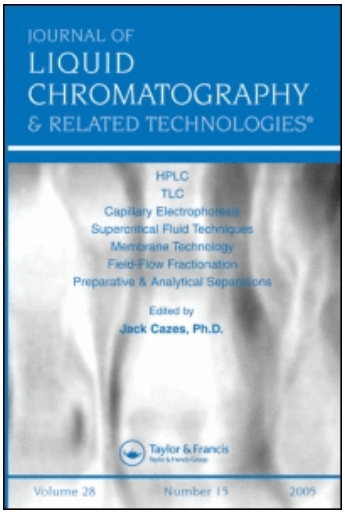


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Rapid LC Method for the Determination of Haloperidol and Its Degradation Products in Pharmaceuticals Using a Porous Graphitic Carbon Column

Lotfi Monser[a]; Hassen Trabelsi[b]
[a] Institut National des Sciences Appliquées et de Technologie, Tunis Cedex, Tunisie [b] S.I.P. Ibn Albaytar, Tunis-Carthage, Tunisie

Online publication date: 02 March 2003

To cite this Article Monser, Lotfi and Trabelsi, Hassen(2003) 'A Rapid LC Method for the Determination of Haloperidol and Its Degradation Products in Pharmaceuticals Using a Porous Graphitic Carbon Column', Journal of Liquid Chromatography & Related Technologies, 26: 2, 261 — 271
To link to this Article: DOI: 10.1081/JLC-120017167
URL: http://dx.doi.org/10.1081/JLC-120017167

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 2, pp. 261–271, 2003

A Rapid LC Method for the Determination of Haloperidol and Its Degradation Products in Pharmaceuticals Using a Porous Graphitic Carbon Column

Lotfi Monser[1,*] and Hassen Trabelsi[2]

[1]Institut National des Sciences Appliquées et de Technologie, Tunis Cedex, Tunisie
[2]S.I.P. Ibn Albaytar, Tunis-Carthage, Tunisie

ABSTRACT

A simple, rapid, and sensitive HPLC method has been developed and validated for the simultaneous determination of haloperidol and its degradation products, such as *cis*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone *N*-oxide, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-hydroxyphenyl)-1-butanone, 4-[4-(4-chlorophenyl)-3,6-dihydro-1(2H)-piperidinyl]-1-(4-fluorophenyl)-1-butanone and *trans*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone *N*-oxide in pharmaceutical formulations. The method was developed using a porous graphitic carbon (PGC) column and an isocratic elution of 55 : 45 v/v tetrahydrofuran/water containing

*Correspondence: Lotfi Monser, Institut National des Sciences Appliquées et de Technologie, Centre Urbain Nord, B. P. 676, 1080 Tunis Cedex, Tunisie; E-mail: lotfi.monser@insat.rnu.tn.

DOI: 10.1081/JLC-120017167
1082-6076 (Print); 1520-572X (Online)

0.5% trichloroacetic acid (TCA). The elution of these compounds was monitored at 254 nm with a flow rate of 1 mL min^{-1}. Linearity was observed in the concentration range from 2 to 50 μg mL^{-1}, with a correlation coefficient (R^2) greater than 0.999. The limits of detection were 0.1 μg mL^{-1} for haloperidol and 0.05 μg mL^{-1} for the degradation products. Parameters of validation prove the precision of the method and its applicability for the simultaneous determination of haloperidol and its degradation products. The method is fast and one chromatogram separation lasts about 9 min.

Key Words: Haloperidol; Degradation products; Isocratic elution; Porous graphitic carbon.

INTRODUCTION

Haloperidol (HA) "4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone", is an antipsychotic drug that has been widely used against positive symptoms (hallucinations and delusions) of schizophrenia. [1] Under different abnormal conditions such as temperature, pH, light, and air exposure, haloperidol degrades forming several degradation products that have undesirable side effects. The oxidation of haloperidol could result in the formation of *cis*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone *N*-oxide (*cis*-PFBoxide) and *trans*-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone *N*-oxide (*trans*-PFBoxide), while its hydrolysis in acidic and basic medium could form 4-[4-(4-chlorophenyl)-3,6-dihydro-1(2H)-piperidinyl]-1-(4-fluorophenyl)-1-butanone (2H-PFB) and 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-hydroxyphenyl)-1-butanone (HydroxyPHB), respectively. Therefore, there is a need for analytical methods that are sensitive to the presence of the drug degradation impurities.

Several analytical techniques have been reported in the literature for the determination of HA in pharmaceuticals, most commonly high performance liquid chromatography (HPLC).[2–7] However, only a few of them have discussed the separation of haloperidol from its degradation products in tablet dosage form using reversed phase silica based columns.[8,9] In addition to the instability of silica based packing materials, most of these methods suffer from the lack of sensitivity and undesirable analysis time. The current official HPLC assay for the determination of HA in tablet forms requires an undesirable analysis time of about 24 min.[10] At present, no HPLC methods have been reported for the simultaneous determination of HA and its degradation products using a porous graphitic carbon column. Porous graphite carbon column has proved its efficacy in the separation of diasteriomers, geometric

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

isomers, and closely related compounds that were hardly separated on alkyl-bonded silica columns.[11–14]

In the previous work,[15,16] PGC has been successfully used for the determination of pharmaceutical formulations in the presence of their closely related compounds. This work describes an isocratic HPLC method using a porous graphitic carbon column in an attempt to measure haloperidol in the presence of its degradation products in pharmaceutical formulations rapidly and precisely. This method could be used as an alternative to the above mentioned HPLC methods.

EXPERIMENTAL

Chemicals and Reagents

Haloperidol, *cis*-PFBoxide, HydroxyPHB, 2H-PFB and *trans*-PFBoxide, were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). Their chemical structures are represented in Fig. 1. Drug samples (Haldol 1 mg and 5 mg) were collected from local pharmacies. High performance liquid chromatographic grade methanol, acetonitrile, and tetrahydrofuran were purchased from prolabo (Paris, France).

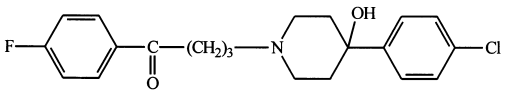
Instrumentation and Chromatographic Conditions

The HPLC system (Beckman Instruments Inc., USA) was equipped with a solvent module 125, a spectrophotometer detector 166, and a rheodyne 7725 injector with a 20 μL loop. The carbon column (100 × 4.6 mm I.D., 7 μm particle size) was packed with Hypercarb porous graphitic carbon (Shandon, Runcorn, UK). The mobile phase consisted of an isocratic mixture of tetrahydrofuran–water containing 0.5% trichloroacetic acid. The flow rate was 1 mL min^{-1}. The detector wavelength was set at 254 nm. Responses were recorded and integrated using Gold Nouveau software.

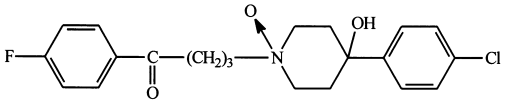
Preparation of Solutions

Stock solutions of haloperidol and its degradation products (200 μg mL^{-1} each) were prepared in methanol and stored at 4°C. The working standards (2–50 μg mL^{-1}) were freshly prepared from the stock solutions by dilution with the appropriate volume of methanol. Haloperidol containing tablets were prepared by crushing 20 tablets and an accurately weighed portion of the mixed powder, equivalent to haloperidol content of one tablet, was transferred

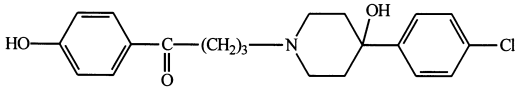
MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016



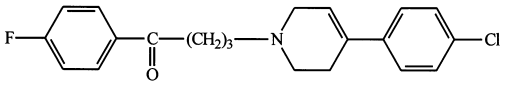
Haloperidol **(HA)**



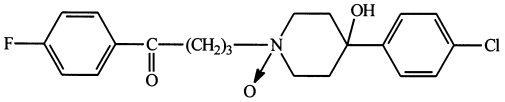
Cis-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, N-oxide
(Cis-PFBoxide)



4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-hydroxyphenyl)-1-butanone
(HydroxyPHB)



4-[4-(4-chlorophenyl)-3,6-dihydro-1(2H)-piperidinyl]-1-(4-fluorophenyl)-1-butanone
(2H-PFB)



Trans-4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, N-oxide
(Trans-PFBoxide)

Figure 1. Chemical structures and names of studied compounds.

to 25 mL volumetric flask and dissolved by sonication. The sample was filtered and diluted to make a final haloperidol concentration range of 5–10 $\mu g\,mL^{-1}$.

Method Validation

Accuracy, precision, and linearity were determined with an intra-day and inter-day procedure on three different days. Recovery of analytes after sample preparation was determined at different concentration levels. The detection limit and the quantification limit were determined as 3 and 10 times the baseline noise, respectively, following the United States Pharmacopeia.[17]

RESULTS AND DISCUSSION

Chromatography

Porous graphite carbon was utilised to develop and optimise the chromatographic method. Injections of haloperidol and related compounds, both one at a time and in mixture, were made to evaluate the capacity of the method in separating all the degradation impurities from each other and from HA. Figure 2, shows tat haloperidol was successfully separated from its degradation products on PGC packing materials. Since retention on PGC is different from that of alkyl-bonded silica, a mobile phase containing tetrahydrofuran (THF) has to be used. The % of THF in the mobile phase was selected to minimise the analytical time, while maintaining baseline resolution of adjacent peak pairs ($Rs > 1.5$). A mobile phase containing 55% THF was selected as the optimum composition with regards to the resolution, analysis time, and column efficiency. The elution order was, *cis*-PFBoxide, HydroxyPHB, HA, 2H-PFB, and *trans*-PFBoxide. The separation of these peaks takes less than 10 min. An additional benefit of the fast separation is rapid column equilibrium when the composition of the mobile phase is changed. These separation conditions are much better than the separation obtained by reversed phase ODS column. Improved resolution of HA from its degradation products was observed with tailing factors and column efficiency within the recommended limits. Results of the chromatographic parameters are shown in Table 1.

The previously described HPLC methods and that of the USP assay for HA, lists reversed phase ODS columns with a total run time of about 20 min and an elution order as following: HydroxyPHB, HA, *trans*-PFBoxide, *cis*-PFBoxide, and 2H-PFB. The elution order obtained with these columns was different to that obtained with PGC column. The difference in the elution order between PGC and ODS columns, suggest that molecular interactions deter-

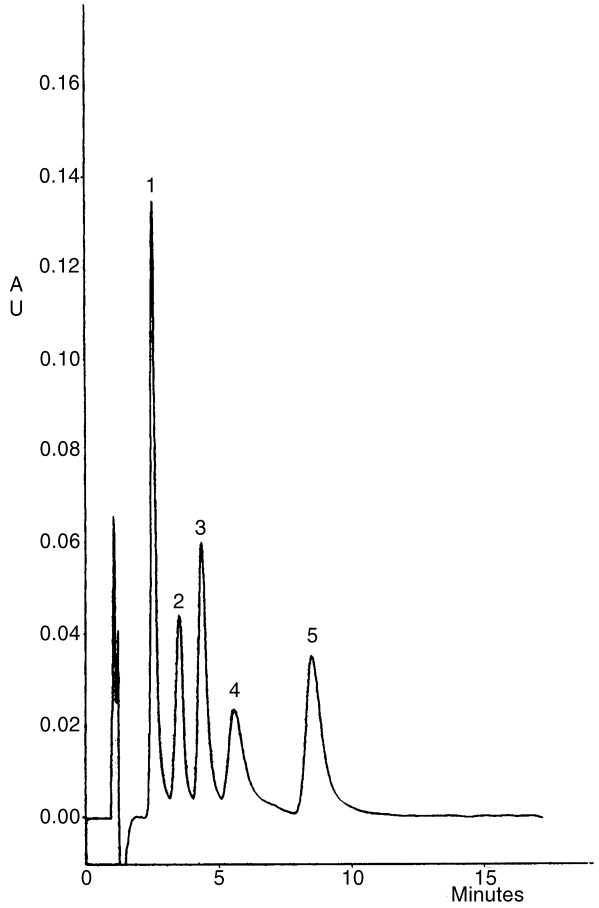


Figure 2. Separation of HA and its degradation products on PGC column with a mobile phase containing 55 : 45 v/v THF-0.5%TCA, and a flow rate of 1 mL min^{-1}. Peaks: 1 = *cis*-PFBoxide; 2 = HydroxyPHB; 3 = HA; 4 = 2H-PFB; 5 = *trans*-PFBoxide.

mining solute retention are different for the two packing materials. This difference was further clarified by the elution order of the two geometric isomers (*cis*-PFBoxide and *trans*-PFBoxide), where on an ODS column they were closely separated (3ed and 4th peak), however on PGC column they were widely separated (1st and 5th peak). These results prove once more that PGC packing materials were more highly selective towards geometrical isomers and closely related compounds than ODS packing materials.

Table 1. Chromatographic parameters for the peaks recorded in analysis of haloperidol and its degradation products.

Compounds	k′	A_s	N	R_s
cis-PFBoxide	1.27	1.75	1227	
				1.83
HydroxyPHB	2.05	1.18	1114	
				1.75
HA	2.86	1.40	1480	
				1.51
2H-PFB	3.8	1.9	840	
				2.72
trans-PFBoxide	6.48	1.33	1337	

The effects of mobile phase pH and organic modifier (nature and concentration) were investigated. Results obtained showed that, as the pH increased from 1.5 to 7.5, the retention of studied compounds increased. These results indicate the usual behaviour of retention vs. pH of basic compounds. The ideal pH for separation, based on good peak shapes and retention times, was below 2.5. The high dipolar properties of THF make it more of an appropriate solvent for the separation of HA and related compounds on PGC than acetonitrile or methanol. Increasing the ratio of THF in the mobile decreases the retention time of all studied solutes. Therefore, a mobile phase of 55 : 45 v/v THF/water containing 0.5% TCA was selected for method validation.

Validation Assay

The selectivity of the proposed method for HA in the presence of its degradation products was determined by analysing a solution of HA containing standard solution of *cis*-PFBoxide, HydroxyPHB, 2H-PFB, and *trans*-PFBoxide. Figure 2 shows that there is adequate resolution among all of these compounds.

Linearity was established by injecting different solutions of haloperidol and related compounds with concentrations ranging from 2 to 50 $\mu g\,mL^{-1}$. These solutions were injected five times, starting with the least concentrated ones. The resulting peak areas were measured and linear regression analysis was performed to evaluate the correlation coefficient (R^2) and the relative standard deviation (RSD) within solution. Linearity was good, with a correlation coefficient greater than 0.999. Results of regression analysis parameters are summarised in Table 2. The limit of detection based on a signal-to-noise ratio of

Table 2. Linear regression (least squares fir of peak area vs. concentration in μg mL^{-1}) calibration data for the analysis of haloperidol and its degradation products ($n = 5$).

Compounds	Concentration range ($\mu g\,mL^{-1}$)	Equation of the line	RSD of the slope (%)	RSD of the intercept (%)	R^2
cis-PFBoxide	2–50	$Y = 51942x + 46360$	0.62	0.80	0.9999
HydroxyPHB	2–50	$Y = 14333x + 2992$	0.80	1.32	0.9995
HA	2–50	$Y = 17186x + 29341$	1.12	1.24	0.9997
2H-PFB	2–50	$Y = 67100x + 18742$			0.9995
trans-PFBoxide	2–50	$Y = 23936x - 12517$	0.88	1.40	0.9999

3 was $0.1\,\mu g\,mL^{-1}$ and $0.05\,\mu g\,mL^{-1}$ for HA and degradation products, respectively.

The within-day and between-days precision of the method, expressed as RSD, were determined for both retention time and peak area by repeat analysis ($n = 5$) of solutions containing HA and its related compounds (Table 3). The within-days values for retention time was 0.60–1.20% and for peak area 1.10–1.80%. The between-days precision values for retention time was 0.82–1.46% and for peak area 1.26–1.82%.

Accuracy of the proposed method for determination of HA was examined for HA alone and in the presence of the known impurities. The method was evaluated from the recovery assay as the % error. The average recovery was calculated as the mean value, obtained by spiking HA drug tablets with a mixture of the studied solutes at three levels within the working range (6, 12, and $18\,\mu g\,mL^{-1}$). The range of the overall recovery for all compounds was between 98.9 and 102% (Table 3). The proposed method provides satisfactory precision and accuracy for the analysis of HA in the presence of its related compounds.

Method Applicability for the Analysis of Haloperidol Drug

The method was applied to different pharmaceutical formulations (Haldol tablets) for determining their content in HA. The values of the drug percentage

Table 3. Method reproducibility, recovery and limit of detection for the studied compounds.

Compounds	Retention time[a] mean ± S.D. (min)	RSD (%)	Peak area[b] RSD (%)	recovery[c] (%)	Limit of detection (μg mL^{-1})
cis-PFBoxide	2.50 ± 0.03	1.2	1.21	98.9	0.05
HydroxyPHB	3.35 ± 0.02	0.60	1.10	99.0	0.05
HA	4.25 ± 0.05	1.17	1.20	100	0.1
2H-PFB	5.30 ± 0.06	1.13	1.80	102	0.05
trans-PFBoxide	8.23 ± 0.08	0.97	1.65	99.2	0.05

[a]Chromatographic conditions as in Fig. 2.
[b]20 μL injection of 6 and 12 μg mL^{-1} solutions ($n = 5$).
[c]20 μL injection of Naproxen samples spiked with 6, 12 and 18 μg mL^{-1} standard solutions ($n = 5$).

with respect to the label claimed (Table 4), ranged within 98 and 102%. According to the United State Pharmacopeia, these values were acceptable.

CONCLUSION

The method proposed herein, based on PGC-HPLC can reliably determine HA and its degradation products (*cis*-PFBoxide, HydroxyPHB, 2H-PFB, and *trans*-PFBoxide) in pharmaceutical formulations, with good sensitivity, accuracy, and precision. This method is straightforward, simple, and feasible, enabling the determination of haloperidol in the presence of its degradation products in less than 10 min. Taking advantages of its robustness

Table 4. Content of HA in tablets expressed as % with respect to label amount claim.

	Haloperidol (mg)		
Drug	Claimed	Found	%
Haldol (1)	1.0	1.00	100
Haldol (1)	1.0	0.98	98
Haldol (5)	5.0	5.10	102

and high selectivity, PGC should be considered for official use in the USP compendia assays.

REFERENCES

1. Beasley, C.; Tollefson, G.; Tran, P.; Satterlee, W.; Sanger, T.; Hamilton, S. Olizapine versus placebo and haloperidol double blind olizapine trial. Neuropsycho-Pharmacology **1996**, *14*, 111–123.
2. Driouich, R.; Trabelsi, H.; Bouzouita, K. Stability-indicating assay for haloperidol syrup by HPLC. Chromatographia **2001**, *53*, 629–634.
3. Trabelsi, H.; Raouafi, F.; Saddem, A.; Bouzouita, K. Separation and determination of haloperidol, methyl and propyl paraben by HPLC. J. Pharm. Belg. **1997**, *52*, 145–148.
4. Fang, J.; Gorrod, J. HPLC method for detection and quantitation of haloperidol and seven of its metabolites in microsomal preparations. J. Chromatogr. **1993**, *614*, 267–272.
5. Lea, A.; Dailey, D.; Duguid, P. Analysis of haloperidol tablets by HPLC—an inter-laboratory study. J. Chromatogr. **1982**, *250*, 3542.
6. Tsuneyosh, T. High. Resolut. Chromatogr. Commun. **1986**, *9*, 254–259.
7. Raggi, M.; Casamenti, G.; Mandrioli, R.; Sabbioni, C.; Volterra V. A rapid LC method for the idendification and determination of CNS drugs in pharmaceuticals. J. Pharma. Biom. Anal. **2000**, *23*, 161–167.
8. Xu, X.; Stewart, J. HPLC analysis of haloperidol and its related compound in drug substance and tablet dosage form using non-porous silica octadecylsilane column. J. Liq. Chrom. & Rel. Technol. **1999**, *22*, 1857–1866.
9. Trabelsi, H.; Bouabdallah, S.; Bouzouita, K.; Safta, F. Determination and degradation study of haloperidol by HPLC. J. Pharma. Biom. Anal. **2002**, *29*, 649–657.
10. *The United State Pharmacopeia*, 23rd Ed.; National Formulary 18, Rockvilie, MD, 1995; 731–733.
11. Vial, J.; Hennion, M.; Fernandez-Alba, A.; Aguera, A. Use of porous graphite carbon coupled with mass detection for the analysis of polar phenolic compounds by liquid chromatography. J. Chromatogr. A **2001**, *937*, 21–39.
12. Lim, C. Porous graphitic carbon in biomedical applications. Adv. Chromatogr. **1992**, *32*, 1–19.
13. Mercier, J.; Morin, Ph.; Dreux, M.; Tambute, A. Liquid chromatographic analysis of phosphonic acids on porous graphitic carbon stationary phase with evaporative light scattering and mass spectrometry detection. J. Chromatogr. A **1999**, *849*, 197–207.

14. Karlsson, A.; Berglin, M.; Charron, C. Robustness of the chromatographic separation of alprenolol and related substances using silica based stationary phase and metoprelol on porous graphitic carbon. J. Chromatogr. A **1998**, *797*, 75–82.
15. Monser, L.; Darghouth, F. Rapid liquid chromatographic method for simultaneous determination of tetracycline antibiotics and 6-epi-doxycycline in pharmaceuticals using porous graphitic carbon. J. Pharm. Biomed. Anal. **2000**, *23*, 353–362.
16. Monser, L.; Darghouth, F. Simultaneous LC determination of paracetamol and related compounds in pharmaceuticals using porous graphitic carbon correspondence. J. Pharm. Biomed. Anal. **2002**, *27*, 851–860.
17. *The United State Pharmacopeia*, 24th Ed.; United States Pharmacopeial Convention Rockville, MD, 2000; 2150–2151.

Received August 12, 2002
Accepted September 19, 2002
Manuscript 5932