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Short Communication

Determination of cimetidine and related impurities in pharmaceutical formulations by high-performance liquid chromatography

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ABSTRACT

The analytical characteristics of cimetidine tablets were studied. A high-performance liquid chromatographic method was developed in order to assay cimetidine and its related impurities simultaneously. A reversed-phase system and diode-array detector were used.

INTRODUCTION

Cimetidine is a histamine H_2-receptor antagonist. It inhibits gastric acid secretion and other actions of histamine, mediated by H_2-receptors. Clinical trials have shown cimetidine to be of value in the treatment of gastric and duodenal ulcers and in other conditions where gastric acid is involved [1–14]. It is widely used by the oral, intramuscular and intravenous routes. Also, a large number of cimetidine products are commercially available, especially oral products, and in particular tablet formulations. The impurities profiles in these preparations have been widely investigated [15–17]; the maximum acceptable limits of these impurities are reported in some Pharmacopoeias [18–20].

In the pharmaceutical dosage forms, some degradation products can be present. Cimetidine undergoes decomposition through two pathways: hydrolysis and oxidation [21], with the production of N-cyano-N′-methyl-N″-[2-(5-methyl-1*H*-imidazol-4-yl)methyl]sulphinylethylguanidine (**I**), N-methyl-N′-[2-(5-methyl-1*H*-imidazol-4-yl)methylthio]ethylguanidine (**II**) and N-carbamoyl-N′-methyl-N″-[2-(5-methyl-1*H*-imidazol-4-yl)methylthio]ethylguanidine (**III**). As a related impurity, commercial cimetidine contains also N-cyano-N′-[2-(5-methyl-1*H*-imidazol-4-yl)methylthioethyl]-S-methylisothiourea (**IV**), which is the immediate precursor in the synthesis [15].

Different high-performance liquid chromatographic (HPLC) methods have been utilized for determining compounds **I**–**IV** in cimetidine dosage forms [15–17]. Nevertheless, these are not suitable for determining all the mentioned impurities simultaneously [16–17] and they also need two different mobile phases for their separation [15]. Therefore, it was deemed of interest to develop an HPLC method in order to determine simultaneously the above-mentioned impurities and the drug, using only one mobile phase. An HPLC system equipped with a photodiode-array UV detector was utilized for the on-line determination of impurity profiles. The method was used to verify the quality of cimetidine

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tablet formulations commercially available in Italy with respect to their drug and impurity contents.

EXPERIMENTAL

Materials

Twelve different formulations of cimetidine which are commercially available in Italy were studied. The tablets were composed of 200, 400 or 800 mg of cimetidine and several other ingredients.

Cimetidine and related impurities (**I–IV**) were supplied by Smith Kline & French (Milan, Italy). Sodium 1-pentanesulphonate was from Fluka (Buchs, Switzerland). Acetonitrile (HPLC grade) and all the other reagents were from Carlo Erba (Milan, Italy). All solvents used in the HPLC system were solubilized with distilled water treated with a Milli-Q system (Millipore, Milford, MA, USA).

Apparatus

Analytical HPLC was performed using an LKB Model 2249 gradient pump and an LKB Model 2140 rapid spectral detector (Pharmacia–LKB, Uppsala, Sweden) connected to a personal computer (Personal System 2, mod. 30, IBM, Portsmouth, UK). The column was a μBondapak C_{18} (10 μm) (30 cm × 3.9 mm I.D.) from Waters–Millipore (Milford, MA, USA).

Chromatographic conditions

The separation of the tested compounds was achieved using a linear gradient. Solvent A was 0.025 *M* sodium acetate, adjusted to pH 3.50, containing 0.003 *M* sodium 1-pentanesulphonate. Solvent B was 0.025 *M* sodium acetate (pH 3.50) containing 0.003 *M* sodium 1-pentanesulphonate plus 20% (v/v) of acetonitrile. The gradient was linear for 25 min (from 10% to 90% B), then returned to 10% B to allow the column to re-equilibrate. The elution of the compounds was carried out at room temperature with a flow-rate of 1.0 ml/min. The volume injected was 5–50 μl. Detection was effected at 220, 230, 240 and 250 nm.

Sample preparation

Ten tablets were accurately weighed and ground to a fine powder. An amount of the powder that contained *ca.* 100 mg of cimetidine was weighed and transferred to a volumetric flask where it was stirred with 180 ml of mobile phase A. After sonication for 15 min, the mixture was made up to volume (200 ml) with mobile phase A and filtered.

The impurities **I–IV** were dissolved in mobile phase A (**I**, **III** and **IV** at 2 μg/ml and **II** at 4 μg/ml).

Assay procedure

Volumes of 5 μl of the sample solution were injected into the chromatograph under the conditions described. For comparison, and identical amount of the cimetidine standard solution was injected. The standard solution contained the same concentrations of the drug (based on the label claim). The impurities were determined by injecting 50 μl of the sample solution and comparing the areas of impurity peaks with those of the peaks obtained by injecting known amounts of each impurity.

RESULTS AND DISCUSSION

The method described for determining cimetidine and related impurities, employing HPLC with a photodiode-array detector, was shown to be selective and sensitive. Simultaneous detection, at different wavelengths, and measurements of the UV spectrum of each separated compound during elution make it possible to identify the impurities easily and rapidly.

A representative chromatogram illustrating the resolution of a standard mixture and a sample are

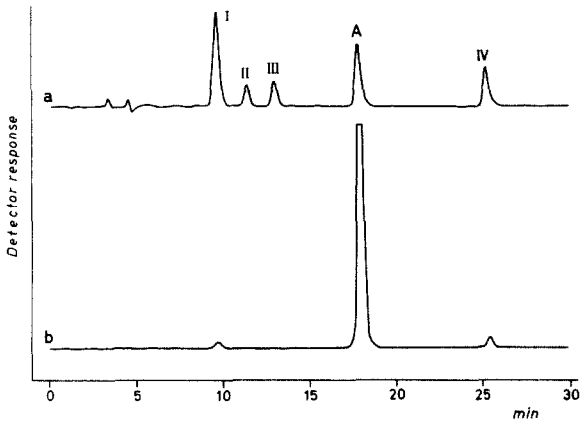

Fig. 1. Chromatograms of (a) standard mixture of cimetidine (a) (0.4 μg), **I** (0.4 μg), **II** (0.8 μg), **III** (0.4 μg) and **IV** (0.4 μg); (b) sample L. Column: μBondapak C_{18}. Detection at 220 nm.

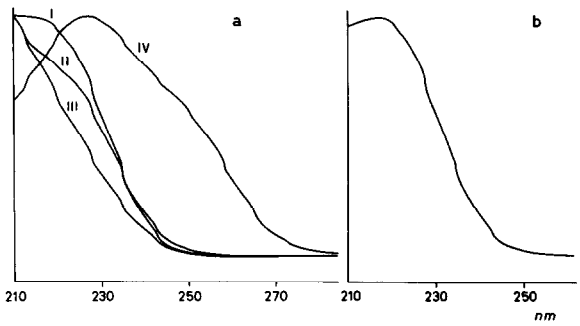

Fig. 2. Photodiode-array UV spectra of the components of a standard mixture after chromatographic separation (see Fig. 1). (a) Spectra of impurities **I**, **II**, **III** and **IV**; (b) spectrum of cimetidine.

TABLE I

REPRODUCIBILITY OF DETERMINATION OF CIMETIDINE IN TABLET FORMULATIONS

Sample	Cimetidine concentration ± S.D. (%)	Coefficient of variation (%)	
		Within-assay[a]	Between-as
A	101.2 ± 1.6	1.8	2.3
B	91.7 ± 1.1	2.1	2.9
C	92.6 ± 1.8	2.7	3.2

[a] Average of five determinations.
[b] Average of ten determinations.

shown in Fig. 1. Fig. 2 shows the spectra of the main compound cimetidine and of the impurities **I–IV** as obtained by photodiode-array detection. Although the spectra are similar, the slight but characteristic differences are of diagnostic value in the identification of **I–IV**.

The reproducibility of the method was satisfactory, as shown in Table I, which reports the response to repeated injections of three of the samples lysed.

Linearity was checked statistically, and Tab shows the data obtained for the calibration gr of cimetidine and the impurities.

Recovery was determined by preparing synt mixtures, simulating three tablet formulations, taining known amounts of standard and impu

TABLE II

STATISTICAL DATA FOR CALIBRATION GRAPHS FOR CIMETIDINE AND IMPURITIES **I, II, III** AND **IV**

Compound	Range tested (μg)	Correlation coefficient	Slope	Intercept
Cimetidine	0.40–20.0	0.9996	3.057	0.058
I	0.02–1.60	0.9997	1.770	0.006
II	0.04–3.20	0.9986	0.518	0.029
III	0.02–1.60	0.9983	1.403	0.030
IV	0.02–1.60	0.9951	2.301	0.127

TABLE III

RECOVERY STUDY

Synthetic mixture	Amount injected (μg)					Recovery (%) ± S.D.[a]				
	Cimetidine	**I**	**II**	**III**	**IV**	Cimetidine	**I**	**II**	**III**	**IV**
a	12.5	0.04	0.08	0.04	0.04	99.5 ± 2.1	96.3 ± 4.5	97.6 ± 3.9	95.8 ± 4.1	98.1 ±
b	15.0	0.04	0.08	0.04	0.04	98.7 ± 2.7	95.6 ± 3.1	94.9 ± 4.3	96.1 ± 3.7	97.1 ±
c	17.5	0.05	0.10	0.05	0.05	97.8 ± 1.8	93.8 ± 3.5	96.8 ± 4.4	95.1 ± 4.3	94.9 ±

[a] Average of five determinations.

TABLE IV

ASSAY (% OF DECLARED) OF CIMETIDINE AND RELATED IMPURITY LEVELS IN COMMERCIAL DOSAGE FORMS

Each value represents the mean (±S.D.) of five determinations. The impurity levels not reported were less than 0.01%.

Sample	Cimetidine concentration ±S.D. (%)	Impurity concentration (%)				
		I	II	III	IV	I+II+III+IV
A	101.2 ± 1.6	–	–	–	0.05	0.05
B	91.7 ± 1.1	–	–	–	–	–
C	92.6 ± 1.8	–	–	–	0.20	0.20
D	90.0 ± 0.4	0.20	–	–	–	0.20
E	91.3 ± 2.0	0.13	–	–	–	0.13
F	99.2 ± 0.8	–	–	–	0.09	0.09
G	102.8 ± 1.2	0.09	–	–	–	0.09
H	109.5 ± 0.9	–	–	–	–	–
I	110.0 ± 0.8	–	–	–	0.20	0.20
L	91.5 ± 2.1	0.11	–	–	0.10	0.21
M	93.7 ± 2.2	0.05	–	–	0.07	0.12
N	93.1 ± 0.8	–	–	–	0.17	0.17

and subjecting them to the procedure (Table III).

The detection limit was *ca.* 2 ng, calculated on a response of twice the noise level.

The results obtained for cimetidine and impurities in commercial dosage forms are reported in Table IV. The amount of active ingredient found experimentally is within 10% of the amount declared, in agreement with Italian regulations. The percentages of the related impurities, for all the formulations examined, met pharmacopoeial standards.

The described HPLC method appears to be reproducible and sensitive and provides a reliable quality control of cimetidine tablet formulations.

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