DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 12(5), 733-746 (1986)

SPECTROPHOTOMETRIC AND DIFFERENTIAL PULSE POLAROGRAPHIC METHODS OF ANALYSIS FOR KETOTIFEN HYDROGEN FUMARATE

Mohamed E. Mohamed and Hassan Y. Aboul-Enein.
Department of Pharmaceutical Chemistry,
College of Pharmacy, King Saud University,
Riyadh, Saudi Arabia.

ABSTRACT

Spectrophotometric and differential pulse polarographic methods have been proposed for the assay of ketotifen hydrogen fumarate in authentic powder and in capsule dosage forms (Zaditen$^{(R)}$), using acetate buffer of pH 5.0 as a solvent as well as a supporting electrolyte. Ketotifen hydrogen fumarate exhibits relatively strong absorption in the ultra-violet region with maximum absorption at 300 nm; the molar absorptivity, ε_{max}, and specific absorbance, A (1 per cent, 1 cm), being 1.38 X 10^4 L mole^{-1} cm^{-1} and 325 respectively. Beer's Law was verified; the absorbance, (A_{max}), was found to be linearly related to concentration, C, over the range 2 to 30 µg ml^{-1}. The mean percentages of recovery for

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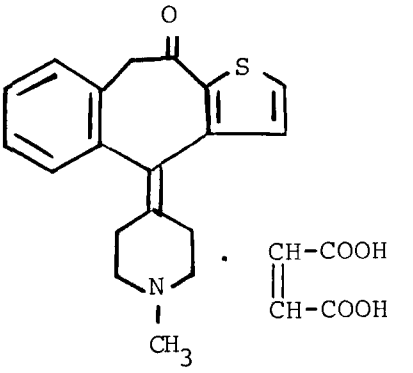
ketotifen hydrogen fumarate powder and in capsule form obtained spectrophotometrically were 100.85 ± 0.56 and 99.75 ± 0.85 respectively.

Differential pulse polarograms were recorded at room temperature under constant pulse amplitude of 50 mV superimposed on a linearly increasing DC-voltage ramp. The peak current, i, of the polarogram was measured at the peak potential of -1.06 V on the dropping mercury electrode, (dme), versus Ag/AgCl reference electrode. A linear relationship between the peak current and concentration, C, was observed over the range 5 to 70 $\mu g ml^{-1}$. The mean percentages of recovery obtained polarographically for ketotifen hydrogen fumarate in bulk and in capsule form were 99.33 ± 1.12 and 101.15 ± 0.70 respectively.

The purity of authentic ketotifen hydrogen fumarate was checked by non-aqueous potentiometric titration using 0.1 N - perchloric acid.

INTRODUCTION

Ketotifen, 4,9-dihydro-4-(1-methyl-4-piperidylidene)-4H-benzo [4,5] cyclohepta [1,2-b] -thiophene-10(9H)-one, is used clinically as hydrogen fumarate for the prevention of bronchial asthma (Zaditen$^{(R)}$ Wander-Berne-Switzerland) (1). Bioavailability studies for ketotifen were conducted using sustained-release oral formulations



Ketotifen hydrogen fumarate.

of the deuterated drug (2). Kennedy (3) investigated the metabolism and pharmacokinetics of ketotifen in adults and children. Capillary gas-chromatography for the elucidation of the metabolism of ketotifen in monkey and man was reported by Guerret et. al. (4). Chromatographic methods have been reported by Daldrup et al (5).

In this report two independent methods based on differential pulse polarography and spectrophotometry have been described for the determination of ketotifen hydrogen fumarate in authentic powder and in capsule dosage forms. The small dose (1 mg) of the drug in the commercial capsules, entails the use of a sensitive method for the analysis of the drug. The spectrophotometric method, based on the relatively strong U.V.-absorption of the chromophores of the basic radical is sensitive as indicated by 1.38×10^4 L $mole^{-1}$ cm^{-1} and 325, being values of the molar absorptivity and specific absorbance respectively.

The differential pulse polarography, being more sensitive than the conventional polarography, is based on the electro-activity of the fumarate radical. Fumaric acid, being an unsaturated dicarboxylic acid, undergoes reduction on the (dme) at the appropriate potential (6).

EXPERIMENTAL

Apparatus

Spectrophotometry: Pye Unicam SP8-100 double beam U.V.-Visible spectrophotometer was used with 1 cm quartz cuvettes.

Differential Pulse Polarography (DPP): Metrohm polarograph, consisting of a polarecord unit 626 and a polarograph stand model E505, was used. The electrode assembly consisted of dropping mercury indicator electrode (dme), a platinum auxiliary electrode and silver-silver chloride reference electrode. The (dme) was a fine capillary, of internal diameter 0.05 mm, and dezigned with a drop controller giving a steady flow of mercury droplets at a frequency of 1 sec^{-1}.

Potentiograph model E576, Metrohm, was used for the non-aqueous potentiometric titrations.

REAGENTS AND MATERIALS

(a) Acetate buffer pH 5.0 (B.P.) : Dissolve 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient distilled water to produce 1000 ml.

(b) Gelatin solution (0.2 % w/v): Prepared fresh daily in the acetate buffer and used a maxima suppressor.

(c) Ketotifen hydrogen fumarate was supplied by Sandoz-Ltd., Basel, Switzerland and the capsules (Zaditen$^{(R)}$ - 1 mg), Batch No. 013D9P, were kindly donated by, Wander Ltd., Berne, Switzerland.

(d) Standard hydrogen fumarate solution: Freshly prepared 0.02 % w/v in the acetate buffer.

For non-aqueous potentiometric titrations, potassium hydrogen phthalate and glacial acetic acid used were analytical grade whereas perchloric acid was a general purpose reagent.

Preparation of sample solution: Carefully empty 20 capsules; weigh the mixed contents and calculate the average mass of powder per capsule. Transfer quantitatively a quantity of powder accurately weighed and equivalent to 10 mg of ketotifen into 50 ml calibrated flask containing about 40 ml of the acetate buffer. Shake for 30 min. Adjust to volume with the buffer, centrifuge for 10 min. and decant the supernatant layer.

Procedures

(a) The non-aqueous potentiometric determination of authentic ketofifen hydrogen fumarate:-

A sample containing about 215 mg of the drug was accurately weighed, transferred to a 100-ml titration vessel and about 25-ml glacial acetic acid were added. The potentiometric titration curve for the sample was recorded using the potentiograph. The volume of titrant (0.1 N - acetous perchloric acid) at the end point was estimated graphically by the method of parallel tangents (7).

The purity of ketotifen hydrogen fumarate was computed using the following expression:

$$\% \text{ of ketotifen hydrogen fumarate} = \frac{V \times F \times 42.55}{\text{Weight of sample}} \times 100,$$

where v is the volume (ml) of 0.1 N - perchloric acid at the end point, F is the factor of the standard acid and 42.55 is the number of milligrams of ketitifen hydrogen fumarate chemically equivalent to 1 ml of 0.1 N-perchloric acid.

(b) The spectrophotometric method: Transfer by pippette 4 ml of sample solution into 50 ml - calibrated flask and adjust to volume using the acetate buffer. Measure absorbance at 300 nm against buffer as a blank. The concentration of ketotifen hydrogen fumarate is calculated using a calibration curve or an equivalent linear equation worked out from absorbance measurements of

1, 2, 4, 6, 8 and 10 ml of standard ketotifen hydrogen fumarate solution treated similarly as the sample.

(c) The differential pulse polarographic method: Pippette 8 ml of sample solution into 50 ml - calibrated flask, add 2 ml of 0.2 % w/v gelatin (maxima suppressor) and complete to volume using the acetate buffer. Transfer about 30 ml of the final solution into the polarographic vessel. De-aerate with a stream of pure nitrogen for 10 min. Record the differential pulse polarogram, using the set of the following polarographic conditions:

Range of polarizing voltage	:	-0.8 to -1.5 V
Scanning rate	:	-5 mV sec^{-1}
Mode	:	DP50
Drop time	:	1.0 sec.
Current sensitivity	:	20 nA/mm
Damping	:	2

Measure the peak current, i, from the base line and calculate the concentration of ketotifen hydrogen fumarate using a calibration curve or an equivalent linear equation. The calibration curve may be prepared by adopting the above procedure using 2, 4, 8, 10, 16 and 16 ml of ketotifen hydrogen fumarate standard solution. Alternatively the standard addition method may be applied.

RESULTS AND DISCUSSION

Ketotifen hydrogen fumarate shows relatively strong U.V. - absorption with 300 nm as absorption maximum (Fig. 1); the molar absorptivity, ε_{max}, and the specific absorbance, A (1 per cent, 1 cm), being 1.38 X 10^4 L $mole^{-1}$ cm^{-1} and 325 respectively. These values indicate the sensitivity of the spectrophotometric method. The absorption characteristics of ketotifen hydrogen fumarate showed no change in 0.1 N - sulphuric acid or in distilled water as solvents. The acetate buffer was chosen in order to employ the same medium as that used in the differential pulse polarographic method.

A linear relationship was shown between A_{max} and concentration, C, over the range 2 to 30 µg ml^{-1}; the regression line equation being

$A_{max} = 3.55 \times 10^{-2} C - 0.003$, with a correlation coefficient, r, of value equal to 0.9993. When the spectrophotometric method was applied to authentic ketotifen hydrogen fumarate and the pharmaceutical formulation, the percentages of recovery were 100.85 ± 0.56 and 99.75 ± 0.85 respectively. For the added recovery the result was 100.80 ± 0.60 (Table 1.).

Elving and Teitelbaum (6) studied the polarographic behaviour of fumaric acid in buffer solution covering

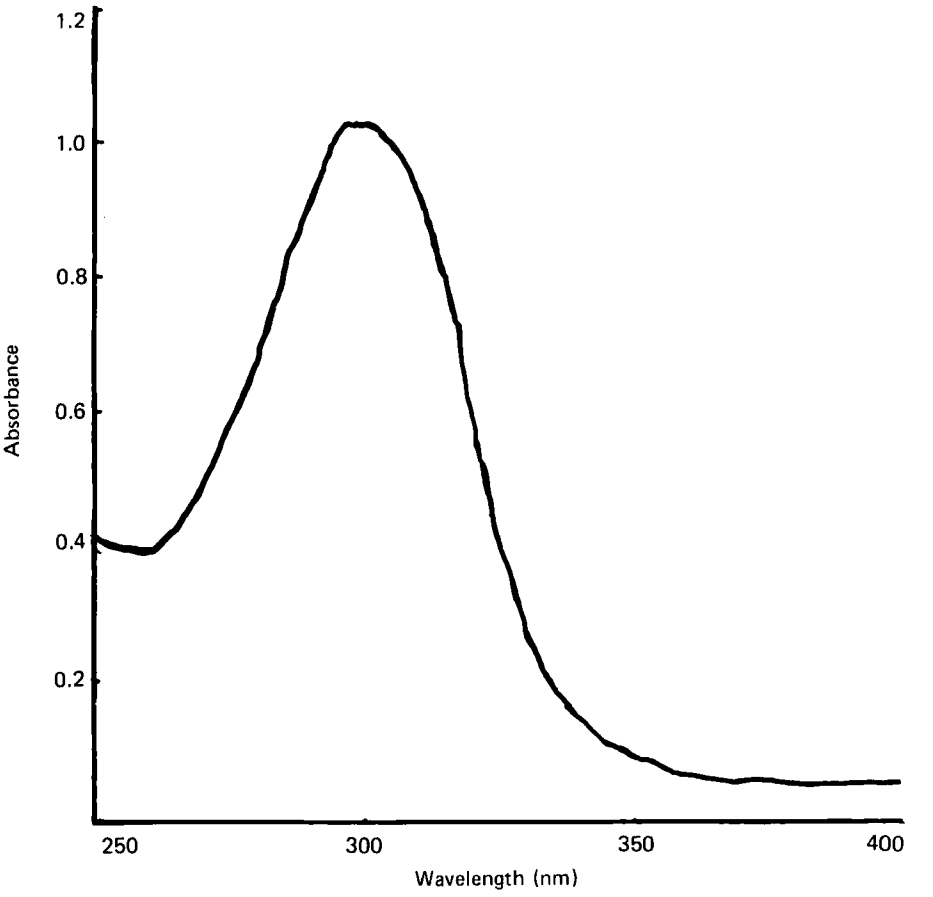


FIG. 1. Absorption spectrum of ketotifen hydrogen fumarate in acetate buffer of pH 5.0.

the pH range 2 to 9 and obtained a single well-defined step with $E_{\frac{1}{2}}$ (half-wave potential) increasing with pH.

Preliminary DC-polarographic investigation of fumaric acid in acetate buffer as a supporting electrolyte gave $E_{\frac{1}{2}}$ values (Fig. 2) agreeing with those cited in the literature. Repetition of the same experiments using keto-

Table 1. Spectrophotometric and Differential Pulse Polarographic Analysis of Ketotifen Hydrogen Fumarate.

Pharmaceutical compound	Method	% Recovery ± S.D.	% Added recovery ± S.D.
Authentic ketotifen hydrogen fumarate	Non-aqueous potentiometric titration	99.8	-
Authentic ketotifen hydrogen fumarate	Spectrophotometry	100.85 ± 0.56	-
Authentic ketotifen hydrogen fumarate	Differential pulse polarography.	99.33 ± 1.12	-
1 mg capsules (Zaditen(R))	Spectrophotometry	99.75 ± 0.85	100.80 ± 0.60
1 mg capsules (Zaditen(R))	Differential pulse polarography	101.15 ± 0.70	99.28 ± 1.35

tifen hydrogen fumarate gave identical CV-curves suggesting that the fumarate radical was responsible for the electro-activity observed under the applied potential. When ketotifen base was isolated and polarographed under identical conditions, it did not give a peak in the applied voltage range -0.8V to -1.5V. Under the polarographic conditions employed, the relationship between the peak current, i (nA), obtained from the differential pulse polarogram (Fig. 3), and concentration, C, was linear in the range 5 to 70 $\mu g ml^{-1}$. The regression

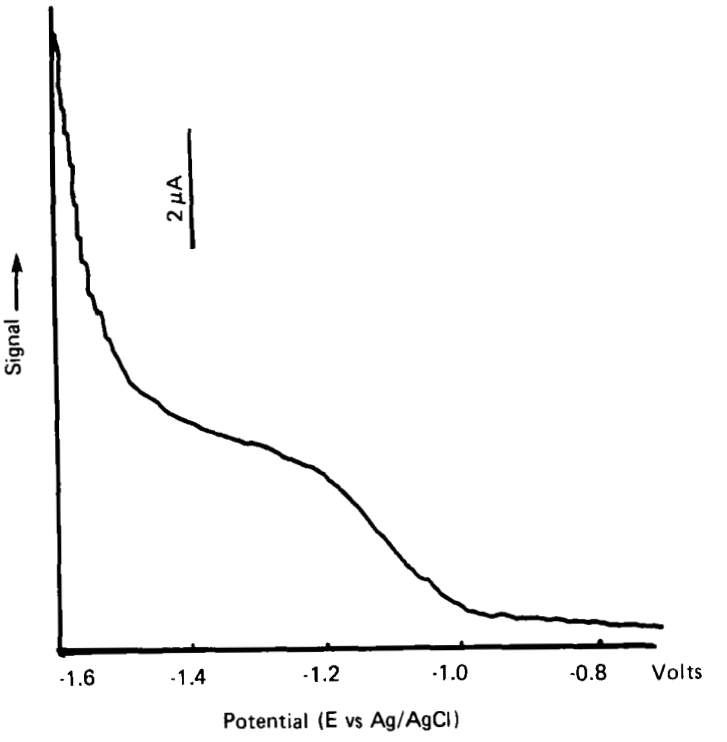


FIG. 2. DC-Polarogram of ketotifen hydrogen fumarate (4 $\mu g ml^{-1}$) in acetate buffer of pH 5.0.

line equation worked out is i = 35.62 C + 3.02, and correlation coefficient, r, is equal to 0.996.

When the differential pulse polarographic method was applied to authentic ketotifen hydrogen fumarate and the capsules, the percentages of recovery were 99.33 ± 1.12 and 101.15 ± 0.70 respectively. For the added recovery the result was 99.28 ± 1.35 (Table 1).

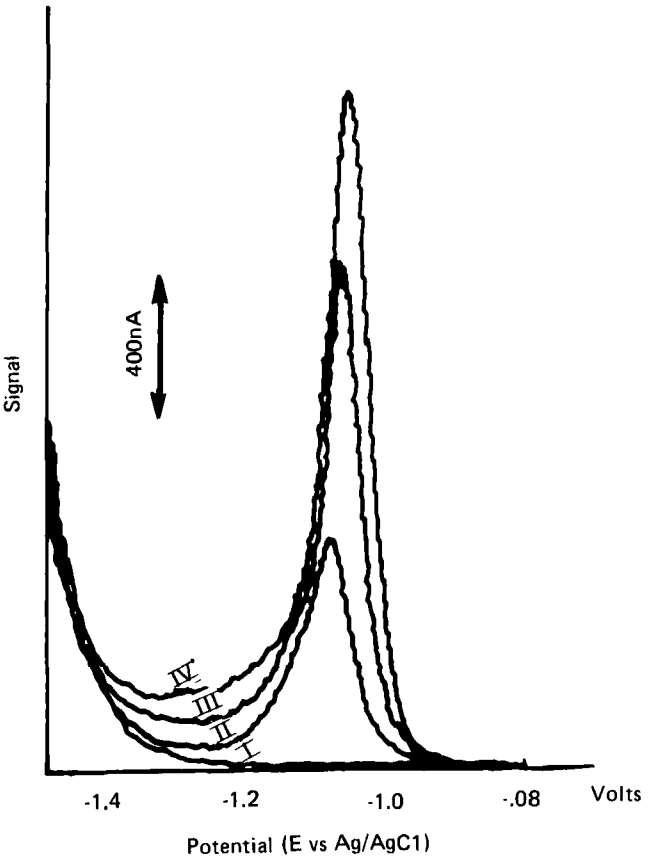


FIG. 3. Differential pulse polarograms of ketotifen hydrogen fumarate in acetate buffer of pH 5.0.

I = Supporting electrolyte.
II = 20 $\mu g ml^{-1}$ ketotifen hydrogen fumarate,
III = 40 $\mu g ml^{-1}$ ketotifen hydrogen fumarate,
IV = 60 $\mu g ml^{-1}$ ketotifen hydrogen fumarate.

Table 2. F- and t- tests statistical analysis.

Pharmaceutical compound	Method	Number of experiments	Varience ratio ($F_{0.05}$ -test)	Student's t ($t_{0.05}$ - test)
Authentic ketotifen hydrogen fumarate.	Spectrophotometry	6	4.00* (5.05)**	2.10* (2.32)**
	Differential pulse polarography	6		
1 mg capsules (Zaditen(R))	Spectrophotometry	6	1.47* (5.05)**	2.19 (2.32)**
	Differential pulse polarography	6		

*Calculated levels.

**Significant levels.

A statistical comparison between the spectrophotometric and the differential pulse polarographic methods using the variance ratio (F-test) and the Student's t-test are shown in Table 2.

According to the F-test, the calculated values were 4.00 and 1.47 for authentic ketotifen hydrogen fumarate and the capsules respectively. The theoretical value for $\phi_1 = 5$ and $\phi_2 = 5$ at 95% level of significance is 5.05. This suggests that there was no significant difference between the precision of the two methods.

The use of t-test to compare the two means, gave values of 2.10 and 2.19 for authentic drug and the capsules respectively. The theoretical t at ϕ = 10 is 2.23 at 95% level. This indicates that there was no significant difference between the two methods with regard to accuracy.

The two methods have comparable sensitivities; however the spectrophotometric method is simpler and less laborious than the differential pulse polarographic method.

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