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Note

High-performance liquid chromatographic determination of cimetidine and related compounds (raw material and tablets)

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Numerous high-performance liquid chromatographic (HPLC) methods for the determination of cimetidine[1–3] and its major metabolite cimetidine sulphoxide[4] in body fluids have been reported. Colorimetric methods for cimetidine in pharmaceutical preparations have also been proposed[5–7] but these methods may not be specific in the presence of cimetidine-related impurities. Related impurities may include N-cyano-N′-[2-(5-methyl-1H-imidazol-4-yl)methylthioethyl]-S-methylisothiourea (I) which, by some routes of synthesis, is the immediate precursor of cimetidine[8]. Possible degradation products of cimetidine due to hydrolysis are N-carbamoyl-N′-methyl-N″-[2-(5-methyl-1H-imidazol-4-yl)methylthio]ethyl guanidine (II) and N-methyl-N′-[2-(5-methyl-1H-imidazol-4-yl)methylthio]ethyl guanidine (III)[8,9]. Cimetidine sulphoxide, N-cyano-N′-methyl-N″-[2-(5-methyl-1H-imidazol-4-yl)methyl]sulphinyl ethyl guanidine (IV), may be present as a result of the oxidation of compound I or the drug[10]. The purpose of this note is to report an HPLC method for the determination of compounds I–IV, 4-methyl-5-imidazolemethanol (V) and other related compounds in cimetidine. Two mobile systems differing only in the proportion of the constituents are used, one for compounds I, IV and V, the other for compounds II and III. A method for the determination of cimetidine in tablets is also described.

EXPERIMENTAL

Apparatus and reagents

Liquid chromatograph. The system consisted of a Varian Model 5010 liquid chromatograph equipped with a Rheodyne Model 7125 10-μl loop injector, a Waters Model 441 UV detector (229 nm), a Whatman Partisil PXS-1025 PAC 10-μm cyanoamino column (250 × 4.6 mm) and a Perkin-Elmer Model LCI-100 computing integrator.

Drugs and chemicals. Cimetidine tablets were purchased locally and cimetidine raw materials were from various sources. Cimetidine related compounds I, II, III and IV were gifts of Smith Kline & French Canada; V was from Aldrich. Other materials were: diphenhydramine (internal standard), Parke-Davis, ammonium phosphate monobasic and 85% phosphoric acid, both HPLC grade, Fisher Scientific, methanol and acetonitrile, both HPLC grade, J. T. Baker, and deionized water.

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Solutions. Buffer: 0.01 *M* phosphoric acid and 0.01 *M* ammonium phosphate monobasic in deionized water, pH adjusted to 3.5. Mobile phase 1: buffer–methanol–acetonitrile (15:15:70). Mobile phase 2: buffer–methanol–acetonitrile (19:19:62). Internal standard: 0.1 mg/ml diphenhydramine in methanol. Standard solution A: 1.5 mg/ml cimetidine and 0.0015 mg/ml of compound IV in mobile phase 1. Standard solution B: 0.05 mg/ml cimetidine in internal standard solution. Standard solution C: 5.0 mg/ml cimetidine and 0.05 mg/ml of each of compounds II and III in mobile phase 2.

System suitability

Mobile phase 1, impurities. Five 10-μl aliquots of standard solution A were injected and the peak responses measured. The resolution of the peaks was greater than 1.5 and the relative standard deviation of the peak response due to compound IV was less than 5%.

Mobile phase 1, assay. Five 10-μl aliquots of standard solution B were injected and the peak responses measured. The resolution was greater than 4.0 and the relative standard deviation of the ratios of the drug to internal standard peak response was less than 1%.

Mobile phase 2, impurities. Five 10-μl aliquots of standard solution C were injected and the peak responses measured. The resolution of the peaks due to compounds II and III was greater than 1.0 and the relative standard deviations of the peak heights were less than 3 and 5%, respectively.

Procedures

Impurities, mobile phase 1 (compounds I, IV and V). The test solutions were prepared by dissolving 15 mg of cimetidine raw material in 10 ml of mobile phase 1. Equal amounts (10 μl) of the test solution and standard solution A were chromatographed and peak responses were recorded and measured. The level of compound IV in the test solution was calculated by comparison of the peak heights to the peak height due to compound IV in standard solution A. Other peaks can be estimated by comparison of response to that due to compound IV, making allowance for absorptivity differences where necessary, or standard solutions containing known amounts of compounds I and V can be prepared.

Impurities, mobile phase 2 (compounds II and III). The test solutions were prepared by dissolving 50 mg of cimetidine raw material in 10 ml of mobile phase 2. Equal amounts (10 μl) of the test solution and standard solution C were chromatographed and peak responses were recorded. The levels of compounds II and III in the test solution were estimated by comparison of the peak heights to the height of the peaks due to compounds II and III in standard solution C.

Assay. Twenty cimetidine tablets were ground in a tablet mill, a portion equivalent to about 25 mg of cimetidine was accurately weighed into a 25-ml extraction tube, and 10 ml of internal standard solution were added. The tube was shaken on a horizontal shaker for 10 min, centrifuged and 2 ml of the clear solution diluted to 100 ml with internal standard solution. Duplicate 10-μl aliquots of the test solution and standard solution B were chromatographed and the peak responses measured. Cimetidine was determined by comparison of the drug to internal standard peak area ratio of the standard solution to that of the test solution.

RESULTS AND DISCUSSION

Initial work was done with mobile phase 1. This gives satisfactory resolution of compounds I, II, III, IV and V from each other and from the drug, but the retention times of compounds II and III, at about 60 and 75 min, respectively, are inconveniently long. Increasing the proportions of methanol and buffer in the mobile system from 15% to 19% (mobile phase 2) reduced the retention times of compounds II and III to less than 20 min. The resolution of compounds I, IV and V from cimetidine and of compounds II and III from cimetidine is shown in Figs. 1 and 2, respectively.

The linearity of the response of compound IV was determined in the presence of cimetidine because the peak, although well resolved, is on the tail of the large cimetidine peak. In three standards ranging from 7.23 to 28.9 ng of compound IV on the column (corresponding to about 0.05 to 0.20% of compound IV in 15 μg cimetidine) the retention time of compound IV relative to cimetidine was about 1.3 and the resolution was between 1.5 and 1.8. A plot of peak height, corrected for the approximately 0.02% of compound IV in the cimetidine, *versus* the amount (ng) of compound IV on the column gave a slope of 2.12 mm/ng and an intercept of −1.5 mm. The responses for compounds I and V, not in the presence of cimetidine, were linear over the range from about 15 to 150 ng on the column. For five standards, slopes and intercepts were 1.75 and 0.24 mm/ng and 0.82 and 0.12 mm, respectively. For compounds II and III in mobile phase 1, the minimum quantifiable amounts would be about 0.2% in cimetidine.

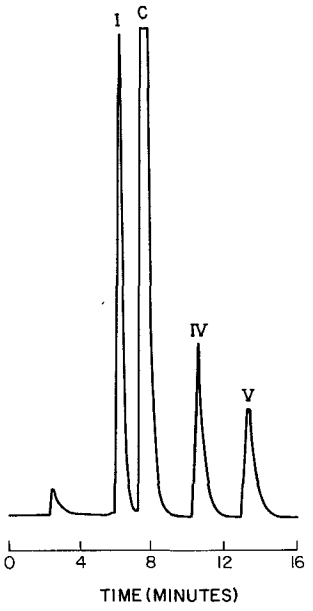

Fig. 1. Resolution of compounds I, IV and V from cimetidine in mobile phase 1. The peak labelled C is cimetidine. Amounts on column were 150 ng of cimetidine and 15 ng of compounds I, IV and V.

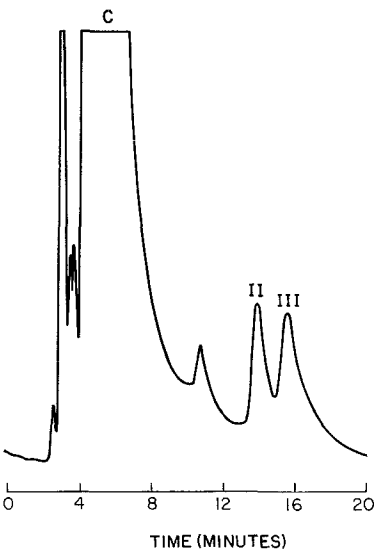

Fig. 2. Resolution of compounds II and III from cimetidine in mobile phase 2. The peak labelled C is cimetidine. The amounts on column were 50 ng of compounds II and III and 50 μg of cimetidine.

The linearity of the responses of compounds II and III in mobile phase 2 was determined over the ranges between 26.8 and 107 ng and 29.8 and 119 ng, respectively, corresponding to levels of about 0.05 to 0.20% in 50 μg cimetidine. The retention times relative to cimetidine were 3.2 and 3.6, respectively, and the mean resolution of compounds II and III was about 1.05. In three standards containing compounds II and III, a plot of the peak height *versus* weight on the column gave slopes of 0.68 and 0.58 mm/ng and intercepts of −0.6 and 1.6 mm, respectively. The mean values of the ratios of peak height to weight on the column were 0.69 and 0.61 mm/ng with relative standard deviations of 6.5 and 7.1%, respectively.

From the point of view of a test method, each impurity can be estimated against a known standard but this necessitates the general availability of a series of relatively rare compounds. An alternative is to use the drug itself as a standard, say at a level of 0.1% of the amount of cimetidine to be injected to estimate impurities. This approach should be coupled with knowledge of the molar absorptivities at the wavelength in question. These are about 25,000 l mol^{-1} cm^{-1} for compound IV, 18,000 l mol^{-1} cm^{-1} for compound I and cimetidine, 6400 l mol^{-1} cm^{-1} for compounds II and III and 3700 l mol^{-1} cm^{-1} for compound V. Although assumption of direct proportionality of peak response will lead to large errors in estimating individual peaks, these errors may be acceptable as part of a thoughtfully designed limit test.

Four lots of cimetidine raw materials were examined with mobile phase 1 for the presence of related compounds (Table I). The impurity levels were calculated by comparison of peak areas to the area of a cimetidine peak at a level of 15 ng, (corresponding to 0.1% of the amount of drug injected) assuming direct proportionality. Levels of compounds II and III were found to be 0.02% or less when examined by mobile phase 2. The chromatogram obtained from lot D of cimetidine raw material in mobile phase 1 is presented in Fig. 3.

TABLE I

RELATED COMPOUND LEVELS IN SELECTED RAW MATERIAL LOTS

Mobile phase 1.

*Compound**	*Relative retention time***	*Impurity level (%)****			
		Lot A	*Lot B*	*Lot C*	*Lot D*§
	0.37	0.03			
	0.40		0.05	0.47	0.50
	0.41	0.12			
	0.67		0.08	0.02	0.11
I	0.82				0.05
IV	1.35	0.05			
V	1.80	0.05			0.11
Total		0.27	0.13	0.49	0.77

* Identification by retention time only.

** Relative to cimetidine at a retention time of about 8 min.

*** Estimated by comparison of peak area to the area of a peak due to cimetidine at a level of 0.1% of the amount of drug injected.

§ Unintegrated peak at about 45 min.

TABLE II

ASSAY RESULTS FOR 300-mg CIMETIDINE TABLETS

Product	*Per cent of label claim*					*Mean per cent of label claim ± S.D.*	*Mean per cent of label claim U.S.P. method*
	Shaking time (min)						
	5	*10*	*10*	*10*	*15*		
	Drug weight (mg)						
	25	*15*	*25*	*40*	*25*		
E	95.7	94.4	93.9	94.4	93.5	94.3 ± 1.48	94.3
	97.4	95.6	93.9	91.9	92.5		
F	97.7	99.2	96.5	96.6	97.0	97.2 ± 1.01	96.1
	96.9	98.6	97.2	95.2	95.6		
G	95.3	96.7	93.5	94.1	95.4	95.6 ± 1.21	96.4
	95.4	96.4	94.4	96.1	98.4		
H	95.2	94.7	95.2	92.5	93.2	94.0 ± 1.60	96.2
	94.4	95.8	95.2	91.0	92.2		

Diphenhydramine hydrochloride was chosen as the internal standard for the assay procedure because it is stable under the conditions of use for at least 8 h, well resolved from the drug and has a similar response at 229 nm, the assay wavelength. Linearity was established by chromatographing five known solutions covering the

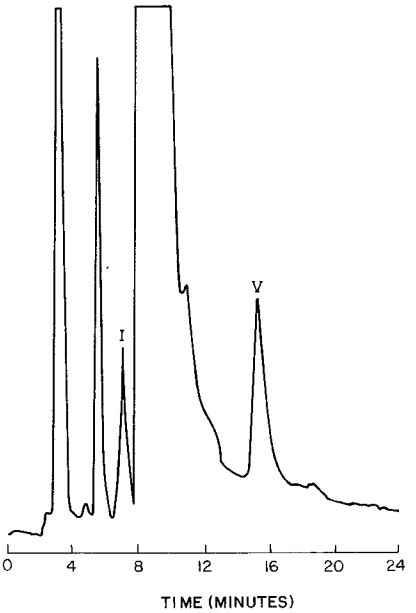

Fig. 3. Cimetidine raw material lot D in mobile phase 1. The related compound peaks integrated to an area equivalent to 0.77% of cimetidine based on comparison to the area of a cimetidine peak corresponding to a 0.1% level. Several small peaks were not integrated. Peak identification by retention time only.

concentration range 0.23–2.36 μg cimetidine on the column (corresponding to 50 to 500% of label claim). The slope of a plot of the ratio of the area response of drug to internal standard *versus* the corresponding weight ratio was 3.87 with an intercept of 0.06 and $r^2 = 0.999$. The mean ratio of area response to weight was 3.85 with a relative standard deviation of 3.4%. For five injections of a solution corresponding to about 100% of label claim (0.4658 ng on the column) the mean of the area–weight ratios was 3.76 with a relative standard deviation of 0.9%.

The effectiveness of the tablet extraction procedure was demonstrated by shaking tablet matrixes in methanol for 5, 10 and 15 min and by varying the amount of drug matrix to correspond to 15, 25 and 40 mg of drug. Four commercial formulations were analysed in duplicate and also analysed by the proposed U.S.P. procedure[11]. The results are presented in Table II. No significant variations were observed. The method calls for shaking an amount of matrix equivalent to 25 mg for 10 min.

The assay method would be a useful alternative to the U.S.P. method in situations where mobile phase 1 was in use for purity evaluation.

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