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Effect of Maleic Acid in Compendial UV Absorption Assays for Antihistamine Maleate Salts

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Abstract □ Standard recoveries averaging 89.9% are reported for the NF XIII brompheniramine maleate tablet assay procedure, which employs UV comparison of unidentical sample and standard molecular species in the same medium. The data presented indicate that these low assay values are attributable to the chromophoric properties of the maleic acid moiety and its ability to protonate the α-pyridyl chromophore of the brompheniramine molecule in a neutral organic solvent.

Keyphrases □ Maleic acid—effect on compendial UV absorption assay for antihistamine maleate salts □ Maleate salts of antihistamines—effect of maleic acid on compendial UV absorption assay □ Antihistamine maleate salts—effect of maleic acid in compendial UV absorption assays □ UV absorption—effect of maleic acid in compendial assays for antihistamine maleate salts

Several current compendial monographs contain assay procedures for organic nitrogenous base maleate salts in which the UV absorbance of a sample solution of an extracted base is compared with that of a standard solution of its maleate salt in the same solvent. Recoveries of about 97–98% were previously reported (1) for dexbrompheniramine, dexchlorpheniramine, and chlorpheniramine maleate reference standards obtained by using one method of the Association of Official Analytical Chemists (2) and three monograph assays of NF XIII (3), all of which employ such methodology. These erroneously low assay values, resulting from UV comparisons of unidentical molecular species, have been shown to be caused by the UV absorption properties of maleic acid in 0.1 *N* HCl and 0.1 *N* H_2SO_4, where measurements are made at about 264 nm (1).

In extending these studies of similar antihistamine maleate assays in official monographs, the NF XIII assay for brompheniramine maleate (I) tablets (3, p. 107) was tested for percent recovery values. This NF procedure involves sample extraction of the free amine from alkaline aqueous solution into chloroform. The UV absorbances of the sample solution and of a standard solution of I, diluted directly to the desired concentration in the same medium, are then determined and compared. This method differs from other antihistamine maleate assays previously tested by the authors in that UV measurements of the extracted sample amine base and directly diluted standard amine salt are made in an organic solvent (chloroform) rather than in aqueous acid solution.

Recoveries ranging from 89.3 to 90.5% were ob-

Table I—Recovery Data for Brompheniramine, Chlorpheniramine, and Pheniramine Maleates Reference Standards Using the NF XIII Brompheniramine Maleate Tablet Assay

Salt	Source	Solvent Used for Standard Dissolution	Recovery, %
Brompheniramine maleate	Commercial	Chloroform	90.2
Brompheniramine maleate	Commercial	Chloroform	89.3
Brompheniramine maleate	Commercial	Chloroform (sodium hydroxide treated)	89.7
Brompheniramine maleate	NF	Chloroform (sodium hydroxide treated)	90.5
			Average 89.9
Chlorpheniramine maleate	USP	Chloroform	88.6
Chlorpheniramine maleate	USP	Chloroform (sodium hydroxide treated)	89.0
			Average 88.8
Pheniramine maleate	Commercial	Chloroform	89.5
Pheniramine maleate	Commercial	Chloroform (sodium hydroxide treated)	89.9
			Average 89.7

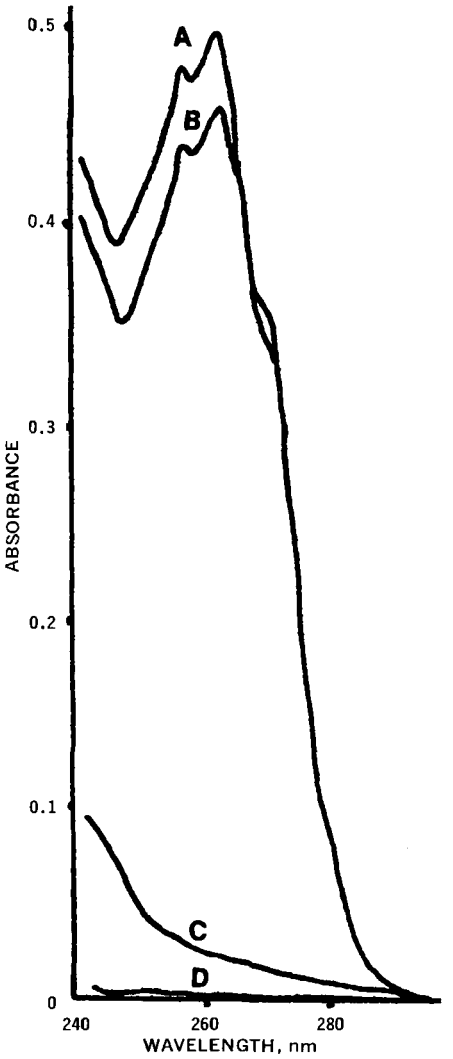

Figure 1—*UV spectra of: A, brompheniramine maleate diluted directly to final concentration of 0.0412 mg/ml in chloroform, alkali treated as per NF assay (3, p. 107); B, brompheniramine at a concentration equivalent to 0.0420 mg/ml of the maleate salt in chloroform, after extraction of brompheniramine maleate by NF assay (3, p. 107); C, maleic acid (0.0112 mg/ml) in chloroform, alkali treated as per NF assay (3, p. 107); and D, chloroform, alkali treated as per NF assay (3, p. 107),* versus *similarly treated chloroform.*

tained for reference standards by the assay for I tablets. These results are of particular significance because of the 95–105% assay limits established by the NF for label-declared amounts of I tablets. In addition, the content uniformity test in this monograph utilizes the same analytical method employed for the composite assay.

The purposes of this paper are to: *(a)* report analytical recovery data for antihistamine maleates obtained by compendial assays which may give erroneous results due to the maleic acid effect (1), and *(b)* show that the significantly and uniquely lower recoveries obtained in the NF assay for I tablets may be explained by bonding (protonation) effects between maleic acid and the α-pyridyl chromophore in the brompheniramine molecule, in addition to the chromophoric properties of the maleic acid moiety itself.

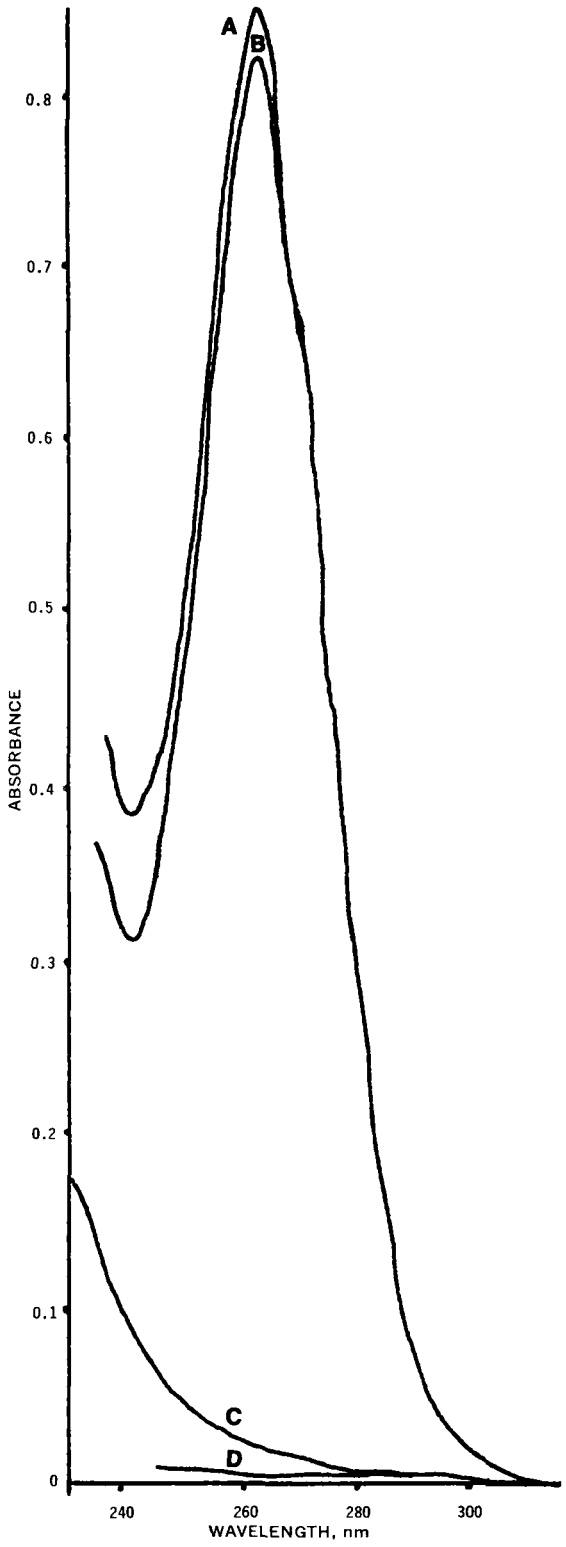

Figure 2—*UV spectra of: A, chlorpheniramine maleate directly diluted to final concentration of 0.0406 mg/ml in hydrochloric acid (1 in 100), solvent treated as per USP assay (4); B, chlorpheniramine at a concentration equivalent to 0.0402 mg/ml of the maleate salt in hydrochloric acid (1 in 100) after extraction of chlorpheniramine maleate by USP assay (4); C, maleic acid (0.01205 mg/ml) in hydrochloric acid (1 in 100), solvent treated as per USP assay (4); and D, hydrochloric acid (1 in 100), solvent treated as per USP assay (4)* versus *similarly treated hydrochloric acid (1 in 100).*

EXPERIMENTAL

Monograph-specified amounts of NF and commercially obtained brompheniramine maleate reference standards were analyzed by the NF XIII assay procedure for I tablets. The commercial standard employed assayed 99.8% by direct dilution and UV comparison with the NF reference standard.

Maleic acid solutions in equimolar concentrations in the appropriate solvents were prepared to determine the contribution of maleic acid to total antihistamine maleate UV absorbance at wavelengths specified in the monograph. The maleic acid employed assayed 99.7% by the NF XIII titrimetric method (3, p. 921).

Spectrograde solvents, when available, were employed in the UV determinations. All spectra were obtained on a recording spectrophotometer[1], using 1-cm matched quartz spectrophotometric cells. All recovery and standard solutions were scanned *versus* their corresponding solvents.

RESULTS AND DISCUSSION

Recoveries averaging 89.9% were obtained in four separate determinations, using the NF assay for I tablets (Table I). UV examination of additional solvent extracts indicated that the free base was completely removed from alkaline aqueous solution by the five 35-ml chloroform extractions specified in the method. Typical UV spectra obtained are presented in Fig. 1.

The NF specifies that UV measurements of sample and standard are to be made "in the same medium." Since this phrase may be subject to interpretation, recovery extracts were compared to standards directly diluted to the desired concentration in both chloroform and chloroform treated with alkali as specified in the monograph. No difference in results was noted with either medium (Table I).

Maleic acid in equimolar concentrations in chloroform and alkali-treated chloroform was found to contribute 3.5–5% to the total UV absorption of I at 263 nm (Fig. 1). (Because of its difficult solubility in chloroform, the maleic acid was initially dissolved in about 1 ml of methanol and diluted to volume with chloroform or alkali-treated chloroform.)

Recoveries of chlorpheniramine maleate and pheniramine maleate reference standards by the NF assay for I tablets were also found to be 88–90% (Table I); maleic acid itself contributed about 4% to the total UV absorbance of the maleate salts of each compound in equimolar amounts in sodium hydroxide-treated chloroform.

Aliquots of the chloroform recovery extracts for all three antihistamines from the I tablet assay were back-extracted with 0.1 *N* H_2SO_4, and the UV absorbances (at 264 nm) of the 0.1 *N* H_2SO_4 extracts were compared to those obtained for the respective antihistamine maleate standards diluted directly to final concentration with chloroform-saturated 0.1 *N* H_2SO_4. Recoveries ranging from 95.8 to 98.6% were obtained. Similarly, 0.1 *N* H_2SO_4 solutions obtained from back-extractions of I–chloroform recovery solutions, when compared with I standards originally directly diluted with alkali-treated chloroform and back-extracted with 0.1 *N* H_2SO_4, gave recoveries of 96.0–96.7%. These results parallel those obtained by the NF XIII assay for dexbrompheniramine maleate tablets (3, p. 213) and for other monograph assays previously tested (1). A possible explanation is that sample (recovery) and standard molecular species may be equally protonated by the highly acidic media. The only effect on UV absorption (and thus the cause of low recoveries) under these conditions is the absence of the maleic acid chromophore from the sample solution. [The authors also obtained standard recoveries averaging 97.6% by the USP XVIII assay for chlorpheniramine maleate elixir (4), in which UV absorptions of sample free amine and standard maleate salt in aqueous acid are also compared. Equimolar amounts of maleic acid were found to contribute 1.8–3.2% of the total absorption at 264 nm in hydrochloric acid (1 in 100). Typical UV spectra are reproduced in Fig. 2.]

These data indicate that elimination of the maleic acid chromophore from the final brompheniramine sample solution as a result of the extraction procedure is not alone sufficient to account for the approximately 10% low recoveries obtained. An investigation

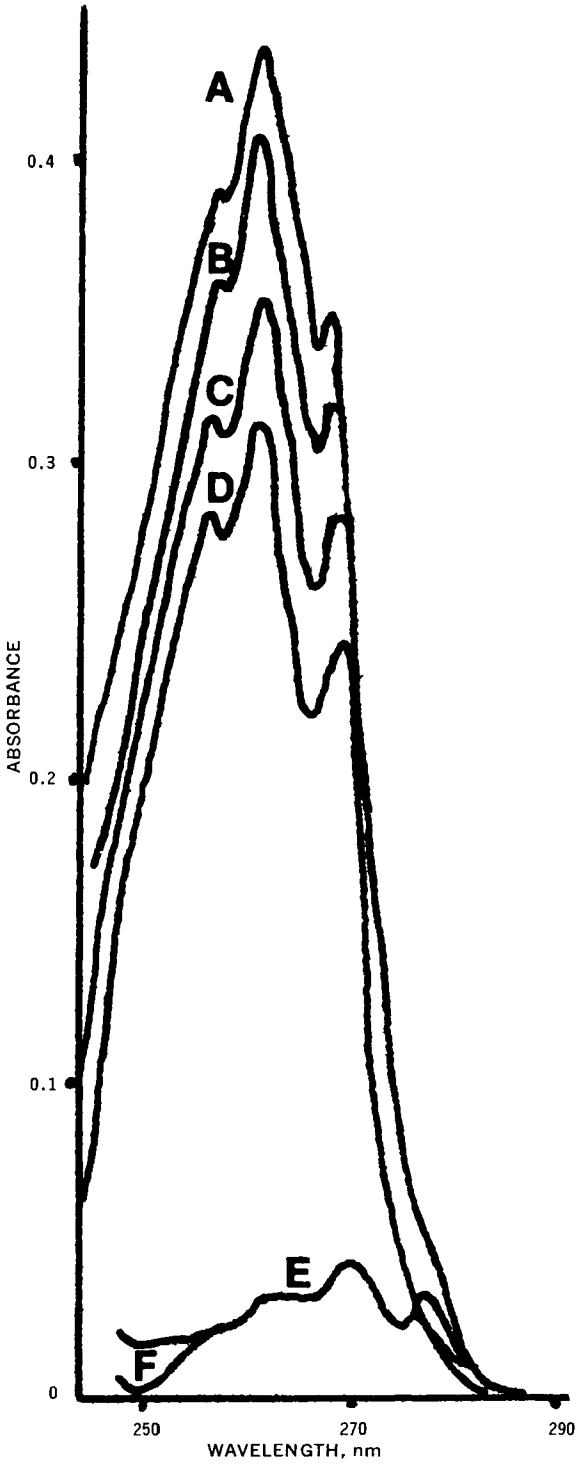

Figure 3—*UV spectra of: A,* p-*bromotoluene (0.0169 mg/ml), α-methylpyridine (0.00933 mg/ml), and maleic acid (0.0116 mg/ml)* versus *maleic acid (0.0116 mg/ml); B, α-methylpyridine (0.0169 mg/ml) and maleic acid (0.0116 mg/ml)* versus *maleic acid (0.0116 mg/ml); C,* p-*bromotoluene (0.0169 mg/ml) and α-methylpyridine (0.00933 mg/ml)* versus *chloroform; D, α-methylpyridine (0.00933 mg/ml)* versus *chloroform; E,* p-*bromotoluene (0.0169 mg/ml) and maleic acid (0.0116 mg/ml)* versus *maleic acid (0.0116 mg/ml); and F,* p-*bromotoluene (0.0169 mg/ml)* versus *chloroform. Spectra of chloroform* versus *chloroform and of maleic acid (0.0169 mg/ml)* versus *maleic acid (0.0169 mg/ml) follow the wavelength axis. All solutions are in chloroform. Concentrations are approximately* 1×10^{-4} *mM.*

[1] Cary model 15, Cary Instruments, Monrovia, Calif.

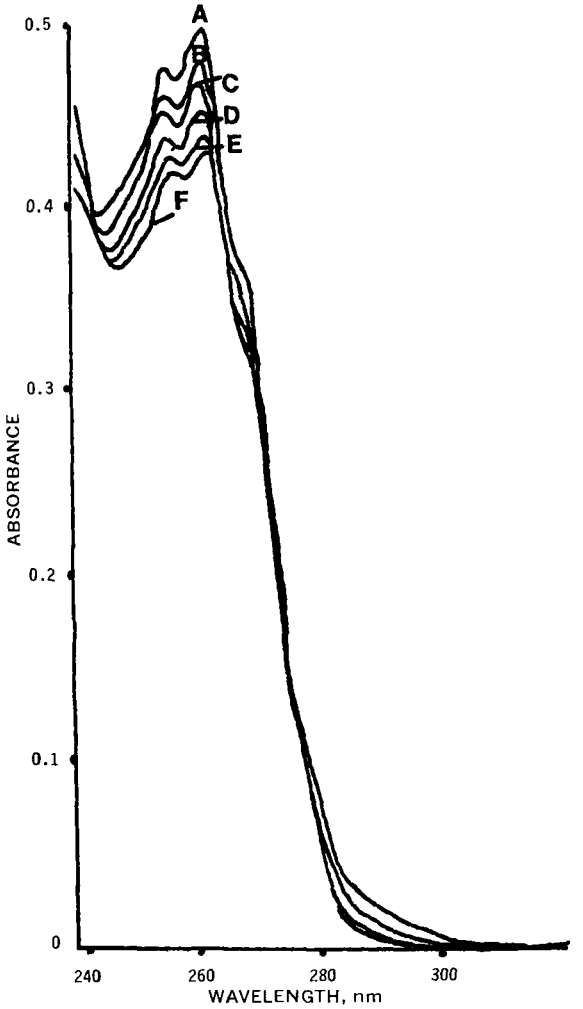

Figure 4—*UV spectra of: A, brompheniramine (0.938 × 10^{-4} mM) and maleic acid (10.05 × 10^{-4} mM) versus maleic acid (10.05 × 10^{-4} mM); B, brompheniramine (0.938 × 10^{-4} mM) and maleic acid (5.023 × 10^{-4} mM) versus maleic acid (5.023 × 10^{-4} mM); C, brompheniramine (0.938 × 10^{-4} mM) and maleic acid (0.942 × 10^{-4} mM) versus maleic acid (0.942 × 10^{-4} mM); D, brompheniramine (0.938 × 10^{-4} mM) and maleic acid (0.628 × 10^{-4} mM) versus maleic acid (0.628 × 10^{-4} mM); E, brompheniramine (0.938 × 10^{-4} mM) and maleic acid (0.314 × 10^{-4} mM) versus maleic acid (0.314 × 10^{-4} mM); and F, brompheniramine (0.938 × 10^{-4} mM) versus chloroform. Spectra of chloroform versus chloroform and of maleic acid (0.942 × 10^{-4} mM) versus maleic acid (0.942 × 10^{-4} mM) follow the wavelength axis. All solutions are in chloroform.*

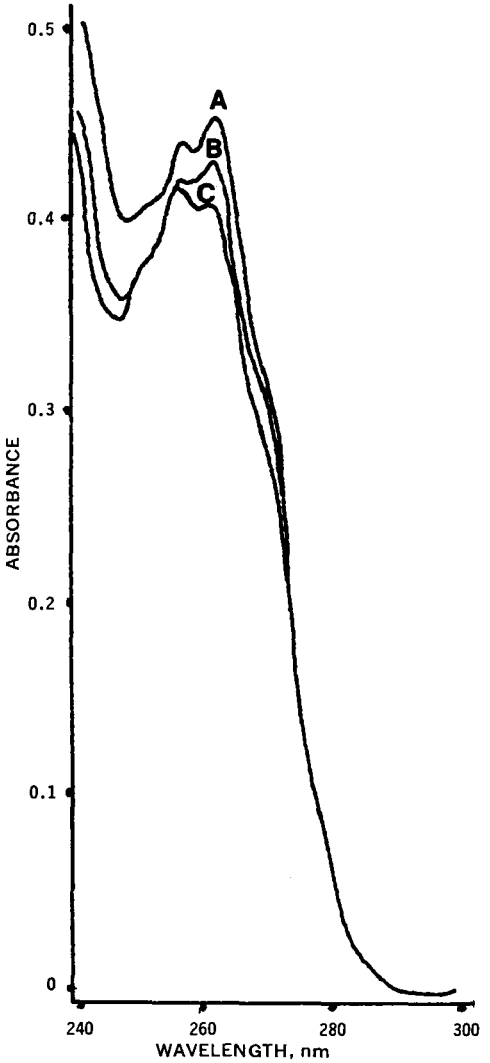

Figure 5—*UV spectra of brompheniramine at a concentration equivalent to 0.0410 mg/ml of the maleate salt in: A, chloroform; B, methylene chloride; and C,* n-C_6H_{14}.

was undertaken to explain the greater reduction in UV absorption observed for the free amine as compared to the maleate salt form when a neutral solvent rather than aqueous acid is employed as the analytical medium.

An unshared pair of electrons on the nitrogen atom in the pyridine ring of the brompheniramine molecule serves as a potential site for proton addition with formation of a pyridinium ion (5). Protonation of pyridine has been reported to enhance the intensity of the $\pi \rightarrow \pi^*$ band (B-Band) (6, 7). Halverson and Hirt (8) obtained a somewhat increased UV absorption in the 250–260-nm region for the ethbromide salt of pyridine as opposed to the parent compound when measured in ethanol, and they found increasing pyridine UV absorption in the same region in solvents of increasing hydrogen-bonding ability. Levine (9) reported that alkaloids containing a basic nitrogen atom in the chromophoric group should be completely protonated in quantitating such drugs by UV measurements, since absorption intensity and wavelength of the maxima vary with degree of ionization. Also, Furman (10) reported increased pyridine and chlorpheniramine UV absorptivities in aqueous acid as opposed to base due to pyridine nitrogen protonation in acid. These data suggest that, should maleic acid be capable of protonating the α-pyridyl chromophore in the I molecule, a hyperchromic effect on UV absorptivity would be expected, accounting for a portion of the observed increased absorption of the salt over the free base form in neutral solvents.

Brompheniramine is a dibasic amine. The nitrogen in the aliphatic side-chain tertiary amine group is more strongly basic than the pyridine nitrogen and would be expected to be preferentially protonated to form the amine salt (11). However, this nitrogen is sufficiently far removed from the active chromophoric site so that any protonation effect here will little influence the UV absorption of the compound. However, brompheniramine maleate is a salt of a dicarboxylic acid with only one of the acidic hydrogens entering into salt formation with the amino group (11). Under certain conditions, such as dissolution in neutral organic solvents, the one proton from the dicarboxylic acid, which is not bonded to the aliphatic amine, might be expected to be available to weakly protonate a chromophoric pyridine nitrogen. Experiments conducted in this laboratory support that argument.

Brompheniramine contains two chromophoric groups, the *p*-bromotoluyl and α-methylpyridyl moieties, both of which absorb in the same UV region. UV spectra of equimolar concentrations of commercially obtained *p*-bromotoluene and α-methylpyridine in chloroform show that the *p*-bromotoluyl moiety contributed less than 10% to the total UV absorption of the combined species at

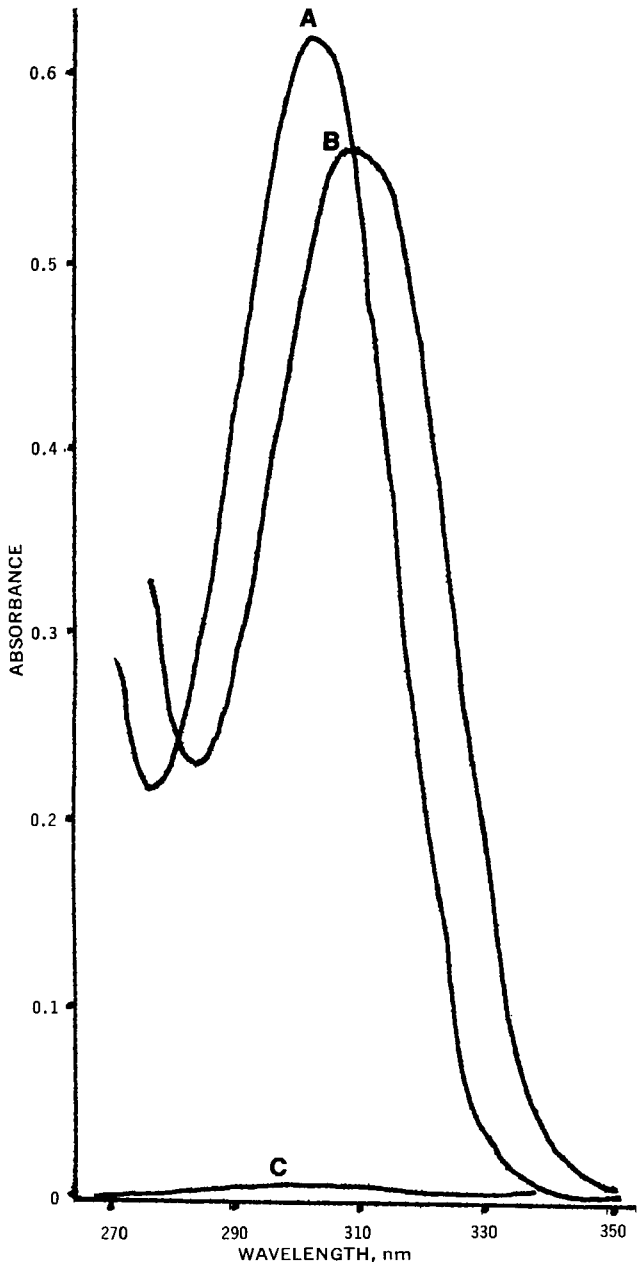

Figure 6—*UV spectra of: A, methapyrilene hydrochloride at a concentration of 0.0398 mg/ml in chloroform; B, methapyrilene at a concentration equivalent to 0.0398 mg/ml of methapyrilene hydrochloride in chloroform; and C, chloroform* versus *chloroform.*

263 nm (Fig. 3). Addition of equimolar quantities of maleic acid to equimolar amounts of both species resulted in a significant increase (about 28%) in the absorption of the α-methylpyridine chromophore at 263 nm and a negligible increase in the UV absorption of *p*-bromotoluene (Fig. 3). The spectra were obtained by scanning *versus* the same maleic acid–chloroform solution to eliminate UV absorption contributed by the maleic acid chromophore. These data indicate that the affected chromophore as well as the site of protonation is the pyridine portion of the brompheniramine molecule. Moreover, they show that the weak carboxylic acid functions of maleic acid are able to exert a measurable effect on the chromophoric properties of the α-methylpyridine system by protonating, at least partly, the pyridine nitrogen.

To provide proof that maleic acid can protonate the pyridyl system in brompheniramine slightly and thus enhance absorptivity at about 263 nm in chloroform, maleic acid in chloroform was added in increasing amounts to the free amine in chloroform and the UV

Table II—Effect of Various Amounts of Maleic Acid on UV Absorption of Brompheniramine

Molar Ratio, Maleic Acid to Brompheniramine (Approximate)	Absorbance of Brompheniramine (Maleate)	Absorbance Increase, %
0.00	0.435	—
0.33	0.442	1.6
0.67	0.455	4.6
1.0	0.469	7.8
5	0.482	10.8
10	0.499	14.7

spectra were recorded. The brompheniramine solution was prepared by dissolving reference standard I in chloroform, extracting with aqueous base to remove the maleic acid, and diluting to volume with chloroform. Increasingly larger aliquots of maleic acid solution were added to a fixed volume of the brompheniramine solution to achieve several molar ratios of maleic acid to brompheniramine, ranging from 0.0 to about 10. All maleic acid–brompheniramine solutions were diluted to the same final volume with chloroform prior to UV scanning. Maleic acid solutions of corresponding concentrations were placed in the reference compartment of the spectrophotometer as previously noted, and the UV spectra of the brompheniramine–maleic acid solutions were recorded by difference measurement (Fig. 4). (Maleic acid was initially dissolved in 1 ml of methanol and diluted to volume with chloroform. Methanol at this concentration was found not to alter the UV spectrum of chloroform.)

Increasing absorption was obtained for brompheniramine with increasing amounts of maleic acid (Table II and Fig. 4). Increased absorption at λ_{max} 263 nm was most pronounced at maleic acid–

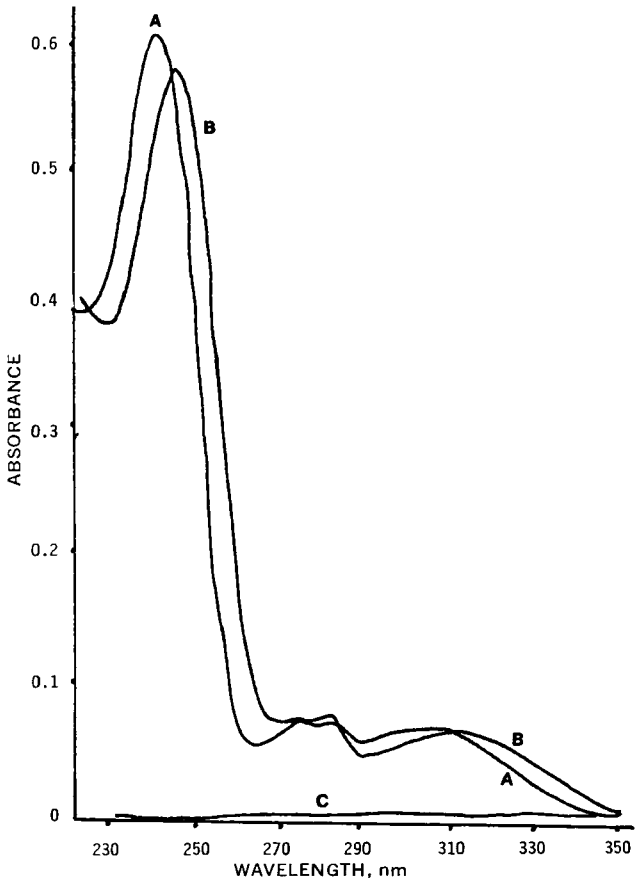

Figure 7—*UV spectra of: A, thonzylamine hydrochloride at a concentration of 0.00835 mg/ml in methylene chloride; B, thonzylamine at a concentration equivalent to 0.00835 mg/ml of thonzylamine hydrochloride in methylene chloride; and C, methylene chloride* versus *methylene chloride.*

brompheniramine ratios of 0.0:1.0. At ratios >1.0, an absorption increase was still exhibited but was lower in magnitude per unit change in ratio. Since the spectra were obtained in such a manner as to cancel out UV absorption due to the maleic acid chromophore, the enhanced absorption observed is explained by interaction of maleic acid with the α-pyridyl chromophore of brompheniramine.

The increase in UV absorption is about four times greater for equimolar amounts of α-methylpyridine and maleic acid in chloroform than for equimolar amounts of brompheniramine and maleic acid in chloroform (Figs. 3 and 4). The difference is apparently due to the presence of the aliphatic amine in brompheniramine, which reduces the degree of protonation of the pyridine nitrogen by preferentially bonding with maleic acid. However, the second ionization of the maleate monosalt is apparently of sufficient magnitude to accomplish weak protonation of the pyridine nitrogen and to produce a measurable effect on the UV absorption of brompheniramine.

To observe the effect of varying degrees of protonation on the UV absorption of brompheniramine, UV spectra were obtained for brompheniramine base in three organic solvents of differing H-donating (H-bonding) abilities (Fig. 5). Identical aliquots from the same I standard stock solution made basic with sodium hydroxide were separately extracted with chloroform, methylene chloride, and *n*-hexane as in the NF assay for I tablets. The extracts were diluted to the same final concentration with the appropriate solvent, and their UV spectra were recorded by scanning *versus* similarly treated solvent blanks. UV tests for completeness of extraction showed total extraction of the brompheniramine base with each solvent using the five 35-ml portions of solvent specified in the method.

Increased UV absorption was exhibited with increasing H-bonding ability of the solvent. Highest brompheniramine absorption at 263 nm was obtained using the best H-donating solvent, chloroform; the absorption was about 5 and 10% lower in the poorer H-bonding solvents, methylene chloride and *n*-hexane, respectively. In addition, brompheniramine and its maleate salt exhibit about twice the UV absorption (with loss of spectral detail) in more highly protonated environments, such as 0.1 *N* HCl, than in chloroform. This is evidenced by the concentrations of the UV solutions used in the NF assay for dexbrompheniramine compared to those for brompheniramine maleate tablets (see also Figs. 1 and 2). These data indicate that the greater the "degree of protonation" of the pyridine chromophore by its surrounding environment, the greater is the increase in UV absorption of the brompheniramine molecule.

Other organic nitrogenous base drugs exhibit different UV spectra for their free amine and salt forms when both are determined in the same neutral organic solvent. Methapyrilene hydrochloride and thonzylamine hydrochloride exhibit increased UV absorptions at somewhat lower wavelengths than their free base forms when measured in chloroform and methylene chloride, respectively (Figs. 6 and 7). In these studies, aqueous base was employed to remove the hydrochloric acid from aliquots of standard stock solutions of the drugs prepared in organic solvents. The free amine-containing solutions were diluted with corresponding organic solvents to a final concentration equivalent to that of their respective standard salt solutions and the UV spectra were recorded. Since these drugs are salts of a nonchromophore-containing acid, the only influence on UV absorption must be due to bonding effects between the proton donating hydrochloric acid and amine chromophores.

CONCLUSIONS

The data indicate that low standard recoveries obtained by the NF XIII assay for brompheniramine maleate tablets are attributable to the chromophoric properties of the maleic acid moiety and to the ability of maleic acid to protonate the α-pyridyl chromophore when in the amine maleate salt form and measured in neutral organic solvents.

Two recommendations appear to offer the best remedies for achieving increased analytical accuracy for present compendial assays of antihistamine maleate preparations that compare the UV absorptions of sample and standard solutions of unidentical molecular species at wavelengths below 280 nm: *(a)* employ an experimentally determined factor in the calculations which relates the absorbance of the standard maleate salt to the absorbance of an equivalent weight of the free amine[2], or *(b)* extract standards in the same manner as samples[3].

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ˣ To whom inquiries should be directed.

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[3] The assay procedures for brompheniramine maleate tablets, dexbrompheniramine maleate tablets, dexchlorpheniramine maleate syrup, and dexchlorpheniramine maleate tablets were revised in the Fifth Supplement to the National Formulary, 13th ed., to include extraction of standards in a similar manner as samples.