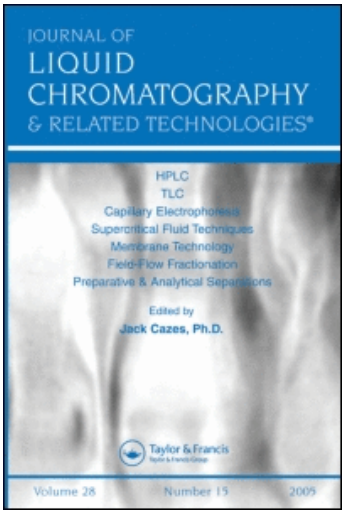

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Evaluation of an HPLC Stability-Indicating Assay for Bulk 2-[4-(1-Hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic Acid

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Evaluation of an HPLC Stability-Indicating Assay for Bulk 2-[4-(1-Hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic Acid

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Abstract: A stability-indicating assay method using high performance liquid chromatography was developed and evaluated for monitoring 2-[4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid hydrochloride bulk compound. A sample of the compound was dissolved in mobile phase and chromatographed using an ODS (C_{18}) column. The mobile phase composition was 65/35 (v/v) acetonitrile/water made 0.012 M in sodium phosphate buffer and 0.004 M in sodium perchlorate at apparent pH 2.3. Ultraviolet detection at 220 nm was used for the assay. Solutions containing the bulk compound 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 method. The validation, evaluation, and other aspects of this assay method will be discussed.

Keywords: Stability-indicating assay, pharmaceutical analysis, drug analysis

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.

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Stability-indicating assays for pharmaceuticals have been reviewed[1] and their development has often been described in the literature.[2–8] The stability-indicating assay can also be a method, which would be used for the analysis of stability samples studied within pharmaceutical research and development. With the advent of the International Conference on Harmonisation (ICH) and its associated guidelines, the stability-indicating assay method is a mandatory requirement of pharmaceutical research. The U.S. Food and Drug Administration (FDA) define the stability-indicating assay as: "... a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating assay accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities."[9,10] Long term storage under various temperature and humidity conditions can affect the stability of a drug substance, and this requires accurate methods to verify the active drug content of the stored material. Light degradation can be avoided by the storage container used; ambient temperature and humidity are often not as readily controlled. The shipment of bulk drug material can introduce many uncontrolled variations during transport and should be considered,[4] even if the ambient conditions of the storage facilities can be well controlled.

The drug described in this study, 2-[4-(1-hydroxy-4-(4-(hydroxyl-diphenyl-methyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid (Figure 1A), is under study as an antihistamine agent. It is a second generation class type H-1 antihistamine, which is an area of active research within pharmaceutical drug research.[11–13] This compound has a carboxylic acid function and has the possibility of some peak tailing problems when using standard reversed-phase HPLC. It was the objective of the current reported work to develop

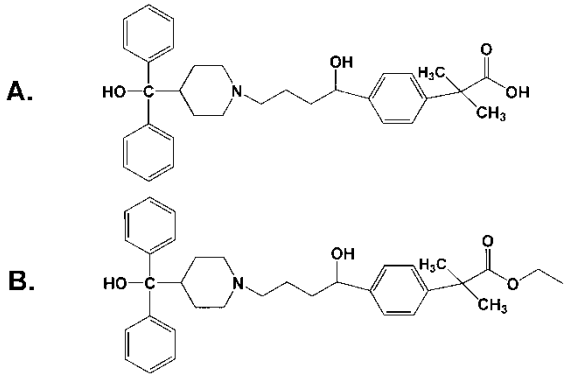

Figure 1. The structure of (A) 2-[4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid, the drug substance and its analog and synthetic precursor, (B) the ethyl ester. The relative retention time (RRT) of B to the parent drug peak is 1.7 using the described HPLC conditions.

and validate[14–16] an HPLC test method assay to have in place to evaluate different bulk lots of drug substance placed under storage conditions. For simplicity, a regular reversed-phase ODS (C_{18}) column using an isocratic mobile phase was finally chosen for this study. The general considerations behind the selection of reversed phase stationary phases has been described in the literature.[17] The presented HPLC study was designed as an assay procedure; that is, the focus of the procedure was the accurate and precise determination of the active drug as compared to a stable and certified reference standard. It was not an impurity test, which would focus the accurate and precise quantification of impurities and would be a different goal of an analytical test procedure. The ethyl ester analog of the drug compound (Figure 1B) is the immediate synthetic precursor of the drug and was used initially for chromatographic performance evaluation and performance. The ethyl ester analog was retained longer than the parent drug in all reversed-phase systems evaluated in the earlier work of this study, and was finally used as a system suitability test spike compound. Forced decomposition studies included with this reported work included oxidative and pH stress with concurrent thermal stress. Because this work was for bulk drug compound, light stress was not considered important since storage containers were opaque; thus, the bulk compound would not be light stress under actual conditions. These conditions and the results of the chromatographic validation will be discussed in detail.

EXPERIMENTAL

Reagents

High purity HPLC water was provided by a Barnstead (Boston, Massachusetts, USA) NANOpure system, followed with an ultraviolet radiation treatment by a Barnstead ORGANICpure system. High performance liquid chromatographic grade acetonitrile was purchased from Burdick and Jackson (Muskegon, Michigan, USA). Sodium dihydrogen phosphate monohydrate, phosphoric acid (85%), and sodium perchlorate monohydrate were ACS reagent grade (Fisher Scientific, Fair Lawn, New Jersey, USA). Concentrated hydrochloric acid, solid sodium hydroxide and 30% hydrogen peroxide were commonly available ACS grade reagents. The 2-[4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid hydrochloride and its ethyl ester analog were obtained "in-house."

Chromatographic Conditions and Apparatus

A Spectra-Physics (San Jose, California, USA) Model SP8800 liquid chromatograph equipped with a Rheodyne (Coatati, California, USA) Model

7010 injector valve and an Applied Biosystems (PE Biosystems, Norwalk, Connecticut, USA) Model 757 detector was used for all the HPLC experiments at a detection wavelength of 220 nm. A Chromanetics (Vineland, New Jersey, USA) 5 μm Spherisorb ODS (C_{18}) column with dimensions of 250 mm × 4.6 mm was used with the optimized conditions described. Mobile phase consisted of 65/35 (v/v) acetonitrile/water made 0.012 M in sodium phosphate buffer and 0.004 M in sodium perchlorate (1.0 g of sodium dihydrogen phosphate monohydrate, 0.5 g of concentrated phosphoric acid (85%), and 0.5 g of sodium perchlorate monohydrate were dissolved in 350 mL of water; then 650 mL of acetonitrile were added to make approximately 1 liter of mobile phase). The flow rate was 0.9 mL/min. Sample solution injection volume was 20 μL. The final sample solution concentration for injection at the normal assay level was 0.05 mg/mL. All final dilutions of the sample solutions were prepared in mobile phase. An H.P. Model 1040A (Agilent Technologies, Palo Alto, California, USA) 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 obtained by first preparing a stock solution of the drug substance in 75/25 (v/v) acetonitrile/water at an approximate concentration of 1.0 mg/mL. Five milliliter aliquots of this stock solution were treated with either 5.0 mL of 1% hydrogen peroxide, 1 M HCl, or 1 M sodium hydroxide solutions. The resulting solutions were stressed at 90°C for 1, 3, 6, 24, and 72 hours using an ordinary laboratory oven. No light stress testing was performed. After the stress conditions, the solutions were diluted with mobile phase to a theoretical concentration of 0.05 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:

$$(A_{sample}/A_{std})(W_{std}/W_{sample})(100) = \%\text{assay}$$

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

RESULTS AND DISCUSSION

Chromatographic Conditions

The basic chromatographic conditions used for this method were designed to be simple and easy to use and reproduce. Isocratic conditions were chosen for this assay procedure, and the Chromanetics 5 μm ODS column was found to be useful for this procedure. The ODS column showed very little peak tailing when using the phosphate buffer mobile phase using 0.004 sodium perchlorate, despite the functional groups of the drug compound. This chromatographic system appeared to separate the degradation products of the drug substance, as well as the synthetic precursor (Figure 1B) from the parent compound. A detection wavelength of 220 nm was finally used for this assay; other wavelengths were studied. At 200 nm, the linear range was not ideal and at 260 nm (a relative absorbance maximum for the drug substance) gave too low of a response for a useful assay. The 220 nm wavelength detection offered high response, good linearity, and the best option for detection conditions. The drug substance dissolved in mobile phase was found to be very stable; after 48 hours of standing, solutions displayed no measurable loss of assay value nor developed any degradation peaks measured at any wavelength using a diode array detector. Assay values from the 48 hour samples were statistically identical to samples prepared freshly. This demonstrated a reasonable working time to chromatograph solutions of the drug substance.

Chromatographic run times of twenty minutes proved to be adequate to elute the known compounds described previously. The parent drug eluted in 8 to 9 minutes using the optimized conditions and the various Chromanetics ODS columns used during this evaluation. The ethyl ester analog of the drug substance eluted later at a relative retention time (RRT) of 1.7 using the described optimized chromatographic conditions. Calculated resolution of spiked drug substance solution with 1% levels of the ethyl ester analog exceeded 10 for all ODS columns used during this method's development and evaluation; therefore, this compound was not particularly useful in evaluating system performance. Actual generated degradation products were less well resolved from the parent drug peak than the analog, and are more important for the stability-indicating performance of the method. It should be noted that all degradation peaks produced during this study were resolved from the parent peak using the described HPLC conditions.

Method Validation Criteria

The linearity and limit of detection were established for the optimized chromatogarphic conditions. Linearity of this assay method was verified using sample solutions at concentration levels of 0.01, 0.02, 0.06, 0.08, 0.05, 0.06,

and 0.07 mg/mL, which represented a range of 20 to 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. The calculated instrumental limit of detection (LOD) was determined in the traditional way; three times the average noise level.[14] The average noise level was based on 100 data points, short term noise in the chromatographic system, divided by the slope of peak height calibration curve. The limit of detection was found to be approximately 0.00003 mg/mL concentration of the parent or 0.06% level of the normal assay concentration level of the drug (0.05 mg/mL). The LOD could be expected to be different between UV detectors and by the age and noise generated by their deuterium lamps. As this is a stability-indicating assay, those low levels of active drug would not have to be accurately determined in a practical application. If a drug substance had decayed more than 10% in a formulation or more than 2% in bulk compound; regulatory requirements would require the discard of that particular lot of drug formulation or bulk, respectively.

Accuracy and precision of this test method was evaluated using a three day recovery study utilizing two identical Chromanetics ODS columns. Nine sample solutions were prepared over the three day period to contain approximately 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 99.7% with a relative standard deviation of 1.0%. This is well within an acceptable level of accuracy and precision for an assay method. Peak height data was similarly accurate for these nine

Table 1. Three-day recovery study

Day/column	Approximate percent assay level	Concentration of prepared sample solution (mg/mL)	Concentration found by assay (mg/mL)	Recovery as assay value (%)
1/1	80	0.04019	0.04027	100.2
	100	0.04996	0.05001	100.1
	120	0.06026	0.06128	101.7
2/1	80	0.04035	0.01008	99.0
	100	0.05029	0.01247	98.6
	120	0.06039	0.01504	98.5
3/2	80	0.04010	0.01008	99.8
	100	0.05038	0.01253	100.1
	120	0.06179	0.01525	99.4
			Mean =	100.3
			Std. dev. =	0.8

samples; mean recovery was 100.1% with a relative standard deviation of 1.5%. Generally, peak area data is preferred for quantification, especially when advanced integration systems are available. Also, the use of two different batch lots of Chromanetics ODS columns would seem to demonstrate some method robustness; the method appeared to give similar results between columns of different production lots. This recovery study would also demonstrate that the chromatographic conditions appear to be repeatable from day-to-day operation. Specificity of the method was verified by use of stressed samples and the use of 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 appear to be resolved from the parent peak using the described optimized conditions. The stressed sample study will be discussed in more detail later.

Stress Condition Results

Chromatograms of the stressed drug samples are shown in Figure 2. Figure 2A shows a chromatogram of hydrogen peroxide stressed drug substance; treatment was for 1 hour at 90°C. Degradation was slight, with roughly 95% of the drug substance remaining as is shown in this chromatogram. Also, the peak appearing near the dead volume of this chromatogram was seen in blank hydrogen peroxide solutions and is not related to degradation of the drug substance. A small degradation peak eluting immediately before the drug substance can be seen in this chromatogram (Figure 2A), but it was resolved the parent drug peak in the stress solutions. Figure 2B shows a chromatogram

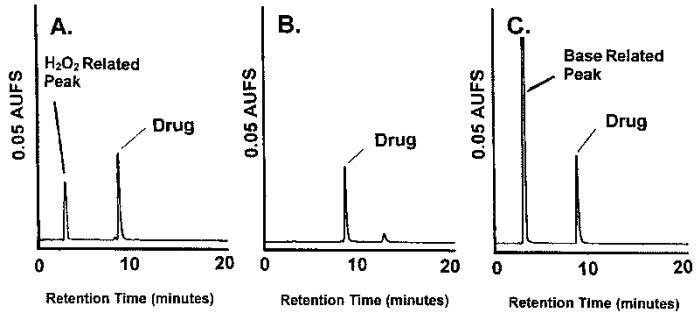

Figure 2. Chromatograms of stressed 2-[4-(1-hydroxy-4-(4-(hydroxy-diphenylmethyl)-1- piperidinyl)-butyl)-phenyl]-2-methylpropionic acid hydro-chloride at the one hour interval and 90°C. A: Chromatogram of the drug treated with 1% hydrogen peroxide solution, 95% of the drug substance remains. B: Chromatogram of the drug treated with HCL, 79.5% of the drug remains. C: Chromatogram of the drug treated with NaOH, 98.4% of the drug remains. Degradation product peaks are not labeled, system related peaks are labeled.

stressed under acidic conditions for 1 hour at 90°C. Some degradation has occurred, 79.5% of the drug remains and a degradation peak is clearly shown at retention time of 12.5 minutes. Finally, Figure 2C shows a chromatogram of a sodium hydroxide stressed sample for 1 hour at 90°C. No degradation has apparently occurred; the drug peak represents an assay value of 98.4% of theory. This chromatogram also shows a peak at the dead volume, which is related to the sodium hydroxide solution, not a degradation product. All degradation peaks generated by this study were chromatographically resolved from the parent drug peak. Again, this would indicate that the chromatographic conditions are valid for a stability-indicating assay. The assay values for the complete stress study are shown in Table 2. Table 2 displays the data from stressed conditions from 1, 3, 6, 24, and 72 hour periods. The acid and peroxide stress conditions showed significant degradation over the period of the test. After 24 hours, most of the drug substance had been degraded under the acid and peroxide conditions. The sodium hydroxide, however, produced very little evidence of drug degradation. After 72 hours, 95.9% of the drug substance remained. 2-[4-(1-Hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid appears to be very stable under the base stress conditions, which included the temperature of 90°C. The most important aspect of this study is that all chromatograms showed complete separation of all degradation compound peaks. As mentioned previously, a photodiode array 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.

Table 2. Stressed samples

Time (hours)	Stress conditions	Assay percent of drug
1	H_2O_2	95.6
	HCl	81.0
	NaOH	98.4
3	H_2O_2	66.5
	HCl	20.9
	NaOH	100.4
6	H_2O_2	27.3
	HCl	5.0
	NaOH	100.4
24	H_2O_2	5.2
	HCl	1.4
	NaOH	98.1
72	H_2O_2	3.7
	HCl	2.3
	NaOH	95.9

Only a few other points need to be discussed. Light degradation studies were not conducted with the drug substance. Since this assay method was designed for bulk drug substance, and storage would be in opaque 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 also be helpful, as well as the availability of actual reference compounds to investigate chromatographic systems. Again, acid and hydrogen peroxide stress conditions showed degradation product peaks, which need to be identified; basic stress conditions showed only slight degradation after 72 hours. Identification of the degradant peaks 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 2-[4-(1-hydroxy-4-(4-(hydroxydiphenylmethyl)-1-piperidinyl)-butyl)-phenyl]-2-methylpropionic acid hydrochloride drug substance. A Chromanetics ODS (C_{18}) column with dimensions of 250 × 4.6 mm was used with a 65/35 acetonitrile/water mobile phase 0.012 M in phosphate buffer and 0.004 M in sodium perchlorate at apparent pH 2.3. The assay method was found to be accurate and precise. Recovery of nine samples was 99.7% with a relative standard deviation of 1.0% using peak area data. Two different Chromanetics ODS columns were used, indicating the robust nature of the method. Stressed samples showed resolution of all degradation peaks and a photodiode array HPLC detector verified homogeneity of the parent drug peak.

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