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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1116–1121
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

^{19}F NMR as a powerful technique for the assay of anti-psychotic drug haloperidol in human serum and pharmaceutical formulations

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Received 27 May 2006; received in revised form 15 September 2006; accepted 24 September 2006
Available online 2 November 2006

Abstract

^{19}F nuclear magnetic resonance was used as a suitable analytical tool for the identification and selective determination of haloperidol in human serum and pharmaceutical preparations. The method is based on the integration of appropriate signals of haloperidol and trifluoroacetic acid as an internal standard. The proposed method is a rapid and facile, while without any sample pretreatment, manipulation of large samples and lengthy instrument time. The regression equation for haloperidol in human serum showed a good linearity in the range of 60–600 μg ml^{-1} with a detection limit of 1.4 μg ml^{-1}. The mean recovery results on human serum samples ranged from about 96–103%, with relative standard deviations <8%. The method was also applied successfully to the determination of haloperidol in real pharmaceutical samples, and compared with the results obtained by a reference method. The drug's degradation was studied by the proposed method in hydrochloric acid media and main products were identified.

Keywords: Haloperidol; ^{19}F NMR analyses; Serum; Pharmaceuticals; Degradation

1. Introduction

Haloperidol, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol], belongs to the butyrophenone group of drugs and is widely used in the treatment of schizophrenia, mania and similar psychotic states [1]. Haloperidol causes often-extrapyramidal side effects (motor disturbances) including acute dystonic reactions, akathisa, drug-induced Parkinsonism and following chronic treatment, tardive dyskinesia [2]. Therefore, a serum-concentration-guided drug dosage might help to reduce the number of patients suffering from such side effects due to overdosing.

As haloperidol is practically insoluble in water (1.4 mg/100 ml) [1], pharmacologically active doses of the drug are administered in acidic aqueous media (in which its solubility increases) with a pH range of 2.5–3.8 (injectable form) and 2.5–4.5 (oral form) [1]. It has been reported that the haloperidol is unstable when exposed to elevated temperature and light [3,4].

A number of methods have been reported for the assay of haloperidol in dosage forms. They include acidimetric titration in non-aqueous medium [5], ultraviolet spectrophotometry [6,7], derivative spectrophotometry [8], fluorimetry [9], colorimetry [10,11], polarography [12], conductometry [13], ^{1}H NMR [14], densitometry [15], gas chromatography [16], high-performance liquid chromatography [17–23], micellar electrokinetic chromatography [24] and capillary electrophoresis [25]. The existing methods usually necessitate sample pretreatment and/or time-consuming extraction steps prior to analysis of the drug to remove impurities contained in plasma or serum and required relatively large sample amounts of serum. Withdrawal of large blood volumes might, however, cause problems in medical treatment, especially if many samples are required for frequent drug monitoring or pharmacokinetic analysis.

As a basis for quantitative determination of fluorinate species, fluorine-19 nuclear magnetic resonance (^{19}F NMR) offers several potential advantages compared to chromatography:

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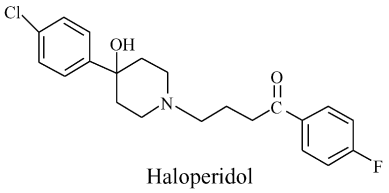
doi:10.1016/j.jpba.2006.09.038

(i) ^{19}F is the only naturally occurring isotope of fluorine.
(ii) ^{19}F NMR spectroscopy has a large spectral window associated with it (300 ppm); consequently, the wide range of fluorine chemical shifts minimized signal overlap.
(iii) The spectra are simple and independent of the complexity of the molecule.
(iv) The spectroscopic sensitivity of ^{19}F NMR is 83% that of ^{1}H NMR.

On the other hand, since fluorine atoms are not naturally present in biological fluids, the ^{19}F NMR spectra of these matrices containing desirable F-substituted analytes and their metabolites are generally highly simplified, in comparison with the corresponding ^{1}H NMR spectra. In addition, in the case of ^{1}H NMR spectra, a water suppression method is also necessary for the analysis of haloperidol in biological fluid matrices, to eliminate the intense water signal observed in these matrices. Thus, the simultaneous identification and determination of this substance by ^{1}H NMR spectroscopy method seems to be very difficult, if not impossible. The advantages and drawbacks of ^{19}F NMR for the quantitative analysis of fluorinated drugs compared to chromatographic methods have already been reported in the literature [26–29]. In this work, the presence of fluorine atom in the haloperidol structure enables the use of ^{19}F NMR for the analysis of drug contents of human serum samples and pharmaceutical formulations.

A number of previously published results revealed that ^{19}F NMR spectroscopy could be employed as a powerful selective tool to analyze different fluorinated drugs [26,30–32]. Thus, in this work, we were interested to apply ^{19}F NMR to the simultaneous identification and quantification of haloperidol in human serum samples and in pharmaceutical formulations. Experimental parameters for ^{19}F NMR analysis of haloperidol were selected to optimize the NMR method with respect to accuracy, precision and analysis time. Furthermore, to investigate the degradation of haloperidol under stressed conditions, a forced degradation studies of haloperidol was performed in acidic media and the degradation products were identified by the proposed method.



Haloperidol

2. Experimental

2.1. Chemicals and reagents

Pure reference standard of haloperidol with chemical purity of >99.3% was obtained from Apotex Inc. (Canada's Pharmaceutical Co.) and was used without any further purification. Trifluoroacetic acid, as internal standard, and formic acid were of analytical grade and supplied by Merck (Darmstadt, Germany). Deuterium oxide (>99.8%) was purchased from Fluka (Buchs, Switzerland). Reagent grade hydrochloric acid was obtained from Merck and used as received.

The pharmaceutical formulations of Haldol tablet (Apotex Pharmaceutical Industries, Canada), Haloperidol tablet (Sobhan Pharmaceutical Company, Tehran, Iran) and Haloperidol injectable as lactate solution (Iran Pharmaceutical Development and Investment Company (I.P.D.I.C.), Rasht, Iran) were purchased from local pharmacies. Drug-free human serum was obtained from the Pathobiology Laboratory Center (Tehran, Iran).

2.2. Instrumentation

All ^{19}F NMR spectra were recorded on a BRUKER DRX 500 AVANCE (11.7 T) spectrometer operating at 470.59 MHz ^{19}F observation frequency, equipped with a dedicated 5-mm QNP probehead and running XWIN-NMR 2.6 software using 500 μl of samples. In all experiments, a known amount of D_2O was added as an internal field frequency lock. The spectra were acquired using 90° pulses with 16–512 scans collected into 128k data points over a spectral width of 61,162 Hz (130 ppm). The 90° pulse width was measured to be 12.10 μs for human serum. The acquisition time was 1.07 s followed by a relaxation delay of 10 s, to ensure full T_1 relaxation. The spectra were recorded at 278 K and ^{19}F NMR chemical shifts were reported relative to trichlorofluoromethane ($CFCl_3$) at $\delta_F = 0.0$ ppm.

The NMR processing for final solutions of all samples included phase correction (performed manually for each replicate) and baseline correction over the entire spectral range. In all instances, the baseline was additionally corrected over the integrated regions. Areas of the peaks were determined by electronic integration of the expanded regions around diagnostic resonances, using an integral limit of ±20 Hz around the corresponding signals. The percentage of error (reported in %R.S.D.) was found to be less than 1%.

2.3. Preparation of calibration and validation samples

Stock solutions of haloperidol and the internal standard were prepared separately at a concentration of 6 and 1.5 mg ml^{-1}, respectively, in doubly distilled deionized water. Formic acid was added dropwise to properly dissolve the haloperidol (final pH of the stock solution = 3.3).

Drug-free serum samples from healthy donors were used for calibration and validation studies. The calibration solutions were prepared in human serum samples on the day of analysis by dissolving appropriate amounts of the stock solutions of haloperidol and internal standard in deuterium oxide (500 μl final volume), to yield spiked solutions of 60–600 μg ml^{-1} of haloperidol and 30 μg ml^{-1} of internal standard. In all NMR measurements, 20% (w/w) of deuterium oxide was added to lock the field frequency of the instrument. Serum samples spiked with different concentrations of the analyte (i.e. 280 and 320 μg ml^{-1}) were prepared to test the accuracy and precision of the method.

2.4. Preparation of real samples

Three pharmaceutical formulations containing haloperidol such as Apotex tablet (5 mg tablet^{-1}), Sobhan tablet (0.5 mg tablet^{-1}) and I.P.D.I.C. injection (5 mg ml^{-1}) were analyzed using the proposed method. The assays of these formulations were also performed by a HPLC standard USP method [33].

For tablets, 5 units were weighed and powdered. An accurately weighed portion of the well-mixed powder was placed in a 25-ml volumetric flask containing 15 ml deionized water and formic acid. The content of the flask was sonicated for about 10 min and then made up to the volume with deionized water and the solution was filtered. For injection sample, 1 ml of the sample was placed into a 5-ml calibrated flask and the content of the flask was made up to the volume with deionized water. The appropriate volumes of these solutions with internal standard stock solution and D_2O were transferred into analytical NMR tubes (500-μl final volume) and the spectra were recorded. Haloperidol-spiked real samples were prepared by adding 0.04–0.16 mg haloperidol to real samples. In all cases, five determinations were performed.

2.5. Forced degradation of standard haloperidol

A 35 mg portion of standard haloperidol was mixed separately in 35 ml of 1.0 M HCl. The mixture, obtained with hydrochloric acid, was refluxed for 5 h. The resulting solution was cooled at room temperature and filtered. This solution was analyzed using the proposed method.

3. Results and discussion

3.1. Evaluation of longitudinal relaxation time, T_1, of haloperidol

To measure the longitudinal relaxation time (T_1) of haloperidol, the longitudinal relaxation delay of the fluorine was determined by the inversion recovery pulse sequence method, using T_1cal BRUKER program which fitted the data to the exponential equation $I = I_0 + P\exp(-\tau/T_1)$, where I is the intensity of haloperidol resonance at inversion delay time τ and I_0 at the equilibrium state and P is a constant. Inversion delay time was 0.10, 0.50, 0.75, 1.00, 2.50, 5.00, 7.50, 10.00, 15.00 and 20.00 s. The T_1 value was determined to be 1.23 ± 0.01 s for haloperidol.

3.2. Optimization of ^{19}F NMR parameters for the assay of haloperidol

The ^{1}H-decoupled ^{19}F NMR spectrum of haloperidol, possessing a sharp singlet ^{19}F signal at −104.8 ppm, which is well separated from that of trifluoroacetic acid, used as an internal standard, located at −75.4 ppm. The high sensitivity of ^{19}F nucleus in conjunction with the wide range of fluorine chemical shifts provided a suitable and simple method for the identification and determination of haloperidol.

To provide quantitative information needed for the haloperidol assay, many NMR data collection parameters have been optimized. The magnitude and duration of the applied RF pulse are important parameters, which affect both the signal-to-noise ratio and the quantitative accuracy of the signal integrals. The other parameter for optimizing sensitivity is the recycling delay time (D_1), necessary to return all magnetizations to equilibrium between pulses. This is obviously related to the spin-lattice relaxation time (T_1) of the nuclei. To carry out quantitative determinations, we first measured the T_1 value of haloperidol by the inversion recovery pulse sequence method, and the resulting value found to be 1.23 ± 0.01 s.

It has been suggested that a good approach for the quantitative analysis of complex mixtures containing nuclei with a short range of T_1 values is to use large pulse angles (70–90°) and a repetition time higher than the maximum T_1 by three- to five-fold. After performing optimization studies on the haloperidol, a standard pulse angle of 90° and a relaxation delay of 10 s associated with an acquisition time of 1.07 s were selected, which allowed the accurate quantification of haloperidol in real samples.

3.3. Potential applicability of the proposed method to the assay of haloperidol in human serum

Fig. 1a shows the ^{19}F NMR spectrum of a blank serum sample, which contains no ^{19}F NMR signal in chemical shift win-

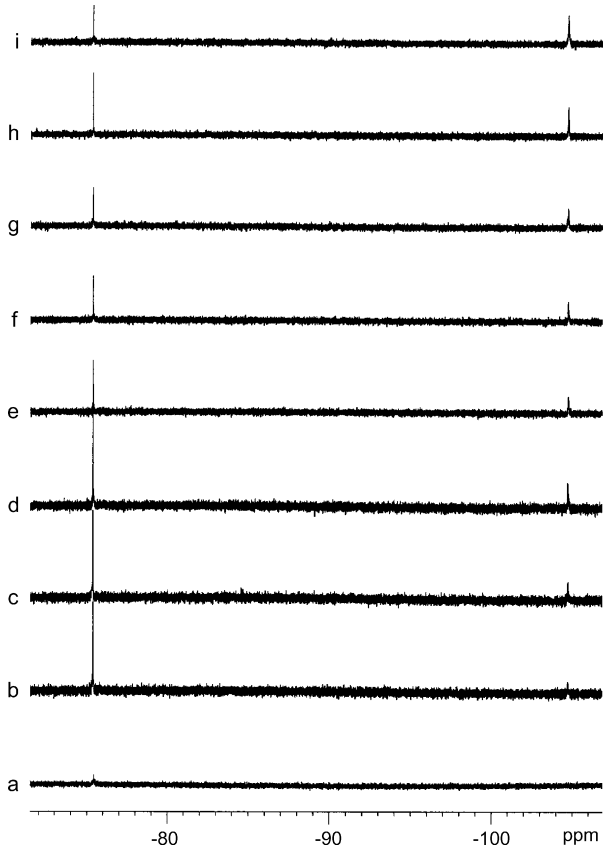


Fig. 1. The ^{19}F NMR spectra of (a) blank human serum, and serum spiked with (b) 60 μg ml^{-1}, (c) 135 μg ml^{-1}, (d) 180 μg ml^{-1}, (e) 240 μg ml^{-1}, (f) 275 μg ml^{-1}, (g) 320 μg ml^{-1}, (h) 400 and (i) 600 μg ml^{-1} haloperidol (each containing trifluoroacetic acid as internal standard).

Table 1
Limit of quantitation (LOQ) of haloperidol as a function of scan number

Number of scans	Scan time (min)	LOQ (mg ml^{-1})
16	3	0.18
64	12	0.06
128	24	0.04
256	48	0.03
850	120	0.02

dows employed for the quantitative studies of the haloperidol. The quantitative behavior of the proposed method was evaluated by spiking known concentrations of the analyte into control serum samples. Fig. 1b–i shows the ^{19}F NMR spectra of serum-spiked samples over a concentration range of 60–600 μg ml^{-1}. Calibration graphs were constructed using a least-squares linear regression of the haloperidol-to-trifluoroacetic acid internal standard integral ratios versus the corresponding concentration of the drug. The resulting regression equation was $I_{(\text{haloperidol})}/I_{(\text{internal standard})} = 531.08C - 1.057$ ($n = 11$, $r = 0.9968$), where $I_{(\text{haloperidol})}/I_{(\text{internal standard})}$ is the integral ratio and C is the haloperidol concentration. The detection limit calculated from the calibration graph was1.4 μg ml^{-1} (S/N = 3).

The S/N ratio for a peak in the NMR is known to increase with scan number as the $(S/N)^{1/2}$. Therefore, minimum quantitable limit of analyte may be attained with ^{19}F NMR by increasing the number of scans. This limit of quantitation (LOQ) would be obtained without any pre-concentration step. The LOQ for the analyte examined (at an S/N = 10) are summarized in Table 1. As is obvious, the limitation for reaching lower LOQs is the number of scans.

The precision of proposed method (expressed as relative standard deviation, R.S.D.) for spiked samples at 280 and 320 μg ml^{-1} concentration levels was studied. The obtained R.S.D. values were at the most 6% and the recovery was found to be almost quantitative. A summary of results of the precision and accuracy experiments is given in Table 2.

Table 2
Precision and accuracy of the analysis of haloperidol spiked to human serum ($n = 3$)[a]

Spiked (mg ml^{-1})	Found[b] (mg ml^{-1})	C.V. (%)[c] (mg ml^{-1})	Recovery (%)[b]
0.28	0.29 ± 0.02	6.9	102.5 ± 7.2
0.32	0.31 ± 0.01	3.2	96.7 ± 2.4

[a] Equation of calibration curve for haloperidol in serum: $I = 531.08C - 1.057$ ($r = 0.9968$), where I is the relative integral (arbitrary unit) and C is the concentration of analyte (mg ml^{-1}).
[b] Mean ± S.D. ($n = 3$).
[c] Coefficient of variation.

However, since the plasma haloperidol content in the drug-treated patients is much lower (by a factor of about 100) than the sensitivity of the NMR apparatus (LOQ = 0.02 mg ml^{-1}), the pre-concentration of the plasma samples is necessary before their haloperidol assay by ^{19}F NMR. We are currently involved on work on the development of a solid-phase microextraction (SPME) method for the efficient pre-concentration of low level haloperidol content of human serum samples and its subsequent determination by the proposed ^{19}F NMR method.

3.4. Assay of haloperidol in pharmaceutical formulations

The determination of haloperidol content of three pharmaceutical samples was performed according to the procedure described previously. The results show that the contents of active ingredient of tablet and injection samples are consistent with their declared values. The relative standard deviations were less than 5%. Furthermore, the results obtained for three real samples are compared with those obtained by the USP reference method, which are summarized in Table 3. The calculated t-test values did not exceed the theoretical values, indicating the absence of any significant difference in terms of precision and accuracy.

In order to further validate the method, recoveries were tested under the conditions mentioned above. These experiments were

Table 3
Analysis of haloperidol in pharmaceutical preparations

Drug	Label claim (mg tablet^{-1})	Proposed method[a]	USP method[a]
Apotex tablet	5	4.88 ± 0.05	4.96 ± 0.01
Sobhan tablet	0.5	0.489 ± 0.02	0.495 ± 0.04
I.P.D.I.C. injection	5	4.92 ± 0.04	4.81 ± 0.03

[a] Mean ± S.D. ($n = 3$).

Table 4
Results of spike recoveries in pharmaceutical preparations

Drug	Added (mg ml^{-1})	Found[a] (mg ml^{-1})	Recovery (%)[a]
Apotex tablet	0.10	0.10 ± 0.01	99 ± 5
	0.20	0.21 ± 0.01	104 ± 3
	0.29	0.30 ± 0.01	105 ± 3
I.P.D.I.C. injection	0.07	0.070 ± 0.006	99 ± 6
	0.20	0.19 ± 0.01	97 ±4
	0.29	0.30 ± 0.01	103 ± 3

[a] Mean ± S.D. ($n = 5$).

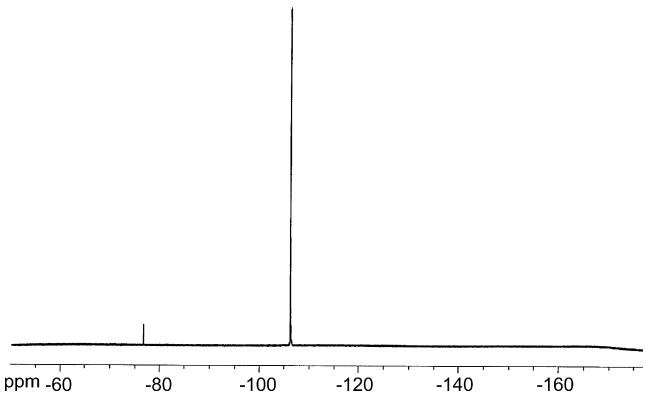


Fig. 2. A 500 MHz ^{19}F NMR spectrum of a 1.0 mg ml^{-1} crude haloperidol sample in D_2O/formic acid solvent. Operating conditions: number of scans, 32; D_1, 10 s; pH of solution, 3.4.

carried out by adding haloperidol to the above mentioned pharmaceutical sample solutions (Apotex tablet and I.P.D.I.C. injection), at three concentration levels, followed by the haloperidol assay using the proposed procedure. The obtained results for five replicate measurements are listed in Table 4. As is obvious, the average percent recoveries varied from 97 to 105.

3.5. Study of degradation of haloperidol by ^{19}F NMR

The ^{19}F NMR spectrum obtained from a crude haloperidol sample revealed an additional signal at −75.8 ppm (Fig. 2); therefore, to identify the source of this additional signal, the degradation under stressed conditions was studied.

The resulting spectra for haloperidol standard solution with those of haloperidol solution obtained under stressed condition are shown in Fig. 3. Degradation signals were identified by their chemical shifts. In the spectrum obtained from the acidic medium, two degradation products appeared at −105.3 and −75.7 ppm, the former of which is related to 4-fluorobenzoic acid compound, as in the spectrum of 4-fluorobenzoic acid in acidic medium the fluorine signal is appeared at −105.8 ppm. The latter corresponds to dehydrated compound. Since, in acidic medium, haloperidol is reported to be dehydrated [34], and based on the previous results reported on decomposition of some other hydroxylated compounds via dehydration in acidic medium [35], the signal observed at −75.7 ppm could be possibly due to the formation of dehydrated haloperidol.

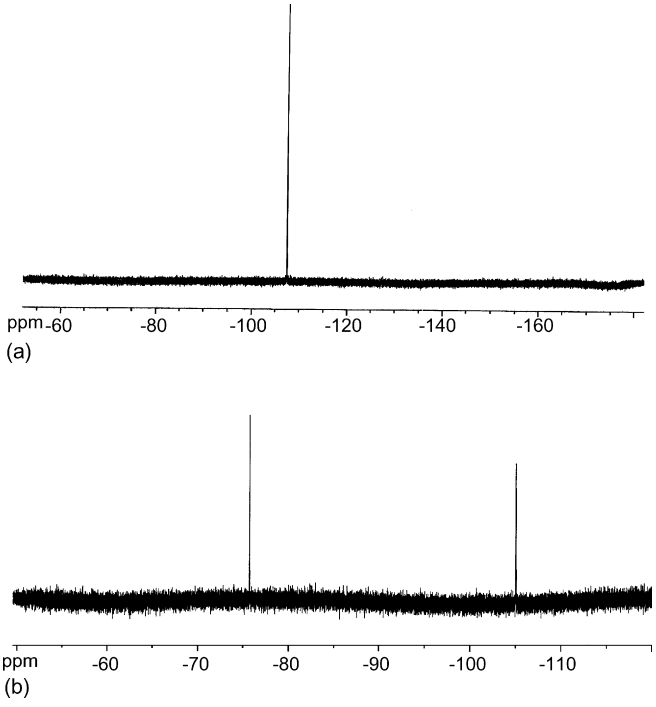


Fig. 3. A 500 MHz ^{19}F NMR spectra from haloperidol degradation study. (a) Haloperidol standard: operating conditions—concentration of haloperidol, 0.8 mg ml^{-1}; number of scans, 64; D_1, 10 s. (b) Acidic degradation: operating conditions—concentration of refluxed standard haloperidol, 1.0 mg ml^{-1}; number of scans, 256; D_1, 10 s.

4. Conclusion

A simple, precise and selective ^{19}F NMR spectroscopic method was developed for determining haloperidol in pharmaceutical and human's serum samples using trifluoroacetic acid as internal standard. The described technique requires minimal sample preparation and only a few analytical reagents, while it does not need any sample pretreatment, manipulation of large samples and lengthy instrument time.

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