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Determination of the Structure of a Synthetic Impurity in Guaifenesin: Modification of a High-Performance Liquid Chromatographic Method for Phenylephrine Hydrochloride, Phenylpropanolamine Hydrochloride, Guaifenesin, and Sodium Benzoate in Dosage Forms

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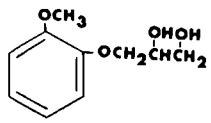
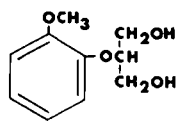
Abstract □ An impurity present in all commercial guaifenesin-containing dosage forms examined was isolated and identified as 2-(2-methoxyphenoxy)1,3-propanediol (VI). The eluant of a previously developed stability-indicating liquid chromatographic method for phenylephrine hydrochloride (I), phenylpropanolamine hydrochloride (II), and guaifenesin (III) was modified to yield a better separation between phenylpropanolamine and the impurity. The method was expanded to include sodium benzoate (IV), a preservative found in some liquid formulations.

Keyphrases □ Guaifenesin—synthetic impurity, HPLC with phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and sodium benzoate

A stability-indicating reverse-phase high-performance liquid chromatographic (HPLC) method for phenylephrine hydrochloride (I), phenylpropanolamine hydrochloride (II), and guaifenesin (III), primarily in capsule formulations, was recently presented (1). However, on further examination of a wide variety of capsule, tablet, and liquid formulations from various manufacturers, it became evident that all chromatograms of products containing III (and even standard III solutions) yielded an impurity that eluted immediately following the II peak. In early method development work involving the capsule formulations, the peak area of this III impurity was only slightly more than 2% of the area of II (~0.2% of the III area) and barely discernable in the tail of the II peak (*cf.* Fig. 2 of Ref. 1). The separation was sufficient to yield good analytical data. However, impurity peak areas were later observed in other commercial formulations that were >20% of the area of the II peak. Because of the relatively large amount of the III impurity in some formulations, the ubiquitous nature of the impurity, and the danger of loss of resolution between II and the impurity for less efficient columns, both elucidation of the impurity structure and modification of the chromatographic eluant system to yield better resolution between II and the impurity were sought.



III



VI

EXPERIMENTAL SECTION

Reagents and Chemicals—All reagents and chemicals were ACS, USP or NF quality and were used without further purification. Compounds I, II, and III were used as received[1].

2-(2-Methoxyphenoxy)-1,3-propanediol (VI) was synthesized according to a literature procedure (2). Recrystallization from ethyl acetate–hexane, yielded a white powder, mp 62–64°C [lit. (2) mp 61–62°C]. IR (KBr): 3325 (O—H), 3041 (ArC—H), 3000–2850 (aliphatic C—H), 2837 (methoxy C—H), 1600–1450 (Ar deformations), 1240 and 1100–1000 (C—O), and 750 cm^{-1} (1,2-substituted Ar ring C—H, out of plane deformation); ^{1}H-NMR (acetone-d_6): δ 7.07 (ArH), 4.25 (—OCH), 3.87 (—OCH_3) and 3.85 ppm (—CH_2OH).

The *para* isomer of III, 3-(4-methoxyphenoxy)-1,2-propanediol, was pre-

[1] Norwich Eaton Pharmaceuticals, Inc.

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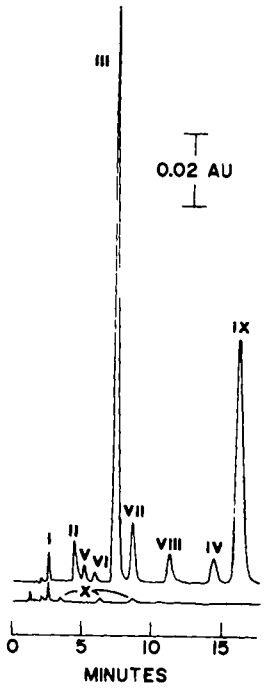

Figure 1—*Chromatogram of 40 μg/mL of phenylephrine hydrochloride (I), 400 μg/mL of phenylpropanolamine hydrochloride (II), 1.8 mg/mL of guaifenesin (III), 40 μg/mL of benzoic acid (IV), and possible impurities and degradants: 1 μg/mL of α-aminopropiophenone (V), 20 μg/mL of 2-(2-methoxyphenoxy)-1,3-propanediol (VI), 4 μg/mL of* m*-hydroxybenzaldehyde (VII), 100 μg/mL of guaiacol (VIII), 40 μg/mL of benzaldehyde (IX), and forced degraded phenylephrine hydrochloride products (X).*

pared according to a general procedure for synthesis of α-glyceryl phenyl ethers (3). Recrystallization from ethyl acetate–hexane yielded a white powder, mp 80–82°C [lit. (4) mp 76–78°C]. IR (KBr): 818 cm^{-1} (*para*-substituted benzene ring); otherwise, the spectrum was similar to that for III.

Degradation products of I were formed by storing I in 0.5 M KOH at 80–90°C for 1 week (1).

Apparatus—The HPLC apparatus described previously (1) was used. The system used for quantitative assay of I, II, III, and the preservative sodium benzoate [as benzoic acid (IV)] consisted of an absorbance detector at 254 nm, a 20-μL loop injector, and a C_8 column[2] pumped at 2.0 mL/min with an eluant containing 50 mL of methanol, 170 mL of acetonitrile, 755 mL of water, and 25 mL of pentanesulfonic acid sodium salt in glacial acetic acid[3]. The resulting eluant was 5 mM in ion-pairing salt and 1.7% in acetic acid. The HPLC system used for collection of the impurity consisted of a C_{18} column[4] with water–methanol (80:20) as eluant at a 1.5-mL/min flow rate. To collect the impurity, 10 fractions from 100-μL injections of a 107-mg/mL aqueous solution of III were collected from the chromatographic region just prior to the elution of the III peak. The combined fractions were evaporated to a total volume of 1 mL.

A GC apparatus[5] was used with a photoionization detector at 10.2 eV and a 1.8 m × 2 mm i.d. glass column packed with 3% OV-17 on Chromasorb G-HP. Both III and the collected impurity were treated with *n*-butylboronic acid for 30 min. One microliter or less of both derivatized and underivatized solutions were injected at a column temperature of 200°C and nitrogen flow rate of 25 mL/min.

^{1}H-NMR spectra were obtained on an 80-MHz Fourier-transform instrument[6]. Samples were dissolved in 99.96 atom-%D hexadeuterated acetone with tetramethylsilane as internal standard. IR spectra were obtained from KBr pellets with a Fourier-transform instrument[7].

Standard Solution Preparation—The final HPLC method used aqueous standard solutions containing 0–0.1 μg/mL of I, 0.24–0.45 μg/mL of II, 2–5.6 μg/mL of III, and 0–0.12 μg/mL of IV, depending on the label claim of the dosage form assayed.

Sample Preparation—For the capsule and tablet samples not containing I, 200 or 300 mg of ground sample was leached with water or mobile phase and diluted to 50 mL. For capsule samples containing I, to obtain a more homogenous sample, 1–1.3 g of ground sample was leached with water and diluted to 250 mL. The samples were injected directly after a 10-min sonication and 5-min centrifugation at 2500 rpm. For the liquid samples, 4.0–25.0 mL (depending on label claim) was diluted to 250 mL with water and then injected directly.

Table I—Area Linearity

Parameter	Phenylephrine Hydrochloride	Phenylpropanolamine Hydrochloride	Guaifenesin	Sodium Benzoate[a]
Correlation coefficient	0.99996	0.99997	0.9999	0.9999
Standard error of the estimate ($S_{y/x}$)	270	800	24,000	3700
Intercept, %[b]	−0.3	0.7	1.4	1.4
Variation, %[c]	0.7	0.6	1.2	1.2

[a] As benzoic acid. [b] (y-Intercept/$\bar{y}$) × 100, where $\bar{y}$ is the average y. [c]($S_{y/x}$ / $\bar{y}$) × 100.

RESULTS AND DISCUSSION

Elucidation of Impurity Structure—Gas chromatograms of both derivatized and underivatized III and collected impurity samples revealed that cyclic butylboronate derivatization increased the retention time of both compounds by ~50%. This indicated that, like III, the impurity required two hydroxyl groups, no more than one carbon atom apart, for formation of the cyclic butylboronate derivative (5). Logical possibilities included the *para* isomer of III and 2-(2-methoxyphenoxy)-1,3-propanediol (VI). The former was immediately excluded since it was found to possess nearly the same HPLC retention time as III.

^{1}H-NMR of both the collected impurity and the synthesized compound (VI) in hexadeuterated acetone yielded practically identical spectra, provided the concentration of the synthesized compound was dilute (≤1 mg/mL)[8]. Intermolecular hydrogen bonding resulted in peak shifts in the spectra at higher concentrations.

The collected impurity sample was lyophilized to remove the eluant and pressed into a KBr pellet. The IR spectrum of the impurity was practically identical to that of the synthesized compound, again provided that the concentration of the synthetic compound was low enough to minimize hydrogen bonding effects[8].

HPLC of the synthesized compound using both C_8 and C_{18} columns yielded a retention time identical to that for the impurity observed in III samples. The ratio of molar absorptivities of III to VI at 254 nm was found to be 1:1.

Modification of the Liquid Chromatographic Stability-Indicating Assay—Any HPLC system developed should be specific for the analytes I, II, III, and IV with respect to the following known and postulated impurities and degradation products (1): α-aminopropiophenone (V), a precursor and postulated (but so far undetected) trace impurity of II; 2-(2-methoxyphenoxy)-1,3-propanediol (VI), the identified III impurity; *m*-hydroxybenzaldehyde (VII), an observed degradation product of I; guaiacol (VIII), an observed hydrolysis product of III; benzaldehyde (IX), an observed hydrolysis product of II; forced-degradation products of I (X).

To achieve the desired resolution between II and VI and maintain the separation from the above compounds, the organic modifier of the original eluant system (1) was changed from 30% methanol to 5% methanol and 17% acetonitrile (Fig. 1). The separation was maintained on several C_8 columns, including one that had seen heavy use. An internal standard was not deemed necessary, due to the high precision obtained with fixed-loop injectors.

The linearity data for I, II, III, and IV, determined by plotting peak areas *versus* standard weights, are presented in Table I. Linearity was observed over the ranges of 0.4–3.4 μg for I, 3.3–31 μg for II, 14.5–138 μg for III, and 1–9.3 μg for IV.

Assay of 12 synthetic capsule samples made by spiking placebo with solutions containing known amounts of standard I, II, and III at levels of 50–150% of the theoretical values yielded average recoveries and *RSD* values of 99.8

[2] Whatman Partisil-10.
[3] PIC-B5; Waters Associates.
[4] μ-Bondapak C_{18}; Waters Associates.
[5] HNU Model 401.
[6] Bruker WP-80.
[7] Nicolet MX-1.
[8] Spectra furnished on request.

Table II—HPLC Assay of Capsule [a] and Liquid [b] Samples

Sample Weight or Volume	Phenylephrine Hydrochloride, mg/g	Phenylpropanolamine Hydrochloride, mg/g or mL	Guaifenesin, mg/g or mL	Sodium Benzoate, mg/mL[c]
Weight, g				
0.651	9.61	82.6	375	
0.661	9.61	85.0	382	
0.666	9.34	83.7	376	
1.046	9.36	82.2	373	
1.078	9.73	81.7	371	
1.303	9.48	82.2	373	
1.331	9.65	84.0	375	
1.328	9.45	83.9	375	
1.927	9.45	84.0	375	
1.922	9.54	84.3	375	
1.137	9.83	82.9	365	
1.912	9.62	83.4	365	
Mean, mg/g	9.56	83.7	372	
RSD, %	1.5	0.6	1.4	
Volume, mL				
15.0		2.52	19.3	1.16
20.0		2.51	19.1	1.15
25.0		2.48	19.1	1.14
30.0		2.50	19.2	1.15
35.0		2.50	19.2	1.16
Mean, mg/mL		2.50	19.2	1.15
RSD, %		0.6	0.5	0.7

[a] Entex Capsules, Norwich Eaton Pharmaceuticals, Inc. Label is 9.4 mg/g for phenylephrine hydrochloride, 85 mg/g for phenylpropanolamine hydrochloride, and 377 mg/g for guaifenesin. [b] Head & Chest Liquid, Procter & Gamble. Label is 2.56 mg/mL for phenylpropanolamine hydrochloride, 20 mg/mL for guaifenesin, and 1.20 mg/mL for sodium benzoate. [c] As benzoic acid.

Table III—HPLC Assay of Various Commercial Dosage Forms

Dosage Form	Phenylephrine Hydrochloride		Phenylpropanolamine Hydrochloride		Guaifenesin		2-(2-Methoxy-phenoxy)-1,3-propanediol, % of Guaifenesin Conc.
	Assay, mg/mL	Label, %	Assay, mg/mL or g	Label, %	Assay, mg/mL or g	Label, %	
Liquid[a]	—	—	2.36	94.4	19.5	97.7	1.5
Liquid[b]	—	—	9.0	99.9	31.6	105.2	2.3
Liquid[c]	—	—	2.52	99.8	20.0	99.8	0.2
Liquid[d]	—	—	1.26	100.3	9.8	97.9	0.3
Liquid[e]	—	—	2.66	106.4	9.8	97.7	2.0
Capsule[f]	9.56	101.7	83.7	98.5	372	98.7	0.2
Tablet[g]	—	—	39.2	100.2	299	97.9	0.2

[a] Triaminic (Dorsey). [b] Naldecon Ex (Bristol). [c] Robitussin CF (A. H. Robins). [d] Dorcol (Dorsey). [e] Vicks Formula 44D (Vicks). [f] Entex Capsules (Norwich Eaton Pharmaceuticals, Inc.); from Table II. [g] Head & Chest Tablets (Procter & Gamble).

± 1.1%, 99.7 ± 1.2%, and 99.3 ± 1.0%, respectively. Similarly, assays of 12 synthetic liquid samples for II, III, and IV yielded average recoveries and *RSD* values of 99.7 ± 1.5%, 100.1 ± 1.5%, and 100.5 ± 1.5%, respectively.

The assay results for I, II, III, and IV in several commercial dosage forms representing liquid, tablet, and capsule preparations are presented in Tables II and III. In addition, assay values for VI (as percentage of III concentration) in seven different formulations from six different manufacturers are also presented in Table III. The impurity VI was observed in each formulation examined at concentrations ranging from 0.2 to 2.3% of the III concentration.

The widespread distribution of VI in formulations containing III prompted assessment of the potential toxicity of VI. According to literature data (16), when administered to mice, impurity VI is actually less toxic [mean paralyzing dose (PD_{50}) = 800 mg/kg, mean lethal dose (LD_{50}) = 1710 mg/kg] than III (PD_{50} = 320 mg/kg, LD_{50} = 1200 mg/kg). This information, coupled with the similarity in structure between III and VI, indicates the potential toxicity of VI is minimal.

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