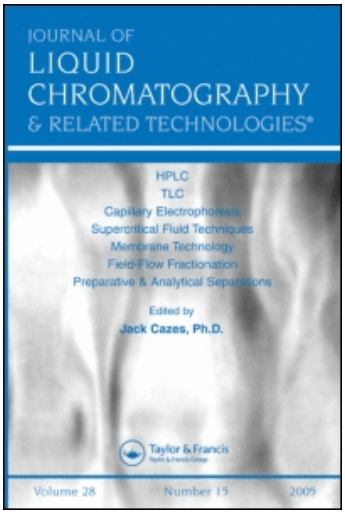

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 03 December 2003

To cite this Article Segall, A. I. , Collado, E. A. , Ricci, R. A. and Pizzorno, M. T.(2003) 'Reversed-Phase HPLC Determination of Sibutramine Hydrochloride in the Presence of its Oxidatively-Induced Degradation Products', Journal of Liquid Chromatography & Related Technologies, 26: 6, 977 — 986
To link to this Article: DOI: 10.1081/JLC-120018897
URL: http://dx.doi.org/10.1081/JLC-120018897

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 6, pp. 977–986, 2003

Reversed-Phase HPLC Determination of Sibutramine Hydrochloride in the Presence of its Oxidatively-Induced Degradation Products

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ABSTRACT

The determination of sibutramine hydrochloride in the presence of its oxidatively-induced degradation products by reversed-phase HPLC is described. The method was validated as stability-indicating by forced decomposition of sibutramine hydrochloride in acid, base, water, oxidative, thermal, and photochemical media. The chromatographic conditions employed a reversed-phase C_{18} column (Microsorb-MV^{TM} 100 A, 5 µm, 25 cm × 4.6 mm). The mobile phase consisted of methanol : water : triethylamine (80 : 20 : 0.3) and the pH was adjusted to 4.5 with 85% phosphoric acid. The flow rate was maintained at 1.1 mL per minute. Chromatographic separation was monitored by ultraviolet detection at a wavelength of 225 nm. The peak area vs. sibutramine hydrochloride

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DOI: 10.1081/JLC-120018897 1082-6076 (Print); 1520-572X (Online)

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concentration proved linear over the 50–160% range of the working analytical concentration of 0.06 mg/mL. Mean absolute recovery of sibutramine hydrochloride, using the described method, was $100.4 \pm 1.8\%$ (mean $\pm$ SD, $n = 9$). The precision, expressed as relative standard deviation (RSD), of six replicate injections of sibutramine hydrochloride reference solution, remained below 0.4%.

Key Words: Stability-indicating HPLC method; Sibutramine.

INTRODUCTION

Sibutramine *N*-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl-*N*, *N*-dimethyl-amine (Fig. 1) is a serotonin–norepinephrine reuptake-inhibitor for the treatment of obesity. It produces a dose-related weight loss. It is used in the management of obesity, as the hydrochloride, in an initial daily dose of 10 mg and 15 mg, usually taken in the morning. Many clinical trials have been described.[1–7]

A computer search (Medline, Analytical Abstracts, and International Pharmaceutical Abstract) disclosed no HPLC method for the assay of sibutramine hydrochloride in pharmaceuticals.

This report describes a sensitive, accurate, reproducible, and stability-indicating reversed phase HPLC method for the determination of sibutramine hydrochloride in tablets. Mobile phase pH influence on resolution and retention time were examined.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a dual piston reciprocating Spectra Physics pump (Model ISO Chrom. LC pump), a UV–Vis Hewlett

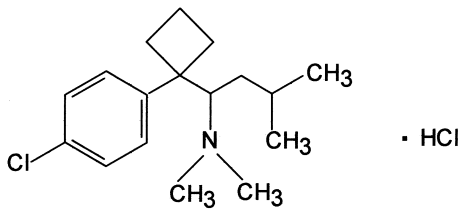

Figure 1. Sibutramine hydrochloride.

Packard detector (Model 1050), a Hewlett Packard integrator (Series 3395), and a Rheodyne injector (Model 7125).

Chemicals and Reagents

Methanol used in the mobile phase was HPLC grade. Distilled water was passed through a 0.45-micron membrane filter. Triethylamine and phosphoric acid were AR grade. Solutions and mobile phase were prepared just before use and all solvents and solutions for HPLC analyses were filtered through a Micron Separations N04SP04700 nylon membrane filter (pore size 0.45 μm) and vacuum degassed before use.

Sibutramine hydrochloride was kindly donated by Laboratorios Dallas S.A. (Argentina) and used as working standard without further purification.

A commercial local tablet formulation was used in this study. Its composition was: sibutramine hydrochloride 15.0 mg, in a matrix of: microcrystalline cellulose, lactose, colloidal silicon dioxide, and magnesium stearate.

Chromatographic Conditions

Resolution solutions of sibutramine hydrochloride at different pH and mobile phase composition were analyzed. The mobile phase chosen was methanol : water : triethylamine (80 : 20 : 0.3) and the pH was adjusted to 4.5 with 85% phosphoric acid.

The analytical column was a Varian Microsorb-MV^{TM} 100 A, 5 μm, 25 cm × 4.6 mm. All analyses were performed under isocratic conditions at a 1.1-mL/min flow rate, and 7-min run time, at room temperature. In these conditions sibutramine hydrochloride retention time (t_R) was roughly 4 min. Detector sensitivity was set at 1 a.u.f.s. and eluents monitored at 225 nm. The volume of each injection was 20 μL.

Calibration Curve

Solutions ranging from 30 to 96 μg/mL of sibutramine hydrochloride were prepared in mobile phase from a methanol stock solution of sibutramine hydrochloride standard. The calibration curve was constructed by plotting peak areas against micrograms injected.

Preparation of Solutions

A standard stock solution of sibutramine hydrochloride, 0.6 mg/mL, was prepared in methanol. The standard preparation was obtained by diluting the

sibutramine hydrochloride stock solution with mobile phase to yield a concentration of 0.06 mg/mL.

Resolution Solution

Thirty milligrams of sibutramine hydrochloride were dissolved in 10 mL of H_2O_2 100 vol, refluxed for 30 min and diluted to 50 mL with methanol. The solution was diluted to a concentration of 0.06 mg/mL.

System Suitability

System suitability results were calculated according to the USP 24 ⟨621⟩ from typical chromatograms.[8] Instrument precision, as determined by six successive injections of the standard preparation, should provide a relative standard deviation (RSD) below 1.0%. The tailing factor did not exceed 1.0 at 5% peak height. Column efficiency was greater than 300 theoretical plates. Finally, 20 μL of the resolution solution were injected. The resolution between sibutramine hydrochloride and the nearest adjacent peak was greater than 3.8 (Fig. 2D)

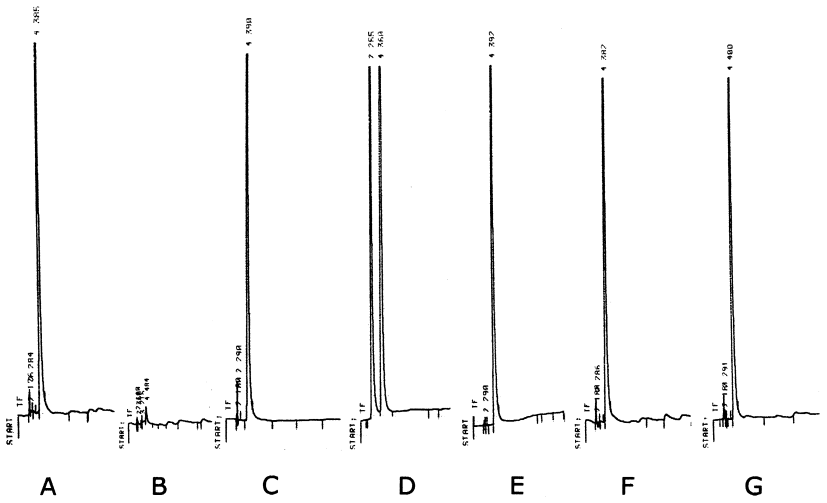

Figure 2. Chromatograms of sibutramine hydrochloride. (A) sibutramine hydrochloride standard; (B) alkaline degradation; (C) acid degradation; (D) oxidative degradation; (E) water degradation; (F) photolysis; and (G) heat dry degradation.

Stability-Indicating Validation

The HPLC method was validated as stability-indicating by forced degradation of sibutramine hydrochloride. Samples were prepared by transferring 30 mg of sample into 50 mL volumetric flask. Intentional degradation was attempted using 10 mL of HCl 1 N, NaOH 1 N, H_2O_2 30 vol, water and refluxing for at least 15 min. Sibutramine hydrochloride was subjected to thermal (in an open container in an oven at 110°C, 24 h) and photochemical degradation (in an open container exposed to daylight for 24 h). After degradation treatments were completed, samples were allowed to cool to room temperature and diluted to the same concentration as the standard preparation, after being neutralized with acid–base if required. Samples were then analyzed against the standard.

Accuracy

Twenty tablets from the same lot of a commercial formulation were weighed and finely powdered. Assay accuracy was assessed at 80, 100, and 120% of sibutramine hydrochloride by recovery experiments.

Precision

Method precision was evaluated by two repeated assays of the same lot of one commercial formulation, by different analysts.

Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Prior to injecting solutions, the column should be equilibrated for at least 30 min with mobile phase flowing through the system. Quantitation was accomplished by using an external standard method. Each solution was injected in triplicate and the RSD was required to remain below 1.5% on a sibutramine hydrochloride peak area basis.

RESULTS AND DISCUSSION

Effect of Mobile Phase pH on Resolution and Retention Time

Table 1 shows the effect of mobile phase pH and composition on resolution and retention time, both of which were significantly affected. Based on these results, a pH range of 4.5 ± 0.1 was selected. (Table 1).

Table 1. Effect of pH and composition of mobile phase solution on retention time and resolution for sibutramine hydrochloride and its oxidative-induced degradation products.

Mobile phase	Sibutramine retention time (min)	Resolution	Degradation products retention time (min)
Methanol : water : triethylamine 85 : 15 : 0.3 pH: 4.5	3.760	2.4	2.292
Methanol : water : triethylamine 75 : 25 : 0.3 pH: 4.5	5.109	4.0	2.292
Methanol : water : triethylamine 80 : 20 : 0.3 pH: 4.0	4.237	2.3	2.327
Methanol : water : triethylamine 80 : 20 : 0.3 pH: 5.0	4.219	3.5	2.269

Specificity

Sibutramine hydrochloride was stressed by thermal, acidic, basic, oxidative, and photochemical degradation for up to 24 hours. No interfering peaks at the retention time of sibutramine hydrochloride were observed in any of the stressed samples. (Fig. 2)

Stability determinations were conducted to assess method specificity for the assay of sibutramine hydrochloride without interference from the oxidatively-induced degradation products. The forced degradation experiment described above yielded a reduction in intact sibutramine hydrochloride (retention time 4.360 min) with the formation of a new peak eluting at circa 2.255 min. (Fig. 2)

None impurities were detected with alkaline, acid, water, photolysis, and heat dry degradation.

Precision

Precision was considered at two levels as ICH suggest: repeatability and intermediate precision. Repeatability was evaluated by analyzing six replicate injections of sibutramine hydrochloride reference solution, giving a RSD of 0.4% and minimal variation in retention time. Intermediate precision was determined by carrying out two precision assays of one lot of a commercial formulation. For the precision assays, the results were as follows: mean values 100.4 and 99.2%, standard deviations 1.2 and 1.1 and RSD 0.7% and 0.3%. Test "*t*" comparing two samples with 95% confidence for 10 degrees of

freedom disclosed that both results were not significantly different inter se $(t_{n-2,\alpha:0.05}) = 2.23$. (Table 2)

Linearity

Five solutions containing sibutramine hydrochloride at concentrations ranging from 30 to 96 μg/mL were analyzed. The curve of peak area vs. micrograms injected proved linear. Response linearity was evaluated by regression analysis and the regression equation ($Y = 13889126X + 249328$) presented a correlation coefficient (r) of 0.9995 while intercept values were not significantly different from zero, ($p = 0.05$). (Table 3)

Microsoft Excel software was used to plot peak areas vs. micrograms injected. (Fig. 3)

Accuracy

Assay accuracy was assessed at 80, 100, and 120% of sibutramine hydrochloride by recovery experiments, using tablets from the same lot of one commercial formulation.

Individual recovery ranged from 97.0 to 103.1 (Table 4). Recovery at all levels was 100.4% with an RSD value of 1.8%.

Method accuracy was demonstrated by plotting the amount recovered vs. the amount added (in mg). Linear least squares analysis of the data yielded a correlation coefficient (r) value and slope of 0.9965 and 1.0303, respectively. The r value indicates that the method is linear over the concentration range investigated. The slope value was close to unity and the intercept was not

Table 2. Precision of the assay method.

	Analyst 1		Analyst 2	
Sample no	Amount determined (%)	RSD	Amount determined (%)	RSD
1	98.9	0.09	98.3	0.08
2	99.7	0.17	99.9	0.04
3	99.9	0.01	98.1	0.33
4	100.3	0.16	99.1	0.51
5	101.1	0.02	101.1	0.08
6	102.4	1.60	98.6	0.65
Means	100.4	0.68	99.2	0.28

Table 3. Linearity of sibutramine hydrochloride response.

% w/w	Injected (μg)	Average peak area response	RSD (%)
50	0.5980	8499023	0.50
80	0.9568	13344749	1.1
100	1.1960	17237045	1.1
120	1.4352	21059915	1.4
160	1.9136	26724021	0.5
Slope[a] = 13889126 ± 710727			1.8
Intercept[b] = 249328 ± 922585			

[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).

significantly different from zero (t test, $p = 0.05$) which confirmed the accuracy of the method over the range investigated.

Sibutramine hydrochloride recovery achieved showed that there was no interference from excipients present in the tablets.

Range

Assay method range was set at 80–120% of the finished product label claim, since the method proved precise, accurate, and linear within these limits.

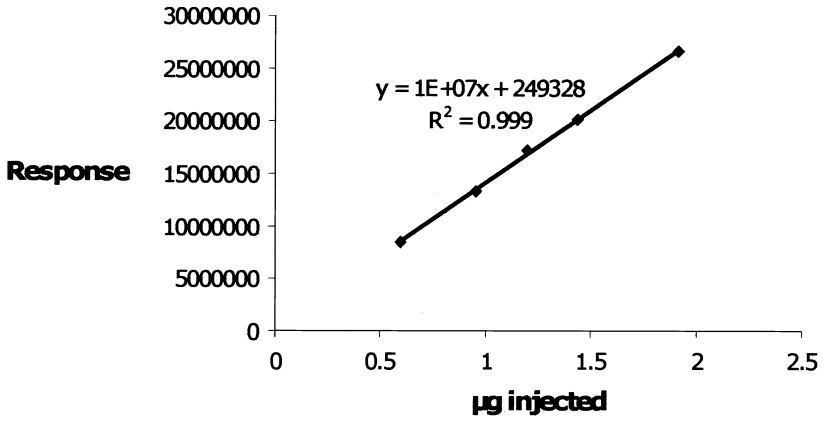

Figure 3. Peak area response vs. μg injected of sibutramine hydrochloride.

Table 4. Recovery analysis.

% w/w	Amount added (mg)	Amount determined (mg)	Amount recovered (%)	Average recovered ($n = 3$)	RSD (%)
80	12.19	11.83	97.0	100.2	3.1
	12.17	12.55	103.1		
	11.92	11.97	100.4		
100	14.81	14.68	99.1	100.0	1.2
	15.35	15.28	99.5		
	14.99	15.19	101.4		
120	18.18	18.27	100.5	101.1	0.8
	18.14	18.29	100.8		
	18.26	18.65	102.1		
Overall recovery ($n = 9$)				100.4	1.8

CONCLUSION

The HPLC method described in this work is specific, precise, accurate, and useful for quantitation of sibutramine hydrochloride as an alternative for routine analysis, for evaluation of its chromatographic purity and stability studies.

ACKNOWLEDGMENTS

The authors thank Laboratorios Dallas S. A. (Argentina) for assistance and donation of drugs. This work was supported by grant B005 to A. I. S. from UBA.

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Received November 1, 2002
Accepted November 20, 2002
Manuscript 6008