Rapid Analysis of Phentolamine by High-Performance Liquid Chromatography

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Abstract

A rapid liquid chromatographic method is validated for the quantitative analysis of phentolamine. Phentolamine exists in three forms for this investigation: as a mesylate salt, hydrochloride salt, and free base. In solution, phentolamine dissociates from its salt and is chromatographed as free phentolamine. This validation confirms the analysis of each form, which is simply based upon molar mass differences encountered in weighing. As such, both the United States Pharmacopeia hydrochloride and mesylate standards are used throughout this validation to demonstrate this equivalency. The validation demonstrates that this method may be used to quantitate phentolamine, regardless of its salt form.

Introduction

Phentolamine mesylate is an antihypertensive agent sold by Ciba Geneva (Summit, NJ) under the registered trade name of Regitine. The drug produces an alpha-adrenergic block of relatively short duration. Phentolamine mesylate also has vasodilator effects on vascular smooth muscle.

Chromatographic analysis of phentolamine has consisted of gas (1), thin-layer (2), and liquid chromatographic (LC) (3–11) determinations. Gas chromatographic (GC) analyses require a derivatization step in order to increase the volatility of phentolamine. As such, LC has a distinct advantage over GC in terms of sample preparation.

Mollica et al. (3) developed an LC method scheme for the analysis of imidazoline drugs. For phentolamine analysis, the scheme used a strong cation exchange column and a pH 11.45 buffer at 0.8 mL/min and UV detection at 254 nm. The method was not investigated for the resolution of the synthesis precursors of phentolamine.

de Bros and Wolshin (4) and Godbillon and Carnis (5) used LC in blood and urine determinations. The de Bros and Wolshin system used a µBondapak C18 column and an ion pairing mobile phase flowing at 2.4 mL/min. The mobile phase was 52% methanol (at an apparent pH of 4.0)/48% of 6.16mM octanesulfonic acid (with 1% acetic acid aqueous phase at pH 4.0). Using a UV detection wavelength of 280 nm and antazoline and naphazoline as internal standards, the de Bros and Wolshin system established an average recovery of 85% over a 15–5000 ng/mL phentolamine range. The Godbillon and Carnis system used a Lichrosorb R-18 or R-8 (10 µm) column for urine samples and a Lichrosorb R-8 (5-µm) column for plasma samples. The mobile phase for this system was 60% 2.6mM orthophosphoric acid–40% acetonitrile (flowing at 3 mL/min for urine samples) and 85% HCl-sodium citrate buffer (at pH 4.0)–15% acetonitrile flowing at 2 mL/min for plasma samples. The Godbillon and Carnis system posted a sensitivity limit of 200 ng/mL phentolamine for plasma samples and 2–3 µg/mL phentolamine for urine samples using UV detection at 254 nm.

Phentolamine was determined in serum and liver homogenate samples by Kerger et al. (6). The system used a Phase-2 ODS column and a mobile phase of 75% of a 0.15M monochlororacetate solution (at pH 3.0)–25% acetonitrile spiked with 0.35 g/L EDTA. The mobile phase flowed at 0.6 mL/min and electrochemical detection was carried out at +900 mV. The Kerger et al. system yielded a detection limit of 5 ng/mL phentolamine in serum and 10 ng/mL in liver.

The chemical stability of phentolamine mesylate and papaverine HCl formulation solutions was studied by LC by Torrado-Valeiras et al. for i.v. injections. The system used a C18 column, UV detection at 254 nm, and a mobile phase consisting of 65% methanol–35% of a 1% acetic acid solution adjusted to pH 4.0 with triethylamine. Torrado-Valeiras et al. found that the chemical degradation of phentolamine and papaverine dissolved in 5% glucose and stored for 2 months at 5, 30, 45 and 60ºC, and after 4 years at room temperature it was less than 10%.

LC has proven to be an effective tool for the quantitative analysis of phentolamine. However, compendial potency determinations are still based on titrimetic determinations (12). Because LC is much more selective, stability-indicating, and can be used to analyze for impurities, a rapid LC analysis for phentolamine was developed to replace the compendial titrimetic assay. Phentolamine was found to be effectively resolved from its synthesis precursors using an ODS-AQ column (Waters, Milford, MA) and an isocratic mobile phase consisting of a heptanesulfonic acid

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buffer, methanol and acetonitrile in a ratio of 35:55:10 (v/v/v).

Phentolamine exists in three forms for this investigation: (*a*) as a mesylate salt, (*b*) a hydrochloride salt, and (*c*) a free base. In solution, phentolamine dissociates from its salt and is chromatographed as free phentolamine. The analysis of each form is simply based upon molar mass differences encountered in weighing. Unlike the United States Pharmacopeia (USP) titration assay, LC presents the opportunity to quantitate all three salt forms. The ability to assay phentolamine regardless of its salt form is important for reaction monitoring and quality control. As such, the use of both the USP hydrochloride and mesylate standards are used throughout this validation to demonstrate this equivalency.

Experimental

LC

The LC data reported was generated using three equivalent systems: (*a*) HP Series 1050 system with an HP Series 1050 PDA photodiode array detector (Hewlett-Packard, Palo Alto, CA), (*b*) HP Series 1050 system equipped with an HP Series 1050 UV detector, and (*c*) Alliance 2690 Module equipped with a Waters 486 UV detector. All three systems ran at ambient temperature and with an injection volume of 10 µL. An ODS-AQ, 5-µm, 120 A, 4.6 × 150 mm C18 column phase I and a Metasil AQ 5-µm, 120 A, 4.6 × 150 mm C18 column phase II (Metachem, Torrance, CA) were used interchangeably in this study. All columns were used with a flow rate of 1.0 mL/min and with the detector wavelength set at 220 nm.

Mobile Phase

The mobile phase buffer (15mM heptanesulfonic acid) consisted of 6.06 g heptanesulfonic acid and sodium salt weighed into a 2-L flask, which contained approximately 1 L of H_20. Upon total dissolution, the solution was adjusted to a pH of 3.0 using 2N H_2SO_4 and diluted to mark with H_2O. For the chromatographic mobile phase, the buffer was diluted with methanol and acetonitrile in a ratio of 35:55:10 (v/v/v), respectively.

Sample and standard preparation

The samples and standards were prepared to a concentration of 0.1 mg/mL in a dilution solution (15mM heptanesulfonic acid buffer and 500 mL methanol in a ratio of 1:1, v/v).

Validation materials

The standards were the current USP standards for phentolamine hydrochloride (Lot F) and phentolamine mesylate (Lot H). The phentolamine free base; phentolamine hydrochloride; phentolamine mesylate; 2-chloromethylimidazoline HCl,4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole; 3-hydroxy-4'-methyldiphenylamine; 3-acetoxy-4'-methyldiphenylamine; and 3-acetoxy-4'-methyldiphenylaminoacetonitrile samples used for the method validations were all synthesized for this study. The structures for these compounds are given in Figure 1.

Results and Discussion

Phentolamine synthesis

The synthesis of 4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-(*p*-methylphenyl)amino-methyl]-1H-imidazole (phentolamine) was reported in 1950 by Urech et al. and patented in the same year by Miescher (15). The patent disclosed two methods of preparing phentolamine hydrochloride. The first example described the reaction of 3-hydroxy-4'-methyldiphenylamine with 2-chloromethylimidazoline hydrochloride to yield phento-

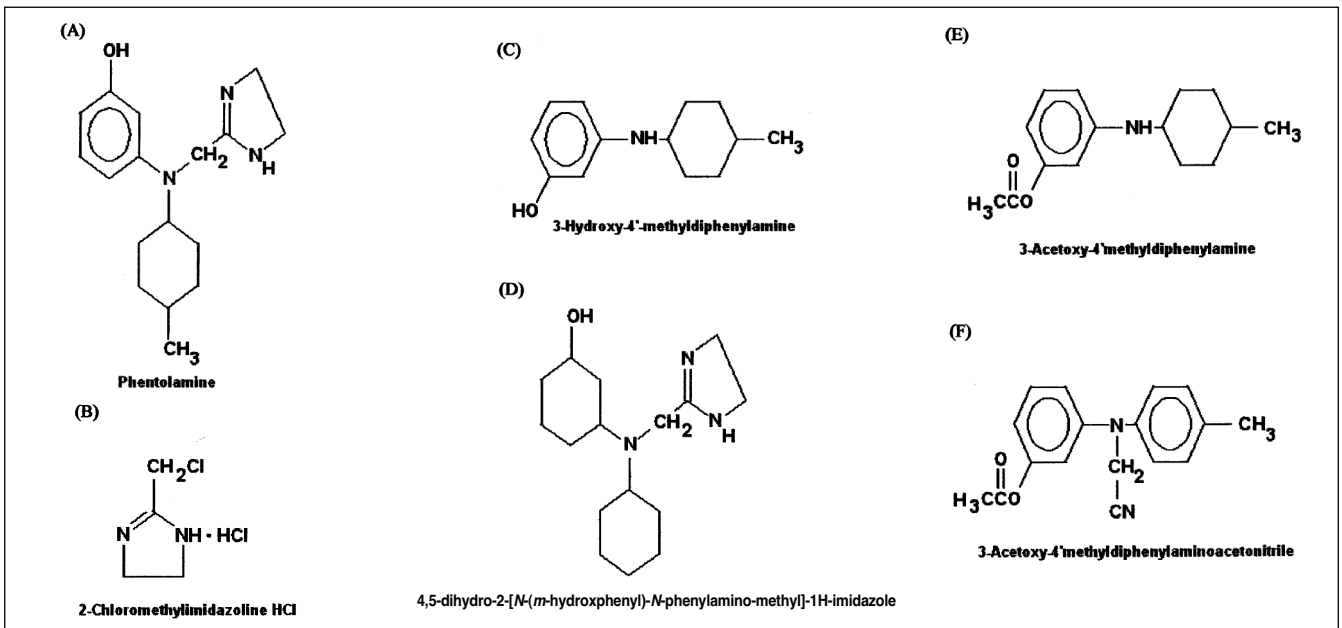

Figure 1. Structures for compounds of interest including phentolamine (A), 2-chloromethylimidazoline HCl (B), 3-hydroxy-4'-methyldiphenylamine (C), 4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole (D), 3-acetoxy-4'-methyldiphenylamine (E), and 3-acetoxy-4'-methyldiphenylaminoacetonitrile.

lamine hydrochloride. Specifically, Miescher described a process in which two equivalents of 3-hydroxy-4'-methyldiphenylamine and one equivalent of chloromethylimidazoline hydrochloride were heated at 150ºC for 16 h under a nitrogen atmosphere. The viscous liquid was then cooled to less than 100ºC and partitioned between water and ethyl acetate. The desired product was obtained from the aqueous layer upon cooling. No yields were cited in the patent.

In U.S. Patent 2,503,059, Miescher described an alternate method of synthesizing phentolamine from 3-hydroxy-4'-methyldiphenylamine. This procedure built the imidazoline ring onto the diphenylamine in three steps. First, the sodium salt of 3-hydroxy-4'-methyl-diphenylamine was acetylated with acetyl chloride to yield 3-acetoxy-4'-methyldiphenylamine. The 3-acetoxy-4'-methyldiphenylamine was then reacted with paraformaldehyde and potassium cyanide in aqueous acetic acid to give 3-acetoxy-4'-methyldiphenylaminoacetonitrile. Finally, the nitrile was heated with ethylenediamine in the presence of a catalytic amount of hydrogen sulfide (or carbon disulfide) to produce phentolamine. The reaction mixture was partitioned between ethyl acetate and dilute hydrochloric acid. Phentolamine hydrochloride was obtained after concentration of the aqueous layer.

The inventors published a variation of the first method (14)

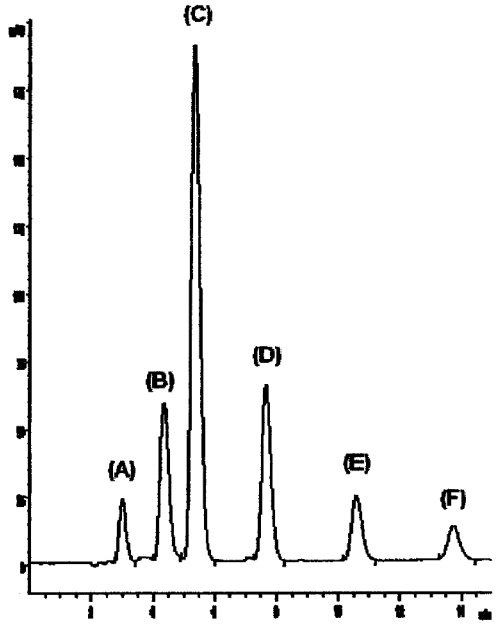

Figure 2. LC analysis of phentolamine in the presence of potential synthesis materials using column phase I. The peaks represent 2-chloromethylimidazoline HCl (A), 4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole (B), phentolamine (C), 3-hydroxy-4'-methyldiphenylamine (D), 3-acetoxy-4'-methyldiphenylamine (E), and 3-acetoxy-4'-methyldiphenylaminoacetonitrile (F).

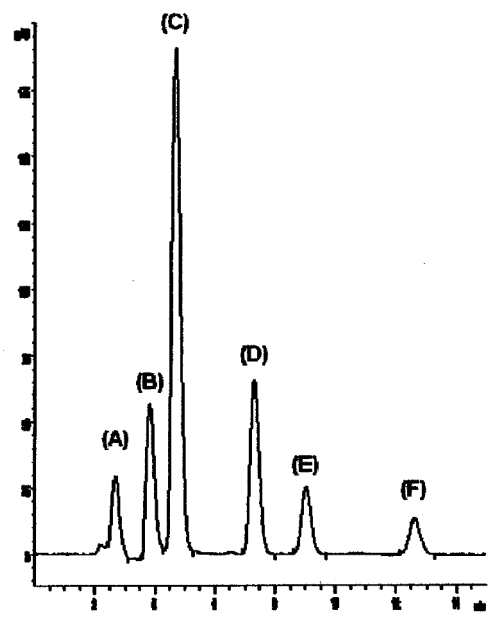

Figure 3. LC analysis of phentolamine in the presence of potential synthesis materials using column phase II. The peaks represent 2-chloromethylimidazoline HCl (A), 4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole (B), phentolamine (C), 3-hydroxy-4'-methyldiphenylamine (D), 3-acetoxy-4'-methyldiphenylamine (E), and 3-acetoxy-4'-methyldiphenylaminoacetonitrile (F).

Table I. LC Accuracy Runs of Phentolamine Salts vs. USP Assay

	LC system 1 column phase I		LC system 2 column phase I		LC system 2 column phase I		LC system 3 column phase II	USP titration assay (%)
Phentolamine salt	HCl standard	Mesyl standard	HCl standard	Mesyl standard	HCl standard	Mesyl standard	HCl standard	
Mesyl-1	100.5	99.9	98.7	99.2	100.5	98.3	98.7	99.0
Mesyl-2	99.1	99.1	98.5	99.0	98.6	97.7	98.7	100.5
Mesyl-3	99.4	99.4	98.9	99.4	99.2	98.3	98.8	100.7
Mesyl-4	99.8	99.3	97.9	98.4	98.5	97.6	98.4	97.7
Base-5	100.1	98.8	98.4	98.4	99.2	98.3	98.5	
Base-6	99.2	97.9	97.9	97.9	97.8	97.0	98.1	
Base-7	100.0	98.7	96.7	96.7	97.5	96.7	97.7	
Base-8	99.5	98.2	97.8	97.8	97.8	97.0	98.0	
HCl-9	96.5	95.0	94.9	95.3	93.6	92.9	95.6	
HCl-10	90.8	89.4	89.6	89.9	89.8	89.1	90.1	
HCl-11	99.3	97.8	97.2	97.5	97.0	96.3	97.4	
HCl-12	94.8	93.3	92.9	93.1	95.2	94.5	93.9	

whereby phentolamine hydrochloride was obtained by reacting equimolar amounts of chloromethylimidazoline hydrochloride and 3-hydroxy-4'-methyldiphenylamine in *o*-dichlorobenzene. After heating at reflux for 6 h, the gummy mass was filtered and recrystallized from water to produce phentolamine hydrochloride (no yield cited). The authors stated that xylenes were an acceptable substitute for dichlorobenzene. Phentolamine mesylate was obtained from the phentolamine hydrochloride by a simple salt switch.

LC analysis/validation

The methods established in the literature (3–7), as well as in-house data from similar studies, were used as a starting point for the analytical development work. The previous methods required higher mobile phase flow rates to yield short analysis times, and it was not established that the LC method would resolve phentolamine from its synthesis precursors. The latter requirement is necessary for a practical quality control procedure.

The de Bros and Wolshin (4) system established that an ion-pairing mobile phase was effective in producing a good peak shape with phentolamine. After optimizing the LC system with heptanesulfonic acid buffer–methanol gradient investigations, as suggested by Snyder et al. (13), the optimal resolution and peak shape of phentolamine was still unsuitable. Acetonitrile was added to the mobile phase and the peak shapes of the test compounds were greatly improved. The mobile phase conditions were optimized to be heptanesulfonic acid buffer, methanol, and acetonitrile in a ratio of 35:55:10 (v/v/v). LC columns from several column manufacturers were tested for performance. The ODS-AQ and an equivalent phase from Metachem were chosen because they produced the best resolution and peak shape performance (Figures 2 and 3).

Linearity of response/detection limits

The system linearity for phentolamine was validated to span approximately 50–150% of the nominal sample preparation used in the standard preparation. Each LC system produced a correlation coefficient (r) greater than 0.99. The worst limit of detection (LOD) (S/N = 3) and limit of quantitation (LOQ) (S/N = 10) were obtained for the photodiode array system. This system had an LOD of 1.75 ng/mL and an LOQ of 5.83 ng/mL of phentolamine using the USP mesylate standard.

Accuracy

The accuracy of the LC method was investigated by comparing the analysis results of the USP titration assay for phentolamine mesylate with the chromatographic result (Table I). Because the USP titration procedure does not work for the hydrochloride or free base salts because of solubility differences in the specified USP assay solvents, the LC and titration results could not be

Table II. Statistical Comparison of LC System Results vs. USP Assay for Phentolamine Mesylate*

LC system	USP	Column	t_{exp}
1	Phentolamine HCl	Phase i	0.24
	Phentolamine mesylate	Phase i	0.08
2	Phentolamine HCl	Phase i	1.79
	Phentolamine mesylate	Phase i	0.88
3	Phentolamine HCl	Phase i	0.34
	Phentolamine mesylate	Phase i	2.30
4	Phentolamine HCl	Phase ii	1.32

* t_{crit} = 3.18.

Table III. Standard Addition Investigations

Phentolamine salt	LC (%)	Sample recovery (%)	Spike recovery (%)
Mesyl-2	101.9		
Mesyl-2 spike	121.4	101.3	99.4
Mesyl-1	100.4		
Mesyl-1 spike	119.3	98.8	98.4
HCl-12	93.8		
HCl-12 spike	108.8	93.6	99.8
HCl-11	97.6		
HCl-11 spike	113.3	97.5	99.9
Base-7	97.8		
Base-7 spike	112.1	95.9	98
Base-5	99.3		
Base-5 spike	113.7	96.9	99.4

Table IV. Method Precision

LC system	Phentolamine salt		USP HCl standard	USP mesylate standard
1	HCl-9	Average	95.41	95.52
		Standard deviation	0.14	0.18
		%RSD	0.15	0.19
	Base-5	Average	98.21	98.81
		Standard deviation	0.2	0.24
		%RSD	0.21	0.25
	Mesyl 13	Average	98.62	99.18
		Standard deviation	0.18	0.18
		%RSD	0.19	0.18
2	HCl-9	Average	95.73	96.04
		Standard deviation	0.21	0.34
		%RSD	0.22	0.35
	Base-5	Average	98.88	99.48
		Standard deviation	0.13	0.14
		%RSD	0.13	0.14
	Mesyl-13	Average	98.42	98.93
		Standard deviation	0.13	0.14
		%RSD	0.13	0.14
3	HCl-9	Average	95.52	94.64
		Standard deviation	0.52	0.47
		%RSD	0.55	0.5
	Base-5	Average	98.8	97.67
		Standard deviation	0.55	0.56
		%RSD	0.56	0.57
	Mesyl 13	Average	100.21	98.83
		Standard deviation	0.21	0.24
		%RSD	0.21	0.24

directly compared for these salts. The accuracy investigations were conducted using both the USP mesylate and hydrochloride standards for comparison.

No significant difference in results was detected for the LC analysis of phentolamine mesylate and the USP titration assay. The analysis results for the phentolamine mesylate accuracy samples run on the three LC systems were investigated using a two-tailed paired *t*-test and a 95% confidence level (Table II). Each system and the corresponding USP standard used were compared with the compendial USP assay (titration) result. For the statistical comparison, the critical *t*-value was 3.1824. Thus, sample sets yielding a paired *t*-value less than this critical value were deemed "not significantly different." The data illustrate that no significant difference was detected using either the USP standard on the chromatographic systems or the USP compendial assay result. No significant difference was detected between the use of either the ODS AQ (Table II, phase I) or Metasil AQ (Table II, phase II) columns versus the compendial USP assay (titration) result.

Table V. System Precision

LC system	Phentolamine salt		USP HCl standard	USP mesylate standard
1	HCl-9	Average	95.3	95.7
		Standard deviation	0.1	0.1
		%RSD	0.1	0.1
	Base-5	Average	98.5	99.1
		Standard deviation	0.7	0.7
		%RSD	0.7	0.67
	Mesyl 13	Average	98.0	98.8
		Standard deviation	0.1	0.1
		%RSD	0.1	0.1
2	HCl-9	Average	95.8	96.2
		Standard deviation	0.1	0.1
		%RSD	0.1	0.1
	Base-5	Average	99.3	99.7
		Standard deviation	0.1	0.1
		%RSD	0.1	0.1
	Mesyl-13	Average	98.4	98.9
		Standard deviation	0.2	0.2
		%RSD	0.2	0.2
3	HCl-9	Average	95.4	94.3
		Standard deviation	0.1	0.1
		%RSD	0.08	0.1
	Base-5	Average	99.7	98.5
		Standard deviation	0.3	0.3
		%RSD	0.3	0.3
	Mesyl 13	Average	100.8	99.6
		Standard deviation	0.8	0.8
		%RSD	0.8	0.8

Standard addition study

Because the USP titration cannot access the potency of the hydrochloride salt or base, the accuracy of the LC method was further investigated for all three salt forms by spiking representative samples with phentolamine mesylate and establishing the recovery. All recoveries, regardless of salt complex, were 98% or better (Table III). Because this investigation illustrates equivalency in the LC assay of phentolamine regardless of its salt (mesylate, free base, or hydrochloride), this method, unlike the USP assay, can be used to quantitate any of these salts whereas the USP titration can be used solely for the mesylate salt.

Precision

Method precision was determined by analyzing five weights of a single phentolamine mesylate, free base, and hydrochloride lot for each LC system (Table IV). System precision for each LC system was obtained by analyzing six consecutive injections of a single phentolamine mesylate, free base, and hydrochloride sample preparation (Table V). All three LC systems exhibited acceptable precision per USP.

Ruggedness

The ruggedness of the LC method was determined by analyzing accuracy (Table I, system 2), method precision (Table IV, system 1), and system precision (Table V, system 1) using separate LC systems, ODS-AQ column, and an analyst. For this section, analyst 2 ran linearity and accuracy on LC system 2 and precision on LC system 1. Method precision for ruggedness was again determined by analyzing five weights of a single phentolamine mesylate, free base, and hydrochloride lot for each LC system. The LC method exhibited acceptable precision per USP.

System suitability parameters

The chromatographic system was also tested to verify that the

Table VI. System Suitability Figures for Phentolamine and its Synthesis Materials

Compound	LC phase	Resolution I*	Resolution II†	Tailing I*	Tailing II†	Theoretical plates I*	Theoretical plates II†
2-Chloromethyllmidazline HCl		–	–	1.5	1.0	890	544
4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole		2.9	2.3	1.3	1.5	1144	892
Phentolamine		1.9	1.6	1.3	1.3	1539	1214
3-Acetoxy-4'-methyl diphenylaminoacetonitrile		4.2	4.8	1.2	1.2	3102	2892
3-Acetoxy-4'-methyl diphenylamine		5.0	3.1	1.2	1.2	4730	3928
3-Hydroxy-4'-methyl diphenylamine impurity		4.8	5.8	1.1	1.0	6080	5897

* ODS-AQ column
† Metasil AQ column

phentolamine peak maintained its integrity in the presence of the potential starting materials used for its synthesis: 2-chloromethylimidazoline; 4,5-dihydro-2-[*N*-(*m*-hydroxyphenyl)-*N*-phenylamino-methyl]-1H-imidazole (an R&D synthesis marker); 3-hydroxy-4'-methyldiphenylamine, 3-acetoxy-4'-methyldiphenylamine; and 3-acetoxy-4'-methyldiphenylamino-acetonitrile. The system suitability parameters for these compounds using column phase i are presented in Table VI.

Specificity study

A forced degradation study was performed to verify the separation of phentolamine and its potential degradation products. To perform this study, a stock solution of phentolamine mesylate [0.25283 g of reference material in 25 mL of 50% heptanesulfonic acid (HSA)–50% methanol dilution solution] was diluted 1:10 in 1N HCl, 15mM heptanesulfonic acid, 1.25N NaOH, and 30% H_2O_2 as stress solvents. After an 18-h storage period at 60ºC, the samples were again diluted 1:10 in dilution solution and analyzed neat by LC. The degradation samples were compared with a freshly prepared standard preparation, and the percentage of phentolamine remaining was determined. The peak purity factor was confirmed using a Hewlett-Packard Series 1050 photodiode array detector. Chromatographic integrity was maintained for each stressed system. It was noted that the degradation peak produced with the HCl matrix eluted near the phentolamine peak; the system retained a resolution of 0.9 between these peaks. Under the degradation conditions used, the peak response for phentolamine remained acceptably homogeneous.

Conclusion

The LC method presented here has been qualified and found acceptable for use in the quantitative analysis of phentolamine as its free base, hydrochloride, and mesylate salts. This method was found to yield assay results not significantly different from the USP compendial titration assay for phentolamine mesylate. Whereas the USP procedure was ineffective at assaying the free base and hydrochloride salt of phentolamine, the LC procedure effectively validated and resolved phentolamine from its potential starting materials and degradants.

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