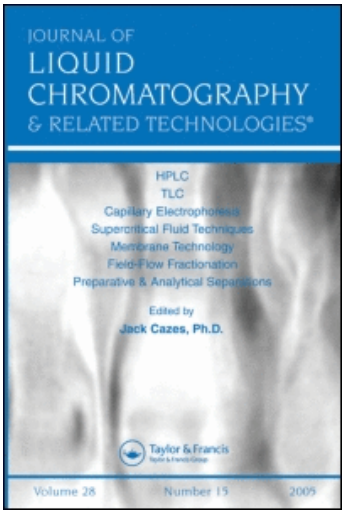

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Development of an HPLC Stability-Indicating Assay for 1-(2-Thienylethyl)-4-imidazoline-2-thione

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ABSTRACT

A stability-indicating assay method using high performance liquid chromatography was developed for monitoring 1-(2-thienylethyl)-4-imidazoline-2-thione bulk compound. A sample of the compound was dissolved in mobile phase and chromatographed using a Zorbax Rx-C8 column. The mobile phase composition was 35/65 (v/v) acetonitrile/water made 0.02 M in sodium phosphate buffer at apparent pH 6.9. Ultraviolet detection at 239 nm was used for the assay. Solutions containing 1-(2-thienylethyl)-4-imidazoline-2-thione were stressed using acidic and basic conditions at elevated temperatures. Oxidative stress using hydrogen peroxide treated solutions were also used to verify the stability-indicating capability of the developed chromatographic

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method. The validation and other facets of this developed method will be discussed.

Key Words: Stability-indicating assay; Zorbax Rx-C8 column; Basic drug analysis; HPLC.

INTRODUCTION

The production and storage of bulk active compound used in the manufacture of pharmaceuticals require high performance liquid chromatographic (HPLC) assay methods, capable of accurately determining the assay level of the active compound and separate all degradation peaks from the parent compound. Stability-indicating assays for pharmaceuticals have been reviewed,[1] and their development has often been described[2–4] in the literature. Long term storage, under various temperature and humidity conditions can affect the stability of a drug substance, and requires accurate methods to verify the active drug content of the material. Light degradation can be avoided by the storage container used; ambient temperature and humidity are not as readily controlled. The shipment of bulk drug material can introduce many uncontrolled variations during transport and should be considered,[5] even if the ambient conditions of the storage facilities can be well controlled.

The drug described in this study, 1-(2-thienylethyl)-4-imidazoline-2-thione (Fig. 1), is under study as a possible antihypertension agent and cardiotonic. Substituted 4-imidazoline-2-thiones and imidazole-2-thiones have been known to exhibit inhibition of dopamine β-hydroxylase.[6–8] Dopamine β-hydroxylase catalyzes the conversion of dopamine to norepinephrine.[9] The possible interference with the biosynthesis of norepinephrine has been thought to be a means for treating cardiovascular disorders, such as congestive heart failure and hypertension.[6]

The compound of interest, 1-(2-thienylethyl)-4-imidazoline-2-thione, as well as two of its known possible degradation products, 2-(2-aminoethyl) thiophene and 1,1-dimethoxy-2-isothiocyanatoethane, is basic in nature (see Fig. 1). Often, organic basic compounds analyzed on alkyl bonded silica HPLC columns elute in broad bands with peak tailing.[10–12] The degree of peak asymmetry varies among manufacturers of silica, and it is commonly believed that the poor chromatographic properties of basic compounds analyzed on silica based reversed-phase columns are due to hydrogen bonding of the ionized basic functional groups of the solute to non-bonded silanol groups on the silica support in the stationary phase.[11–14] Kohler and Kirkland[10] noted in 1987 that certain commercially prepared alkyl bonded silica materials exhibited low base adsorptivity, and they reported the production of a silica polymer exhibiting low basic

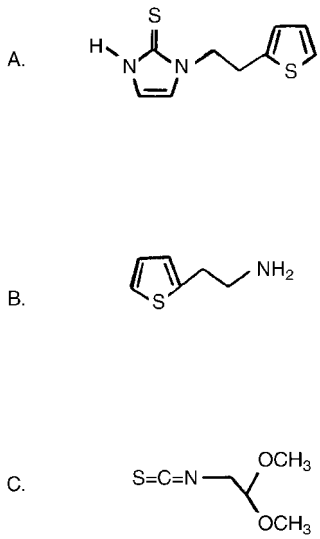

Figure 1. The structure of (A) 1-(2-thienylethyl)-4-imidazoline-2-thione, the drug substance and two of its suspected known degradation products, (B) 2-(2-aminoethyl)thiophene, and (C) 1,1-dimethoxy-2-isothicyanatoethane. The RRT to the parent drug peak of B is 0.6 and that of compound C is 2.

compound adsorptivity. The Zorbax Rx column was first produced by DuPont nearly 20 years ago and currently offered by Agilent Technologies; it contains low base adsorptivity packing. The Zorbax Rx-C8 has been used extensively in drug analysis and reported in the literature, often in the analysis of drug compounds with amine functional groups.[12–19] The Zorbax Rx-C8 packing material consists of a di-isopropyl *n*-octyl ether linkage to the silanol groups of the silica support and has been reported to have a very homogeneous distribution of surface silanol groups.[14] This results in low base adsorptivity and low peak asymmetry of the Zorbax Rx-C8 column.[20] Therefore, the Zorbax Rx-C8 column was ultimately used for the development of this reported HPLC method for the assay of 1-(2-thienylethyl)-4-imidazoline-2-thione.

EXPERIMENTAL

Reagents

High purity HPLC water was provided by a Barnstead (Boston, MA) NANOpure system followed with an ultraviolet radiation treatment by a

Barnstead ORGANICpure system. HPLC grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). Both sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate heptahydrate were ACS grade (Fisher Scientific, Fair Lawn, NJ). Concentrated hydrochloric acid, solid sodium hydroxide, and 30% hydrogen peroxide were commonly available ACS grade reagents. The 1-(2-thienylethyl)-4-imidazoline-2-thione drug substance, 2-(2-aminoethyl) thiophene, and 1,1-dimethoxy-2-isothiocyanatoethane were obtained "in-house."

Chromatographic Conditions and Apparatus

A Spectra-Physics (San Jose, CA) Model SP8800 liquid chromatograph equipped with a Rheodyne (Coatati, CA) Model 7010 injector valve and an Applied Biosystems (PE Biosystems, Norwalk, CT) Model 757 detector was used for all the HPLC experiments, at a detection wavelength of 239 nm. A Zorbax (Mac-Mod Analytical Inc., Chadds Ford, PA) Rx-C8 250 mm × 4.6 mm type column was used with the optimized conditions described. The mobile phase consisted of 35/65 (v/v) acetonitrile/water made 0.02 M in sodium phosphate buffer (2.2 g of sodium dihydrogen phosphate monohydrate, and 1.1 g of disodium hydrogen phosphate heptahydrate) were dissolved in each liter of mobile phase, apparent pH was ~6.9. The flow rate was 0.9 mL/min. Sample solution injection size was 20 uL. The final sample solution concentration for injection at the normal assay level was 0.0125 mg/mL. All final dilutions of the sample solutions were prepared in mobile phase. An H.P. Model 1040A (Agilent Technologies, Avondale, PA) photodiode array (PDA) HPLC detector was used to verify the peak homogeneity of stressed samples of the drug substance.

Stressed Samples

Multiple stressed samples were prepared by accurately weighing out ~25 mg of the drug substance, and then dissolving the drug substance in 5 mL of acetonitrile. Treatment with 5.0 mL of 5 M hydrochloric acid, 5 M sodium hydroxide, or 1% hydrogen peroxide was performed. The acid and base treated samples were heated at 85°C by a standard laboratory oven. The peroxide samples were held at room temperature in darkness. No light stress testing was performed. The solutions were diluted with mobile phase to a theoretical concentration of 0.0125 mg/mL assuming no degradation and chromatographed along with a non-stress standard sample.

Calculations

Peak areas were used to calculate the assay results for the drug substance. The calculation of assay result was as follows:

$$\left(\frac{A_{\text{sample}}}{A_{\text{std}}}\right)\left(\frac{W_{\text{std}}}{W_{\text{sample}}}\right)100 = \%\,\text{assay}$$

where A_{sample} is the area of the drug peak in the chromatogram of the sample solution; A_{std}, the area of the drug peak in the chromatogram of the standard solution; W_{sample}, the weight of the drug substance used for the sample; and W_{std} is the weight of the drug substance used for the standard.

DISCUSSION

The basic chromatographic conditions used for this method are based on previous work on an impurity test for 1-(2-thienylethyl)-4-imidazoline-2-thione.[21] The impurity test used a gradient system and the Zorbax Rx-C8 column. The column was retained and an isocratic mobile phase was used for the assay test method. Other columns, including a Spherisorb ODS-2, were tried in the first stages of this project; however, most gave extreme tailing and poor response for 2-(2-aminoethyl) thiophene, even with the use of an amine modifier in the mobile phase. The Zorbax Rx-C8 column showed very little peak tailing and separated the known compounds from the parent compound. A detection wavelength of 239 nm was chosen for this assay, because it was the $\lambda_{\max}$ for the 1-(2-thienylethyl)-4-imidazoline-2-thione drug in the mobile phase used. An apparent pH of 6.9 for the mobile phase was selected because the drug substance is more stable at neutral levels. Chromatographic run times of 20 min proved to be adequate to elute the known compounds described previously. The parent drug eluted in 8–9 min using the optimized conditions. The 2-(2-aminoethyl)thiophene eluted before the parent drug peak at relative retention time (RRT) 0.6 and 1,1-dimethoxy-2-isothicyanatoethjane eluted much later at RRT 2. Solutions of the drug dissolved in mobile phase and held at room temperature for 24 hr showed no development of degradation peaks. Assay values from the 24-hr samples were statistically identical to samples prepared freshly.

Any test method devised cannot be completed without supporting data on the essential elements of analytical test method validation, which include linearity, accuracy, precision, and specificity.[22] Linearity of this assay method was verified using sample solutions at concentration levels of 0.0025, 0.005, 0.0075, 0.01, 0.0125, 0.015, and 0.0175 mg/mL, which represented a range

of 20–140% of the normal assay concentration. The response curves generated using both peak height and peak area were linear. Correlation coefficients were greater than 0.99, and *y*-intercepts were close to zero. Accuracy and precision of this test method was evaluated using a 3-day recovery study, utilizing two identical Zorbax Rx-C8 columns. Nine sample solutions were prepared over the 3-day period to contain ~80%, 100%, and 120% of the typical assay concentration of the drug substance. The results are summarized in Table 1 using peak areas. The mean recovery was 100.3% with a relative standard deviation of 0.8%. This is well within an acceptable level of accuracy and precision for an assay method. Furthermore, the use of two different Zorbax Rx-C8 columns would seem to demonstrate some method robustness; the method appeared to give similar results between columns. Specificity of the method was verified by the use of stressed samples and a photodiode array (PDA) detector. All stressed samples demonstrated peak homogeneity from scans of the parent drug peak when checked using the PDA detector. All degradation products appeared to be resolved from the parent peak, using the described optimized conditions. The stressed sample study will be discussed in greater detail later.

Chromatograms of the stressed drug samples are shown in Fig. 2. Figure 2(A) shows a chromatogram of acid stressed drug substance; the treatment was for 6 hr at 85°C. Degradation was significant, with only 57% of the drug substance remaining. Figure 2(B) shows a chromatogram stressed under

Table 1. Three day recovery study.

Day/ column	Approximate assay level (%)	Concentration of prepared sample solution (mg/mL)	Concentration found by assay (mg/mL)	Recovery as assay value (%)
1/1	80	0.01014	0.01014	100.0
	100	0.01273	0.01286	101.0
	120	0.01496	0.01501	100.3
2/1	80	0.01003	0.01008	100.5
	100	0.01249	0.01247	99.8
	120	0.01506	0.01504	99.9
3/2	80	0.01018	0.01008	99.0
	100	0.01249	0.01253	100.4
	120	0.01499	0.01525	101.7
				Mean = 100.3
				Std. dev. = 0.8

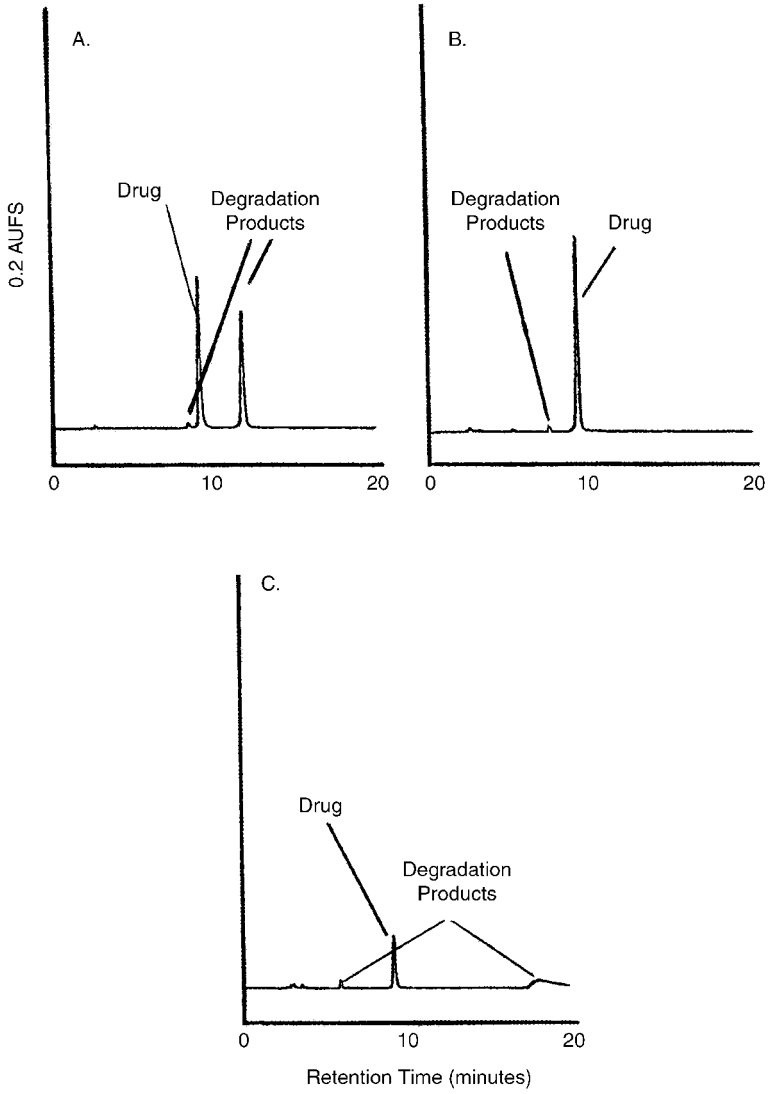

Figure 2. Chromatograms of stressed 1-(2-thienylethyl)-4-imidazoline-2-thione samples. (A) Chromatogram of the drug treated with HCl for 6 hr at 85°C, 57% of the drug remains. (B) Chromatogram of the drug treated with NaOH for 72 hr at 85°C, 73% of the drug remains. (C) Chromatogram of the drug treated with hydrogen peroxide for 1 hr at room temperature, 19% of the drug remains. Degradation product peaks are labeled.

basic conditions for 72 hr at 85°C. Little degradation was noticed as peaks in the chromatogram; however, the parent drug assay level was only 73% of initial theory. Finally, Fig. 2(C) shows a chromatogram of a peroxide stressed sample for 1 hr at room temperature. Only 19% of the drug substance remained for this sample. Assay values for the complete stress study are shown in Table 2. The first part of Table 2 displays the data from acid and base stressed conditions from 1, 6, 24, and 72 hr periods. The acid stress conditions caused rapid loss of the parent compound, and the base stress conditions caused a loss of the active drug more slowly. Under the acid stress conditions, only 14% of the drug remained after 72 hr, while 73% of the drug remained at this time point under the base stress conditions. The hydrogen peroxide treated caused rapid degradation of the drug substance. This treatment nearly degraded the drug fully after 4 hr at room temperature; only an assay value of 0.3% was determined chromatographically. The most important aspect of this study is that all chromatograms showed complete separation of all degradation compound peaks. As mentioned previously, a PDA HPLC detector verified the homogeneity of all parent drug peaks in chromatograms of the stressed samples. Therefore, all data collected would seem to indicate that the described chromatographic conditions are stability-indicating for the drug.

Only a few other points need to be discussed. Light degradation studies were not conducted with the drug substance. As this assay method was designed for bulk drug substance and storage would be in opaque

Table 2. Stressed samples.

Time (hr)	Stress conditions	Drug assay (%)
A. *Acid and base conditions*		
1	HCl/85°C	95.1
	NaOH/85°C	101.1
6	HCl/85°C	59.1
	NaOH/85°C	100.9
24	HCl/85°C	29.8
	NaOH/85°C	97.1
72	HCl/85°C	14.1
	NaOH/85°C	73.1
B. *Hydrogen peroxide conditions*		
1	1% H_2O_2/RT	19.1
2	1% H_2O_2/RT	1.7
4	1% H_2O_2/RT	0.3

containers, light degradation was not considered necessary at this stage of analytical support. Upon actual formulation of the drug into a final pharmaceutical product, the need for light degradation is very important. Separation of formulation excipients, as well as their own degradation products, would be necessary for a formulation assay. Since this method is designed for the bulk drug substance, a separate study and method would be needed to be devised for the specific formulation. As of this time, a formulated drug has not been devised and will represent an area of future study. Also, identification of degradation compounds discovered in this work would be helpful, as well as the availability of actual reference compounds to investigate chromatographic systems. Acid and peroxide stress conditions showed several degradation product peaks, which need to be identified. This is outside the scope of this current study, which was to demonstrate the stability-indicating nature of the described method. Degradation compounds generated under the stressed conditions did not interfere with the parent drug peak in the described chromatographic system.

CONCLUSIONS

An HPLC stability-indicating assay method has been developed for the bulk substance 1-(2-thienylethyl)-4-imidazoline-2-thione. A Zorbax Rx-C8 column with dimensions of 150 mm × 4.5 mm, was used with a 35/65 acetonitrile/water mobile phase 0.02 M in phosphate buffer, at apparent pH 6.9. The assay method was found to be accurate and precise. Recovery of nine samples was 100.3% with a relative standard deviation of 0.8% using peak area data. Two different Zorbax Rx-C8 columns were used, indicating the robust nature of the method. Stressed samples showed resolution of all degradation peaks and a PDA HPLC detector verified homogeneity of the parent drug peak.

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