Direct Differential Pulse Polarographic and Adsorptive Stripping Voltammetric Assay of Ketotifen in Tablets

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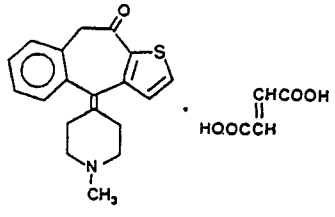
Direct differential pulse polarography allows the determination of the content uniformity of ketotifen (1) in single Zitaden® 1 mg tablets using the reduction wave of the keto moiety. 1N H_2SO_4 is used as solvent and supporting electrolyte.
By adsorptive stripping voltammetry (AdSV) the ketotifen concentration of complex "Positan" (Polfa-Poznan) samples can be determined directly without preliminary separation of excipients. Analysis of (1) in urine is carried out by AdSV after a preliminary solid phase extraction.
In model solutions as little as $2.5 \cdot 10^{-9}$ M ketotifen hydrogen fumarate can be determined by AdSV.

Direkte pulspolarographische und AdSV-Bestimmung von Ketotifen in Tabletten

Direkte differentielle Pulspolarographie erlaubt die Bestimmung des einheitlichen Gehalts von Ketotifen (1) in 1 mg-Tabletten. Dabei wird die Reduktionswelle der Ketogruppe benützt, 1N-H_2SO_4 dient als Lösungsmittel und Grundelektrolyt.
Durch AdSV wird der Ketotifen-Gehalt in "Positan" (Polfa-Posen), einer kompliziert zusammengesetzten Mischung, direkt ohne Abtrennung der Hilfsstoffe bestimmt. Ketotifen wird in Urin durch AdSV nach Festphasenextraktion bestimmt. In Modelllösungen kann durch AdSV $2.5 \cdot 10^{-9}$ Mol Ketotifen-Hydrogenfumarat bestimmt werden.

Ketotifen, (Zatiden®, Wander, Berne, Switzerland)[1)]



=4-(1-Methyl-4-piperidylidene)-4*H*-benzo[4,5]cyclopenta[1,2-b]thiophene-10(9*H*)-one hydrogen fumarate, is used clinically as hydrogen fumarate for prevention of bronchial asthma[2)].

Its chromatographic [TLC, GC, HPLC] and spectral properties, IR, UV, ^1H-, ^{13}C-NMR, and MS have been discussed (cf. e.g. ref.[13)]).

The assay of ketotifen has been performed mainly by chromatographic techniques. There are only two electrochemical papers, one discussing the potentiometric[13)], the other the differential pulse polarographic assay[2)].

1.1. Chromatographic determination of ketotifen and metabolites in bulk, formulated forms and biological media

TLC[3)], GC[3,4,7,8)], GC-MS[5,6)], LC, and HPLC[3,9,10)] have been proposed for the assay of ketotifen in bulk, pharmaceutical forms, and biological fluids.

Selective and sensitive GC methods have been developed for the quantitative determination of ketotifen (1) and its desmethyl metabolite in biological fluids[4)]. Ketotifen is detected by nitrogen F.I.D. or by mass-fragmentography down to 1 ng/ml and 0.05 ng/ml, respectively. The *N*-glucuronide of desmethyl-ketotifen is measured as desmethyl-ketotifen after enzymatic cleavage. The technique was used for the elucidation of the metabolism of the drug in rhesus monkeys, baboons, gibbons, chimpanzees, and man[4)].

Gas chromatography-mass spectrometry with selected ion monitoring was used for the determination of ketotifen and its demethylated, 10-hydroxy, and 10-hydroxy demethylated metabolites in human plasma. The minimal detectable concentrations for ketotifen and its demethylated metabolites were 50 pg/ml and 300 pg/ml for the 10-hydroxymetabolites[5)]. GC-MS was also used for the identification of ketotifen and its metabolites in human urine[6)].

Kennedy[7)] investigated the metabolism and pharmakokinetics of ketotifen in adults and particularly in children. The analyses in urine were performed by GC, measuring the unchanged product, the demethyl and the *N*-glucuronide derivatives. Plasma analyses were performed radio-immunologically before and after action of β-glucuronidase which enabled the unchanged product and its main metabolite, the *N*-glucuronide derivative, to be determined. Recently a GC method for the determination of free and glucuronide-conjugated ketotifen in rabbit blood serum after hydrolysis by β-glucuronidase was presented[8)]. For the determination of the free drug, the hydrolysis was omitted.

Daltrup et al.[9)] used reversed phase LC under isocratic conditions with 31.2% w/w acetonitrile in aqueous buffer, containing 4.8 g 85% orthophosphoric acid and 6.66% of KH_2PO_4/l water, pH 2.3. Retention data of 560 pharmaceuticals, drugs and insecticides are given.

Post-column derivatisation using a fluorimetric ion-pair technique, with sodium 9,10-dimethoxyanthracene-2-sulphonate as reagent was described for the assay of ketotifen[10)]. The combination of an extraction technique using di-(2-ethylhexyl)-phosphoric acid (HDEHP) with HPLC has been shown to be very useful in the routine analysis of Zatiden syrup (276 µg ketotifen hydrogen fumarate/ml syrup or 1 ml aqueous solution). A comparison of recoveries of ketotifen by three extraction techniques (sodium n-octylsulphate; HDEHP and phosphate buffer (pH 10.0)-chloroform) showed that the extraction with HDEHP is the method of choice. The use of an internal standard in order to compensate for drug loss during the extraction procedure is recommended[11)].

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Bioavailability studies for ketotifen were conducted using sustained-release oral formulations of deuterated drug[12].

1.2. Spectrometic and electrochemical assay of ketotifen in bulk, pharmaceutical forms, and biological media

Micotic-Mihun et al.[13] determined ketotifen (**1**) in tablets by UV-spectrometry at 298 nm. For the assay, several tablets are needed. The bulk content of ketotifen base on the other hand was determined potentiometrically using a glass/SCE electrode in glacial acetic acid anhydride with 0.1 N $HClO_4$. Fumaric acid in ketotifen hydrogen fumarate was determined by potentiometry with 0.1 N NaOH in ethanol/water medium[13]. *Mohamed* et al.[2] compared the results of proposed spectrophotometric and differential pulse polarographic determinations performed in their laboratory for the assay of ketotifen hydrogen fumarate in authentic powder and in capsule dosage forms. For both techniques, acetate buffer, pH 5, was used as solvent and as supporting electrolyte for the polarographic assay. For the spectrometric method (λ_{max} at 300 nm), a linear relationship was found between A_{max} and the concentration (c) over the range from 2 to 30 µg/ml. Using differential pulse polarography, a linear dependence was found between signal/peak height (i_p) (exploiting the reduction wave of the fumarate group with a peak potential (E_p) of -1060 mV (*vs.* Ag/AgCl)) and the depolarizer concentration in the range from 5 to 70 µg/ml. Mean percentages of recovery for ketotifen hydrogen fumarate in powder and in capsule forms obtained spectrometrically were 100.85 ± 0.56 and 99.75 ± 0.85%, respectively, as compared to 99.33 ± 1.12 and 101.15 ± 0.70% in bulk and in capsule form for the differential pulse polarographic technique[2].

The present paper describes: first the direct differential pulse polarographic determination of ketotifen hydrogen fumarate (**1**) and its assay in Zatiden® 1 mg tablets exploiting the reduction wave of the keto moiety in the seven-membered ring of the tricyclic drug (cf. ref.[14]); second the determination of ketotifen using the adsorptive stripping technique for the determination of the drug in "Positan" granulates for the preparation of syrup PZF-Polfa and the clinical control of the drug in urine, cf. ref.[15].

Experimental Part

2.1. Reagents and solutions

Zatiden® tablets were purchased in a pharmacy.- The stock solutions of ketotifen hydrogen fumarate and ketotifen base were made by dissolving an appropriate amount of substance in the 1N H_2SO_4 supporting electrolyte. Only fresh stock solutions were used for quantitation by the standard addition technique.

For the assay of ketotifen in "Positan" (granulates containing 0.69 mg ketotifen/g granulate used for the preparation of syrup PZF-Polfa) the stock solution was prepared by dissolving the ketotifen in spectrally pure KCl and redistilled water.

2.2. Instrumentation

2.2.1. For the differential pulse polarographic measurements and some adsorptive stripping assays a Polarecord 626 or 506 in combination with a polarographic stand (model 663) (both from Metrohm) and an Amel multipolarograph (model 471) were used. The working electrode was a dropping Hg electrode (DME) or a hanging Hg drop electrode (HMDE). The auxiliary electrode was a Pt-wire. A saturated calomel electrode (SCE) was used as a reference electrode. It was connected to the cell solution by means of a double salt-agar bridge containing supporting electrolyte.

2.2.2. Cyclic and stripping voltammograms were obtained using a PA-4 polarograph with an XY-4106 recorder (Laboratorni Pristroje, Prague). The working electrode was a hanging Hg-drop electrode (SMDE 1 Laboratorni Pristroje, Prague); as the reference electrode, a saturated Ag/AgCl and as the auxiliary electrode, a Pt-electrode were used.

All solutions were deoxygenated with supporting electrolyte saturated O_2-free N_2 for 5-10 min before measurement.- Instrument settings were as follows: medium drop size/surface area = 0.017 cm^2, potential scan rate v = 100 mV s^{-1}, preconcentration time = 60 s.

2.3. Procedures

2.3.1. *Differential pulse polarographic determination of ketotifen in Zatiden® tablets*

A single tablet was treated for 10 min in 10 ml 1N H_2SO_4 in an ultrasonic bath. After disaggregation of the tablet, the suspension was centrifuged at 3000 r.p.m., the clear supernatant solution was filtered through a 0.45 µm filter (Gelman Acrodisc LC PVDF). 2 ml of this solution were added to 15 ml 1N H_2SO_4 and polarographed after deaeration for 10 min.

2.3.2. AdSV determination of ketotifen in "Positan" (a) and in urine (b)

(a) An aqueous solution of "Positan" containing about $1 \cdot 10^{-5}$ M of ketotifen was prepared. 30 µl of this solution was added to 10 ml 0.1 N KCl supporting electrolyte and the voltammogram recorded.

(b) After washing 0.5 g ODS (bonded silica C_{18} used for the solid phase extraction) with 5 ml methanol and 2.2 ml water 1 ml urine containing about 5 µg ketotifen was passed through and rinsed with 2 ml water. The ketotifen was eluted with a solution of KCl in methanol-water. The methanol was evaporated, the remaining solution was made up to 10 ml with 0.2 M KCl. An aliquot of this solution was added to 0.2 M KCl supporting electrolyte and the AdSV was made as described.

3. *Results and Discussion*

3.1. *Differential pulse polarographic measurements: determination of ketotifen in Zatiden®-tablets*

The ketotifen hydrogen fumarate yields - cf. Mohamed[2] - in acetate buffer, pH 5, a well-defined wave with a peak potential (E_p) of - 1.125 V (*vs.* SCE), due to the reduction of the fumarate group. Under these conditions the ketotifen base gives no peak in the applied voltage range from -0.8 to -1.5 V (*vs.* SCE).

Direct polarographic indication of the tricyclic drug is feasible. Measurements in 1N H_2SO_4 revealed two reduction waves with peak potentials (E_p) of -625 to -675 mV (*vs.* SCE) and -815 mV, which can be assigned to the reduction of the fumarate moiety and the keto group, respectively (Fig. 1).

The peak height (i_p) of the keto reduction wave is linearly dependent on the concentration in the range from 6 µg to 30 µg depolarizer/ml polarographic solution, thus allowing the quantitative determination by standard additions.

The *ketotifen base* gives one well-formed wave at -835 mV (*vs.* SCE) in 1 N H_2SO_4. The ketotifen fumarate (i) and the ketotifen base (ii) exhibit the same slope in the i_p-c diagram (Fig. 2) (c = calculated on the basis of the tricyclic compound).

The keto reduction wave can be exploited for the determination of the content uniformity of Zatiden® 1 mg tablets, provided the amount of tablet material (sample) in the assay lies below 35 mg/l. At higher concentrations, tablet material interferes, resulting in non-linearity of the i_p-c plot of the

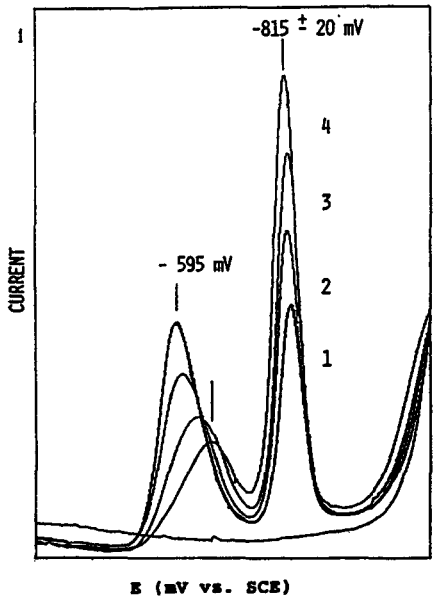


Fig. 1: Differential pulse polarograms of ketotifen hydrogen fumarate in the presence of Zitaden® tablet material, measured in 1 N sulfuric acid. DPP : ΔE = 10 mV; scan = 5 mV/s; t = 0.5 s; Curve 1 = duplicated measurement of the sample. Curves 2-4 = after standard addition of ketotifen hydrogen fumarate; 2 = 1.2 µg/ml; 3 = 2.4 µg/ml; 4 = 3.6 µg/ml polarographic solution

exploited keto-reduction wave. Quantification was performed by three standard additions of ketotifen hydrogen fumarate stock solution, cf. Fig. 1. The results for fully independent measurements of eleven single Zatiden® tablets are summarized in Table I. The values found showed good agreement with the ketotifen content indicated by the manufacturer. The dependence of the signal height (peak height, i_p) *vs.* the added ketotifen standard additions to various test solutions of the above mentioned series is shown in Fig. 3.

3.2. *Adsorptive stripping voltammetric measurements*

In recent years adsorptive stripping voltammetry (AdSV) has become a well-established, viable method in trace and ultratrace analysis (down to $10^{-9}/10^{-10}$ M and less) in the practical and research laboratory. The technique - first developed by *Kalvoda* et al.[16-19] - is currently used for the assay of pharmaceutical forms and in biological media. The AdSV technique and practical applications have been discussed in two modern monographs[20,21] and a recent review by *Kalvoda* and *Kopanica*[19].

Controlled interfacial accumulation has been used for the determination of a number of tricyclic compounds[22]. "Positan", a granulated product for the preparation of syrup PZF-Polfa, contains ketotifen (**1**) as active agent, sodium benzoate, citric acid, bisodium versenate, polyvinylpyrrolidone

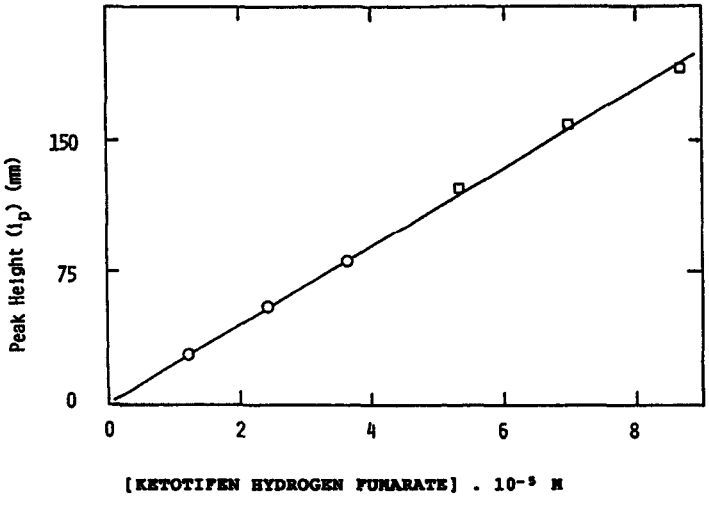


Fig. 2: Peak height (i_p) vs. ketotifen hydrogen fumarate (○) and ketotifen base (□) concentration

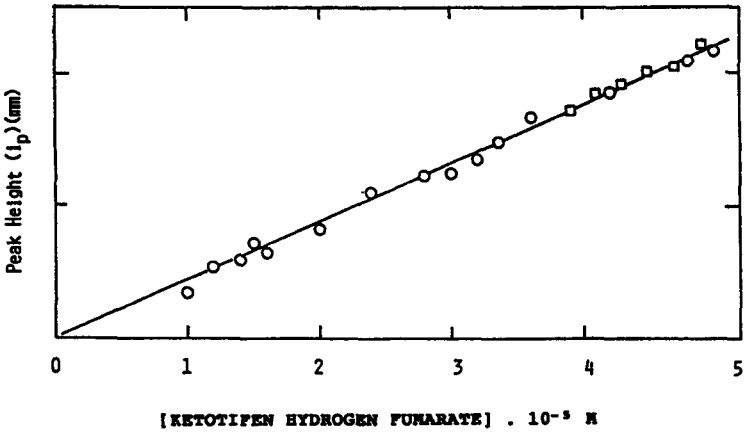


Fig. 3: Peak height (i_p) of ketotifen hydrogen fumarate standard additions to different measured Zatiden® test solutions (cf. Table 1)

Table 1: Differential pulse polarographic determination of ketotifen in single Zatiden® tablets

Tabelet Nr.	mg ketotifen/tablet
1	1.009
2	1.037
3	0.964
4	1.026
5	0.964
6	1.037
7	0.995
8	0.995
9	1.057
10	1.037
11	0.943
Average of all tablets	1.006
RSD	± 3.7%

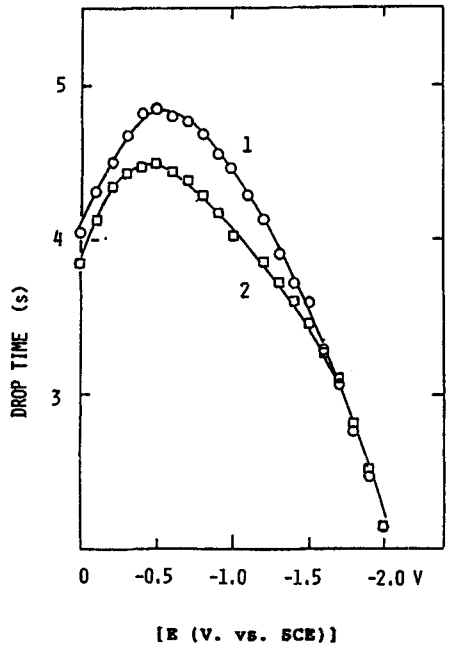


Fig. 4: Electrocapillary curves of ketotifen hydrogen fumarate (2) □ in 0.2 M KCl (1) ○. Ketotifen hydrogen fumarate concentration = 1 mM

and saccharin. These compounds hinder direct spectrophotometric assay, thus necessitating a separation step. From the clinical standpoint it is also important to control the amount of the drug in urine.

Therefore, a direct method was developed for the assay of ketotifen in "Positan" and in urine, based on controlled adsorptive preconcentration of 1 on the hanging Hg-drop electrode, followed by voltammetric determination of the accumulated species.

The electrocapillary curves obtained in 0.2 M KCl show a marked interfacial tension change in the presence of ketotifen hydrogen fumarate (Fig. 4) at potentials between 0 and -1.6 V (*vs.* SCE), indicating adsorption/reaction at the Hg-electrode over this potential range.

The tensammetric wave of ketotifen hydrogen fumarate on the other hand exhibits a rather complex wave pattern with a strong depression of the rest current and concentration dependent numbers of rearrangement and desorption peaks, Fig. 5.

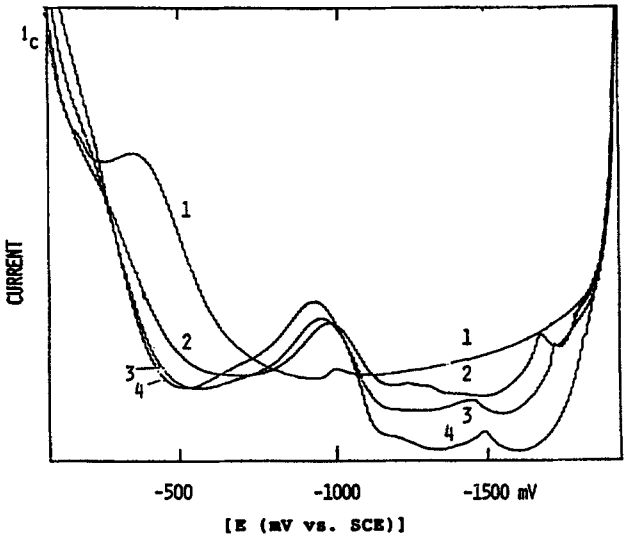


Fig. 5: Alternating current tensammograms of (1) 0.2 M KCl supporting electrolyte; (2) plus 10^{-5} M; (3) plus $3 \cdot 10^{-5}$ M; (4) 10^{-4} M ketotifen hydrogen fumarate. Working electrode = HMDE; $\Delta E = 10$ mV; $\nu = 75$ Hz

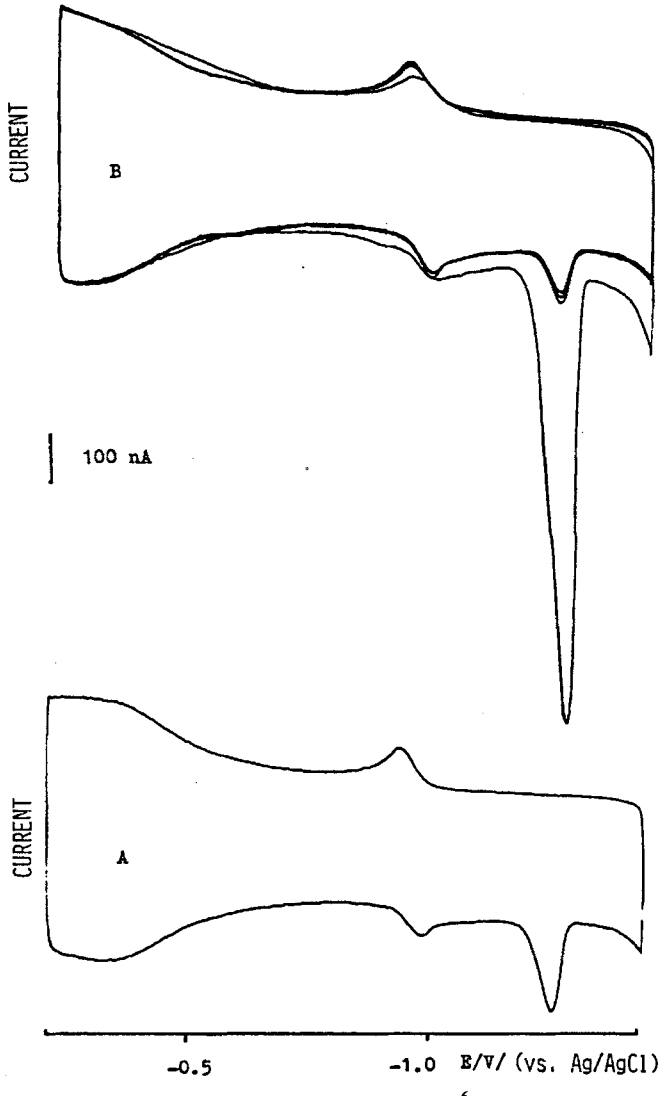


Fig. 6: Repetitive cyclic voltammograms for $1 \cdot 10^{-6}$ M ketotifen hydrogen fumarate, after stirring for 1 min at -0.2 V (vs. Ag/AgCl). Scan rate = 100 mV s^{-1}

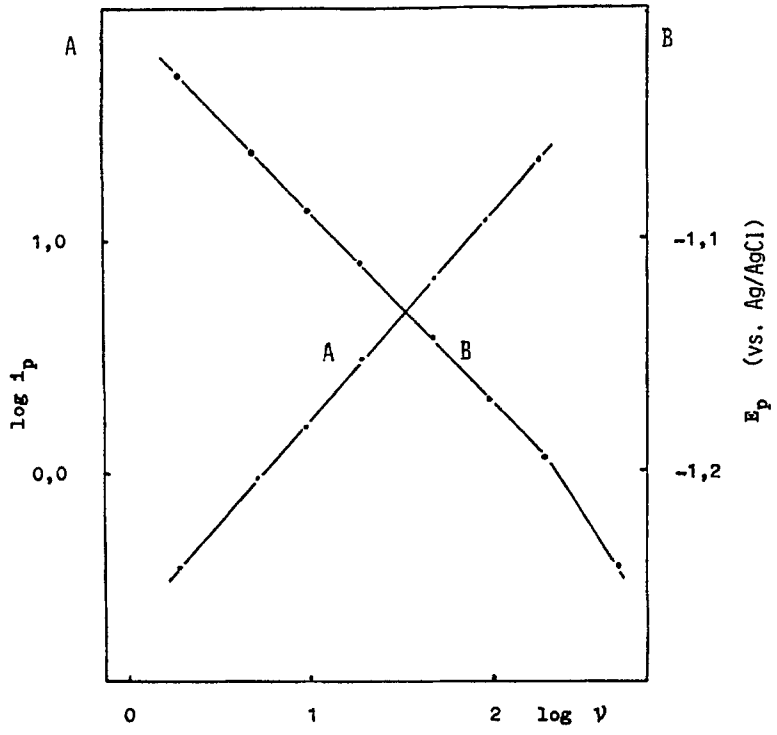


Fig. 7: Dependence of the logarithm of the peak current /A/ and of peak potential /B/ on the logarithm of the potential scan rate

Cyclic voltammetry was also used to explore the interfacial behaviour, Fig. 6 and 7.

Fig. 6 shows repetitive cyclic voltammograms for $1 \cdot 10^{-6}$ M ketotifen hydrogen fumarate after stirring for 1 min at -0.2 V. A large and well-defined cathodic peak, due to the reduction of the adsorbed drug, is observed at the first scan at -1.2 V (*vs.* Ag/AgCl). Subsequent scans exhibited substantially smaller cathodic peaks, indicating rapid desorption from the surface.

Voltammogram A represents an analogous response without accumulation; the voltammetric peak is substantially smaller than those obtained following accumulation.

The effects of potential scan rate (v) on the peak current and potential were evaluated for the surface-bound ketotifen hydrogen fumarate - Fig. 7. A log i_p *vs.* log v plot was linear over 2-500 mV s^{-1} range (A) with a slope of 0.8 (correlation coefficient 0.998). A slope of 1.0 is expected for an ideal reaction of surface active species[23]. The peak potential shifts negatively with increasing scan rate (B) (ca. 40 mV). The plot of the peak potential (E_p) *vs.* log v was also linear (correlation coefficient for the linear range = 0.997).

The adsorption of the ketotifen hydrogen fumarate can be used as an effective preconcentration step prior to voltammetric measurement.

The voltammetric response was examined in the presence of various supporting electrolytes, e.g. ammonium chloride,

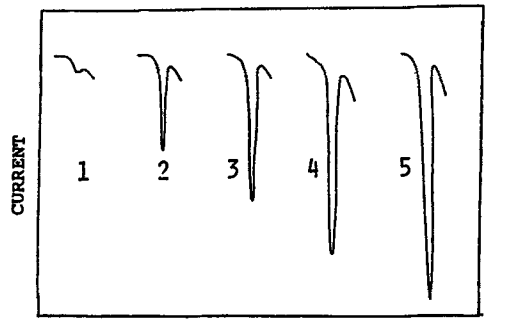


Fig. 8A: Linear scan stripping voltammograms for $5 \cdot 10^{-8}$ M ketotifen in 0.2 M KCl as a function of the accumulation time. Curve (1): accumulation, 0; (2) 20 s; (3) 40 s; (4) 60 s; (5) 80 s.

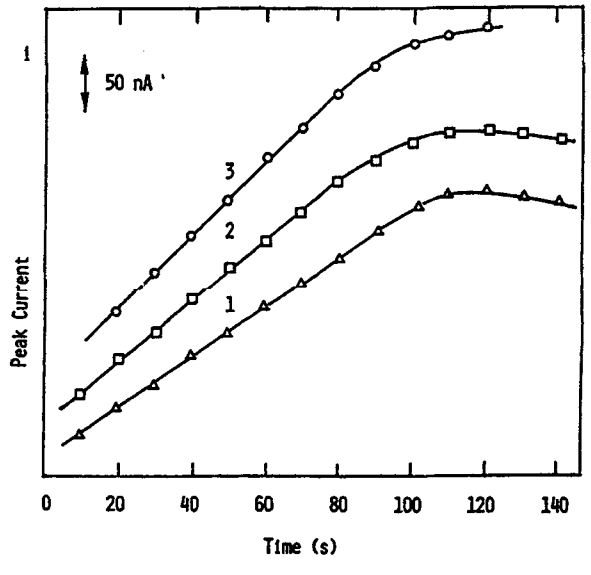
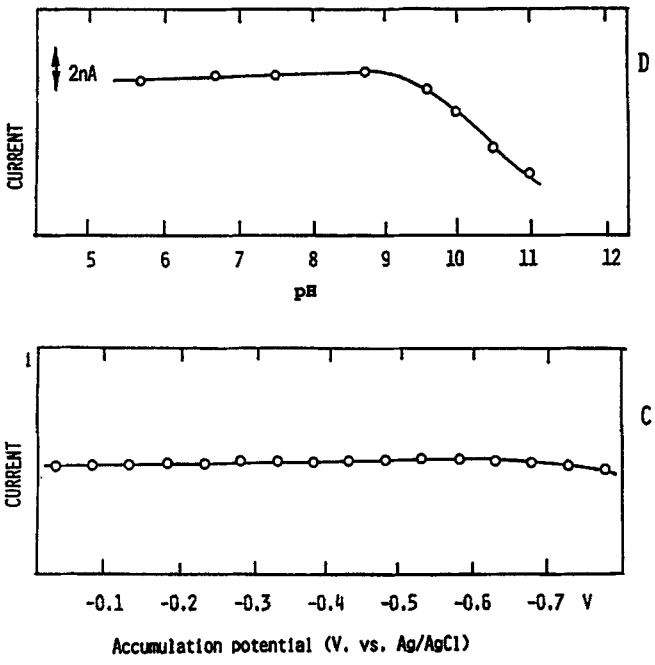


Fig. 8B: Effect of the accumulation time on the voltammetric peak height (i_p): (1) $2 \cdot 10^{-8}$ M; (2) $5 \cdot 10^{-8}$ M; (3) $8 \cdot 10^{-8}$ M ketotifen



Figures 8(C,D): Peak current (i_p) of $5 \cdot 10^{-8}$ M ketotifen in 0.2 M KCl; accumulation, 60 s; scan, 100 mV/s; C: as a function of the accumulation potential; D: as a function of the pH of the 0.2 M KCl supporting electrolyte

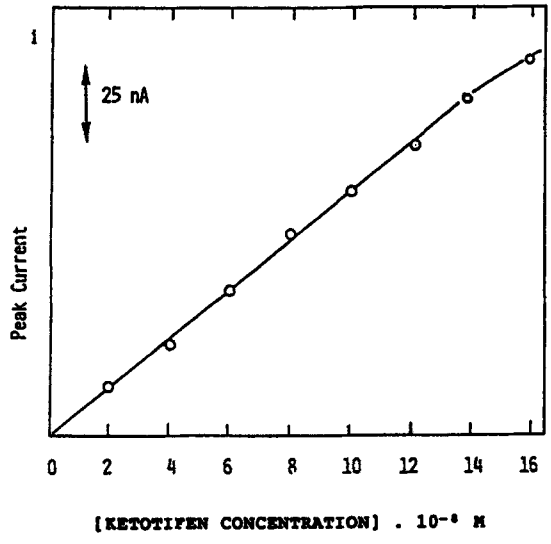


Fig. 9: Peak height (i_p) as a function of the ketotifen concentration. Supporting electrolyte = 0.2 M KCl. Accumulation time, 60 s; accumulation potential = -0.2 V (vs. Ag/AgCl); scan, 100 mV s^{-1}

KCl and phosphate buffer (0.05 M) solutions. The best results (with respect to peak enhancement, base-line current, and reproducibility) were obtained in KCl solution.

The adsorptive response was evaluated with respect to experimental parameters such as preconcentration time, potential, pH, bulk concentration and stripping mode.

Fig. 8A shows linear scan voltammograms for $5 \cdot 10^{-8}$ M after different preconcentration times. The longer the preconcentration time, the larger the peak current. Analytically useful relationships between peak intensity and concentration exist over the t_{acc} range (10-100 sec) - Fig. 8B.

Within 0 to -0.6 V the peak current proved to be independent of the accumulation potential (Fig. 8C). Within the pH range from 5 to 9 no effect of acidity on the peak current was observed. Above pH 9, the peak current decreased (Fig. 8D) and the peak was shifted towards more negative potentials.

A linear dependence of the peak current *vs.* depolarisation concentration (c) was observed in the concentration range of $1 \cdot 10^{-8}$ to $1 \cdot 4^{-7}$ M (Fig. 9), for the accumulation time of 60 sec and a preconcentration potential of - 0.2 V (vs. Ag/AgCl).

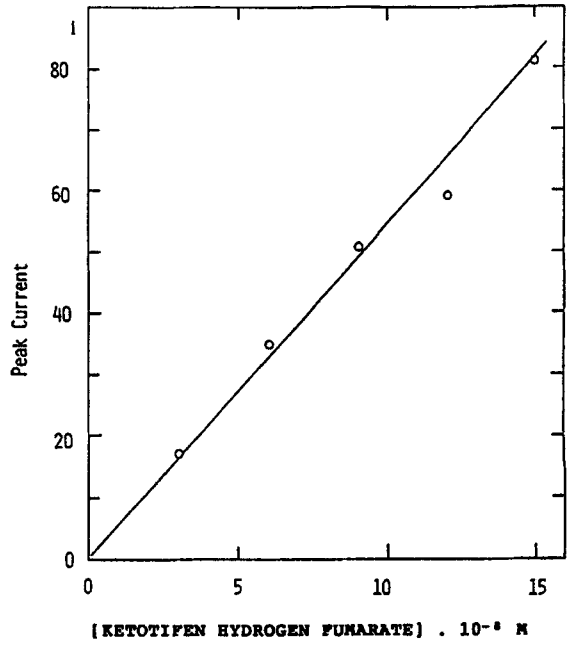


Fig. 11: Height of differential pulse voltammograms (i_p) of ketotifen as a function of the ketotifen hydrogen fumarate concentration. Supporting electrolyte = 0.01 M KCl; accumulation time, 180 s; accumulation potential, -0.2 V (vs. SCE); DPV : ΔE, 50 mV; scan, 7.5 mV/s; (t), 0.4 s

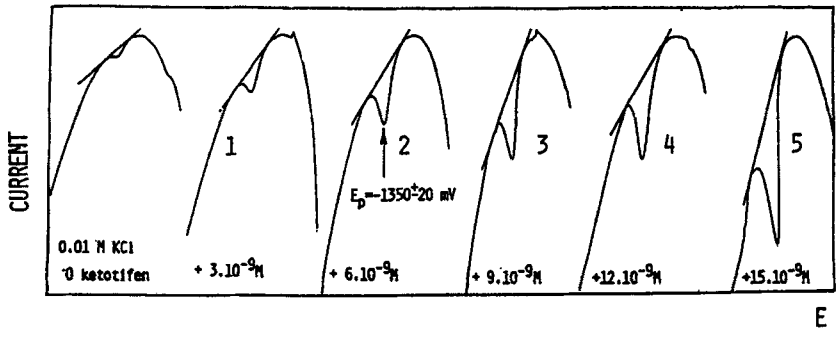


Fig. 10: Differential pulse voltammograms for (1) $3 \cdot 10^{-9}$ M; (2) $6 \cdot 10^{-9}$ M; (3) $9 \cdot 10^{-9}$ M; (4) $1.2 \cdot 10^{-8}$ M; (5) $1.5 \cdot 10^{-8}$ M ketotifen hydrogen fumarate in 0.01 M KCl. Accumulation time, 180 s; accumulation potential, -0.2 V (vs. SCE); scan, 7.5 mV/s; (t), 0.4 s; ΔE, 50 mV

Determination of lower concentrations of ketotifen was possible when differential pulse voltammetry was used. In model solutions with 0.01 M KCl as supporting electrolyte and an accumulation time of 180 s a fair linearity for the i_p-c plot (Figs. 10, 11) was found with a detection limit of about $2.5 \cdot 10^{-9}$ M.

AdSV assay of ketotifen in "Positan" and in urine: Using the adsorptive stripping technique described, direct determination of the ketotifen content of a complex sample such as "Positan" was feasible using the method of multiple standard additions. The value of 0.684 mg found agreed well with the concentration of 0.69 mg ketotifen (in 1 g of "Positan") indicated by the manufacturer.

For 6 determinations of ketotifen in "Positan" a relative standard deviation of ± 3% was found. Fig. 12 shows the dependence of i_p on c of ketotifen standard obtained for the sample of the compound "Positan" after subsequent standard addition. Value of the slope = -9.3 nA; correlation coefficient = 0.998. For the assay following parameters were used: preconcentration potential = -0.2 V (*vs.* Ag/AgCl), preconcentration time = 60 s, scan rate = 100 mv s^{-1}.

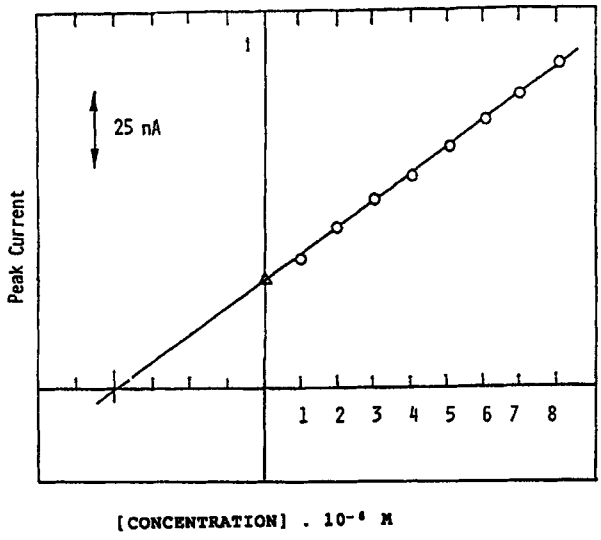


Fig. 12: Determination of ketotifen in "Positan" after solid phase extraction, using standard additions of ketotifen. Mode: adsorptive stripping voltammetry. Accumulation time, 60 s; accumulation potential, -0.2 V (vs. Ag/AgCl); scan, (LSV mode), 100 mV/s

The adsorptive stripping technique can also be used for the assay of ketotifen in urine.

In a urine sample containing 4.255 µg ketotifen/ml urine, 4.051 µg/ml were determined with a standard deviation of ± 7.8% for 5 determinations, using the adsorptive stripping technique.

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