ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 281–289

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Use of mixed anhydrides for the determination of terfenadine in dosage forms and spiked human plasma

A.A. Al-Majed *, J. Al-Zehouri, F. Belal

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia

Received 30 September 1999; received in revised form 29 December 1999; accepted 29 December 1999

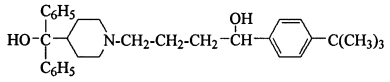
Abstract

Terfenadine reacts with mixed anhydrides (malonic and acetic anhydrides) producing a yellow-coloured product with intense fluorescence. Based on this fact, a spectrophotometric method was developed for the determination of terfenadine in dosage forms. The relation between the absorbance at 395 nm and the concentration is rectilinear over the range 0.5–5 μg ml^{-1} (molar absorptivity is 1.405×10^5 l mol^{-1} cm^{-1}). The reaction product was also measured spectrofluorimetrically at 435 nm after excitation at 395 nm. The fluorescence intensity was directly proportional to the concentration over the range 0.5–4 ng ml^{-1} with minimum detectability (S/N = 2) of 0.07 μg ml^{-1} ($\sim 1.5 \times 10^{-10}$ M). The different parameters affecting the development and stability of the reaction product were carefully studied and incorporated into the procedure. The proposed spectrophotometric method was successfully applied to the determination of terfenadine in tablets and suspensions; the percentage recoveries were 99.83 ± 0.75 and 99.65 ± 0.83, respectively. The proposed spectrofluorimetric method was applied to the determination of terfenadine in spiked human plasma. The percentage recovery was 99.35 ± 2.19. The method is highly sensitive and specific. No interference was noticed from co-formulated drugs, such as pseudoephedrine and ibuprofen.

Keywords: Terfenadine; Malonic anhydride; Plasma; Dosage forms; Fluorimetry

1. Introduction

Terfenadine is a selective H_2-receptor antagonist administered orally for the symptomatic treatment of allergic rhinitis and various skin allergies. It has the advantage of being free of the undesirable effects (sedation and anticholinergic effects) caused by many antihistamine drugs [1].



Terfenadine

Terfenadine is the subject of a monograph in the USP XXIII [2]: the raw material is assayed by non-aqueous titration, while the dosage forms are analysed by HPLC. Reviewing the literature re-

* Corresponding author. Fax: +966-1-4677383.
E-mail address: almajed99@yahoo.com (A.A. Al-Majed).

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00301-0

vealed that several methods have been reported for the determination of terfenadine in dosage forms and biological fluids, viz. non-aqueous titration [2,3], spectrophotometry [3–7], TLC [3,8,9], GC [10–12], HPLC [13–22], radioimmunoassay [23] and fluoroimmunoassay [24]. All these methods are either insufficiently sensitive [3–9] or tedious and require highly sophisticated instrumentation [10–24].

Terfenadine undergoes extensive pre-systemic metabolism by cytochrome P 450-3A4 (CYP 3A4) in the gut and liver [25]. As more than 99% of terfenadine undergoes pre-systemic biotransformation, peak plasma level of the drug following therapeutic doses of 60 mg once or twice daily are typically below 5 ng ml^{-1} [26]. Plasma concentrations of terfenadine are of clinical interest because the parent drug is cardiotoxic. Incidents of cardiotoxicity have been described in patients suffering from over-dosage [27] or when metabolism of terfenadine is inhibited by liver diseases [28].

A radioimmunoassay for the drug in plasma has been reported [23] but the antiserum used is not commercially available. An assay based on HPLC has also been published [21], but its limit of detection is 10 μg ml^{-1}, which is not sensitive enough to measure the drug level after therapeutic doses. Other HPLC methods were also reported [16,17].

It is clear that there is a need for a method that is sensitive enough to monitor the drug level after therapeutic doses. Fluorimetry by virtue of its high sensitivity meets this requirement. The proposed method has some distinct advantages that render it a promising substitute for HPLC methods.

2. Materials and methods

2.1. Materials and reagents

Terfenadine was purchased from Sigma (Poole, UK). Teldane tablets (Batch No. 6289) labelled to contain 60 mg of terfenadine/tablet and teldane suspension (Batch No. 6770) labelled to contain 30 mg of terfenadine/5 ml were obtained from commercial sources. Plasma was obtained from King Khalid University Hospital, Riyadh, Saudi Arabia.

Malonic acid anhydride (MAA) reagent was prepared by dissolving 10 g of malonic acid in 100 ml of acetic anhydride by gentle heating at 60°C.

Standard solution of terfenadine was prepared in methanol to contain 1.0 mg ml^{-1} then further diluted with the same solvent as appropriate.

2.2. Apparatus

The apparatus used consisted of a UV-Visible Spectrophotometer, Shimadzu, UV-160, IPC with 1-cm quartz cells and a spectrofluorimeter, Kontion SFM 25.

2.3. Procedures

2.3.1. Preparation of calibration graphs

Transfer aliquots containing suitable amounts of terfenadine in methanol into a set of 10-ml screw capped test tubes. Evaporate the solvent till dryness using a water bath. Cool under tap water, then add 2 ± 0.2 ml of MAA reagent. Screw cap the test tubes then heat at 80 ± 5°C for 25 ± 5 min, using water bath. Cool, transfer the solution quantitatively into 10-ml measuring flask, and dilute with methanol to the mark. Measure the absorbance of the solution at 395 nm or measure the fluorescence intensity at 435 nm after excitation at 395 nm. Plot the absorbance readings or the fluorescence intensities versus the concentration to get the calibration graphs. Alternatively, derive the corresponding regression equations.

2.3.2. Procedure for the tablets

Weigh and pulverise 20 tablets and then transfer a quantity of the powder equivalent to 60.0 mg of terfenadine into a small flask. Extract with 3×30 ml of methanol by shaking for 15 min, then filter into a 100-ml measuring flask. Wash the residue and filter with methanol and pass the washing into the same measuring flask. Complete to the mark with the same solvent. Transfer 10 ml of the filtrate to 100-ml measuring flask, and complete to the mark with methanol. Transfer 1.0 ml of this solution to the test tube. Proceed as described under Section 2.3.1, measuring the ab-

sorbance of the solution at 395 nm. Determine the nominal content of the tablets either from the calibration graph or using the corresponding regression equation (Table 1).

2.3.3. Procedure for suspension

Transfer 10 ml of the suspension (equivalent to 60 mg of terfenadine) into a 100-ml measuring flask, shake for 15 min and then complete to the mark with methanol and filter. Dilute 5 ml of the filtrate to 50 ml with methanol in a measuring flask. Transfer aliquots containing suitable amounts of terfenadine (Table 1). Proceed as described under Section 2.3.1, measuring the absorbance solution at 395 nm. Determine the nominal content of the suspension either from the calibration graph or using the corresponding regression equation (Table 1).

2.3.4. Procedure for plasma

Dilute 100 μl of spiked plasma to 1.0 ml with acetonitrile. Centrifuge the solution at 3000 rpm for 10 min. Transfer 100.0 μl of the clear supernatant into the screw-capped tube, dilute with 900 μl of methanol. Proceed as described under Section 2.3.1, measuring the fluorescence of the solution at 395/435 nm. Calculate the concentration of terfenadine in plasma from a previously plotted calibration graph.

Table 1
Performance data for the spectrophotometric and fluorimetric methods

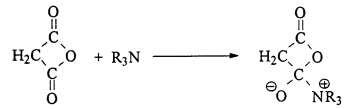
Parameters	Spectrophotometric method	Fluorimetric method
Wavelength	395 nm	395/435 nm.
Concentration range	0.05–5 μg/ml	0.5–4 ng/ml
Regression equation[a]	$C=0.49+3.88A$	$C=-0.28+0.026F$
Correlation coefficient	0.9974	0.9961
Standard deviation of the residuals Sy/x	0.0074	0.413
Standard deviation of the intercept	0.01	0.61
Standard deviation of the slope	0.0018	0.12
Application	Dosage forms	Plasma

[a] C is the concentration in μg/ml, A is the absorbance and F is the fluorescence intensity.

3. Results and discussion

Terfenadine weakly absorbs light in the UV region, and its molar absorptivity at 260 nm in methanol is 660 l mol^{-1} cm^{-1} [29]. However, upon reaction with MAA reagent, a highly coloured product with intense fluorescence was obtained. Fig. 1 shows the absorption spectrum of the reaction product while Fig. 2 displays its fluorescence spectrum.

Malonic acid anhydride is proposed to condense with terfenadine producing an internal salt. The high pK_a value of terfenadine, i.e. 10 [29], indicates that the rate of the condensation reaction is dependent on the basicity of the tertiary amine. The reaction is proposed to proceed as follows:



A number of organic acid anhydrides are capable of forming coloured products with tertiary amines [30]. Malonic acid was preferred because it forms the most stable fluorescent product. The citric acid product exhibits a steady increase in fluorescence over a period of time. Aconitic acid, on the other hand, was unsatisfactory as invariably a purple solution resulted on heating to dissolve the acid [31]. A yellow solution occasionally results when malonic acid is dissolved in acetic anhydride; a fluorescent product is still obtained with tertiary amines, but it is preferable to prepare a fresh solution. This coloration is possibly due to traces of alkali on the glassware catalysing the condensation reaction [30]. On ageing, the MAA reagent develops a yellow colour, which does not affect the fluorescence of the reaction product. Moreover, the blank value does not increase significantly for this aged solution. A 10% solution of malonic acid in acetic anhydride yields a more stable fluorescent

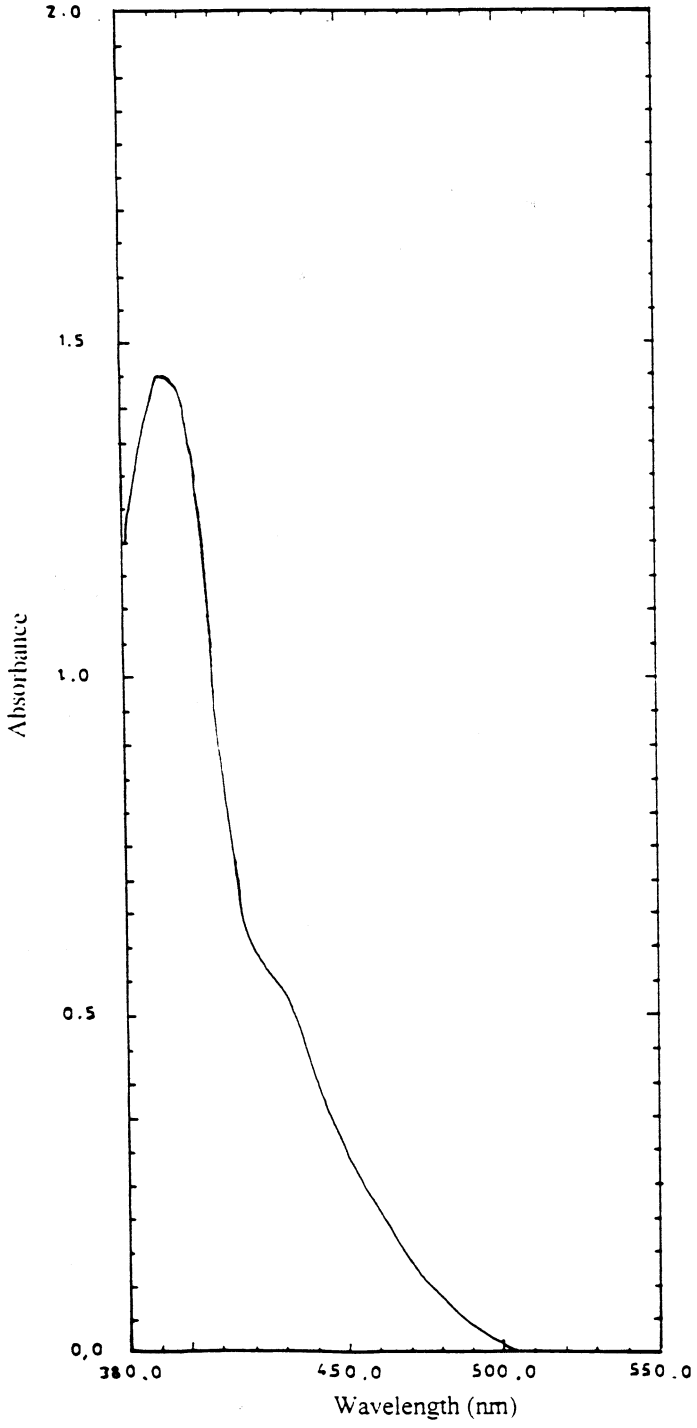


Fig. 1. Absorption spectrum of the reaction product of terfenadine with mixed anhydride.

product than the 2% solution recommended by Fiegel [30] for spot tests.

The variation of fluorescence intensity with heating time and heating temperature is shown in Figs. 3 and 4, respectively. Heating at 80 ± 5°C for 25 ± 5 min gave the highest fluorescence intensity. The obtained fluorescent product was found to be stable for at least 5 hours.

Table 1 presents the performance data for both the spectrophotometric and fluorimetric methods and their applications. The limit of detection adopting the fluorimetric method (S/N = 2) was 0.07 μg ml^{-1} ($\sim 1.5 \times 10^{-10}$ M). This ultra-high sensitivity of the method renders it suitable for the

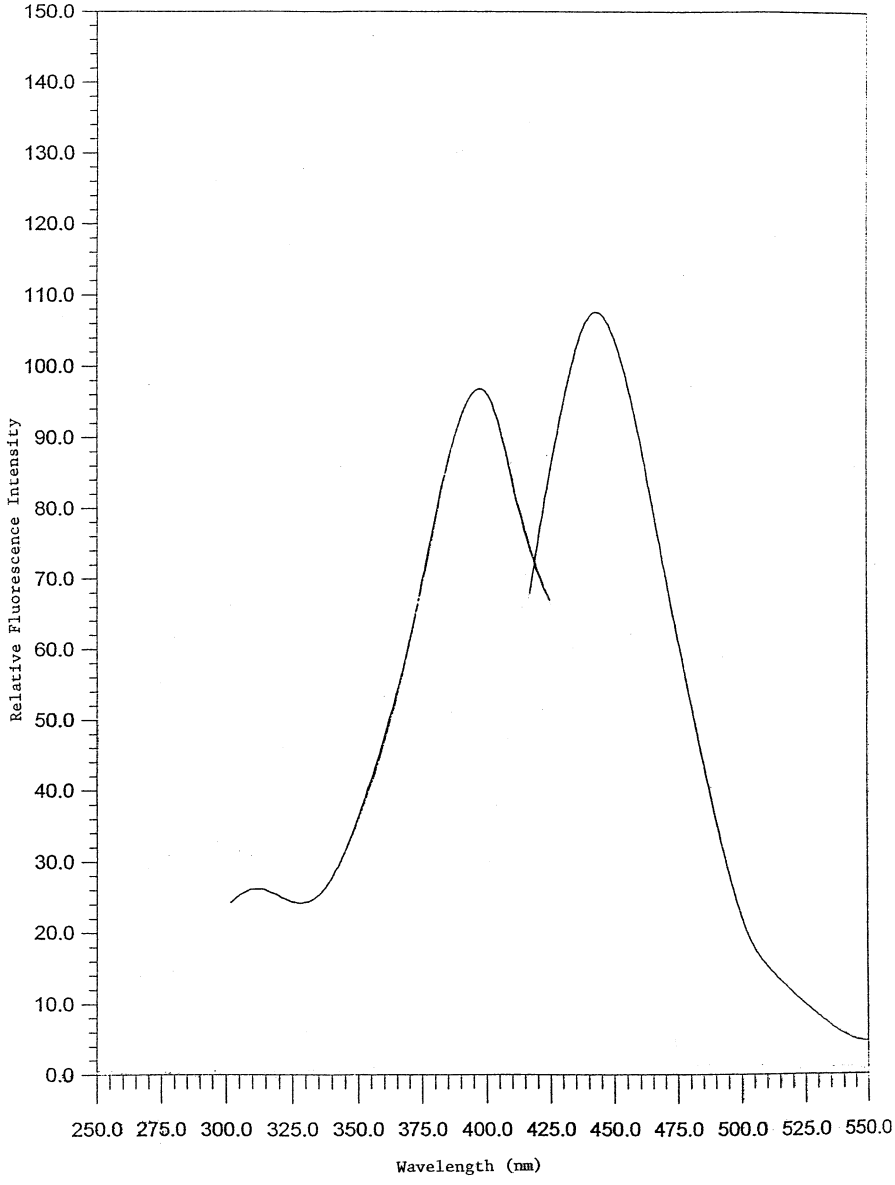


Fig. 2. Fluorescence spectra of the reaction product of terfenadine with mixed anhydride: (a) excitation spectrum; (b) emission spectrum.

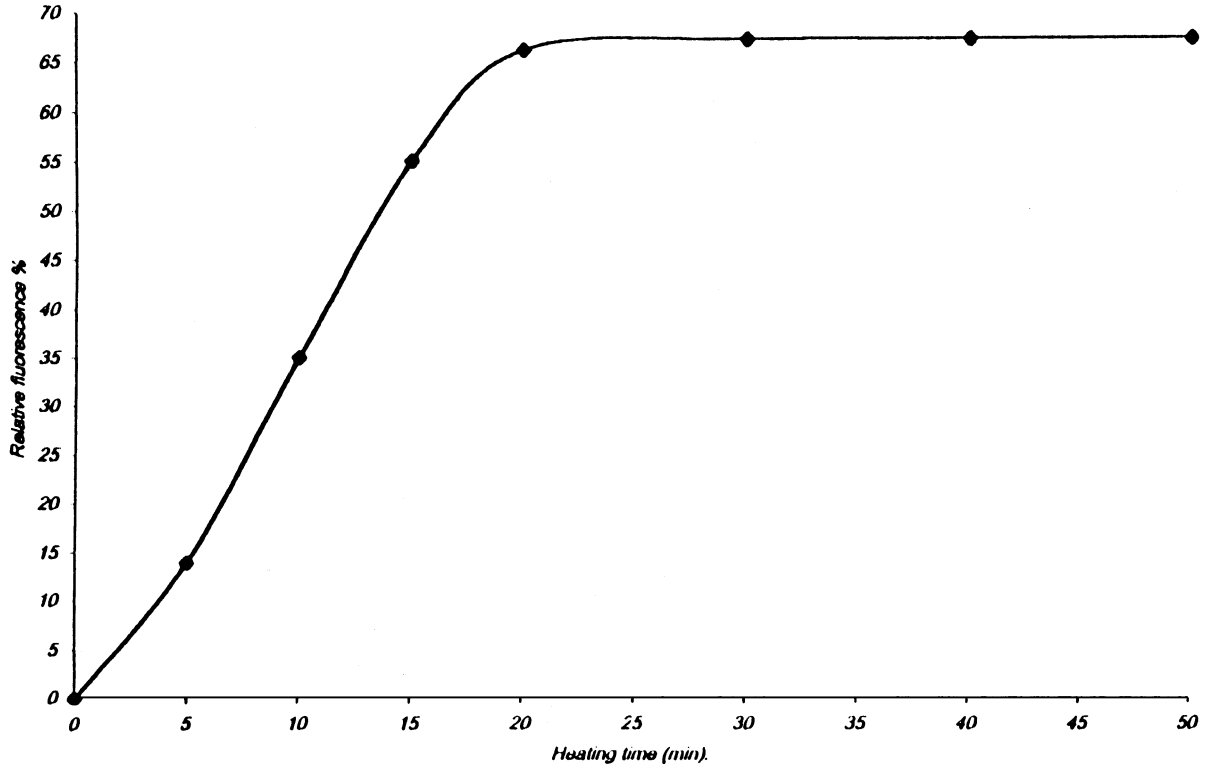


Fig. 3. Effect of heating time on the fluorescence intensity of the reaction product of terfenadine with mixed anhydride.

detection and determination of terfenadine in biological fluids.

To assess the validity of the method, it was applied to the determination of authentic samples of terfenadine. The results obtained adopting both the spectrophotometric and the fluorimetric methods were in good agreement with those obtained using the official USP [2] method as revealed by statistical analysis of the data [34]. Applying Student's t-test and variance ratio F-test, it is evident that there is no significant difference in the performance of the two methods regarding accuracy and precision (Table 2).

The ruggedness of the proposed method was studied by evaluating the within-day and between-day precision for both the spectrophotometric and fluorimetric techniques. The within-day precision was evaluated through five replicate analyses of the samples at different concentration levels: the mean percentage recoveries were 99.55 ± 0.34 and 99.65 ± 0.69 for the spectrophotometric and fluorimetric techniques, respectively. Similarly, the between-day precision was evaluated on several days up to 6 days. The percentage recoveries were 99.83 ± 0.45 and 99.60 ± 0.71 for the spectrophotometric and fluorimetric techniques, respectively. The robustness of the method is demonstrated by the high versatility of the experimental factors affecting the fluorescence intensity.

The proposed method was then applied to the determination of terfenadine in its dosage forms, tablets and suspension. No interference was encountered from common tablets and suspensions excipients such as talc, starch, lactose, avisil, and magnesium stearate. The results in Table 3 are in good agreement with those obtained by a reference spectrophotometry method [29]. Drugs co-formulated with terfenadine, such as ibuprofen and pseudoephedrine, did not interfere with the assay, as they are derived from the tertiary amino group.

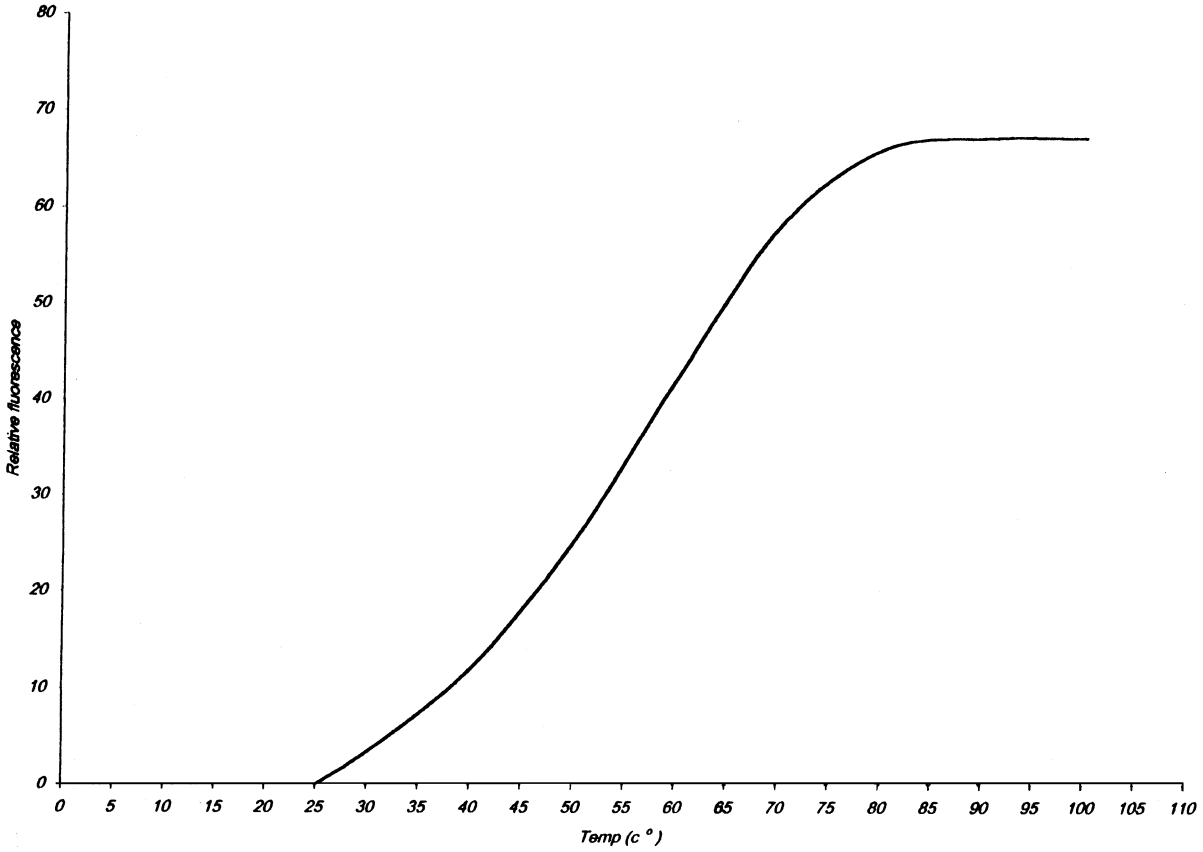


Fig. 4. Effect of temperature on the fluorescence intensity of the reaction product of terfenadine with mixed anhydride.

Table 2
Application of the proposed method to the determination of terfenadine in its pure form

Spectrophotometric method			Spectrofluorimetric method			Official method [2]
μg Taken	μg Found	% Recovery	ng Taken	ng Found	% Recovery	% Recovery
1.0	0.995	99.50	0.5	0.505	101.0	97.6
2.0	2.030	101.50	1.0	0.984	98.7	96.2
3.0	2.963	98.76	1.5	1.479	98.6	99.8
4.0	4.038	100.97	2.0	1.990	99.5	98.0
5.0	4.930	98.00	2.5	2.465	98.6	96.6
			3.0	2.970	99.0	
			3.5	3.524	101.2	
			4.0	3.960	99.0	
$\bar{X}$		99.75			99.45	97.55
S.D		±1.196			±1.09	±1.41
		$t = 1.425$ (2.78)[a]			$t = 2.15$ (2.37)[a]	
		$F = 1.3$ (6.39)[a]			$F = 2.47$ (4.12)[a]	

[a] Tabulated values of t and F-test at 95% confidence limit.

Table 3
Application of the proposed method to the determination of terfenadine in dosage forms

Preparation	% Recovery	
	Proposed method	Method [29]
Teldane[a] tablets mg of terfenadine/ tablet)	(5099.83 ± 0.75	98.05 ± 0.85
		$t = 3.25$ (5.83)[b] $F = 1.857$(9.25)[b]
Teldane[a] suspension (30 mg of terfenadine/5 ml)	99.65 ± 0.85	97.15 ± 0.58
		$t = 3.99$(5.84)[b] $F = 3.35$(9.28)[b]

[a] Product of Marrion Merrel Dow (France).
[b] The figures in brackets are the tabulated values of t and F at 95% confidence limit.

The proposed method was further applied to the determination of terfenadine in spiked human urine. Terfenadine is administered orally twice daily in a dose of 60-mg tablets (120 mg/day). Assuming that 60% of the administered terfenadine is excreted and 40% is absorbed into blood circulation (volume ~5 l), the maximum concentration (mean ~5 μg/ml) appears in sera 2–2.5 h after the oral administration of 120 mg of the drug [24]. This concentration level (5 ng/ml) lies above the working concentration range (0.5–4 μg/ml) of the proposed method, thus it is suitable for bioanalytical routine applications. The results in Table 4 are satisfactorily accurate and precise.

Table 4
Application of the proposed method to the determination of terfenadine (2 ng) in spiked human plasma[a]

ng Found	% Recovery
1.952	97.60
1.971	99.55
2.015	100.75
1.990	99.50
1.972	98.6
1.981	99.05
2.051	102.55
1.943	97.15
1.975	98.75
2.020	101.00

[a] $\bar{X} = 99.45$; S.D. = ± 1.63.

The major metabolic pathway of terfenadine is the cleavage of the *N*-alkyl chain to yield the parent azacyclonol (α,α-diphenyl-4-piperidine-methanol, AZA) which is formed by the action of the enzyme cytochrome P 450 [32]. This product is devoid of the tertiary amino group and would not react with MAA, thus confirming specificity of the method. Similarly, the precursor of terfenadine, 4-hydroxyldiphenylmethylpiperidine, which is secondary amino derivative would not interfere with the assay [33].

In conclusion, a highly sensitive and simple method was developed for the determination of terfenadine in pharmaceuticals and plasma. The method is suitable for monitoring terfenadine at therapeutic levels and can be considered as an alternative for HPLC.

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