A Gradient HPLC Test Procedure for the Determination of Impurities and the Synthetic Precursors in 2-[4-(1-Hydroxy-4-[4-(Hydroxydiphenylmethyl)-1-Piperidinyl]-butyl)-Phenyl]-2-Methylpropionic Acid

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Abstract

A gradient high-performance liquid chromatographic (HPLC) test procedure is developed and evaluated for its ability to establish the levels of impurities and remaining synthetic precursors in 2-[4-(1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid. A gradient program with a mobile phase of 0.02M sodium phosphate buffer and 0.004M sodium perchlorate in acetonitrile–water (~ pH 2.5) is used with a Spherisorb C6 column. The acetonitrile composition is increased linearly from 40% to 65% over a 45-min period and held at 65% for 20 min. UV detection at 210 nm is used to quantitate all components. The procedure is validated for accuracy using spiked levels (0.1% to 1.5%, w/w) with two suspected impurities, the synthetic precursors. A multiday repeatability study using two different Spherisorb C6 columns and HPLC systems shows consistent impurity quantitation results with one production lot of the bulk compound.

Introduction

High-performance liquid chromatography (HPLC) is the separation technique of choice for the testing of bulk drug substance for impurities and for use as a stablity-indicating assay of drug products as evidenced by the number of methods published in the literature (1–15) and in the current United States Pharmacopeia (16). The main advantage of HPLC is its ability to have reproducible and reliable analysis. When compared with gas chromatography (GC), HPLC has the advantage in its reliance on the solubility of the analyte versus the analyte volatility required for GC analysis. HPLC also offers useful detection systems; the UV detector is generally inexpensive when compared with the mass spectrometric detector. UV detection is adequate for routine analysis and is well suited for many pharmaceutical compounds, which have UV chromophores.

Reversed-phase HPLC has been cited extensively in the analysis of pharmaceutical compounds in the literature (1–15) and was chosen for this work. 2-[4-(1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid hydrochloride (Figure 1A) is a drug under study and exhibits many antihistamine properties. It is one of many second generation type H-1 antihistamines and is an area of active research within pharmaceutical drug research (17,18).

The objective of the presently reported work was to develop and validate (19–22) an HPLC test method to have in place to evaluate different synthetic lots of this drug substance. Although isocratic HPLC systems have been used often for the determination of impurities in bulk drug (10,11), as well as stability indicating assays (12–15), a gradient method offered a better ability to resolve and detect low-level impurities over an isocratic system. Because the design emphasis of the impurity test is in the detection and accurate quantitation of impurities, the gradient appoach was taken during this study. A standard analytical Spherisorb C6 (25-cm × 4.6-mm i.d.) column was used with a gradient mobile phase composed of phosphate buffer and acetonitrile as the organic modifier. Because UV chromophores were present in the drug compound and the suspected impurities (Figure 1), UV detection was chosen as the detection system for this routine test procedure. Two possible impurities were of concern in this study. The ethyl ester analog (Figure 1B) and the ketone–ethyl ester analog (Figure 1C) of the parent compound were included in the validation work of this test procedure. These impurities were possible because of their use as precursors in the synthesis of the drug. The recovery data and other validation results of this study will be discussed.

Experimental

Reagents

High-purity HPLC water was provided by a Barnstead (Boston, MA) NANOpure system followed with a UV radiation

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treatment by a Barnstead ORGANICpure system. HPLC-grade acetonitrile was purchased from Burdick & Jackson (Muskegon, MI). Concentrated phosphoric acid, sodium perchlorate monohydrate, and sodium hydroxide were American Chemical Society grade (Fisher Scientific, Fair Lawn, NJ). The drug substance, 2-[4-(1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid hydrochloride, and related precursor analogs in the form of hydrochloride salts (see Figure 1), 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 Biosystem, Norwalk, CT) Model 757 detector was used for most of the HPLC experiments. Some limited work was performed using a Waters pumping system (Waters, Milford, MA) consisting of a Model 510 pump and Model 680 controller and the same models of injection valve and detector. Spherisorb C6 (4.6-mm × 250-mm, 5-µm particle) (Waters) type columns were used during the development and evaluation of this test procedure. Mobile phase A consisted of 40:60 acetonitrile–water (v/v) made 0.004M in sodium perchlorate and 0.02M in sodium phosphate buffer; 2.4 g of concentrated phosphoric acid (85%) was added to each liter of mobile phase; and 10M sodium hydroxide was added dropwise to obtain the approximate pH 2.5. Mobile phase B consisted of 65:35 acetonitrile–water (v/v), also made 0.004M in sodium perchlorate and 0.02M in sodium phosphate buffer (approximate pH 2.5 made as described previously). The gradient program was linear, starting at 100% A to 100% B during a 45-min period, and then a hold at 100% B for 20 min was used. A 3-min return ramp to mobile phase A was followed by a 10-min re-equilibration time at 100% A before the next chromatographic run. The flow rate was 1 mL/min and detection was at 210 nm. Sample solution injection size was 20 µL. The sample solution concentration was 1.0 mg/mL prepared in mobile phase A. A reference solution at 1% concentration (a 1 to 100 dilution in mobile phase A or approximately 0.01 mg/mL) was made for each sample weight. The 1% reference solution was injected and chromatographed, then followed by the corresponding sample solution (1.0 mg/mL).
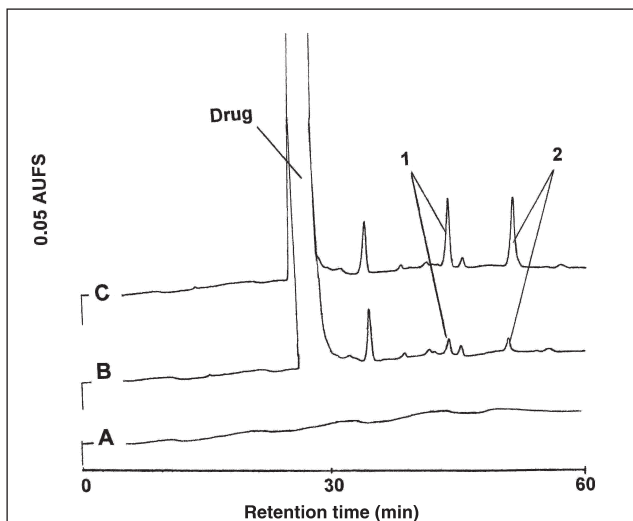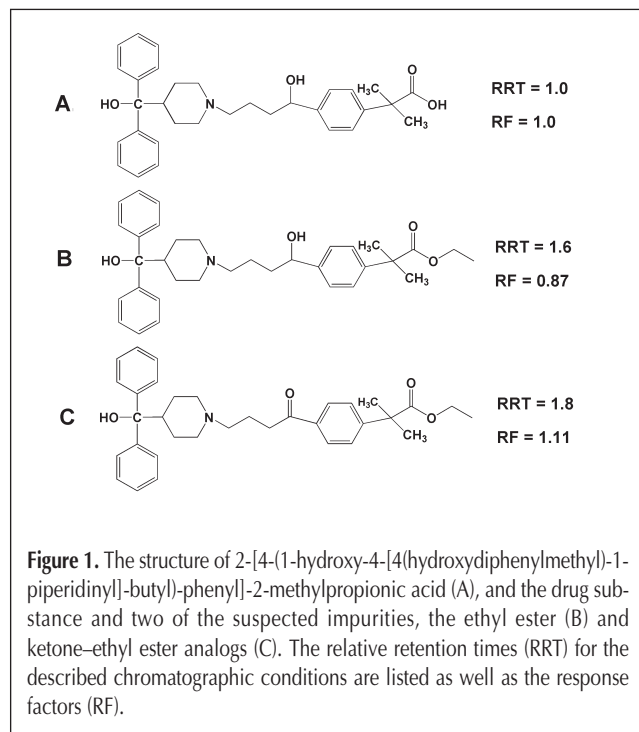
Spiked sample solutions containing the parent drug compound 2-[4-(1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid and the two related analogs (Figure 1) were prepared at the 0.1%, 0.2%, 0.6%, 1.0%, and 1.5% (w/w) equivalent level. Response factors were determined by comparing the peak area of 0.01 mg/mL level solutions of each known component to the same concentration of parent compound. A 1% (w/w) spike with the ethyl ester analog was used to evaluate the performance of the different columns by calculating the resolution between it and the drug substance peak.

Calculations

The peak areas for all impurity peaks and the peak area of the 1% reference peak for the drug were determined by integration. The known impurity percent (w/w) was determined by the known components in the sample or by following the equation:

$$(1/F) \cdot A_i /(A_r + \Sigma A_i /100) = \% \text{ impurity (w/w)} \qquad \text{Eq. 1}$$

where F was the response factor of the identified component (F = 1.0 for unknown components for percent A/A. The parent drug had a response factor of 1.0; its ethyl acetate analog had a response factor of 0.87, and the ketone–ethyl ester analog had a response factor of 1.11. The response factor was for calculating percent w/w). A_i was the area of the impurity peak. ΣA_i

Figure 1. The structure of 2-[4-(1-hydroxy-4-[4(hydroxydiphenylmethyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid (A), and the drug substance and two of the suspected impurities, the ethyl ester (B) and ketone–ethyl ester analogs (C). The relative retention times (RRT) for the described chromatographic conditions are listed as well as the response factors (RF).

Figure 2. Chromatograms of blank injection (A) and spiked sample solutions containing 0.1% (B) and 0.5% (w/w) (C) of the two suspected impurities. Peak 1 is the ethyl ester analog, and peak 2 is the ketone–ethyl ester analog. The unlabeled small peaks are unidentified impurities in the batch lot of drug substance. The largest unknown impurity at retention time 35 min (RRT 1.3) is approximately 0.4% (A/A).

was the sum of the areas of all the impurity peaks in the chromatogram, and A_r was the area of the drug peak in the 1% reference chromatogram.

Resolution between the parent drug and the ethyl ester analog peaks were calculated by the traditional equation:

$$R_s = 2(t_2 - t_1)/(w_1 - w_2) \qquad \text{Eq. 2}$$

where t was the retention time of the peak, and w was peak width at the baseline.

Results and Discussion

Chromatographic system

The separation of the two impurity analytes from the parent, as well as other low-level unidentified impurities, was easily accomplished using this chromatographic procedure. Chromatograms of one lot of the drug compound are shown in Figure 2, which is a blank injection chromatogram and two chromatograms of the lot of drug compound spiked at 0.1 and 0.5 % (w/w) with the two suspected impurities. Resolution of the analogs from the parent peak was acceptable with three different C6 columns used in the development of this procedure. Calculated resolution of a 1% spiked level solution of the ethyl ester analog (Figure 1B) was consistently greater than 6.0 with three different manufacturing lots of C6 columns. This was more than adequate resolution for estimation of the analog impurity. Other reversed-phase HPLC columns were evaluated during the early development phase of this study. Early chromatographic work was done using Spherisorb ODS-1 (Waters) columns, but consistent resolution of the minor impurities in the batch of bulk drug studied was not achieved with the use of different production lots of these columns. A Zorbax Rx C-8 (Agilent Technologies, Palo Alto, CA) and a Supelcosil Suplex pKb-100 (Supelco, Bellefonte, PA) column gave less than optimal baseline stability under the gradient conditions at 210 nm detection. The 5-μm Spherisorb C6 column gave the best results for this gradient method. The pH and concentration of sodium phosphate and sodium perchlorate were optimized for the best peak shape and best theoretical plate count for this chromatographic procedure.

A detection wavelength of 210 nm was chosen for this method for several reasons. There was no true wavelength maximum lower than 260 nm for the drug compound; its UV absorbance spectrum increases as the wavelength approaches 200 nm. A maximum response was desired under gradient LC conditions, and 210-nm detection worked well. Also, by using a detection wavelength of

210 nm, response factors for the two analogs (Figure 1B and 1C) were close to 1.0, which was convenient for impurity peak estimation and quantitation. The ethyl ester had a response factor of 0.87 and the ketone–ethyl ester analog had a response factor of 1.11. Any other analog of the parent drug would likely have a response factor close to one and would be accurately estimated, should it be found in future work with other lots of the drug compound.

Method validation criteria

Accuracy, precision, and reproducibility

The accuracy of this method to estimate the level of known impurities was verified by chromatographing solutions containing known levels of the two analog compounds. Spike levels of 0.1%, 0.2%, 0.6%, 1.0%, and 1.5% (w/w) in the parent solution were run. The HPLC method was found to be accurate,

Table I. Known Impurity Recovery Study Using Spiked Solutions*

Level of spike	Impurity analog	Actual weighed spike level (% w/w)	Measured level*	Recovery (%)
0.0	Ethyl ester	0	0	–
	Ketone–ethyl ester	0	0	–
0.1	Ethyl ester	0.11	0.13	118
	Ketone–ethyl ester	0.10	0.10	100
0.2	Ethyl ester	0.22	0.21	95
	Ketone–ethyl ester	0.20	0.19	95
0.6	Ethyl ester	0.66	0.63	95
	Ketone–ethyl ester	0.59	0.59	100
1.0	Ethyl ester	1.09	1.07	102
	Ketone–ethyl ester	0.98	0.99	101
1.5	Ethyl ester	1.62	1.59	98
	Ketone–ethyl ester	1.46	1.42	97

* Corrected by response factors.

Table II. Reproducibility Study of the Test Procedure

Sample	Instrument	Column	Total impurities (area %)	Four major impurities detected area % (RRT)*
1	Spectra Physics	1	1.12	0.38 (1.3), 0.10 (1.4), 0.17 (1.55), 0.13 (1.6)
2	Spectra Physics	2	0.86	0.38 (1.3), 0.10 (1.4), 0.10 (1.55), 0.14 (1.6)
3	Spectra Physics	2	0.89	0.40 (1.3), 0.11 (1.4), 0.10(1.55), 0.14 (1.6)
4	Waters	1	1.06	0.39 (1.3), 0.15 (1.4), 0.13 (1.55), 0.14 (1.6)
5	Waters	1	0.97	0.37 (1.3), 0.15 (1.4), 0.13 (1.55), 0.11 (1.6)
	Mean =		0.98	0.38 (1.3), 0.12 (1.4), 0.13 (1.55), 0.13 (1.6)
	Standard deviation = 0.11			

* RRT is the relative retention time with the parent drug peak.

as the data in Table I clearly show. Percent recovery for most levels was generally between 95% to 102%, with the exception of the 0.1% level spike. The ethyl ester analog had a slightly high recovery of 118% at that low level. The lot of drug compound used in the study had no detectable quantity of the known suspected impurities, as is shown at the zero spike level in Table I. The reproducibility of this test procedure was demonstrated by analyzing the same lot of drug five times on five separate trial days using two different C6 columns and two different HPLC pumping systems. The total impurity level was consistent, estimated to be between 0.86% and 1.12% (A/A) over the study period (Table II), and had an average total impurity level of 0.98% (A/A). The standard deviation was 0.11% (A/A) for the total impurity level on these five analyses. The four major impurity peaks were also reproducible over this study (Table II).

Method robustness

The robustness of this test procedure (i.e., its characteristic to remain unaffected by small changes) was verified by using the two different pumping systems and was worth noting. The two HPLC systems used had both high (Waters) and low (Spectra Physics) pressure solvent mixing over the gradient run. As can be seen in the data presented in Table II, there was no significant difference in the impurity levels estimated using either HPLC pumping system. The data generated in Table II, also included the use of C6 columns of different production lots. Early development of this procedure also included a third C6 column of a different production lot.

Linearity and limit of detection

Linearity of response for the parent compound was also verified during this work. Chromatographed solutions having concentrations of 0.001, 0.01, 0.1, 1.0, and 1.4 mg/mL (0.1% to 140% concentration of the normal working range of the sample solution) gave peak area responses that were linear. The y-intercept of the five-point peak-area response curve was nearly 0 [$y = 65.0x + 0.096$, slope = absorbance units at full scale – s – mL/mg, and y-intercept = AUFS – s], and the correlation coefficient was 1.0. The calculated instrumental limit of detection (LOD) was determined in the traditional way (three times the average noise level) (12). The average noise level was based on 100 data points, short-term noise in the chromatographic system, divided by the slope of the peak height calibration curve. This instrumental LOD was found to be approximately 0.00015 mg/mL concentration of the parent or 0.015% level of the normal assay concentration level of the drug (1.00 mg/mL). The LOD could be expected to be different between UV detectors and by the age and noise generated by their deuterium lamps. It was desired that this method give an accurate determination of any impurity of at least 0.1% (A/A), which the LOD and the limit of quantitation (three times the LOD) would indicate.

Solution stability

The stability of the drug substance dissolved in mobile phase A was established. A sample solution held at room temperature for 3 days did not show any appreciable increase or change in impurity peaks; therefore, the sample solution appears to be reasonably stable upon short-term standing. This was more than an adequate time interval to perform an analysis and not be concerned with the possibility of degradation during analysis, which would bias results.

Further considerations

This procedure will be used to evaluate future synthetic lots of bulk drug compound as they become available, and this aspect was outside the scope of this manuscript. The immediate need for a validated test method for the current known impurity compounds has been successfully completed and demonstrated.

Conclusion

An HPLC procedure to measure the impurities in 2-[4-(1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl)-phenyl]-2-methylpropionic acid hydrochloride bulk drug substance was developed and found to be both accurate and reproducible. This chromatographic system easily separated the two known impurities from the parent compound. Recovery of spiked sample solution from 0.1% to 1.5% (w/w) of these impurities gave accurate results; recovery was generally between 95% and 102% for most levels, with the 0.1% spike being the least accurate. Two different Spherisorb C6 columns gave reproducible results for impurity levels of one sample lot of the drug compound. In this sample, total impurities were consistently measured near 1% (A/A) using the developed HPLC procedure with reproducible results for individual impurities detected.

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