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Three simple Spectrophotometric Methods for the Assay of Ketotifen in Pharmaceutical Formulations

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Three simple and sensitive spectrophotometric methods for the determination of ketotifen based on the formation of a charge- transfer complex between ketotifen and chloranilic acid (method A, $^{\lambda}$max: 550nm), by the inner molecular complex with sodium nitroprusside (method B, $^{\lambda}$max : 770 nm) or oxidation with excess potassium permanganate and the determination of unconsumed permanganate using Fast Green FCF (method C, $^{\lambda}$max : 625 nm) have been developed.

A survey of literature revealed only a few reported methods which include a single tirimetric[1], three UV[1-3] and two visible[4,5] spectrophotometric and three HPLC[6-8]. This paper describes three visible spectrophotometric methods for the determination of Ketotifen by exploiting characteristic properties due to the presence of tertiary amino group (formation of charge-transfer complex[9] with chloranilic acid, method A or inner molecular complex[10] with sodium nitroprusside, method B) and unsaturation[11] (oxidation with excess potassium permanganate and the determination of unconsumed permanganate using Fast Green FCF, method C).

* For correspondence

Table - 1 : Optical Characteristics and Precision

Parameter	Methods		
	A	B	C
λmax (nm)	550	770	625
Beer's law limits (μg/ml)	16.6 - 166.6	2.0 - 20.0	0.4 - 4.0
Molar absorptivity (1 $mole^{-1}$ Cm^{-1})	1.42×10^3	1.16×10^4	5.34×10^4
Sandell's sensitivity (μg cm^{-2}/0.001 absorbance unit)	0.299	0.037	0.009
Regression Equation (Y)*			
Slope (b)	3.36×10^{-3}	2.74×10^{-2}	1.25×10^{-1}
Intercept (a)	-3.32×10^{-4}	-5.0×10^{-4}	6.0×10^{-4}
Correlation coefficient	0.9999	0.9999	0.9999
Relative standard deviation (%)**	0.60	0.86	0.60
% Range of error** (95% confidence limit)	0.63	0.91	0.63

* : Y = a + bc where c is concentration

** : Six replicate samples : (concentration of 100.0, 12.0 and 3.2 ug ml-1 of pure drug for methods A,B and C respectively)

A systronic model 106 and Milton Roy Spectronic 1201 spectrophotometers with 1 cm matched Quartz cells and Elico LI- 120 digital pH meter were used. All the reagents were analytical grade and all solutions were prepared in doubly distilled water.

A solution of chloranilic acid (2.39×10^{-2} M) was prepared by dissolving 500 mg in isopropanol and making upto 100 ml with chloroform for method A. Aqueous solutions of sodium nitroprusside (5%), hydroxyl ammonium chloride (5%) and sodium hydroxide (1 M) were prepared for method B. Aqueous solutions of sodium sulphate (1.0 M), potassium permanganate (2.0×10^{-3} M) in 2.0 M H_2SO_4 and FG-FCF (1.23×10^{-5} M) in 1.0 M H_2SO_4 were prepared for method C.

The stock solution of Ketotifen was prepared by dissolving an appropriate amount of its fumarate equivalent to 25 mg of free base in 10 ml of distilled water, adding 2 ml of 0.1 M sodium hydroxide and extracting the free base with chloroform (5 x 20 ml). The stock solution (250 μg/ml) was directly used as working solution for method A. For methods B and C, the residue obtained after evaporating 50 ml of stock solution was initially dissolved in 10 ml of 0.01 M sulphuric acid, diluted stepwise with distilled water to get working solutions (100 μg/ml, method B, 20 μg/ml, method C). Tablet powder or syrup equivalent to 25 mg ketotifen was treated with 10 ml of distilled water and filtered if insoluble portion left. The filtrate was treated in the same manner as under standard solutions preparation for methods A, B and C and assayed as per the procedure.

Into a series of 15 ml calibrated tubes containing different aliquots of ketotifen solution (250-2500 μg), 0.2 ml of chloranilic acid was added and the volume made upto the mark with chloroform. The absorbance of the coloured species was measured at 550 nm against a reagent blank during the stability period. The amount of ketotifen present was calculated from its calibration graph.

Table 2
Analysis of Pharmaceutical formulations by proposed and reference procedures

Pharmaceutical preparation	Labelled amount (mg)	Amount found* (mg)			Found Reference 2 method	Recovery by proposed methods**		
		Proposed methods						
		A	B	C		A	B	C
Tablets I	1.0	0.996±0.006 t = 0.55 F = 2.62	0.998±0.008 t = 0.63 F = 1.52	0.991±0.005 t = 0.46 F = 3.65	0.995±0.010	99.3±0.1	99.6±0.4	99.0±0.4
Tablets II	1.0	0.996 ± 0.005 t = 0.61 F = 4.21	1.002 ± 0.010 t = 1.19 F = 1.01	0.999± 0.008 t = 1.11 F = 1.82	0.999±0.010	99.5±0.6	99.8±0.3	99.7±0.7
Syrup I	1 mg/5ml	0.997 ± 0.007 t = 1.70 F = 3.02	0.999±0.008 t = 0.32 F = 2.24	0.996±0.007 t = 1.99 F = 2.64	1.002±0.012	99.6±0.7	99.8±0.8	99.3±0.5
Syrup II	1 mg/5 ml	1.003±0.006 t = 0.69 F = 4.36	0.996±0.004 t = 0.80 F = 2.53	1.001±0.009 t = 0.08 F = 1.73	1.004±0.012	100.1±1.0	99.3±1.0	99.9±0.8

* : Average ± standard deviation of six determination, the t- and F-values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limits F=5.05, t=2.57.
** : Recovery of 10 mg added to the pharmaceutical formulations (average of three determinations)
I & II : Formulations that are manufactured by two different pharmaceutical companies.

Aliquots of drug standard solution (0.5-5.0 ml, 100 μg/ml) were transferred in to a series of 25 ml calibrated tubes. One ml each of hydroxyl ammonium chloride and sodium nitroprusside were successively added to the test tubes and shaken for 2 min. Then 1 ml of sodium hydroxide was added and shaken for 15 to 20 min. The contents were diluted to the mark with distilled water and the absorbances were measured at 770 nm against a reagent blank. The amount of drug was computed from a calibration curve.

To a series of 25 ml calibrated tubes containing aliquots (0.5- 5.0 ml) of ketotifen (20 μg/ml), 0.5 ml of $KMnO_4$ solution was added to each and the total volumes were adjusted to 10.0 ml with distilled water. After allowing to stand for 15 min at room temperature, 0.4 ml of FG-FCF solution and 4.0 ml of Sodium Sulphate solution were added successively. After 10 min, the volume was adjusted to 25 ml with distilled water. The absorbances were measured against a reagent blank at 625 nm within 3 h. The amount of drug was computed from a calibration curve.

Beer's law limits, molar extinction coefficient, sandell's sensitivity and regression characteristics of proposed methods are presented in Table 1. The relative standard deviation and % range of error at 95% confidence level are also given in Table 1. The values obtained by the proposed and reference (UV) method for the estimation of KTF in Pharmaceutical

formulations are compared in Table 2 and are in good agreement.

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Diuretic activity of aqueous extract of Orthosiphon thymiflorus in rats

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Aqueous extract of Orthosiphon thymiflorus have been tested for diuretic activity in rats. The parameters taken for each individual rat were; body weight before and after test period, total urine volume (corrected for water intake during the test period), urine concentration of Na^+, K^+ and Cl^-. The extract given orally does not act as an aquaretic. The values of urine volume are only slightly elevated. However, the cation and Cl^- excretion is increased.

TWIGS of the genus **Orthosiphon** provide the base for a widely used herbal tea reported to have diuretic and blood purifying activity. Different **Orthosiphon** aqueous extracts have been reported to be diuretic[1-4] and antiinflammatory[5-6]. The present study focused on diuretic activity of the aqueous extract of **Orthosiphon thymiflorus** in albino rats.

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Orthosiphon thymiflorus was collected from Tirunelveli district of Tamil Nadu and confirmed in Central siddha research unit, Tirunelveli, Tamil Nadu and found to comply with all specifications. The aqueous extract was obatined by macerating 5 kg of whole plant of **Orthosiphon thymiflorus** with 50 l of boiling water. The filtrate was reduced to about 4 l in vacuo at about 35° and freeze-dried afterwards. The yield was about 750 g of freeze dried extract (17 %). Male albino rats with body weights between