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Estimation of impurity profiles of drugs and related materials Part 15. Identification of minor impurities in cimetidine[1]

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Abstract

Besides several known impurities in cimetidine, two additional compounds at levels below 0.1% were detected by ion-pair reversed-phase high-performance liquid chromatography (HPLC). The impurities were isolated from crude cimetidine using normal-phase preparative HPLC. ^{1}H and ^{13}C NMR and mass spectrometric investigations revealed the structures of the impurities to be 2,5-bis[(*N'*-cyano-*N''*-methyl)guanidinoethylthiomethyl]-4-methylimidazole (**VII**) and 1,8-bis[(*N'*-cyano-*N''*-methyl)guanidino]-3,6-dithiaoctane (**VIII**). These structures were verified by synthesis of the impurities and comparison of the spectra and chromatographic (HPLC and TLC) retention data of the isolated and synthesized materials.

Keywords: Cimetidine; Impurity profiling; High-performance liquid chromatography; NMR spectroscopy; Mass spectrometry

1. Introduction

Thin-layer chromatographic (TLC) [2] and high-performance liquid chromatographic (HPLC) [3] methods are described in the principal pharmacopoeias for testing the purity of cimetidien bulk material. Table 1 summarizes the structures of related impurities (degradation products) reported in the literature. Impurity **II** is an oxidative degradation product [4–7], impurities **IV** and **V** are hydrolytic decomposition products [4–7] whereas **III** is the last intermediate in the synthesis of cimetidine [4,6,7]. Numerous data can be found in the literature for the separation and quantification of these impurities by TLC [4] and HPLC [4–7] methods.

The purity requirement of the USP XXIII [3] specifies that the proportion of total impurities and that of individual impurities expressed in terms of cimetidien must not exceed 1% and 0.2%, respectively. The British Pharmacopoeia (BP) [2] sets the same limit for the proportion of individual impurities and does not allow the presence of

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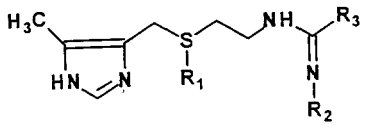
Table 1
Structures of cimetidine and some of its impurities



No.	R_1	R_2	R_3	Name	Origin
I	–	CN	$NHCH_3$	Cimetidine	–
II	=O	CN	$NHCH_3$	Cimetidine sulphoxide	Oxidation product
III	–	CN	SCH_3	–	Last intermediate
IV	–	$CONH_2$	$NHCH_3$	–	Hydrolysis product
V	–	H	$NHCH_3$	–	Hydrolysis product

more than two impurities at levels above 0.1% expressed in terms of cimetidine.

The presence of minor impurities at levels below 0.1% is not limited but, as a consequence of the high dose (up to 800 mg per day) [8], drug registration authorities are increasingly interested in the identificaion of impurities even at this trace level. The aim of this paper is to describe the isolation and identification of two impurities in cimetidine present at a level not exceeding 0.10%.

2. Experimental

2.1. Samples

The investigated bulk and crude cimetidine samples were products of the Chemical Works of Gedeon Richter Ltd. (Budapest).

2.2. High-performance liquid chromatography (analytical)

A Hewlett-Packard Model 1090A instrument equipped with an HP-1040 diode-array UV detector was used. A 250 × 4 mm i.d. column was packed with 10 μm Nucleosil C-18 (Macherey–Nagel).

The eluent and the chromatographic conditions were adapted from those of USP XXIII [3] with the slight modification: that the eluent was composed of 270 ml of methanol, 0.3 ml of 85% (w/w) phosphoric acid and 0.94 g of sodium 1-hexanesulphonate, made up to 1000 ml with water.

2.3. High-performance liquid chromatography (preparative)

An ISCO Model 2350 pump was used, equipped with a Rheodyne Model 7125 injector and an ISCO Model V^4 UV detector. A 250 × 16 mm i.d. column was packed with 10 μm LiChrosorb Si-100 (Bio Separation Technologies, Budapest). The eluent was chloroform–methanol–glacial acetic acid–25% (w/w) aqueous ammonia solution (81:15:3:1.5 v/v/v/v). The flow-rate was 7 ml min^{-1}. Detection was at 238 nm. After the evaporation of chloroform and methanol under reduced pressure, the impurities were obtained from the separated fractions by repeated lyophilization.

2.4. Thin-layer chromatography

The system of the BP 1993 [2] was used.

2.5. Mass spectrometry

Mass spectra were run on Kratos MS 25 (70 eV, direct insertion), VG Quattro MS/MS and VG-ZAB 2SEQ mass spectrometers.

2.6. NMR spectroscopy

NMR measurements were carried out on a Varian UNITYplus 500 instrument (500 MHz for ^{1}H and 125 MHz for ^{13}C) at 30°C in DMSO-d_6.

The chemical shift values listed in Fig. 1 and in the Experimental section are given for 30°C ($\delta_{TMS} = 0.00$ ppm). Assignments were confirmed by two-dimensional chemical shift correlation experiments (COSY, HSQC).

2.7. Synthesis of VII

2,5-Bis(hydroxymethyl)-4-methylimidazole [9] was allowed to react with cysteamine hydrochloride in aqueous solution followed by treatment

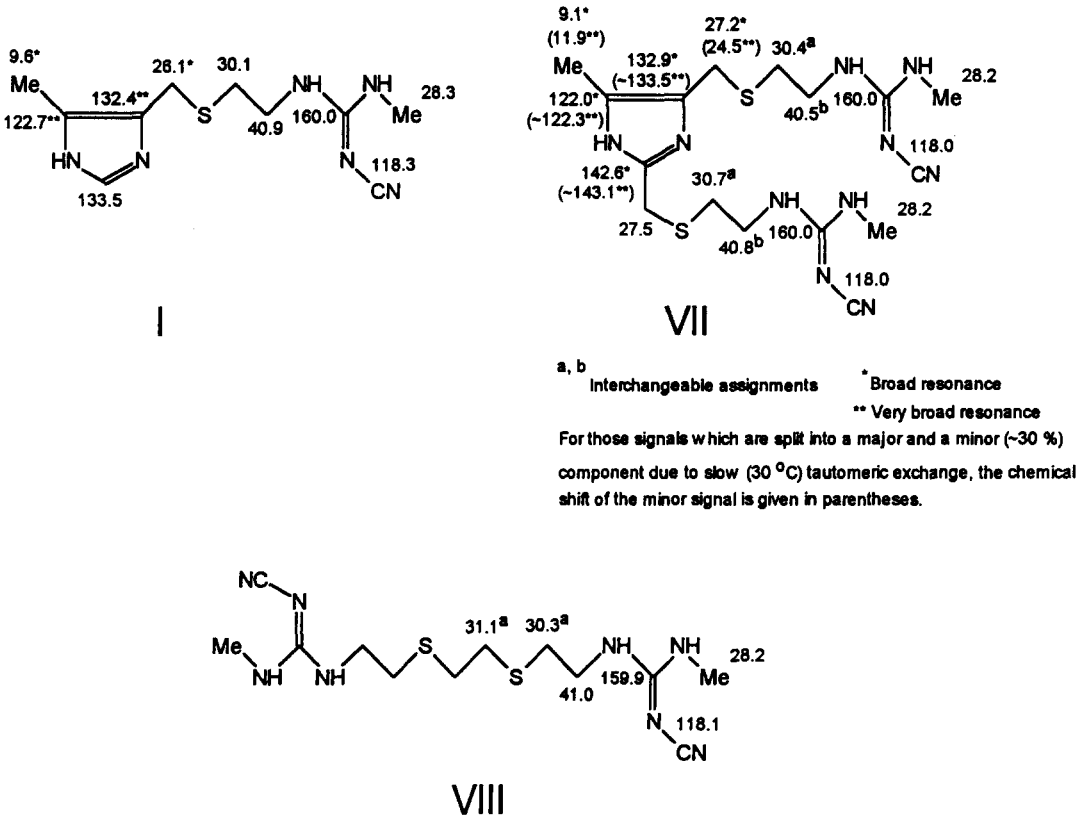


Fig. 1. Structures and ^{13}C NMR chemical shifts of cimetidine (**I**) and impurities **VII** and **VIII**.

with dimethyl *N*-cyanodithioiminocarbonate to obtain 2,5-bis[(*N*-cyano-*S*-methyl)thioureidoethylthiomethyl]-4-methylimidazole as a minor product. This was separated by column chromatography on Kieselgel 40 with ethyl acetate–acetone–water (4:1:1, v/v/v). The bisthiourea derivative was converted into the corresponding bisguanidine (**VII**) with 40% aqueous methylamine.

MS: *m/z* (EI; rel. int.,%) 125 (15.0), 116 (100.0), 99 (20.0), 82 (33.0), 70 (38.0), 57 (74.0), 42 (55.0). FAB (NBA matrix): $MH^+ = 423$.

^1H NMR: 2.1 s (imidazole–*Me*); 2.56 t and 2.63 t (2 × SCH_2–CH_2N); 2.68 d and 2.70 d (2 × NH*Me*); 3.27 q and 3.29 q (2 × SCH_2–CH_2N); 3.61 br s and 3.68 s (2 × imidazole–CH_2–S); 6.95–7.20 br (2 × CH_2–N*H* and 2 × N*H*Me); 11.40 br (imidazole NH). [Compound **I**: 2.16 s (imidazole–*Me*); 2.60 t (SCH_2–CH_2N); 2.73 d (NH*Me*); 3.34 q SCH_2–CH_2N); 3.68 s (imidazole–CH_2–S); 7.18 br t (CH_2NH); 7.33 br (N*H*Me); 7.52 s (HN–C*H* = N); 11.65 br s (imidazole NH)].

For ^{13}C NMR data, see Fig. 1.

2.8. Synthesis of *VIII*

Compound **VIII** was prepared by condensation of 1,8-diamino-3,6-dithiaoctane [10] with 2 mol of dimethyl *N*-cyanodithioiminocarbonate in ethanol followed by substitution of the methylthio group in the obtained 1,8-bis[*N*-cyano-*S*-methyl)thioureido]-3,6-dithiaoctane using ethanolic methylamine solution.

MS: *m/z* (EI; rel. int.,%) 185 (38.0) 158 (100.0), 125 (40.0), 111 (33.0), 85 (54.0), 57 (68.0), 42 (57.0). APCl: $MH^+ = 343$.

^1H NMR: 2.64 t (SCH_2–CH_2N); 2.66 d (NH*Me*); 2.73 s (SCH_2–CH_2S); 3.27 q (SCH_2–CH_2N); 6.95–7.06 br (CH_2–N*H* and N*H*Me).

For ^{13}C NMR, data see Fig. 1.

3. Results and discussion

3.1. Detection of impurities VII and VIII

Fig. 2 shows the liquid chromatogram of a typical industrial production batch of bulk cimetidine obtained by using the ion-pair reversed-phase system of the USP XXIII [3] slightly modified to improve the peak shapes. Of the well known impurities summarized in Table 1, impurities **II** and **III** are detectable. The peak of the latter is merged with another peak designated **VI**, which was earlier identified as 1,6-bis[*N'*-cyano-*N''*-methyl)guanidino]-3,4-dithiahexane; this originated from the disulphide-type oxidation product of cysteamine, one of the reagents in the synthesis.

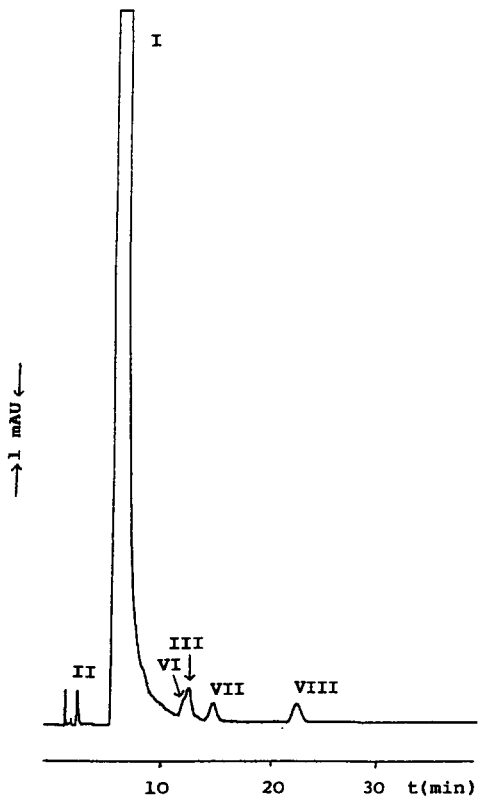


Fig. 2. Liquid chromatogram of an industrial batch of bulk cimetidine. See Section 2.2 for chromatographic conditions. UV detector set at 220 nm. For the structures of **I**, **VII** and **VIII**, see Fig. 1; for **II** and **III**, see Table 1. **VI** is 1,6-bis[(*N'*-cyano-*N''*-methyl)guanidino]-3,4-dithiahexane (see Section 3.1).

The identification of impurities **VII** and **VIII** was the subject of this study. It should be noted that the total proportion of impurities calculated from the chromatogram in Fig. 2 was 0.3% expressed in terms of cimetidine as described in the USP XXIII [3].

Since no conclusion about their structures could be drawn from the cimetidine-like diode array UV spectra of impurities **VII** and **VIII**, these compounds were isolated by preparative HPLC for further MS and NMR studies.

3.2. Isolation of the impurities by preparative HPLC

The reversed-phase HPLC system of the USP XXIII [3], with a high concentration of ion-pairing reagent in which the impurities to be isolated are eluted very much later than the main component, is not suitable for their preparative isolation. For this reason, a normal-phase system was developed (see Section 2.3). In this system cimetidine was eluted at 17–22 min while the retention times of **VIII** and **VII** were about 12 and 16 min, respectively.

Crude cimetidine was subjected to the preparative HPLC procedure. The isolation of **VIII** in a sufficiently pure form for the spectroscopic investigations did not present problems. In the case of **VII**, however, partly owing to the close vicinity of the main component and partly because of **IV** and **V** which are not present at a measurable level in bulk cimetidine but are components of the crude product, repeated fractionation was necessary.

3.3. Structure elucidation of impurities VII and VIII

The structures of **VII** and **VIII** (Fig. 1) were identified by the combined use of mass spectrometry and high-field NMR studies employing two-dimensional measurements. Fig. 1 shows the ^{13}C chemical shifts for **VII** and **VIII** and also for cimetidine (**I**); ^{1}H chemical shifts and MS data are given in Sections 2.7 and 2.8. A comparison of these data provides a straightforward verification of the relevant structures. Low-field ^{1}H NMR data for cimetidine, together with the ^{1}H and ^{13}C

chemical shifts of cimetidine hydrochloride, were reported previously [4]. It is noted that the ^{13}C chemical shift assignments given therein for the two CH_2 carbons flanking the S atom should be reversed.

One interesting feature of **VII** and **I** is that in **I** some NMR signals are substantially broadened whereas in **VII** some resonances are actually split into a major and a minor (~ 30%) component (30°C). This indicates the presence (at 30°C in DMSO) of a relatively slow exchange process between the two tautomeric forms of the imidazole ring and/or may also involve slow motions associated with the intramolecular hydrogen bonding of the N*H*Me proton to the basic imidazole nitrogen. The presence of such a bonding has been described on the basis of IR [11] and X-ray studies [12]. Extensive exchange broadening affects mainly the imidazole carbons; this suggests that the two-site tautomeric process is responsible for the observed exchange. For those ^{13}C signal pairs which fall in the slowly exchanging regime in **VII**, the minor signal is typically broadened to a degree which facilitates detection only at very high signal-to-noise ratios.

The full identity of the NMR and mass spectra of the synthesized impurities with those isolated by preparative HPLC furnishes evidence for the validity of the structures of **VII** and **VIII** as presented in Fig. 1.

3.4. Identification of the impurities by chromatographic methods

In addition to the identification of the impurities on the basis of the identiy of the spectra of the isolated and synthesized samples, a prerequisite for the identity is successful retention matching in at least three independent chromatographic systems [13].

Fig. 2 shows the HPLC trace for an industral batch of bulk cimetidine. Spiking the sample with synthesized **VII** and **VIII** increased the height of peaks **VII** and **VIII**, thus providing further evidence for the identity of the impurities originally present at a level below 0.1%. Similarly successful retention matching was carried out in the normal-phase system developed for preparative purposes.

The third chromatographic system for the successful retention matching was TLC system No. 2 of the BP 1993 [2]. The R_f values were cimetidine 0.35, **VII** 0.28 and **VIII** 0.65.

3.5. Origin of the impurities

One of the key steps in the synthesis of cimetidine is the introduction of the hydroxymethyl group at position 4 of the imidazole ring [4]. A side-reaction of this step is the formation of the 2,4-bishydroxymethyl derivative. Although the reactivity of the second hydroxymethyl group at position 2 towards reagents to build up the side-chain of cimetidine is very low [9], this is sufficient for the formation of **VII** at the trace level.

The source of the formation of **VIII** is probably the presence of 1,8-diamino-3,6-dithiaoctane as an impurity in cysteamine, which is one of the reagents in the synthesis of cimetidine [4].

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