only the sharp peak at −830 mV could be quantified.

The cyclic voltammetric peaks of both drugs appeared at distinctly separate potentials and they could be determined together. In addition, no peaks were observed in the anodic scan, which implies an irreversible electrodic process (Fig. 1c).

To determine whether the reduction process is controlled by diffusion, i_p versus $v^{1/2}$ and i_p versus v plots were made. Straight lines were obtained for: acetazolamide 5×10^{-6} M [$i_p = -3.06 + 1.77\, v^{1/2}$ ($r = 0.9936$)]; and todralazine 5×10^{-6} M [$i_p = 0.33 + 3.04\, v^{1/2}$ ($r = 0.8281$); $i_p = 9.60 + 0.22\, v$ ($r = 0.9953$)]. These results confirm that the reduction of acetazolamide in the mercury electrode is controlled by a diffusion process whereas in the case of todralazine adsorption is combined with a diffusion process. This confirms the difficulty of using adsorptive techniques (AdsV) for the joint determination of both substances. Therefore a technique such as DPP, whose amperometric response is directly related to the electrodic reduction process controlled by diffusion, must be used.

Increasing the pulse amplitude resulted in a greater peak height and there was a linear relationship for both waves. In addition, the peak potential was displaced towards more positive potentials and the resolution of the polarographic peak was decreased owing to the broadening of the peak. A pulse amplitude of −140 mV was chosen as the optimum value after examination of these opposite effects.

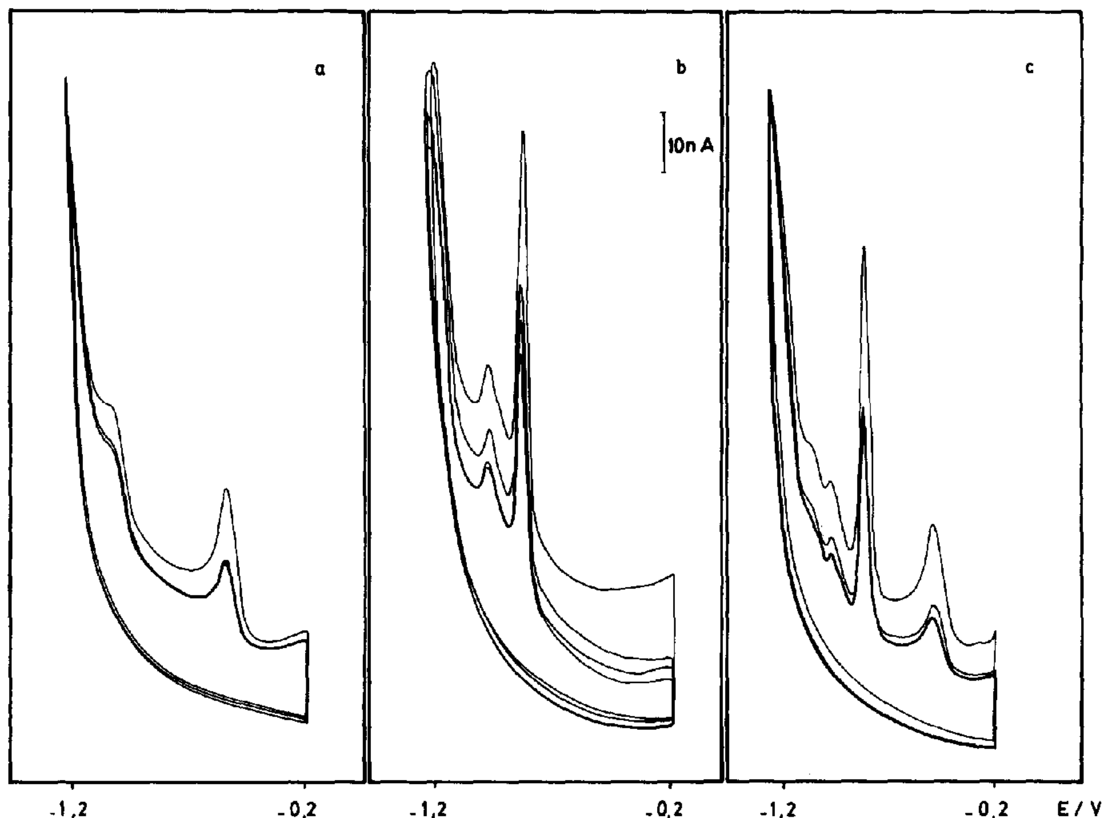

Figure 1
Repetitive cyclic voltammograms at HMDE for: (a) 5×10^{-6} M acetazolamide; (b) 5×10^{-6} M todralazine; and (c) 5×10^{-6} M acetazolamide and todralazine. Scan rate: 100 mV s^{-1}. Britton–Robinson buffer (pH 1.65).

The effect of pH on the voltamperometric study was investigated by recording DP polarograms of todralazine and acetazolamide (5×10^{-7} M) at several pH values ranging from 1.0 to 3.0. The highest intensity of the two peaks observed always corresponds to pH 1.65. Diminution of both peaks was observed at a higher pH. The peak of acetazolamide at pH 3.0 disappeared.

The i_p varied slightly with the ionic strength of the medium. The most intense peak current was obtained for an ionic strength of 0.015 M which was generated by the buffer components.

The straight line calibration curve of todralazine corresponded to the equation: $i_p = -69.55 + 6.13 \times 10^8$ [Tod] ($r = 0.9992$) for concentrations of 5.0×10^{-8}–7.0×10^{-6} M, where i_p is expressed as nanoamperes and concentration as M. Under the optimum working conditions a detection limit of 4.0×10^{-8} M for todralazine was obtained with a RSD at 5.0×10^{-7} M of 0.55% ($n = 10$).

In the case of acetazolamide, the straight-line plot corresponded to the equation: $i_p = 22.10 + 2.20 \times 10^8$ [Acet] ($r = 0.9988$) for the same range of concentrations and where units of peak current and concentration are expresed as in the equation for todralazine. Where acetazolamide was determined in the presence of todralazine, the detection limit was 5.0×10^{-8} M with a RSD at 5.0×10^{-7} M of 0.86% ($n = 10$).

Application to the analysis of samples of human serum

The presence of other surface-active compounds can affect the polarographic response of todralazine and acetazolamide, especially through competitive adsorption, and was therefore investigated. The influence of surfactants commonly found in biological samples was also tested.

In the presence of chloride ions, gelatin and albumin, decreases in the peak current of acetazolamide were observed. The addition of 300 mg l^{-1} of chloride ions caused a significant depression of 35% in the peak current; 10 mg l^{-1} of albumin resulted in the same depression of the peak current. Addition of 10 mg l^{-1} of

gelatin decreased the peak to 12.9%. However, the addition of these substances did not result in a decrease in the peak current of todralazine. That is why the use of the liquid–liquid extraction procedure is necessary for the elimination of the matrix effect for the joint determination of both substances. On the other hand, the metabolites are not active in voltammetric techniques and do not affect the analytical determination.

The quantitative determination of acetazolamide and todralazine in human serum was carried out by DPP, under the conditions described above. The results obtained for a spiked serum sample with the lower mean recovery are shown in Fig. 2. Using the standard addition method, an acetazolamide recovery of 102.24%, with a RSD of 0.86% and a todralazine recovery of 94.72% with a RSD of 1.72% was obtained for five successive samples of spiked serum (0.56 μg ml^{-1} of acetazolamide and 0.67 μg ml^{-1} of todralazine).

The high mean recovery obtained (Table 1) at low concentrations allows detection limits for todralazine of 0.107 μg ml^{-1} and for acetazolamide of 0.111 μg ml^{-1} in human serum. If the extracted serum volume in the polarographic cell is modified, the detection limits change. However, the expected serum concentrations are higher and the limits obtained are acceptable. In fact, after a simple oral dose of 70 mg, a mean peak plasma concentration of todralazine of 0.28 μg ml^{-1} was attained [14]. Similarly after a single oral dose of 250 mg, a peak plasma concentration of acetazolamide of 14 μg ml^{-1} was attained [15].

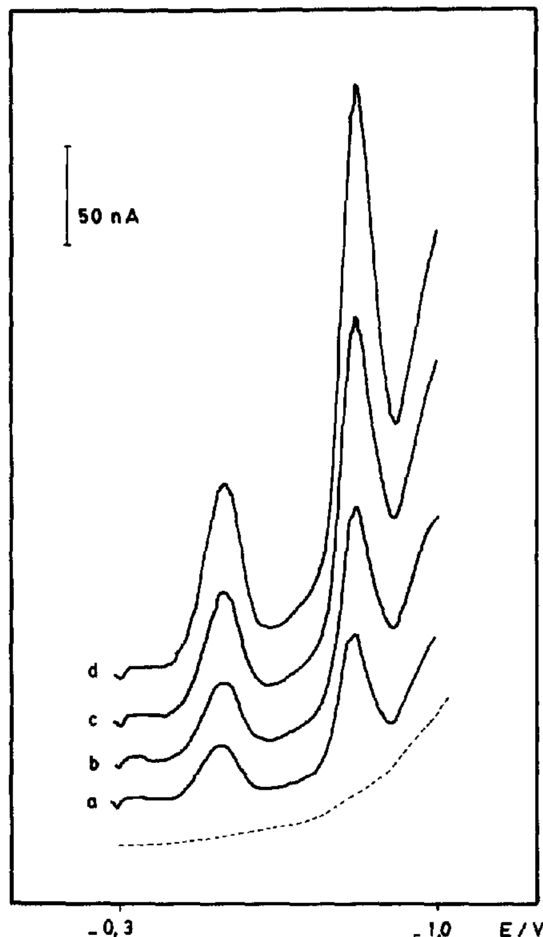

Figure 2
Voltammetric curves (DPP) obtained for the determination of todralazine and acetazolamide extracted from human serum. Cell volume: 20 ml of Britton–Robinson buffer (pH 1.65). (a) sample: 2 ml of serum spiked with 0.67 μg ml^{-1} of todralazine and 0.56 μg ml^{-1} acetazolamide. (b), (c) and (d) standard additions of 20 μl of 26.87 μg ml^{-1} of todralazine and 22.22 μg ml^{-1} of todralazine. Dotted line: blank.

Conclusions

A polarographic method using differential-pulse voltammetry was developed for the joint determination of todralazine and acetazolamide in human serum. The procedure is simple and specific and is not subject to interference from many substances commonly

Table 1
Recovery studies by DPP of spiked samples of blank serum

Spiked serum (mg ml^{-1})		Mean recovery (%)	
Todralazine	Acetazolamide	Todralazine	Acetazolamide
0.16	0.13	95.58	100.27
0.40	0.33	94.96	100.20
0.54	0.44	100.90	100.00
0.67	0.56	94.72	102.24
1.08	0.89	97.09	100.00
2.02	1.67	101.46	101.78
5.37	4.44	99.87	101.75

found in serum. The detection limits obtained are comparable to or lower than those of the accepted or most frequently used methods. The method offers significant advantages in assay time over other reported methods.

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