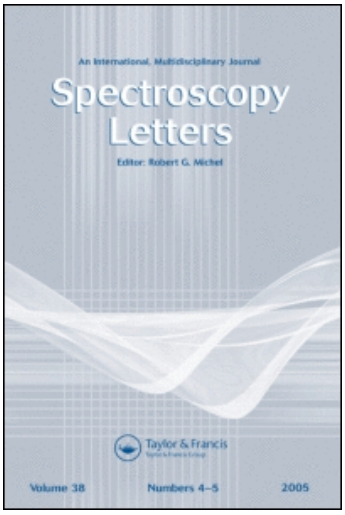

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Stability-Indicating Methods for the Determination of Cimetidine Using Derivative and Fourier-Transform Infrared Spectrophotometry

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Abstract

Two different techniques, that is, second (D_2) and third (D_3) derivative spectrophotometry and fourier transform infrared (FTIR) spectrophotometry, are investigated for the selective determination of the intact cimetidine in presence of up to 25% and 75% of its degradates, respectively. The procedures determine 2-10 $\mu g\ ml^{-1}$ at 224 nm and 217.5 nm by D_2 and D_3, respectively, and 1-6 mg per 250 mg in K Br by the FTIR with mean accuracies of 100.0 $\pm$ 1.07%, 100.5 $\pm$ 0.87% and 100.3 $\pm$ 1.16%, respectively. The drug is successfully analysed in its pharmaceutical formulations.

Introduction

Cimetidine (I) {2- Cyano – 1 – methyl – 3 -{2 – (5 – methyl imidazol – 4 – ylmethylthio) ethyl} guanidine} is a histamine – H_2

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receptor antagonist and is widely used in the treatment and prophylaxis of GI ulcers [1,2].

In acid medium, it hydrolyses into the amide derivative (II) at ambient temperature, and into the guanidine derivative (III) at elevated temperature[2,3]; scheme 1.

Various procedures have been reported for the assay of the drug, including titrimetry[1,4], spectrophotometry[5,6] and polarography[7]. Nevertheless, chromatography[3,8] is the only applicable technique to study and quantitate the degradation of the drug.

This article demonstrates the potential of derivative spectrophotometry and FTIR spectroscopy as useful analytical tools for the direct, selective determination of the intact cimetidine molecule in presence of its acid-hydrolysis products in pharmaceutical formulations.

Experimental

Materials and Reagents

All chemicals used were of AR grade and solvents were of spectroscopic grade.

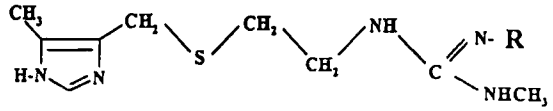
Cimetidine reference standard, kindly supplied by Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt. Its purity was checked by TLC[1,9].

Tagamet tablets, each containing 200 or 400 mg of cimetidine base per tablet, B.N. 2661 or 210189 (Kahira Pharm. and Chem. Ind. Co.), respectively, and Histodil injections containing 200 mg of cimetidine base as hydrochloride per 2 ml, B.N. 70195 (Chemical Works of Gedeon Richter Ltd., Budapest, Hungary) were purchased from the local market.

Standard cimetidine solutions; 5 mg ml^{-1}, and 50 μg ml^{-1} of cimetidine base dissolved in methanol.

Apparatus

Shimadzu UV-Vis (UV – 160IPC) and Perkin-Elmer FTIR (System 2000) spectrophotometers were used. Jenco digital pH / MV / Temp. ATC Meter (Model 5005) with a double junction glass electrode was also used.



Cimetidine (I) $-C\equiv N$ (R)

Amide (II) $-C(=O)-NH_2$

Guanidine (III) H

Scheme 1

Procedures

1- Preparation of cimetidine degradation products[2,3]:

A. The amide derivative (II) – Dissolve 0.5 gm of pure cimetidine (I) in 20 ml of 1 N HCl acid and set aside at room temperature in a stoppered flask for 7 days, protected from light. Neutralize with 1 N NaOH and evaporate to dryness under vacuum, taking care to avoid prolonged heating (not more than 15 min.), otherwise the formed amide (II) will be further hydrolysed into the guanidine derivative (III). Extract the residue twice, each with 40 ml of methanol, filter into a 100-ml volumetric and complete to volume with methanol.

B. The guanidine derivative (III). Dissolve 0.5 gm of pure cimetidine (I) in 20 ml of conc. HCl and reflux at 100°C for 10 hrs. Cool, neutralize with 7 N Na OH and evaporate under vacuum. Extract the residue twice, each with 40 ml of methanol, filter into a 100 ml volumetric flask and complete to volume with methanol.

The methanolic solution obtained from procedures "A" and "B", containing the degradates (II) and (III) derived from 5 mg ml^{-1} of cimetidine base, are used directly for the FTIR procedure. To apply the

derivative procedures, dilute one ml of each solution up to 100 ml with methanol, to obtain solutions of each of (II) and (III) corresponding to 50 $\mu g\ ml^{-1}$ of cimetidine.

2- Construction of calibration curves

A. Derivative spectrophotometric procedures- Transfer aliquot volumes of the standard methanolic solution of cimetidine (50 $\mu g\ ml^{-1}$), corresponding to 0.1 – 0.5 mg, into a series of 50 ml volumetric flasks and complete to volume with methanol. Record the second (D_2) and the third (D_3) derivative spectra for each, against methanol as a blank, at ordinate values of ± 0.006 for D_2 and ± 0.001 for D_3 using $\Delta\lambda$ equal to 8. Measure the absolute troughs' amplitude in mm at 224 nm and 217.5 nm, respectively. Construct calibration curves relating the measured heights in mm versus the drug concentration in $\mu g\ ml^{-1}$.

B. FTIR Procedure- From the standard methanolic solution of cimetidine (5 $mg\ ml^{-1}$), pipette a volume corresponding to 1 – 6 mg of (I) into an IR mortar containing an accurately weighed quantity of K Br so as to keep the total final weight equivalent to 250 mg. Evaporate the solvent, with the aid of a current of air, triturating well during drying, and complete drying in a vacuum oven at 50°C (about 5 min.). Prepare 2 discs (each weighing 50 mg and of 13 mm diameter) from the completely dried, well mixed powder. Record the IR spectrum of each, at a resolution of 4 cm^{-1} using strong apodisation and taking about 100 scans.

Calculate the average absorbance of the band at 2178 cm^{-1}, applying the base-line technique[10-12] as illustrated in fig. 2.a, using the formula:

$$A = \log I_o/I$$

Where I_o and I represent the intensities of the incident and transmitted radiations, respectively.

Construct a calibration curve relating absorbances versus drug concentrations in mg (I) per 250 mg total final weight in K Br. The slope of the rectilinear curve is not always reproducible for different runnings.

For this, it is necessary to check the slope every time, by carrying out two experiments of known concentrations; otherwise, a new calibration curve has to be constructed, simultaneously with the unknown sample.

3- Determination of cimetidine in laboratory – prepared mixtures with its degradation products

A. Derivative spectrophotometric procedures- Mix volumes containing 475-325 μg of pure cimetidine from its standard methanolic solution (50 μg ml^{-1}) with mixture of volumes of equal concentrations of its degradates solutions (II) and (III), each corresponding to 12.5 – 87.5 μg of the intact drug (I), into a series of 50 ml volumetric flasks and complete to volume with methanol. Proceed as detailed under "2- A.", starting with the words "Record the second (D_2) and third (D_3) derivative spectra ....".

Determine the content of (I) in the mixtures from the following regression equations:

$$Y_2 = 0.15 + 5.89\,X\,;\ r = 0.998$$

$$Y_3 = 0.81 + 5.05\,X\,;\ r = 0.976$$

Where "Y_2" and "Y_3" are the heights in mm at 224 nm and 217.5 nm in the D_2 and D_3 spectra, respectively, "X" is the concentration of (I) in μg ml^{-1} and "r" is the correlation coefficient.

B. FTIR Procedure- Accurately measure a volume containing 6–2 mg of pure cimetidine (I) in methanolic solution and mix with volumes of each of its degradates (II) and (III) solutions of equal concentrations, each corresponding to 1-3 mg of the intact drug (I), in an IR mortar containing an amount of potassium bromide accurately weighed so as to maintain the total final weight equal to 250 mg . Proceed as described under "2- B." starting with the words "Evaporate the solvent .....".

Determine the content of the drug in each mixture from a previously prepared and checked or from a simultaneously prepared calibration graph.

4- Application to pharmaceutical formulations

Tagamet tablets

Weigh, finely powder and mix thoroughly 20 tablets. Transfer an accurate weight of the powder claimed to contain about 250 mg of (I) into a 50 ml volumetric flask. Add about 40 ml of methanol, shake thoroughly using an ultrasonic shaker for 15 min., then dilute to volume with methanol and mix well. Centrifuge parts of this solution. The clear solution is assumed to contain about 5 mg ml^{-1} of (I); solution 1. Dilute one ml of solution 1 with methanol up to 100 ml to obtain a solution assumed to contain about 50 $\mu g\ ml^{-1}$; solution 2.

Analyse solution 2 and 1 by the two derivative spectrophotometric and the FTIR procedures, respectively, as mentioned under "2- Construction of calibration curves". Determine the drug content as previously mentioned under procedure "3 A and B".

Histodil injections

A- Derivative spectrophotometric procedures- Mix the contents of ten ampoules and transfer an accurate volume corresponding to about 50 mg of (I) into a 50 ml volumetric flask. Dilute to volume with methanol and mix well; this solution is assumed to contain about 1 mg ml^{-1} of (I). By appropriate dilution with methanol, prepare a solution assumed to contain about 50 $\mu g\ ml^{-1}$ of (I); solution 3.

B. FTIR procedure- From the mixed ampoule solution, transfer an accurate volume corresponding to about 50 mg of cimetidine base (1), into a small dish. Allow to dry in a desiccator over $CaCl_2$ for 24 hrs. Dissolve the residue in about 6 ml of methanol and transfer,

quantitatively, into a 10 ml volumetric flask, wash the dish with about 3 ml methanol and combine to the flask, then complete to volume with methanol and mix well; this solution is assumed to contain about 5 mg ml^{-1} of (I); solution 4.

Proceed as detailed under "Tagamet tablets" starting from "Analyse solution 2 and 1 ....." using solution 3 and 4 instead of 2 and 1.

Results and discussion

Although cimetidine (I) is highly stable in neutral aqueous solutions, yet it partially and slowly hydrolyses in 1 N hydrochloric acid for 7 days at ambient temperature yielding the amide analogue (II). Under more drastic conditions, that is refluxing with conc. Hydrochloric acid at 100°C for 10 hrs., it is completely degraded into the guanidine derivative (III)[2,3]; scheme 1.

The drug was degraded in the laboratory according to the reported conditions[2,3], then tested and identified by TLC, using silica gel 60 F_{254} plates (10 × 10 cm) and ethylacetate – methanol – ammonia 25% (65:20:15) as the mobile phase, then visualised by iodine vapour[1,9]. Complete hydrolysis was detected and the hydrolysate gave only a single spot for each degradate with R_f values of 0.61 and 0.1 for (II) and (III), respectively, whereas the R_f value corresponding to the intact drug was 0.67.

The UV spectra of the methanolic solutions of (II) and (III) have shown severe band overlappings with the principle maxima of the intact drug (I) at 220 nm and 232 nm, in both the zero-order and first derivative curves, respectively; figs. 1.a and 1.b. On the other hand, their D_2 and D_3 spectra revealed that (I) has typical troughs at 224 nm and 217.5 nm respectively, at which contributions of both degradates were greatly minimized; figs. 1.c and 1.d. On the bases of such observations, it would be possible to adopt the D_2 and D_3 spectrophotometry for the direct, selective determination of the intact drug in admixtures with its degradates.

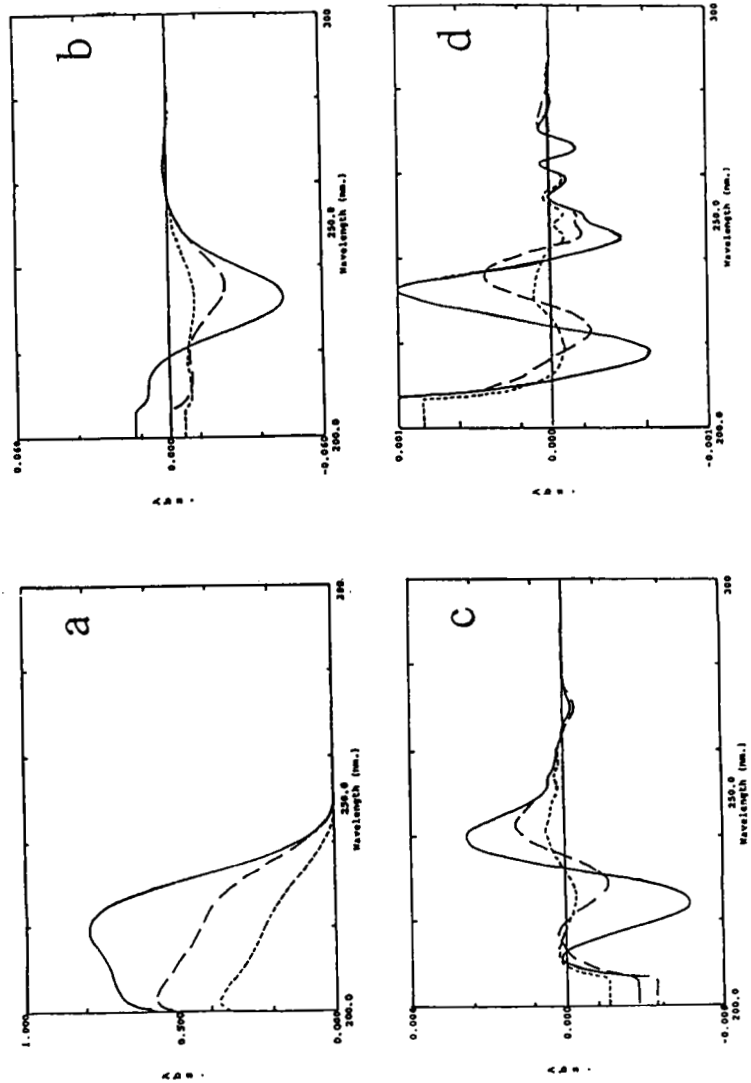


Fig. 1. The UV- spectra [a. zero - order, b. first, c. second and d. third derivative] of 8 μg . ml^{-1} of each of intact cimetidine (——) and degraded cimetidine into amide (– –) or guanidine analogue (- - - -).

Linear relationships between the absolute amplitude heights and the drug (I) concentrations in both the D_2 and D_3 spectrophotometric were found in the range of 2 – 10 $\mu g\ ml^{-1}$.

The IR spectrum of cimetidine (I) has been reported [6]. Among other characteristic bands useful for checking the purity and identity of the drug, a well resolved sharp band is quite obvious around 2178 cm^{-1}, as shown in fig. 2.a corresponding to the cyano group (C ≡ N) in the intact molecule. Fortunately, hydrolysis of cimetidine leads to complete disappearance of this band in the spectra of both degradates; figs. 2.b and 2.c. Thus, the FTIR technique could be advantageously and selectively used for the quantitation of the drug in the presence of its degradates, by calculating the absorbance of the band at 2178 cm^{-1} through the adoption of the base-line technique[10-12].

Application of the three proposed procedures, to different blind experiments of a pure sample of the drug, showed good reproducibilities; 100.0 – 100.5 ± 1.07 – 0.87 % for the D_2 and D_3 techniques and 100.3 ± 1.16 % for FTIR.

To assess the efficiency of the suggested procedures as stability-indicating, both techniques were applied to laboratory prepared mixtures of the intact drug and its two degradates; the amide and guanidine. The results were compared with the procedure reported by Xu et al[5] which depends upon direct measurement of the absorbance of cimetidine solution in absolute ethanol at 219 nm. It is obvious from table (1), that the presence of up to 25% of the mixture of the two degradation products did not affect the accuracies of the two derivative spectrophotometric procedures and up to 75% did not affect the FTIR procedure. However, the compendial procedure[5] gave much higher results.

The drug was successfully analysed by the proposed procedures in its tablets and ampoules, thus revealing no interference by excipients and additives. Upon comparison with the non-aqueous titration of the Indian pharmacopoeial procedure for tablets, the suggested procedures gave results of almost equal accuracies and precisions (table 2); the tablets being recently prepared and undegraded.

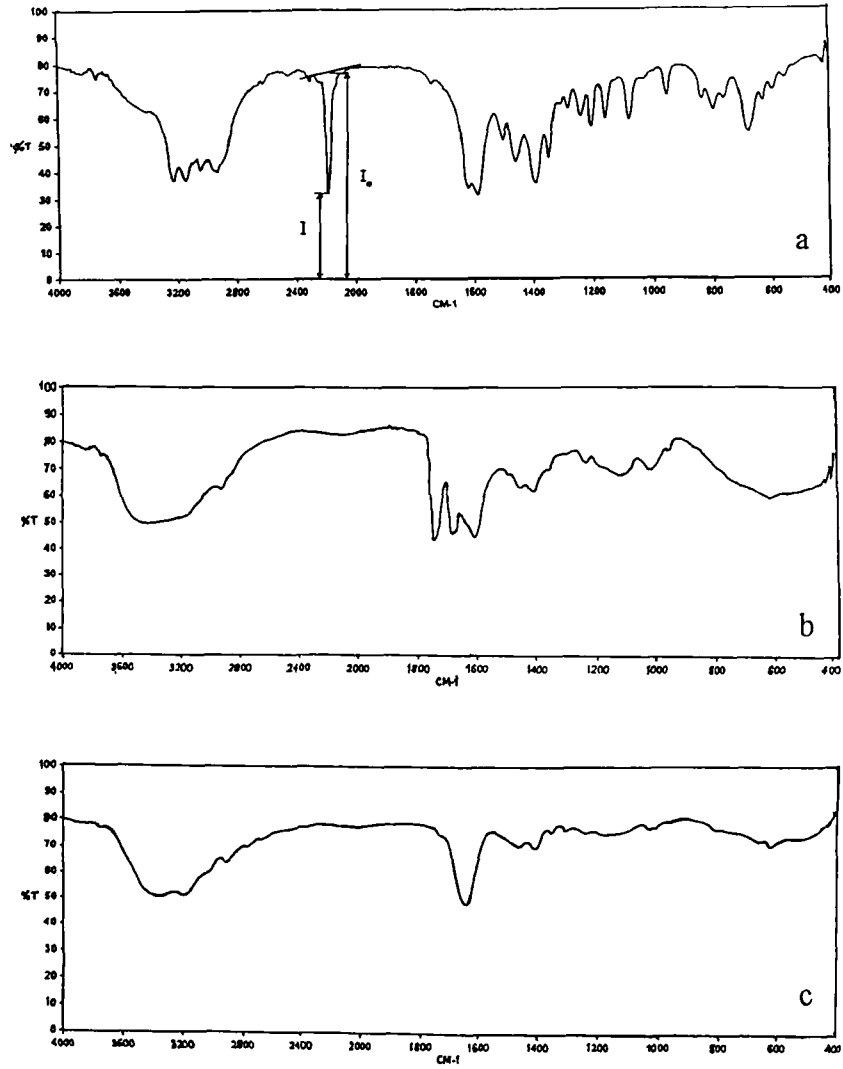


Fig. 2. The FTIR spectra of (4 mg / 250 mg KBr) of each of a. intact cimetidine b. degraded cimetidine into its amide or c. degraded into guanidine analogue.

Table (1) Determination of cimetidine in laboratory prepared mixtures With its degradates by the proposed and compendial[5] procedures.

Derivative spectrophotometric procedures				FTIR procedure			Compendial procedure		
Intact taken $\mu g\ ml^{-1}$	The two * degradates %	D_2 Recovery of intact drug %	D_3	Intact taken mg/250 mg	The two • degradates %	Recovery of intact drug %	Intact taken $\mu g\ ml^{-1}$	The two* degradates %	Recovery of intact drug %
9.5	5	100.7	101.5	6.0	25	97.2	9	10	115.7
9.0	10	99.8	101.3	4.4	45	99.1	8	20	117.6
8.5	15	99.6	100.6	4.0	50	98.5	7	30	132.9
8.0	20	100.5	99.8	3.6	55	97.4	6	40	156.0
7.5	25	101.5	100.1	2.8	65	100.8	5	50	171.4
7.0	30	104.5**	105.9**	2.0	75	99.1	4	60	196.5
6.5	35	113.2**	114.5**						
Mean		100.4	100.7			98.7			
± C.V.		± 0.76%	±0.73%			±1.34%			

* Of total weight
** Rejected

Table (2) The Determination of cimetidine in pharmaceutical Formulations by applying the proposed and the Indian Pharmacopoeial[4] procedures.

Formulation	Derivative spectrophotometric procedures D_2 Means ± C.V	D_3 Means ± C.V	FTIR Procedure Means ± C.V	Official [4] procedure Means ± C.V
Tagamet tablets (200 mg) Assay %	96.0 ± 0.88	95.3 ± 0.76	95.6 ± 1.12	94.0 ± 1.19
Added Recovery %	99.4 ± 0.81	99.9 ± 0.92		
Tagamet tablets (400 mg) Assay %	92.4 ± 0.78	92.9 ± 0.92	93.8 ± 0.67	93.2 ± 1.05
Added Recovery %	100.8 ± 0.87	100.7 ± 1.14		
Histodil injections * (200 mg / 2 ml) Assay %	100.6 ± 0.99	100.9 ± 0.89	101.2 ± 1.33	
Added Recovery %	100.9 ± 0.70	99.6 ± 1.04		

* They could not be also analysed by the official non – aqueous titration procedure[4] since they contain the drug in the form of its hydrochloride.

Standard additions of pure cimetidine to pharmaceutical formulations have shown excellent recoveries by the derivative spectrophotometric technique; table (2). The accuracies of them when applied to two tablet preparations amounted to an average of 99.4 – 100.8 ± 0.81 – 87% by the D_2 procedure and 99.9 – 100.7 ± 0.92 – 1.14% by the D_3 procedure. For the ampoules, the accuracies by the D_2 and D_3 procedures amounted to 100.9 – 99.6 ± 0.70 – 1.04%.

Table (3) Statistical analysis of the results obtained by the Proposed and official[4] procedures upon analysis of pure cimetidine

	Derivative spectrophotometric procedures		FTIR Procedure	Official [4] procedure
	D_2	D_3		
Concentration range	2 – 10 μg ml^{-1}	2 –10 μg ml^{-1}	1-6mg/250mg	250 mg
Mean %	100.0	100.5	100.3	100.1
n	5	5	5	5
S.D.	1.07	0.88	1.16	1.01
Variance	1.145	0.774	1.346	1.020
F (6.4)	1.12	0.76	1.32	-------
t-test (1.86)	0.63	1.8	0.78	-------

Figures in parenthesis are the corresponding theoretical values of F and t (P = 0.05).

Although the derivative spectrophotometric procedures are far more sensitive than the FTIR method, yet the latter is advantageously more selective and more reliable as stability-indicating one; it determines the intact drug selectively, through its cyano group in presence of up to 75% of its degradates.

Statistical analysis of the results obtained by the proposed spectrophotometric procedures and those obtained by the Indian pharmacopeial procedure[4], revealed no significant differences, within a probability of 95% of being correct; table (3). However, the three proposed procedures are far more sensitive than the official one[4], amounting to increased sensitivities of 41.7 – 250 for the FTIR procedure and $2.5 \times 10^4 - 1.25 \times 10^5$ for the D_2 and D_3 procedures. Moreover, they are more selective, since the non aqueous titration of both pharmacopoeias[1,4]does not differentiate between the intact drug and any of its degradates; all being of basic nature.

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