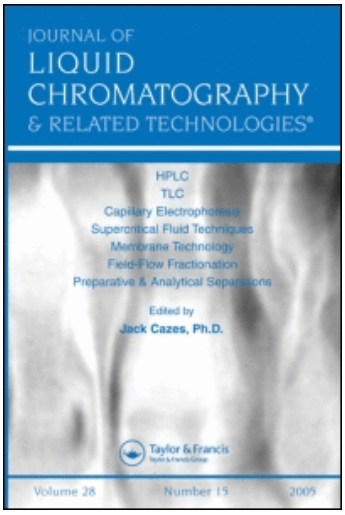

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HPLC ANALYSIS OF HALOPERIDOL AND ITS RELATED COMPOUND IN THE DRUG SUBSTANCE AND A TABLET DOSAGE FORM USING A NON-POROUS SILICA OCTADECYLSILANE COLUMN

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ABSTRACT

A fast and sensitive reverse phase HPLC-UV method for the separation and quantitation of haloperidol and its related compound 4,4'-Bis[4-(p-chlorophenyl)-4-hydroxypiperidino] butyrophenone} in both drug substance and tablet dosage form is reported. A non-porous silica (NPS) ODS-1 column was used as the stationary phase and the mobile phase consisted of 77:23 v/v 50 mM phosphate buffer with 0.2% triethylamine (TEA) pH 2.5/acetonitrile. The flow rate was 1.0 mL/min with detection at 220 nm.

The method was successfully applied to the determination of haloperidol and its related compound in both drug substance and tablet dosage form. The detection limits were 1 ng/mL and 10 ng/mL for haloperidol and its related compound, respectively, based on a signal to noise ratio of 3 and a 10 μL injection.

Linear calibration curves of 10-150 μg/mL and 0.2-15 μg/mL, for haloperidol and its related compound, respectively, showed coefficients of determination of 0.9993 (n=6). Precision calculated as % RSD and accuracy calculated as % error were within 0.087 - 1.52% and 0.22 - 1.41%, respectively, for haloperidol and 0.311-2.561% and 0.60-3.44%, respectively, for the related compound.

INTRODUCTION

Non-porous silica (NPS) columns have emerged as environmentally friendly columns and should be considered for official use in USP compendial assays. Since these fast liquid chromatographic (LC) columns based on 1.5 and 3.0 μm nonporous silica microspheres have become commercially available, there has been great interest in the technique.[1-5]

Haloperidol, 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone, is an antipsychotic drug that has been proven to be effective both in the manic phase of manic-depressive illness and in schizophrenia. Dosage forms are typically tablets, injections, and oral solutions, and patient dosages range from 2 to 20 mg every 8-12 hours.

Haloperidol in a tablet dosage form has been typically analyzed using UV,[6,7] TLC, [8] HPLC with fluorometric,[9] and UV detection.[10,11] The current official USP23 assays for haloperidol drug substance involve a non-aqueous titration of the drug with perchloric acid and an ultraviolet spectrophotometric analysis for its related compound.[12] There is an HPLC assay for the drug substance in the tablet dosage form monograph.[12] The HPLC retention time for haloperidol in the USP monograph is longer than desired (24 min) and sensitivity is not indicated. At present, no HPLC methods have been reported for the determination of both haloperidol and its related compound in the drug substance and tablet dosage form. Current USP specifications allow 1% w/w of the related compound in haloperidol drug substance, but this specification is not included in the haloperidol tablet dosage form monograph.

This paper describes an isocratic reverse phase HPLC method using a NPS-ODS-I column to measure haloperidol and its related compound, an impurity from the synthesis of haloperidol, in both drug substance and tablet dosage form. The USP23 specification of no more than 1% w/w of the related compound in haloperidol drug substance is also shown to be applicable to the tablet dosage form. The NPS-ODS-I method for both analytes showed good sensitivity, selectivity, and a fast chromatographic run time of less than 6 min for both drug substance and tablet dosage form.

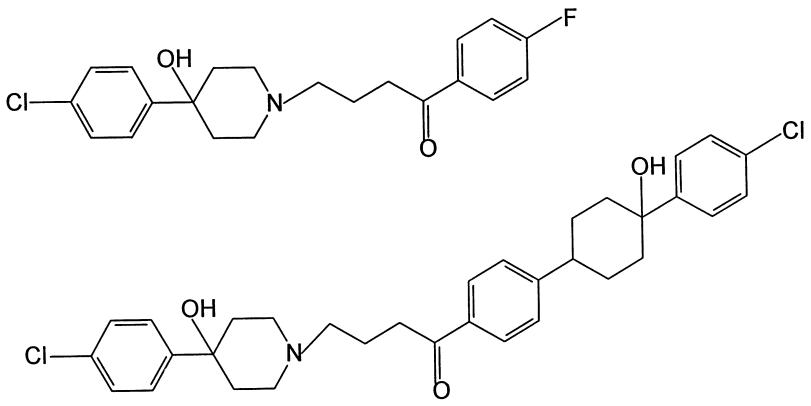

Figure 1. Chemical Structures of Haloperidol (Top) and Its Related Compound (Bottom).

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Figure 1. Haloperidol was purchased from Sigma Chemical Co. (St. Louis, MO 63178). The haloperidol related compound reference standard was obtained from the United States Pharmacopeia (Rockville, MD 20852). Triethylamine was purchased from Eastman Kodak Co., (Rochester, NY). Acetonitrile and methanol (J. T. Baker, Phillipsburg, NJ 08865) were HPLC grade and water was obtained from a Picotech Water System (Research Triangle Park, NC 27708). Concentrated phosphoric acid and potassium dihydrogenphosphate were obtained from J. T. Baker. All chromatographic solutions were filtered through a 0.22 μm nylon filter membrane (Sigma, St. Louis, MO 63178, Lot 77H1734), Haloperidol tablets (Haldol®, 10 mg, McNeil Inc., Fort Washington, PA, Lot J53779T) were purchased from a local pharmacy.

Apparatus

The liquid chromatograph consisted of a Shimadzu HPLC pump (LC10-AT), a Shimadzu Autoinjector (SIL-10A), a Shimadzu System Controller (SCL-10A) (Shimadzu Scientific Instrument, Inc., Columbia, MD), equipped with a 10 μL injector loop and a Waters 996 photodiode array detector (Milford, MA 01757).

The data recording system consisted of a Digital Venturis Pentium Personal Computer with Waters Millennium Software (version v 2.25.01). The column was a non-porous silica (NPS) ODS column (ODS-I 33 x 4.6 mm i.d., 1.5 μm particle) (MICRA Scientific, Inc., Northbrook, IL).

Chromatography Conditions

Liquid chromatography was performed at ambient temperature (23 ± 1°C). The aqueous portion of the mobile phase was prepared by dissolving 3.42 grams potassium dihydrogenphosphate in 500 mL water, adding 1 mL of triethylamine and adjusting with 0.1 M phosphoric acid to obtain a pH of 2.5. This buffer solution was mixed with acetonitrile to obtain a 77:23 v/v solution, which was filtered through a 0.22 μm nylon filter and degassed by sonication. The flow rate was set at 1.0 mL/min and the mobile phase was prepared daily.

Preparation of Stock and Standard Solutions

Individual stock solutions (200 μg/mL) of haloperidol and its related compound were prepared in absolute methanol in low actinic glassware. Both solutions were placed in a 60°C oven for 5 min for complete dissolution of the analytes. The solutions were prepared weekly and were stored at ambient temperature (23 ± 1°C).

Preparation of Calibration Curves for Haloperidol and Related Compound

A calibration curve was prepared for haloperidol by diluting the 200 μg/mL haloperidol stock solution with mobile phase to give final concentrations of 10, 20, 80, 120, and 150 μg/mL (equivalent to 12.5-187.5% of labeled haloperidol). 10 μL of each concentration was injected into the liquid chromatograph.

A calibration curve for the related compound was also prepared by diluting the 200 μg/mL related compound stock solution with mobile phase to give final concentrations of 0.2, 1.0, 3.0, 8.0, 12, and 15 μg/mL (equivalent to 0.03-1.88% w/w of related compound in haloperidol). 10 μL of each concentration was injected into the liquid chromatograph.

Linear regression analysis of analyte peak height versus analyte concentration in μg/mL was performed and the slope and intercept data used to calculate concentration of analytes in drug substance and tablet dosage form.

Assays of Haloperidol and Related Compound

For the assay of related compound in haloperidol drug substance, a solution containing 800 μg/mL of haloperidol drug substance was prepared and 10 μL injected into the liquid chromatograph. The 800 μg/mL solution was further diluted to approximately 80 μg/mL and 10 μL was injected into the liquid chromatograph to determine the haloperidol content.

For assay of related compound in a haloperidol tablet dosage form, a solution containing 800 μg/mL haloperidol was prepared from a 10 mg commercial tablet with the aid of absolute methanol. Shaking, heating at 60°C for 5 min, filtration, and addition of mobile phase, if needed, gave a completely dissolved sample. A 10 μL injection was made into the liquid chromatograph. The solution was further diluted to approximately 80 μg/mL of haloperidol and 10 μL injected to determine the haloperidol content.

RESULTS AND DISCUSSION

If haloperidol were to be quantitated in mixtures with its related compound in its linear range (10-150 μg/mL), the amount of haloperidol injected would have been incompatible with the linear range of its related compound (0.2-15 μg/mL). In order to determine the amount of related compound (≤1% w/w) present in haloperidol drug substance and tablet dosage form, a concentrated solution of haloperidol (800 μg/mL) is initially prepared for assay of the related compound followed by dilution of the sample with mobile phase to the 80 μg/mL level to determine the haloperidol concentration. As a result, separate HPLC assays were developed for each analyte.

Three types of NPS-ODS columns were tested for the separation of haloperidol and the related compound. The ODS-I column is polymeric and not end capped, the ODS-II is monomeric, and not end capped, and the ODS-III column is monomeric, but end capped. Haloperidol was separated from the solvent front on all three columns and its retention was less affected by the strength of the mobile phase as was the related compound. The ODS-II column did not provide symmetrical peak shapes and peak tailing was not improved by the addition of triethylamine to the mobile phase. The ODS-II E column gave good peak shape for both analytes, but at the expense of retention time of the related compound (5.9 min vs 5 min on ODS-I). Overall, the ODS-I column was selected for the assay based on its fast retention times and good peak shapes. It was found that 0.2% triethylamine was a necessary component of the mobile phase for it to achieve desired peak shapes.

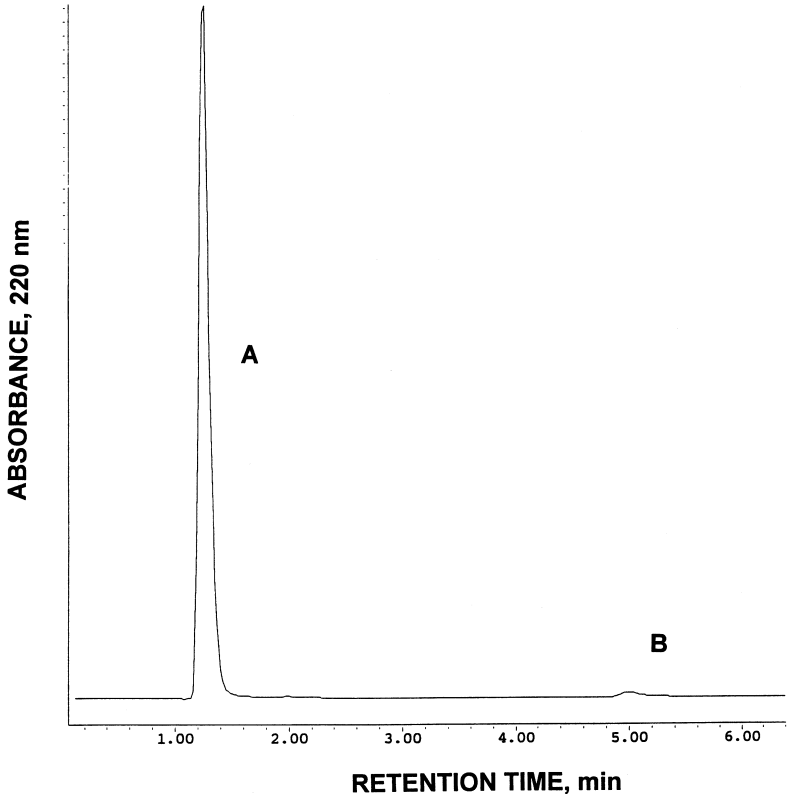

Figure 2. Typical chromatogram of 500 μg/mL haloperidol (A) and 5 μg/mL related compound (B) on an NPS-ODS-1 column using a 23:77 v/v acetonitrile - 50 mM phosphate buffer pH 2.5 containing 0.2% TEA at a 1 mL/min flow rate and detection at 220 nm.

The effects of mobile phase pH and phosphate buffer and organic modifier concentrations were investigated. pH was studied in the 2-4 range. As pH increased, the retention of the haloperidol related compound and tailing factor increased. The ideal pH for the separation was found to be 2.5 based on good peak shapes and fast retention times. Phosphate buffers of 5, 20, 50, and 100 mM were studied. As the buffer concentration increased, the retention time of the related compound decreased and the retention of haloperidol was not significantly affected. Thus, 50 mM phosphate buffer was selected as the best molarity for incorporation into the mobile phase.

Table 1

Analytical Figures of Merit for Haloperidol and Its Related Compound

Analyte	r^{2a}	System Suitability[b]	LOD[c] ng/mL	K'	Theoretical Plates[d]	Tailing Factors[e]
Haloperidol	0.9993	1.2	1	2.9	731	1.0
Related Cmpd.	0.9994	1.8	10	14.2	3380	1.2

[a] Range examined from 0.2 - 15 μg/mL related compound (n=5) and 10 - 150 μg/mL haloperidol; (n = 5).
[b] % RSD of replicate injections at 5 μg/mL related compound and 50 μg/mL haloperidol (n = 6).
[c] Limit of detection, at S/N = 3.
[d] Calculated as $N = 16 (t/w)^2$.
[e] Calculated at 5% peak height.

The current USP 23 HPLC assay for haloperidol lists methanol as the organic modifier with a total run time of about 24 min on a porous ODS column. For the NPS -ODS-I column, it was found that acetonitrile was a better solvent choice since it gave a shorter retention time and better peak shape than methanol for the late eluting related compound. There was also a lower system back pressure with the use of acetonitrile. The final mobile phase consisted of 23:77 v/v acetonitrile-0.05 M phosphate buffer pH 2.5 containing 0.2% TEA.

A typical HPLC chromatogram of haloperidol and its related compound is shown in Figure 2. The retention times for haloperidol and related compound were 1.3 and 5.0 min, respectively. Haloperidol has a very weak absorbance at 336 nm, the λmax of the related compound. Also, the related compound could not be detected at 248 nm, the λmax of haloperidol. However, both analytes showed good absorption at 220 nm and thus, that wavelength was selected as a compromise wavelength for good analyte sensitivity, accuracy, and precision.

The method is linear over the concentration ranges of 0.2 - 15 μg/mL and 10-150 μg/mL for the related compound and haloperidol, respectively. Linear regression analysis performed on the calibration data gave typical correlation coefficients of 0.9993 (n=5). Analytical figures of merit for both analytes are shown in Table 1. The limits of detection based on a signal-to-noise ratio of 3 were 1 ng/mL and 10 ng/mL for haloperidol and its related compound, respectively.

Table 2

Intra- and Inter-day Accuracy and Precision for Haloperidol and Its Related Compound

Analyte	Concentration Added (μg/mL)	Concentration Found[a] (μg/mL)	RSD (%)	Error (%)
Haloperidol				
Intra-day[b]	50	50.11 ± 0.22	0.44	0.22
	100	99.48 ± 0.08	0.087	0.53
Inter-day[c]	50	49.48 ± 0.75	1.52	1.10
	100	98.59 ± 1.10	1.12	1.41
Related Cpd.				
Intra-day[b]	5	5.096 ± 0.02	0.37	1.92
	10	10.08 ± 0.03	0.31	0.81
Inter-day[c]	5	5.17 ± 0.07	1.31	3.44
	10	9.94 ± 0.26	2.56	0.60

[a] Mean ± std. deviation based on n = 3.
[b] n = 3.
[c] n = 9.

The intra- and inter-day accuracy and precision data for both analytes established over one to three days are shown in Table 2. The assay of haloperidol in haloperidol drug substance was calculated to be 99.67 ± 1.78% (n=6).

Assay of the related compound in the same haloperidol drug substance expressed as % w/w of the labeled amount of haloperidol was < 0.03%. The assay of haloperidol in a commercial tablet dosage form expressed as percent of labeled amount of drug was calculated to be 99.81 ± 0.90% (n=6).

Assay of the related compound in the tablet dosage form expressed as % w/w of the labeled amount of haloperidol was 0.046 ± 0.004% (n=6), well below the USP 23 limit of not more than 1% w/w of the related compound.

CONCLUSION

A fast and sensitive HPLC-UV method for the separation and quantitation of haloperidol and its related compound was developed. The separation was achieved on a 33 x 4.6 mm i.d. NPS-ODS-I column at 1 mL/min with detection at 220 nm. The procedure involves a two-step assay since the linear calibration ranges for each analyte do not overlap. The method will easily determine related compound levels down to 0.03% w/w in both drug substance and tablet dosage form.

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