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Sensitive spectrofluorimetric and spectrophotometric methods for the determination of thonzylamine hydrochloride in pharmaceutical preparations based on coupling with dimethylbarbituric acid in presence of dicyclohexylcarbodiimide

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Abstract

Two sensitive and selective spectrophotometric and spectrofluoimetric procedures were developed for the determination of thonzylamine hydrochloride (THAH) in tablets and nasal drops. The methods are based on König reaction which resulted in an orange-yellow fluorescent product. The orange-yellow product of the interaction between the dicyclohexylcarbodiimide (DCC), THAH and dimethylbarbituric acid (DMBA) showed an absorption maximum at 492 nm, a first-derivative signal at 494 nm and a fluorescence emission peak at 518 nm ($\lambda_{ex} = 492$ nm). The orange-yellow color was found to be stable for at least 2 h. The reaction conditions were studied and optimized. The reaction obeys Beer's law over the ranges 8–20 and 0.2–2.0 μg ml^{-1} for the derivative spectrophotometric and fluorimetric measurements, respectively. The detection limits were found to be 0.29 and 0.018 μg ml^{-1} for the spectrophotometric and fluorimetric measurements, respectively. The proposed methods were applied to the analysis of pharmaceutical formulations containing THAH, either alone or in combination with naphazoline nitrate.

Keywords: Thonzylamine hydrochloride; König reaction; Spectrophotometry; Spectrofluorimetry

1. Introduction

Thonzylamine hydrochloride (THAH) is an ethylenediamine derivative with the action and uses of the histamine H_1-receptor antagonists and is given for the symptomatic relief of hypersensitivity disorders [1]. It is co-formulated with naphazoline in a nasal preparation to provide a decongestant action especially in allergic diseases.

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Few analytical procedures are available in the literature for the analysis of THAH. Early on, a gas chromatographic method for determination of several antihistamines in dosage forms has been described [2]. A fluorimetric assay for THAH [3] has been reported: the method is based on the oxidation of THAH with sodium periodate to produce *p*-anisaldehyde which reacts with *o*-aminothiophenol to give 2-(4-methoxyphenyl) benzothiazole. The latter substance exhibits a strong fluorescence in an acid solution. Direct assays for simultaneous evaluation of THAH in presence of either *N*-methyl-5,6-benzoquinoline methylsulfate or naphazoline nitrate in pharmaceutical preparations, based on the third-order UV derivative measurement, have been reported [4,5]. Determination of antihistamines in pharmaceuticals by capillary electrophoresis [6] with on-column UV detection has been published. Another capillary zone electrophoresis procedure [7] has also been reported to screen antihistamines in whole blood.

The cleavage reaction of the pyridine ring to yield glutaconic aldehyde, the so-called König reaction, has been the subject of many studies. Wilchek et al. have developed a colorimetric method for determination of carbodiimides [8]. In such a case the dimethylbarbituric acid reagent (dimethylbarbituric acid dissolved in a mixture of pyridine and water (8:2)) was added to a solution of carbodiimide and the reaction was allowed to continue at room temperature for $\sim$45 min. The vital step in that reaction is the attack of carbodiimide on the pyridine ring and its subsequent cleavage to yield glutaconic aldehyde which reacts with dimethylbarbituric acid to develop a purple red colored chromophore. By using dicyclohexylcarbodiimide as a reagent, a fluorimetric method for the determination of malonic acid has been developed [9]. The reagent combined with the acid to afford *N*,*N*-dicyclohexylbarbituric acid which reacted with glutaconic aldehyde, generated from the reaction of dicyclohexylcarbodiimide (DCC) with pyridine, to form a fluorophore.

Further, Chen has described König reaction for determination of some pyridyl and pyrimidinyl compounds [10] by using DCC and dimethylbarbituric acid (DMBA) as reagents. DCC breaks the pyridine or pyrimidine ring to afford glutaconaldehyde or malonaldehyde and then reacts with DMBA to produce chromophores. The reaction was carried out in methanolic solution at 45°C for 40 min. The preliminary test on the examined compounds (pyridine, pyrimidine, 2-aminopyrimidine, pyridazine, sulfadiazine, sulfamerazine, thiamine hydrochloride, chlorpheniramine maleate and isoniazide) indicated that sulfadiazine afforded the strongest absorption intensity and hence it as used as a model compound to optimize the reaction conditions. The quantitative linear range was also given for each compound. However the reaction has not been applied to the analysis of the interested compounds in pharmaceuticals.

Later, extended application of König reaction for the determination of pyridine derivatives has been reported. Pyridine derivatives with a hydrogen in at least one of the positions adjacent to the heterocyclic nitrogen react with cyanogen bromide [11] to produce a derivative of glutaconic aldehyde. By coupling of the latter with an arylamine, a polymethylene dye can be obtained. Various coupling agents have been examined, including *p*-aminoacetophenone, aniline, *p*-aminophenol, sulphanilic acid and *p*-toluidine. The effect of presence of surfactants on the analytical procedure has also been studied [11]. Aniline was demonstrated to be superior to other coupling reagents when used in sodium dodecyl sulphate micellar solutions. The attack of the pyridine ring with cyanogen bromide to produce a glutaconic aldehyde was not affected by the presence of sodium dodecyl sulphate, but the yield of the coupling reaction was largely increased. The application of the reaction was made to the determination of nicotinic acid in pharmaceuticals.

In our study we present the use of König reaction for the development of colorimetric and fluorimetric assays of THAH. The work aimed to optimize the reaction of THAH, as a pyrimidinyl drug, with DMBA in presence of DCC to attain maximum sensitivity of the analytical signal. The applicability of the developed methods was evaluated through the determination of THAH in bulk form and in pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

Fluorescence spectra and measurements were taken on a Perkin-Elmer 650-10S spectrofluorimeter, equipped with 1-cm quartz cell, a 150-W Xenon arc lamp, excitation and emission grating monochromators and a Perkin-Elmer recorder model 56. Slit-widths for both monochromators were set at 10 nm. The scan speed was set at 120 nm min^{-1}. The emission intensity measuring system of the instrument was calibrated daily by using the Perkin-Elmer set of fluorescent polymer blocks.

Spectrophotometric spectra were performed using a Perkin-Elmer Model 550S UV-VIS spectrophotometer with 1-cm quartz cuvettes and a Hitachi Model 561 recorder.

2.2. Materials and reagents

Thonzylamine hydrochloride and 'Tonamil' tablets (labeled to contain 25 mg THAH per tablet) were kindly donated by ECOBI, Ronco Scrivia, Italy. 'Collirio Alfa' nasal drops (labeled to contain 10 mg of THAH and 8 mg of naphazoline nitrate per 10 ml, the excipients including sodium chloride, sodium citrate, sodium edtate, benzalkonium chloride and menthol) were obtained from the market (Bracco, Italy)

For the dicyclohexylcarbodiimide (Aldrich, Gillingham, Germany) solution, 40 μg μl^{-1} solution in dimethylformamide was prepared. For the dimethylbarbituric acid (Aldrich, Gillingham, Germany) solution, 20 μg μl^{-1} solution in dimethylformamide was freshly prepared. For standard thonzylamine hydrochloride solution, 5 μg μl^{-1} solution in dimethylformamide:water (9:1) was prepared. Further dilutions were made with the same solvents mixture.

2.3. General procedure

A 200-μl aliquot of THAH standard solution (containing 1.0–100.0 μg) in dimethylformamide:water (9:1) was mixed with 250 μl of DCC solution in a glass-stoppered test tube. The mixture was then heated at 95°C for 60 min. Subsequently, 250 μl of DMBA solution was added and the reaction mixture was further heated at 95°C for 45 min. Quantitative transfer to a 5-ml calibrated flask and dilution to final volume was made with dimethylformamide.

2.3.1. Spectrophotometric measurements

The absorbance values (at 492 nm) and the first derivative (^1D) spectra were measured for all the solutions against the corresponding reagents blank. The ^1D spectra were obtained using the following parameters: scan speed, 120 nm min^{-1}; chart speed, 60 mm min^{-1}; mode ^1D; response time, 6 s; wavelength range, 540–420 nm and ordinate maximum and minimum settings, $\pm$ 0.02. The ^1D values were measured at 494 nm.

2.3.2. Spectrofluorimetric measurements

The fluorescence intensities were measured at λ_{em} 518 nm ($\lambda_{ex} = 492$ nm).

2.4. Tablets

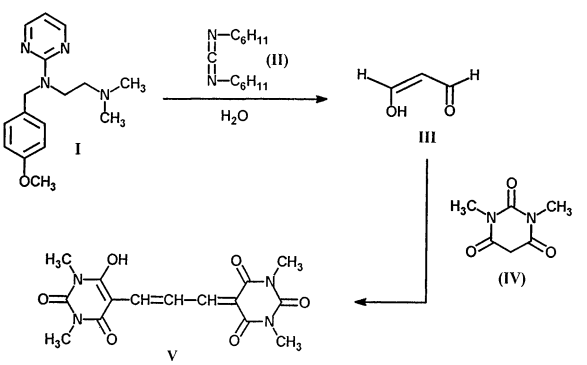
A total of 20 tablets were massed and powdered. To a quantity of the powder containing the equivalent of 250 mg of THAH, 25 ml of dimethylformamide were added. The mixture was stirred for 15 min, then filtered into a 50-ml calibrated flask. The residue was washed with two 10-ml portions of dimethylformamide and washings were added to the filtrate and diluted to volume. Dilution steps were made with dimethylformamide:water (9:1) to obtain a suitable concentration in the range from 1.0 to 100.0 μg per 200 μl of solution. The resulting solutions were analysed as described under Section 2.3.

2.5. Nasal drops

In a 10-ml calibrated flask, a 1.0-ml volume of nasal drops solution, equivalent to 1.0 mg of THAH, was diluted to volume with dimethylformamide (to keep the dimethylformamide:water ratio at 9:1). The practical steps were followed as described under Section 2.3.

3. Results and discussion

The early investigation on the carbodiimides as a cleaving agent on pyridine to afford glutaconaldehyde has been proved by Wilchek et al. [8]. Later, the cleavage of pyrimidine ring, in pyrimidine derivatives, by DCC to give malonaldehyde has been ascertained by Chen [10]. The latter has developed a colorimetric method for determination of some pyrimidinyl compounds based on the chromophore produced from the reaction of DMBA and malonaldehyde derived from cleavage of pyrimidine ring. The reaction conditions have been optimized using sulphadiazine as a model compound. The procedure followed indicated a good sensitivity (2.50 μg ml^{-1} of sulphadiazine gave an absorbance value of 0.3). However, a much lower sensitivity has been obtained with the other examined pyrimidinyl compounds.



Scheme 1. bxsksxk

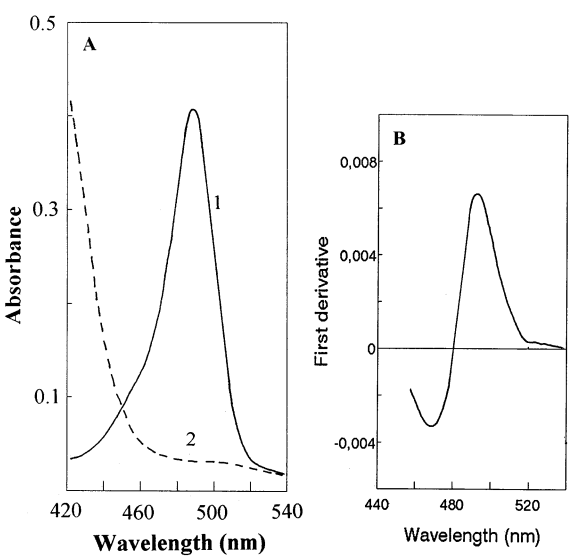


Fig. 1. (A) Absorption spectra of (1) the reaction product of THAH (8.0 μg ml^{-1}) with DCC and DMBA in dimethylformamide and (2) the reagent blank. (B) the corresponding first-derivative spectrum using 1.2 μg ml^{-1} of THAH.

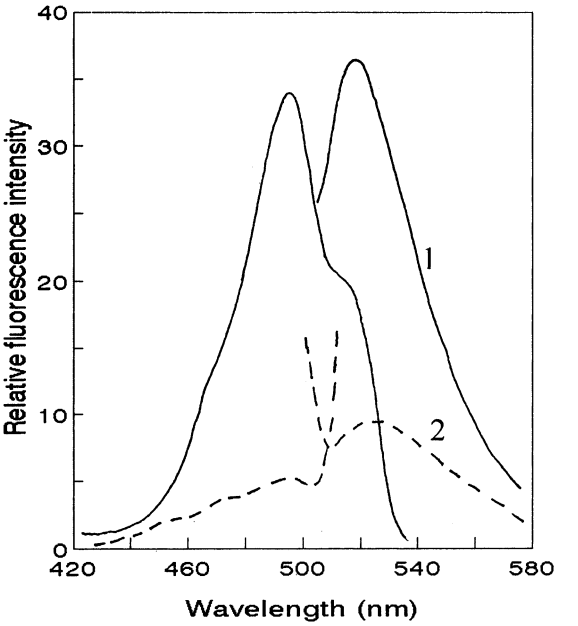


Fig. 2. Excitation and emission spectra of (1) the reaction product of THAH (1.0 μg ml^{-1}) with DCC and DMBA in dimethylformamide and (2) the reagent blank.

Being a pyrimidinyl compound, THAH (I) has been found to react with DCC (II) to give malonaldehyde (III). The latter upon coupling with DMBA (IV) led to the formation of an orange-yellow product (V), see Scheme 1. The absorption spectrum of the reaction product is characterised by a broad peak with a maximum at 492 nm (molar absorbtivity = 1.6×10^4 l mol^{-1} cm^{-1}) (Fig. 1A). The colorimetric method depends on measurement of either absorbance at 492 nm or the first derivative amplitude at 494 nm of the reaction product (Fig. 1B). Considering the spectrofluorimetric method, excitation of this colored reaction product at 492 nm produces fluorescence emission peak at 518 nm with good sensitivity (Fig. 2).

3.1. Optimization of the analytical procedure

Cleavage of THAH with DCC to the corresponding malonaldehyde and coupling of the latter with DMBA to yield a chromophore was studied spectrophotometrically. The preliminary experiments carried out on THAH, following the previously described conditions [10], indicated that no color was developed upon heating the reaction mixture (THAH, DCC and DMBA) in methanol at 45°C for 40 min. Accordingly some modifications in the reaction conditions were set and the influence of some variables on the reaction was tested to establish the most favorable conditions to achieve maximum color development in the determination of THAH.

Table 1
Effect of DCC concentration on the reaction of 200 μl containing 87.2 μg of THAH with 250 μl of 20 μg μl^{-1} solution of DMBA[a]

DCC solution, μg μl^{-1}	A_{492}
10	0.346
15	0.381
20	0.418
25	0.458
30	0.502
35	0.557
40	0.623
45	0.624

[a] The first-step reaction is at 95°C for 30 min and the second-step reaction is at 95°C for 30 min.

Table 2
Effect of DMBA concentration on the reaction of 200 μl containing 87.2 μg of THAH with 250 μl of 40 μg μl^{-1} solution of DCC[a]

DMBA solution, μg μl^{-1}	A_{492}
2.5	0.161
5	0.291
10	0.402
15	0.520
20	0.611
25	0.62

[a] The first-step reaction is at 95°C for 30 min and the second-step reaction is at 95°C for 30 min.

3.1.1. Effect of solvent and water content of reaction mixture

The reaction with DCC reagent is usually influenced by the solvent used in the reaction mixture [9]. Some solvents were examined in the preliminary study, like acetonitrile, methanol, ethanol and dimethylformamide, of which, dimethylformamide gave the highest absorbance value. Accordingly dimethylformamide was chosen as the working solvent to continue the study.

The vital step in the reaction is the attack of DCC on the pyrimidine ring of THAH and its subsequent cleavage to yield malonaldehyde. This step requires the presence of a certain amount of water. The investigation revealed that $\sim 10\%$ water in the analyte solution was optimal. Increasing the water content to 40% resulted in 85% decrease in the color intensity.

3.1.2. Effect of DCC concentration (first-step)

Different concentrations of DCC were tested keeping other conditions constant. As shown in Table 1, 250 μl of 40 μg μl^{-1} DCC is sufficient for the cleavage of pyrimidine ring and formation of malonaldehyde.

3.1.3. Effect of DMBA concentration (second-step)

The effect of DMBA was similarly investigated by taking various concentrations of DMBA. It was observed that 250 μl of 20 μg μl^{-1} DMBA gave maximum absorbance in its coupling with the malonaldehyde derivative formed, as shown in Table 2.

3.1.4. Effect of time and temperature

To study the effect of time and temperature on the cleavage reaction, the reaction mixtures (THAH and DCC) were heated to 60–95°C for 10, 20, 40, 60, 90, or 120 min. Similar procedure was followed after the addition of DMBA reagent to attain optimum condition for the coupling step. Fig. 3A,B represents the time courses of the first- and second-step reactions at different temperatures, respectively. It was found that heating at 95°C for 60 min in the first step and 45 min in the second step gave maximum A_{492} with no further enhancement in color intensity at longer heating time.

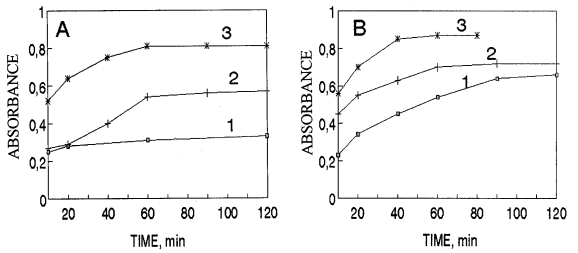


Fig. 3. (A) Time courses of the first-step reaction at different temperatures, 60°C (1), 75°C (2) and 95°C (3) (the second-step reaction was at 95°C for 30 min). (B) Time courses of the second-step reaction at different temperatures, 60°C (1), 75°C (2) and 95°C (3) (the first-step reaction was at 95°C for 60 min). In (A) and (B) 87.2 μg of THAH were used in the reaction.

Although a longer heating time and a higher temperature were needed in the reaction relative to that described in the early study of Chen, a nearly similar sensitivity was attained: 6.5 μg ml^{-1} of THAH gave an absorbance value of 0.3.

3.1.5. Two-steps reaction

It should be noted that in the previous study of Chen [10], both reagents (DCC and DMBA) were mixed at once, in one step, with the analyte. In our work, it was observed that the two-steps reaction is most appropriate and enhances the color intensity (double the absorbance value).

3.1.6. Effect of diluting solvents

Dimethylformamide-ethanol, dimethylformamide-methanol, dimethylformamide and acetonitrile were tested as diluting solvents. Acetonitrile was not suitable as the colored condensation product was turbid. The other solvents media show no significant difference in absorbance readings. However, the fluorescence emission from solutions in dimethylformamide gave the highest measurements. Accordingly dimethylformamide was chosen for the current study.

3.2. Stability

The stability of the reaction product was examined for ~ 2 h. The A_{492} measurements (15-min interval) show no variation over at least 2 h.

3.3. Statistical analysis of results

3.3.1. Concentration ranges and calibration graphs

Using the optimized reaction conditions, the A_{492}, $^1D_{494}$, and the fluorescence emission at 518 nm ($\lambda_{ex} = 492$ nm) were found to be linearly correlated to the THAH concentration. Data recorded in Table 3 summarizes the characteristics of the calibration plots.

Table 3
Analytical features of thonzylamine hydrochloride-reaction product

Parameter	Spectrophotometry		Spectrofluorimetry
	A_{492}	$^1D_{494}$	
Molar absorptivity, l mol^{-1} cm^{-1}	1.6×10^4		
Sandell's sensitivity, μg cm^{-2} $(0.001\ A)^{-1}$	0.02		
Linear range (μg ml^{-1})	8–20	0.5–5	0.2–2
Regression equation ($a+bC$)			
Intercept (a)	−0.0503	−0.00043	−1.045
Slope (b)	0.0489	0.0055	25.801
Correlation coefficient (r)	0.9990	0.9992	0.9990
% RSD[a]	1.97	1.71	1.30
Confidence limit ($P = 0.05$)	±0.013	±0.00016	±0.689
Quantification limit (μg ml^{-1})	0.97	0.5	0.06
Detection limit (μg ml^{-1})	0.29	0.15	0.018

[a] Six replicate analyses.

Table 4
Determination of thonzylamine hydrochloride in the presence of naphazolinenitrate

THAH[a] (μg ml^{-1})	Found ± S.D. (μg ml^{-1})[b]		RSD (%)[c]	E_r (%)[d]
	A_{492}	$^1D_{494}$		
Spectrofluorimetric determination				
10	10.08 ± 0.057		0.57	0.84
2		2.003 ± 0.0.011	0.55	0.14
Spectrofluorimetric determination				
2	2.004 ± 0.011	0.54	0.65	

[a] Naphazoline nitrate was added in a ratio of 1:1 at the indicated concentration level of THAH.
[b] Mean ± S.D. for five determinations.
[c] Percentage relative standard deviation.
[d] Percentage relative error.

3.3.2. Detection and quantification limits

In accordance with the formula given by Miller [12], the limit of detection, LOD = 3 s/k, where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the latter formula, the detection limits obtained for the absorbance, derivative spectrophotometric and spectrofluorimetric measurements were found to be 0.29, 0.15 and 0.018 μg ml^{-1}, respectively. The limits of quantitation, LOQ, defined as 10 s/k, were found to be 0.97, 0.5 and 0.06 μg ml^{-1} for the same measurements, respectively (Table 3).

3.3.3. Specificity, precision and accuracy

Taking the advantage of the specificity of König reaction to the pyridine and pyrimidine rings, selective determination of THAH in the presence of co-formulated drug, naphazoline nitrate could be possible. In order to assess the precision, as percentage relative standard deviation (RSD%) and the accuracy, as percentage relative error (E_r%) of the proposed method, five replicate determinations were carried out on synthetic mixtures. The data shown in Table 4 indicate good accuracy and precision of the proposed procedure for the analysis of THAH. Naphazoline nitrate shows no interference.

Table 5
Assay results for thonzylamine hydrochloride in pharmaceutical preparations

	Spectrophotometric		Spectrofluorimetric
	A_{492}	$^1D_{494}$	
Tonamil tablets (labelled to contain 25 mg THAH per tablet)			
Found[a] (± S.D.)	24.95 ± 0.23	25.03 ± 0.29	24.94 ± 0.17
t (2.3)[b]	0.079	0.58	
F (6.39)[b]	1.92	2.95	
Collirio alfa drops (labelled to contain 1 mg THAH per 1 ml)			
Found[a] (± S.D.)		1.015 ± 0.0055	1.012 ± 0.0034
t (2.3)[b]		1.04	
F (6.39)[b]		2.61	

[a] Average of five determinations.
[b] Values in parenthesis are the theoretical values at $P = 0.05$.

3.4. Analysis of pharmaceutical formulations

The proposed methods were applied to the determination of THAH, either alone or in combination with naphazoline nitrate. From the results shown in Table 5, the methods gave satisfactory recovery data. Also the standard deviations for the assays results show good precision.

The performance of the spectrophotometric measurements were statistically compared with that of the spectrofluorimetric method by Student's t-test and variance ratio F-test. The calculated (experimental) t-values and F-values did not exceed the tabulated (theoretical) values, indicating that there was no significant difference between the methods compared. This suggested that the two methods are equally applicable.

4. Conclusion

In this context, selective and precise methods for colorimetric and fluorimetric determination of THAH were established. Being specific, of good accuracy and high precision, the proposed methods are suitable for the routine analysis and quality control of THAH in various dosage forms.

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