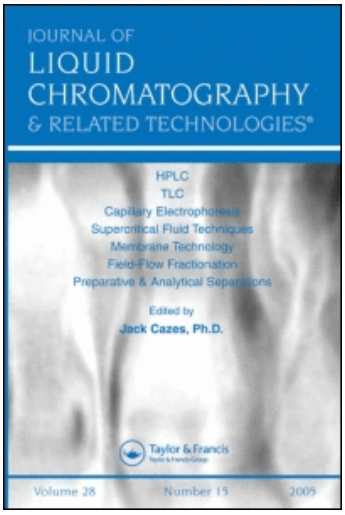

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Hplc Assay for Phenelzine Sulfate Drug Substance and the Decomposition Product Phenethyl Alcohol Using Short Wavelength UV Detection

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To cite this Article George, Garry D. and Stewart, James T.(1988) 'Hplc Assay for Phenelzine Sulfate Drug Substance and the Decomposition Product Phenethyl Alcohol Using Short Wavelength UV Detection', Journal of Liquid Chromatography & Related Technologies, 11: 11, 2399 — 2407
To link to this Article: DOI: 10.1080/01483918808067209
URL: http://dx.doi.org/10.1080/01483918808067209

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(11), 2399-2407 (1988)

HPLC ASSAY FOR PHENELZINE SULFATE DRUG SUBSTANCE AND THE DECOMPOSITION PRODUCT PHENETHYL ALCOHOL USING SHORT WAVELENGTH UV DETECTION

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ABSTRACT

An HPLC procedure for the determination of phenelzine sulfate in the presence of its oxidation decomposition products expressed as phenethyl alcohol has been developed. The method uses a mobile phase consisting of 20:80 acetonitrile - 0.05 M sodium dihydrogen phosphate (pH 2.5) containing 0.02 M heptane sulfonic acid sodium salt, pumped at a flow rate of 1.0 mL/min. through a 100 mm x 4.6 mm i.d. octadecylsilane column (5 um) with UV detection at 209 nm. As little as 0.005% decomposition of phenelzine, expressed as phenethyl alcohol (134.5 pg on column), can also be detected at 209 nm. Peak height or area versus concentration of phenelzine base is linear over the range of 7.5 to 22.5 mg (r^2 = 0.9982 for height and 0.9991 for area; n = 5). Analysis of spiked samples of phenelzine gave mean percent recoveries of 101.31 ± 0.78%. Precision of the method ranged from 0.33 - 1.42% and 0.53 - 0.85% using peak height and peak area data, respectively.

INTRODUCTION

Phenelzine sulfate (2-phenylethylhydrazine sulfate) was introduced in 1959 as a potent monoamine oxidase inhibitor used for the treatment of depression (1). The compound undergoes hydrolytic degradation to yield hydrazine and phenethyl alcohol as decomposition products. Further oxidation of phenethyl alcohol could yield phenylacetaldehyde and/or phenylacetic acid. The literature contains a few references to HPLC and GC procedures for the analysis of phenelzine sulfate and/or its hydrazine decomposition product. Jindal et. al. (2) reported a GC-MS determination for the drug in which the compound was derivatized with pentafluorobenzaldehyde and the derivative extracted into an organic solvent and then analyzed. Sensitivity of the method was reported to be 2 ng/mL with about 10% precision. Concern over the presence of hydrazine in pharmaceuticals led Matsui and coworkers (3) to develop an HPLC procedure for the detection of hydrazine in phenelzine sulfate formulations. Benzaldehyde was used to derivatize the hydrazine to benzalazine, which was then extracted into a chromatographically compatible organic solvent and injected into an HPLC. While this method was sensitive and precise for determining hydrazine, the instability of the phenelzine - benzaldehyde derivative precluded its use in the analysis of phenelzine.

In this paper, an HPLC method for phenelzine based on detection at 209 nm is reported. The method allows the detection of as little as 0.005% decomposition of phenelzine expressed as phenethyl alcohol in phenelzine drug substance. Ultraviolet absorption spectra of phenelzine and phenethyl alcohol in aqueous acetonitrile showed maximum absorption at 209 nm. Phenelzine assays in the literature are usually based on absorption at 254 nm in 50% aqueous methanol (4,5). Since its absorption at 209 nm in aqueous acetonitrile is about 50 times greater than that at 254 nm, this suggested that an HPLC assay for phenelzine employing short wavelength UV detection would provide a more sensitive method not only for the determination of phenelzine, but also for the estimation of phenelzine decomposition expressed as phenethyl alcohol.

EXPERIMENTAL

Reagents and Chemicals

HPLC grade acetonitrile was obtained from the J.T. Baker Chemical Company (Phillipsburg, NJ). Monobasic and dibasic sodium phosphate and phosphoric acid were used in the preparation of the buffer solutions in double distilled water. The mobile phases were filtered through 0.45 um nylon filters and degassed by either stirring under vacuum or by sonication.

1-Heptane sulfonic acid sodium salt was obtained from the Eastman Kodak Company (Rochester, NY).

Phenylacetic acid, phenethyl alcohol, and phenylacetaldehyde were obtained from the Aldrich Chemical Company (Milwaukee, WI).

Phenelzine sulfate was obtained from the Sigma Chemical Company (St. Louis, MO).

All other reagents and chemicals were of the highest grade commercially available.

Apparatus

UV spectra were obtained using either a Bausch and Lomb Spectronic 2000 or a Beckman Model DU-7 spectrophotometer.

pH measurements were made using a Fisher Accument Model 230A pH meter.

Unless otherwise noted, a Brownlee (Santa Clara, CA) octadecylsilane cartridge column (5 um, 100 mm x 4.6 mm i.d.) was employed.

The HPLC system consisted of a Beckman Model 110B pump (Fullerton, CA) equipped with a Rheodyne Model 7125 injector (Cotati, CA) fitted with a 20 uL sample loop. A Kratos Spectroflow Model 757 variable wavelength UV/VIS detector (Ramsey, NJ) was employed. Data collection was achieved using either a Houston Instrument Series 4500 microscribe strip chart recorder (Austin, TX) or a Shimadzu Model C-R3A integrator (Kyoto, Japan).

Mobile Phase

The mobile phase was prepared by dissolving 4.4 g of 1-heptane sulfonic acid sodium salt and 6.9 g of sodium dihydrogen phosphate

in 800 mL of double distilled water. The pH was then adjusted to 2.5 by adding concentrated phosphoric acid dropwise while stirring. A 200 mL volume of acetonitrile was added and the resulting solution was filtered and degassed.

Standard Solutions of Phenelzine Sulfate

Phenelzine sulfate working standards were prepared from a stock solution containing 6.45 mg/mL of phenelzine sulfate in a solution of 50:50 acetonitrile - pH 6 NaH_2PO_4/Na_2HPO_4 buffer. Aliquots of 2, 3, 4, 5, and 6 mL of the stock solution were pipetted into 100 mL volumetric flasks and diluted to volume with HPLC mobile phase to prepare concentrations of phenelzine for the standard curve.

RESULTS AND DISCUSSION

The goal of this study was to develop an HPLC assay for phenelzine sulfate drug substance and its oxidative decomposition products which was not dependent upon derivatization and/or extraction techniques. The observation that the UV detection of phenelzine in aqueous acetonitrile was greatly enhanced by using 209 nm rather than 254 nm led us to believe that the same might be true for its decomposition products. While the phenethyl alcohol, phenylacetaldehyde, and phenylacetic acid degradants exhibited good detectability between 205 and 220 nm, hydrazine failed to show any UV absorbance in this wavelength range. Attempts to derivatize hydrazine *in situ* such that it might be detected along with the other compounds around these wavelengths also failed. Hence, it was decided that the assay for the drug substance should be developed in such a manner as to indicate an estimation of phenelzine decomposition based on the presence of phenethyl alcohol and possibly any phenylacetaldehyde and/or phenylacetic acid present.

Various acetonitrile-water mobile phases (pH 2.5) and phenyl, cyanopropyl, and octadecylsilane columns were investigated during the project. In those mobile phase-column systems which produced reasonable retention times for phenelzine, phenethyl alcohol, phenylacetaldehyde, and phenylacetic acid were observed to co-elute.

Retention of phenelzine was greatly enhanced by ion-pairing of the drug with heptanesulfonic acid sodium salt. This enabled the phenelzine decomposition products to elute earlier than phenelzine such that the abundance of phenelzine in the sample would not interfere with their detection. The pH of the mobile phase was adjusted to 2.5 to prevent the ionization of any phenylacetic acid that might be present in a sample. An octadecylsilane column and a mobile phase consisting of 20:80 acetonitrile - 0.05 M sodium dihydrogen phosphate (pH 2.5) with 0.02 M heptane sulfonic acid sodium salt were finally selected for the assay since they provided the best separation of phenelzine and the co-eluting decomposition products. Under these HPLC conditions, the retention times for the co-eluting decomposition products and phenelzine as shown in Figure 1 were approximately 5.2 and 6.8 minutes, respectively, at a flow rate of 1.0 mL/min.

Although phenethyl alcohol is the dominant degradant present in phenelzine sulfate drug substance, the aldehyde and acid decomposition products have identical retention times in this HPLC assay. This would preclude the method from being used to quantitate each specific degradant. Equimolar concentrations of phenethyl alcohol, phenylacetaldehyde, and phenylacetic acid were chromatographed to compare their detector responses in the flowing stream system at 209 nm. Peak area counts of 449,405, 400,586, and 379,274, for phenethyl alcohol, phenylacetaldehyde and phenylacetic acid, respectively, were obtained indicating that their detector responses at 209 nm are comparable.

Various concentrations of phenethyl alcohol were chromatographed to determine the minimum detectable quantity that could be detected with this method based on a 15 mg phenelzine sample. Relative detectability was determined at both 209 and 254 nm. Based upon a signal to noise ratio of 2, the results shown in Table 1 indicate that there is a 50 fold increase in sensitivity in the detection of phenethyl alcohol at 209 versus 254 nm. This minimum detectable concentration of phenethyl alcohol would be equivalent to as little as 0.005% decomposition of phenelzine base. Therefore,

TABLE 1

Limits of Detection for Phenethyl Alcohol

	Wavelength	
	209 nm	254 nm
Minimum Detectable Phenethyl Alcohol Concentration[a]	55.06 pmoles/mL	2.75 nmoles/mL
Amount Injected on Column[b]	134.5 pg	6.73 ng
% Decomposition Calculated as Phenelzine[c]	0.005%	0.250%

[a]Based on S/N = 2

[b]20 uL loop

[c]Based upon 15 mg of phenelzine base

the assay should be very useful as an estimation of phenelzine decomposition.

For the quantitation of phenelzine, the standard curve was patterned after those concentrations that would be found in a commercially available tablet containing 15 mg of phenelzine base.

Standard solutions containing 7.5, 11.25, 15.0, 18.75, and 22.5 mg of phenelzine base in 100 ml of mobile phase were prepared from a phenelzine sulfate stock solution (6.45 mg/mL). The resulting solutions, equivalent to 50, 75, 100, 125, and 150% of the labeled strength of phenelzine base per tablet (15 mg), were then chromatographed to generate the standard curve data. Linear regression analysis indicated that the curve was linear over the 7.5 - 22.5 mg concentration range with $r^2 = 0.9982$ (n = 5) for peak height versus mg of phenelzine base and $r^2 = 0.9991$ (n = 5) for peak area versus mg of phenelzine base.

Spiked samples of phenelzine sulfate representing 9.375 mg and 20.625 mg of phenelzine base in 100 mL were prepared by diluting

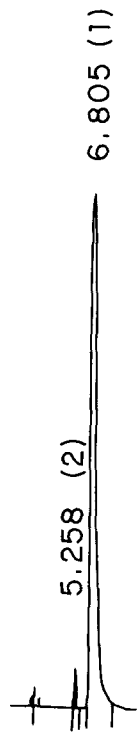

Figure 1. Typical HPLC Chromatogram showing the separation of phenelzine (1) and phenethyl alcohol (2) on a 5 um octadecylsilane column with 20:80 acetonitrile - 0.05 M sodium dihydrogen phosphate (pH 2.5) containing 0.02 M heptane sulfonic acid sodium salt at a flow rate of 1 ml/min and detection at 209 nm. Retention time in min. is indicated above each peak.

appropriate aliquots of the phenelzine sulfate stock solution with mobile phase. Five injections of each solution were made into the liquid chromatograph, and the peak heights and areas obtained were used to calculate recoveries of phenelzine based on the standard curve data (Table 2).

Mean phenelzine percent recoveries of 102.25% and 101.77% were obtained for 9.375 mg and 20.625 mg spiked samples, respectively, based on peak heights, and 101.06% and 100.17% for the samples based

TABLE 2

Accuracy and Precision of Spiked Phenelzine Samples

	Concn Added, mg	Concn Found, mg	% Recovery	
Peak Height	9.375	9.543	101.792	
		9.570	102.080	
		9.600	102.400	
		9.586	102.251	
		9.629	102.709	
		m±sd 9.586±0.032	102.246±0.343	0.334%[a]
	20.625	20.484	99.316	
		21.089	102.250	
		21.058	102.099	
		21.272	103.137	
		21.042	102.022	
		m±sd 20.989±0.297	101.765±1.440	1.415%
Peak Area	9.375	9.413	100.405	
		9.546	101.824	
		9.454	100.843	
		9.458	100.885	
		9.499	101.323	
		m±sd 9.474±0.050	101.056±0.538	0.528%
	20.625	20.455	99.176	
		20.670	100.218	
		20.519	99.486	
		20.868	101.139	
		20.794	100.819	
		m±sd 20.661±0.176	100.168±0.839	0.852%

[a] %RSD.

on peak areas. Relative standard deviations for the peak height data ranged from 0.334 to 1.415%, while peak area values had relative standard deviations ranging from 0.528 to 0.852%.

In summation, the use of short wavelength UV detection for the determination of drugs and their decomposition products can be very useful. However, mobile phases must be composed of those organic solvents which have sufficiently low UV cutoffs of less than 200 nm. Of the commonly used HPLC solvents, only acetonitrile is appropriate for this application in reverse-phase work. While the polarity of acetonitrile mobile phases would generally be sufficient for the elution of most components, selectivity may be lacking for more difficult separations. Finally, for those drugs of interest that may exhibit stronger absorbance properties at UV wavelengths less than 220 nm than at higher wavelengths such as 254 nm, it should be possible to quantitate them at much lower concentrations levels without the need for derivatization and/or extraction steps.

ACKNOWLEDGEMENT

This work was supported by a Fellowship to GDG by the United States Pharmacopeial Convention, Inc.

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