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Fluorimetric determination of some thioxanthene derivatives in dosage forms and biological fluids

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Abstract

A simple and highly sensitive method is proposed for the fluorimetric determination of four thioxanthene derivatives, namely: chlorprothixene, clopenthixol, flupentixol and thiothixene, in dosage forms and biological fluids. The method involves the use of nitrous acid as an oxidant to produce the corresponding fluorescent thioxanthenone sulphoxides. The experimental parameters were carefully studied and incorporated into the procedures. The results obtained compare favourably with those obtained by the official methods. The concentration–fluorescence plots were rectilinear over the range of 0.04–0.4 μg/ml for thiothixene, and 0.02–0.25 μg/ml for the other compounds, with minimum detectability ($S/N=2$) of 2 ng/ml for all the studied compounds except thiothixene which was 4 ng/ml. The proposed method was applied to the determination of the studied compounds in dosage forms. The results obtained were in good agreement with those obtained adopting the USP XIII method. The proposed method was further applied to the determination of flupentixol in spiked human urine and plasma, the percentage recoveries were 94.39 ± 1.81 and 96.46 ± 0.28, respectively. A proposal of the reaction pathway was presented. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Fluorimetry; Thioxanthene derivatives; Chlorprothixene; Thiothixene; Clopenthioxol; Flupentixol; Biological fluids

1. Introduction

Thioxanthenes are effective in the systematic treatment of psychoses mainly in schizophrenia [1]. A review of the literature reveals a variety of analytical methods applicable to the analysis of thioxanthenes. A good guide to the work published for those compounds is found in the review written by Belal et al., [2]. The recently published methods include: ultraviolet and visible spectrophotometric [3–5], chromatographic [6–12] atomic absorption [13] and electrochemical methods [14,15].

As for fluorimetric methods, thioxanthenes have been determined after treatment with different reagents, such as ceric sulphate [16], potassium permanganate [17], phosphoric acid (85%),

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[18] hexa-ammine cobalt (III) tricarbonatocobaltate [19] and 60% sulphuric acid [20]. All these methods involve the use of very strong oxidising agents (standard oxidation potentials of at least + 1.45 V vs. SCE) and, therefore, should be run under strict reaction conditions to avoid side reactions and variable reaction products. The present method involves the use of a mild oxidizing agent, nitrous acid, hence it is more safe, more simple and time-saving. The method can be used as a stability-indicating assay because the major decomposition product of thioxanthenes is: 9-thioxanthone [21,22] or an adduct resulting from the addition of singlet oxygen to the olefin, alternatively a charge-transfer complex with oxygen is formed, which then rearranges to a hydroperoxide [23]. All these species will not react with nitrous acid. The proposed method was successfully applied to the determination of these compounds in dosage forms and biological fluids. The results obtained were satisfactorily accurate and precise.

2. Apparatus

An Aminco-Bowman model J_4-9860-spectrofluorometer with the excitation and emission slit control set at 5 mm, and 1 cm quartz spectrofluorometric cells, were used all over the measurements.

3. Materials and methods

The studied compounds were obtained as gifts from different pharmaceutical companies: Chlorprothixene (Hoffmann-La Roche), thiothixene (Pfizer, USA), clopenthixol and flupentixol hydrochlorides (Lundbeck, England). The dosage forms containing these drugs were obtained from commercial sources in the Egyptian market. Plasma was obtained from Mansoura University Hospital, Mansoura, Egypt. Urine was obtained from healthy volunteers.

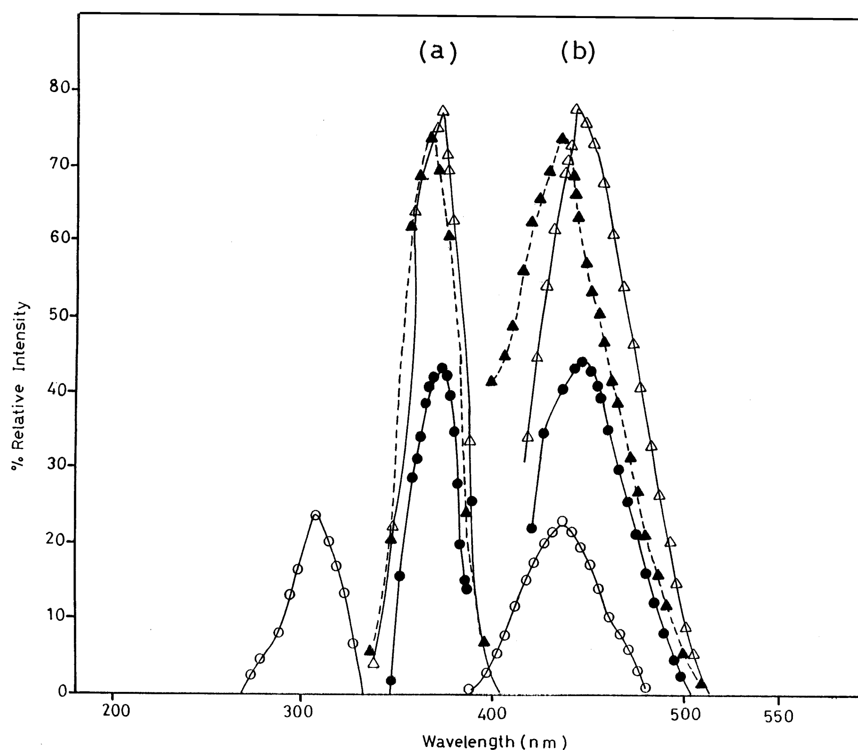

Fig. 1. Fluorescence spectra of the oxidation products of thioxanthenes: (a) Excitation spectra. (b) Emission spectra. ●-● Thiothixene (0.15 μg/ml). △-△ Chlorprothixene (0.12 μg/ml). ▲-▲ Clopenthixol (0.2 μg/ml). ○-○ Flupenthixol (0.2 μg/ml).

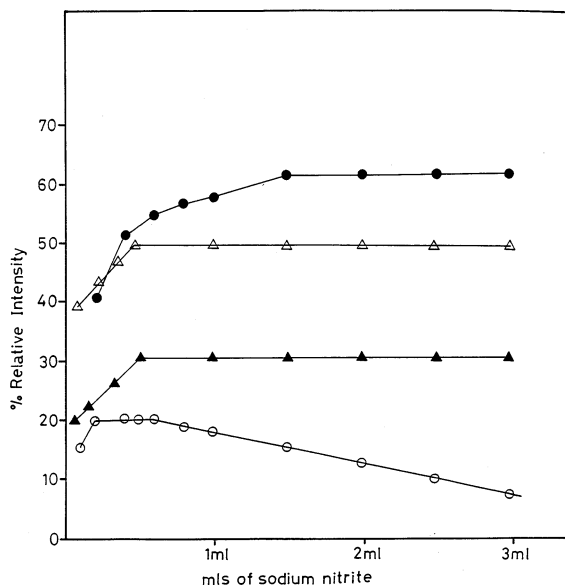

Fig. 2. Effect of sodium nitrite solution (3%) on the intensity of fluorescence. ●-● Chlorprothixene (0.2 μg/ml). △-△ Clopentixol (0.1 μg/ml). ▲-▲ Flupentixol (0.08 μg/ml). ○-○ Thiothixene (0.1 μg/ml).

3.1. Reagents

1. Sodium nitrite (Merck, Germany): 3% aqueous solution.
2. Ammonium sulphamate (Fluka, Germany): 6% aqueous solution.
3. Sodium hydroxide: 1 M and 7 M aqueous solutions.
4. Hydrochloric acid (Prolabo, France): 0.1 M aqueous solution.
5. Glacial acetic acid (Prolabo, France).
6. *n*-Hexane (Prolabo, France).
7. Isobutyl alcohol (Prolabo, France).
8. Methylamine (Prolabo, France).

3.2. Sample preparation:

Stock solutions (1.0 mg/ml) of the thioxanthenes were prepared in 0.1 M hydrochloric acid (chlorprothixene and thiothixene), or distilled water (clopenthixol HCl and flupentixol HCl) and were further diluted with distilled water to obtain the nominal concentrations of the working range (Table 1).

3.3. Analysis of pure samples

Transfer aliquots of thioxanthene stock solutions covering the concentration ranges cited in Table 1 into a 10 ml standard measuring flasks. Add 2 ml of glacial acetic acid, then add a suitable volume of sodium nitrite solution (Table 1), heat in a boiling water bath for 5 min. Cool then add 2 ml of ammonium sulphamate solution, stir till no more effervescence then dilute to the mark with distilled water. Measure the fluorescence intensity at the appropriate emission and excitation wavelengths for each drug (Table 1). Determine

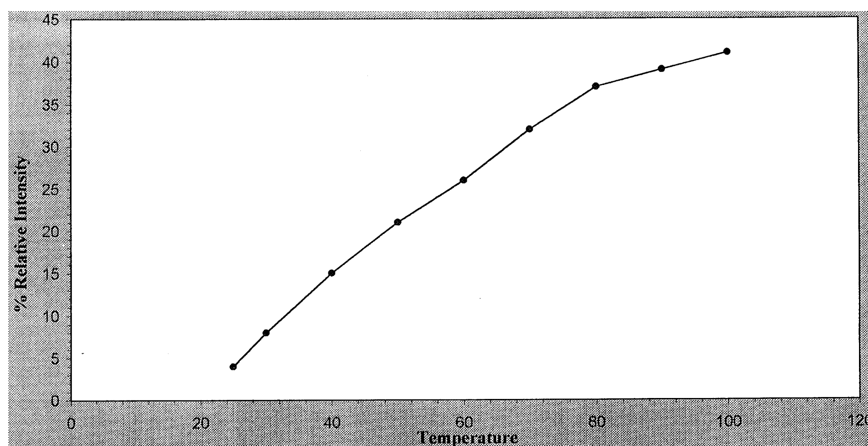

Fig. 3. Effect of temperature on the development of the fluorophore of flupentixol (0.1 μg/ml).

Table 1
Performance data for the fluorimetric determination of thioxanthenes

Compound	λ Maximum excitation (nm)	λ Maximum emission (nm)	Volume of sodium nitrite 3% solution (ml)	Concentration range (μg/ml)	Regression equation[a]	Correlation coefficient[b]	Lower detection limit (M)
(1) Chlorprothixene	374	450	1.6 ± 0.2	0.02–0.25	$C = 0.0005 + 0.0030\%RI$	0.99998	6.35×10^{-9}
(2) Clopenthixol. 2HCl	374	446	1.0 ± 0.1	0.02–0.25	$C = 0.0013 + 0.0029\%RI$	0.99989	5.0×10^{-9}
(3) Flupentixol. 2HCl	370	440	1.0 ± 0.1	0.02–0.25	$C = 0.0002 + 0.0031\%RI$	0.99998	4.6×10^{-9}
(4) Thiothixene	330	440	0.5 ± 0.1	0.04–0.40	$C = 0.0058 + 0.0049\%RI$	0.99991	1.9×10^{-8}

[a] C is the concentration of the analyte in μg/ml, % RI is fluorescence intensity.
[b] Based on seven separate determinations

Table 2
Analysis of pure samples of thioxanthenes by the proposed and official method[a]

	Proposed method			Official method [25]
	μg, Taken	μg, Found	% Found	% Found
(1) Chlorprothixene	0.020	0.020	100.00	
	0.040	0.040	100.00	
	0.060	0.059	98.30	
	0.080	0.079	98.75	
	0.100	0.100	100.00	
	0.200	0.199	99.50	
	0.250	0.247	98.80	
Mean ± SD			99.34 ± 0.71	99.03 ± 0.75
			$t = 0.63$ (2.262)	
			$F = 1.12$ (5.14)	
(2) Clopenthixol.2 HCl	0.020	0.020	100.00	
	0.060	0.061	101.67	
	0.100	0.101	101.00	
	0.200	0.204	102.00	
	0.250	0.251	100.40	
Mean ± SD			101.01 ± 0.84	100.47 ± 0.55
			$t = 1.05$ (2.447)	
			$F = 2.37$ (6.94)	
(3) Flupentixol 2 HCl	0.020	0.020	100.00	
	0.080	0.081	101.25	
	0.100	0.099	99.00	
	0.200	0.202	101.00	
	0.250	0.251	100.40	
Mean ± SD			100.33 ± 0.89	101.00 ± 0.66
			$t = 1.11$ (2.447)	
			$F = 1.80$ (6.94)	
(4) Thiothixene	0.100	0.099	99.00	
	0.200	0.199	99.50	
	0.300	0.300	100.00	
	0.400	0.398	99.50	
Mean ± SD			99.50 ± 0.25	100.00 ± 0.44
			$t = 1.58$ (2.571)	
			$F = 1.19$ (9.55)	

[a] Each result is the average of three separate determinations. Values between brackets are the tabulated values of t and F.

the concentration from the corresponding regression equation.

3.4. Analysis of dosage forms

3.4.1. Tablets

Weigh and pulverize 20 tablets. Transfer an accurately weighed amount of the powder equivalent to 20 mg of the active constituent into a small conical flask. Extract with 3×30 ml portions of the appropriate solvent as used for sample preparation. Filter into a 100 ml volumetric flask. Wash the filter paper and dilute to the mark with the same solvent. Further dilute this solution with distilled water to give an analyte concentration of 1 μg/ml, then proceed as described above. Calculate the nominal content of the tablet from the corresponding regression equation.

Table 3
Analysis of some dosage forms containing thioxanthenes by the proposed and official method[a]

Preparation	% Found	
	Proposed method	Official method [25]
1. Taractan tablets	100.20	
(5 mg of chlor-	101.00	
prothixene per	100.16	
tablet)[b]	100.60 ± 0.57	100.10 ± 0.96
Mean ± SD	$t = 0.78$ (2.776)*	
	$F = 2.88$ (19.00)*	
2. Navane tablets	99.83	
(10 mg of thiothix-	100.06	
ene per tablet)[c]	100.21	
Mean ± SD	100.26 ± 0.44	99.90 ± 0.62
	$t = 0.36$ (2.776)*	
	$F = 2.00$ (19.00)**	
3. Depixol tablets	100.05	
(3 mg of flupen-	101.03	
tixol 2 HCl per	101.24	
tablet)[d]	100.77 ± 0.64	101.00 ± 0.53
Mean ± SD	$t = 0.83$ (2.776)*	
	$F = 1.46$ (19.00)**	
4. Clopixol ampoules	101.240	
(50 mg zu-	102.000	
clopenthixol ac-	101.120	
etate per 1 ml)[d]	101.450 ± 0.48	102.00 ± 0.69
Mean ± SD	$t = 1.13$ (2.776)*	
	$F = 2.09$ (19.00)**	

[a] Each result is the average of three separate determinations.
[b] Product of Hoffmann-La-Roche, Switzerland.
[c] Product of Pfizer, USA.
[d] Product of Lundbeck, England.
* Values between brackets on the tabulated values of t at $P = 0.05$.
** Values between brackets are the tabulated values of F at $P = 0.05$.

3.4.2. Ampoules (clopixol ampoules, zuclopenthixol acetate 100 mg/2 ml)

Mix the contents of 5 ampoules and measure a volume of the mixture equivalent to 25 mg of the drug. Transfer into 25 ml measuring flask, dissolve in diethyl ether, dilute to the mark with the same solvent. Transfer 1.0 ml of this solution into a separating funnel and extract with 3×3 ml of 0.1 M HCl. The acid portion is transferred into 10 ml volumetric flask, complete to the mark, further dilute with distilled water to contain 1 μg/ml, and proceed as described above. Calculate the nominal content of the ampoule from the corresponding regression equation.

Table 4
Flourimetric determination of Flupentixol in spiked urine and spiked human plasma by the proposed method

Sample	Amount taken μg/ml	Amount found μg/ml	% Found
Urine	0.1	0.0948	94.80
	0.1	0.0948	94.80
	0.1	0.0932	93.20
	0.1	0.0932	93.20
$\bar{X} \pm$ SD			94.39 ± 1.81
Plasma	0.0800	0.0775	96.88
	0.1000	0.0963	96.30
	0.2000	0.1924	96.20
$\bar{X} \pm$ SD			96.46 ± 0.28

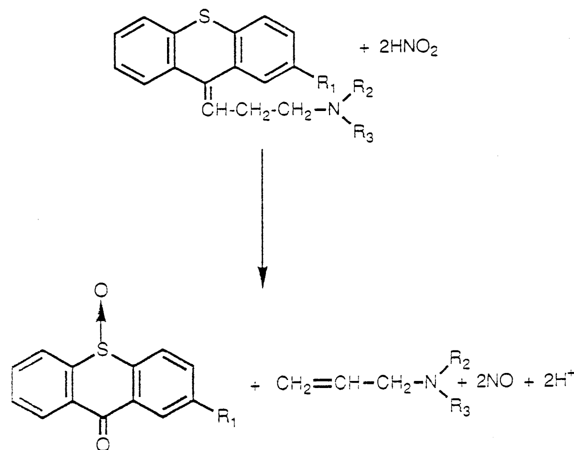

Fig. 4. Proposal of the reaction pathway between thioxanthenes and nitrous acid.

3.5. Determination of flupentixol in spiked human urine

Add a quantity of flupentixol to 5 ml of urine sample to obtain a concentration of 0.5 μg/ml.

Adjust to pH 12 by addition of 1 M NaOH solution then extract using 3 × 10 ml of hexane containing 1.5% isobutyl alcohol. Transfer the hexane phase into 60 ml separating funnel and extract with 5 × 3 ml of 0.1 M HCl. Transfer the acid fraction containing the drug into 25 ml volumetric flask, complete to the mark with water and proceed as described for pure sample.

3.6. Determination of flupentixol in spiked human plasma

To a plasma sample of 2 ml in a stoppered glass tube add 25 μg of flupentixol dissolved in 0.5 ml of water, 0.1 ml of 7 M NaOH solution and 8 ml of *n*-hexane containing 0.1% methylamine. Shake the sample for 15 min and centrifuge at 2400 rpm for 5 min. Transfer the *n*-hexane phase to another tube then add 2 ml of 0.1 M HCl. Shake the sample for 15 min and centrifuge for 5 min then discard the hexane phase. To the HCl phase add 0.2 ml of 7M NaOH and 4 ml of *n*-hexane containing 0.1% methylamine then shake the sample for 15 min then centrifuge for 5 min. Transfer the hexane phase to a conical flask and evaporate to dryness at room temperature. Dissolve the residue in 5 ml of 0.1 M HCl and transfer into 100 ml measuring flask. Complete to the mark with water and proceed as described above.

4. Discussion

The oxidation of thioxanthenes with nitrous acid was found to yield strongly fluorescent products. Fig. 1 shows the excitation and emission spectra of the obtained fluourophores of the standard thioxanthenes. The different experimental parameters affecting the intensity of the fluorophores were studied by changing one parameter while keeping the others constant. Increasing the volume of sodium nitrite solution was found to increase the fluorescence intensity of all compounds up to a certain volume (Table 1), after which it remained constant, except for thiothixene, in which case the fluorescence intensity decreased (Fig. 2). This behaviour of thiothixene may be attributed to the ease of its oxidation to the non-fluorescent sulphone derivative [21]. The fluorescence intensity increased with increasing the temperature applied for hastening the reaction up to boiling (Fig. 3). The effect of boiling time on the fluorescence intensity was also studied; 5 min were found to be sufficient for maximum fluorophore development. Longer heating time (above 20 min) caused decrease in fluorescence intensity of chlorprothixene and fluopenthixol. Acidification with mineral acids rather than acetic acid was found to produce variable results; this may be due to the prompt production and consequent decomposition of nitrous acid. The produced fluorophores were found to be stable at room temperature for at least $1\frac{1}{2}$ h. The calibration graphs were linear over the concentration ranges cited in Table 1. The minimum detectability ($S/N = 2$) was 2 ng/ml for all compounds except for thiothixene which was 4 ng/ml. The validity of the method was tested by analysing pure samples of thioxanthenes. The results in Table 2 show that the method is satisfactorily accurate and precise, as revealed by statistical analysis [26] of the data compared with those obtained by applying the official methods [22].

Thixanthenes are stable when heated for 1 h at 100°C in 0.1 M HCl, 0.1 M NaOH or water. When subjected to UV radiation or strongly basic conditions, they are decomposed to the corresponding thioxanthenes and thioxanthiones [22,23].

The method was further applied to some dosage forms containing those compounds. The results in Table 3 are in accordance with those obtained by the official methods [22]. Statistical analysis of the results using the Student *t*-test and the variance ratio *F*-test showed no significant difference between the performance of the two methods regards accuracy and precision [26].

The determination of flupentixol in biological fluids was studied as a model example. It is administered orally as the hydrochloride salt and intramuscularly as the decanoate ester. The usual

oral dose is 3–9 mg twice daily [27]. That gives a final blood concentration of 0.06–0.18 μg/ml, respectively. This concentration range coincides with the working concentration range for this compound (Table 1), thus, allows its determination in plasma. The results of analysis are shown in Table 4. The results are satisfactorily accurate and precise.

Compared with the other published fluorimetric methods, the proposed method has some distinct advantages. In the method using ceric sulphate as an oxidant [16] the time needed for the fluorescence development is 30 min compared with 5 min in the proposed method. Moreover, the ceric sulphate method is more laborious, an extraction step using benzene is necessary before treatment. In addition, the proposed method is more sensitive, wholly the minimum assay quantity is 0.02 μg for all thioxanthenes – except for thiothixene – is 0.04 μg compared with 0.4 μg in the ceric sulphate method. The method involving sulphuric acid [20] is also very tedious, and needs a great deal of caution, whereby concentrated sulphuric acid is added to the sample followed by heating in a water-bath for 30 min, the fluorophores should be extracted with heptane before measurement. The method involving the use of potassium permanganate [17] needs more carefully controlled conditions, as the permanganate oxidation results in variable calibration curves. Also, the optimal fluorescence is obtained between pH 5 and 8. At higher pH values, side chain oxidation would be incomplete whereas at low pH values random over oxidation could occur. The method based on the use of hexa-ammine cobalt (III) – tricarbonato cobaltate [19] is limited by the unavailability of the reagent, it has to be prepared by a very tedious procedure [19]. In addition, the reagent has a high oxidation potential (E° is + 1.82 V vs. SCE), so it has to be used under very strict conditions to avoid over oxidation to non-fluorescent products. In addition, the reaction time is longer (30 min) with some thioxanthenes.

A proposed mechanism of the reaction pathway is presented in Fig. 4. The reaction products are assumed to be thioxanthenone sulphoxides by analogy with the oxidation pathway reported for use of potassium permanganate as oxidant [17,24]. This analogy is based on the similarity of the excitation (330 nm) and emission (440 nm) wavelengths of the produced fluorophores of thiothixene in both the proposed and the reference method [17].

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