Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 817 (2005) 183–186
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatographic assay of abouthiouzine in plasma and its application to pharmacokinetic studies

Khalid M. Alkharfy[a,*], Rao Muzaffar A. Khan[a], Badraddin M. Al-Hadiya[a], Hisham S. Abou-Auda[a], Rafiq R. Abou-Shaaban[b]

[a] *Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*
[b] *Department of Pharmaceutics, Faculty of Pharmacy and Health Sciences, Ajman University of Science and Technology, Abu Dhabi, United Arab Emirates*

Received 19 September 2004; accepted 1 December 2004
Available online 21 December 2004

Abstract

Abouthiouzine is a newly synthesized antithyroid agent with a proposed less adverse effects profile than other currently used drugs. A simple and rapid reversed phase high performance liquid chromatography assay was developed to determine the concentration of abouthiouzine in human plasma. The procedure involved extraction of the drug and propranolol (internal standard) from the plasma using ethylacetate. The extract was evaporated under nitrogen and the residue was constituted with the mobile phase and injected onto μ-Bondapack phenyl column (10 μm, 3.9 mm × 150 mm). The mobile phase consisted of 10 mM potassium dihydrogen phosphate buffer, acetonitrile, and methanol in the ratio of 60:25:15 (v/v/v, pH = 3.0), which was delivered at a rate of 1.5 ml/min. Abouthiouzine and the internal standard were monitored using UV detection at 240 nm; the run time was less than 5 min. The detection limit of abouthiouzine is 0.5 μg/ml. The within- and between-day coefficients of variation were less than 7%. Our method has been successfully used to measure abouthiouzine plasma concentrations in a rabbit model following an intravenous administration of the drug.

Keywords: Abouthiouzine; HPLC; Assay

1. Introduction

Hyperthyroidism is the clinical syndrome that results from exposure of body tissues to excess circulating levels of thyroid hormones (thyroxine and triiodothyronine) [1,2]. Drugs that are commonly used to treat this condition such as propylthiouracil and methimazole are known to have serious side effects on the immune system and the bone marrow. Abouthiouzine [1-*n*-butyl-3(isonicotinamido)-2-thiourea] [$C_{11}H_{16}N_4OS$; 252.34 mol wt.] (Fig. 1) is a newly designed antithyroid agent with a reportedly less side effects profile compared to other drugs used in the treatment of hyperthyroidism [3]. The relative efficacy of abouthiouzine, after equimolar dose, was found to be 102% and 51.5% of that of propylthiouracil with respect to the rate of 125 l-discharge and 125 l-uptake, respectively. In addition, chemiluminescence studies on polymorphonuclears (PMNs) revealed that abouthiouzine has only slight oxidant property [3]. Such properties may provide advantages in avoiding the iatrogenic hypothyroidism and antithyroid-induced immunological reactions.

The purpose of the current work was to develop a novel and simple high-performance liquid chromatography (HPLC) assay for the determination of abouthiouzine in plasma and to evaluate its application in pharmacokinetic studies.

* Corresponding author. Tel.: +966 1 4677494; fax: +966 1 4677480.
E-mail address: alkharfy@ksu.edu.sa (K.M. Alkharfy).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.12.001

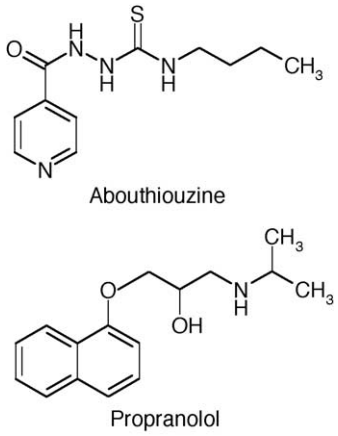

Fig. 1. Chemical structures of abouthiouzine and propranolol.

2. Experimental

2.1. Reagents and chemicals

Abouthiouzine was designed using *electrotopological*-state (E-state) indexes and synthesized in the college of Pharmacy's research laboratories (King Saud University, Riyadh, Saudi Arabia), as it has previously been described [3,4]. Propranolol was purchased from Sigma (St. Louis, MO, USA); methanol, acetonitrile, and ethyl acetate of HPLC grade were obtained from BDH Laboratory (BDH Chemicals Ltd., Poole, UK). All other reagents and chemicals used in the assay were of the highest purity available for analytical research; water was of Milli-Q quality.

2.2. Instrumentation

The HPLC system consisted of a Waters model 717 autosampler, model M-600 dual piston solvent delivery pump, and model M-2487 dual UV absorbance detector (Waters Corp., Milford, MA, USA). The mobile phase consisted of potassium dihydrogen phosphate (10 mM), acetonitrile, and methanol in the ratio of 60:25:15. The pH of the mobile phase was adjusted to 3.0 with phosphoric acid and was delivered at a flow rate of 1.5 ml/min. A Waters μ-Bondapack phenyl column (10 μm, 3.9 mm × 150 mm) was utilized to elute the compound of interest at a λ_{max} = 240 nm. Signal output was captured using Millennium^{32} software, version 3.05 (Waters Corp., Milford, MA, USA).

2.3. Stock solutions

Abouthiouzine stock standard solution was prepared by accurately weighing an amount equivalent to 100 mg of the drug, which was dissolved in 100 ml methanol to produce a concentration of 1 mg/ml. For working standard solution, 1.0 ml of abouthiouzine stock solution (1.0 mg/ml) was transferred to a 10 ml volumetric flask and was completed to the mark with deionized water to produce a final concentration of 100 μg/ml. Propranolol (internal standard, IS) stock solution was prepared by weighing an amount equivalent to 50 mg of propranolol powder, which was then dissolved in 100 ml methanol to give a concentration of 0.5 mg/ml. These working standard solutions were kept at −20 °C for at least 4 weeks.

2.4. Sample preparation and analytical procedure

Abouthiouzine was extracted from the plasma using ethyl acetate as the extraction solvent. To 500 μl of plasma containing abouthiouzine, 25 μl of internal standard (0.5 mg/ml) was added in a 15 ml capped glass tube with vortex mixing. The mixture was shaken with 6 ml of ethyl acetate for 10 min and centrifuged at 3000 × *g* for another 10 min. The ethyl acetate layer was transferred to another glass tube and evaporated to dryness under gentle stream of nitrogen. The residue was reconstituted with 150 μl of mobile phase and 75 μl of this was injected onto the column.

2.5. Calibration and linearity

Calibration curves were obtained daily for 3 days using standards containing eight different concentrations. Curves were constructed by calculating the peak-height ratios of abouthiouzine to that of IS. Calibration curve data points were fit using a weighted least squares linear regression (WLSLR) using $1/y^2$ as weight. WLSLR has been shown to improve the accuracy of the analytical method at the lower end of the calibration curve especially with less homoscedastic data points [5], Sample preparation and analysis were conducted at room temperature. Abouthiouzine working standard (1 mg/ml) was added to 10 ml volumetric flasks in volumes of 0, 5, 10, 50, 100, 200, 300, 400 and 500 μl. Drug-free plasma was used to complete the volume to 10 ml, and was vortex-mixed to yield final calibration standard concentrations of 0.0 (no abouthiouzine added), 0.5,1, 5,10, 20, 30, 40, and 50 μg/ml. Each of these standard solutions were distributed into disposable polypropylene microcentrifuge tubes (1.5 ml, Eppendorf, Hamburg, Germany) in volumes of 0.7 ml and stored at −20 °C pending analysis.

2.6. Precision and accuracy

The precision and accuracy of the assay was determined using quality control (QC) samples of known abouthiouzine concentrations (i.e., 2 and 35 μg/ml), which were processed fresh each validation day as described for calibration curve standards. Six replicates of each QC were analyzed on 3 days and the intra- and inter-assay means, standard deviations, and coefficients of variation (CV) were calculated.

2.7. Long-term stability (freezer storage stability)

Freeze and thaw analytes stability was determined during five freeze (−20 ± 2 °C) and thaw cycles. Freshly prepared

low and the high QC samples (i.e., 2 and 35 μg/ml) were measured at room temperature (22 ± 2 °C) on day one, and the rest were stored at −20 ± 2 °C for 5 weeks. The determination of abouthiouzine was performed at the end of each week for five consecutive cycles.

2.8. Method specificity and application

The specificity of the method was determined by screening 10 different batches of controlled human blank plasma for interfering peaks. In addition, solutions containing some commonly used drugs including aspirin, caffeine, ibuprofen, amoxicillin, theophylline, metformin, sulfamethoxazole, cisapride, phenobarbitone and clonazepam were prepared in the mobile phase and injected onto the column for possible interference.

To demonstrate the applicability of our method in pharmacokinetic studies, abouthiouzine was administered intravenously to a male vole rabbit in a dose of 2 mg/kg. Blood samples were collected at 0,1, 2, 5,10,15, 30, and 60 min, and plasma was separated by centrifugation at 2500 × *g* for 10 min, and subsequently analyzed for abouthiouzine using the developed method.

3. Results

3.1. Chromatographic separation

Representative chromatograms of abouthiouzine in plasma samples are shown in Fig. 2. Abouthiouzine and the internal standard (propranolol) were separated within 5 min of the chromatographic run. The retention times for abouthiouzine and IS were approximately 2 and 3 min, respectively.

3.2. Calibration, precision, and accuracy

Standard curves for abouthiouzine were linear over the range of 0.5–50 μg/ml. The mean coefficient of determination (r^2) for the standard curves was >0.99; precision and accuracy of the calibration curves data points are shown in Table 1. The calculated intra- and inter-day CVs for abouthiouzine QC samples were less than 7% (Table 2); relative recovery of abouthiouzine from human plasma samples was approximately 100% (Table 3).

Table 1
Precision and accuracy of abouthiouzine calibration curve points in human plasma

Added concentration (μg/ml)	Measured concentration (mean ± S.D., μg/ml)	Accuracy (%)	Precision (CV%)
0.5	0.521 ± 0.039	102.35	7.69
1	0.972 ± 0.036	97.21	3.71
5	4.978 ± 0.138	99.56	2.78
10	9.731 ± 0.539	97.31	5.54
20	19.847 ± 0.593	99.23	2.99
30	29.869 ± 0.772	99.58	2.59
40	39.75 ± 1.431	99.38	3.60
50	49.962 ± 1.141	99.25	2.28

Regression line equation: $y = 0.0558x - 0.1162$; $r^2 = 0.9954$ ($n = 8$).

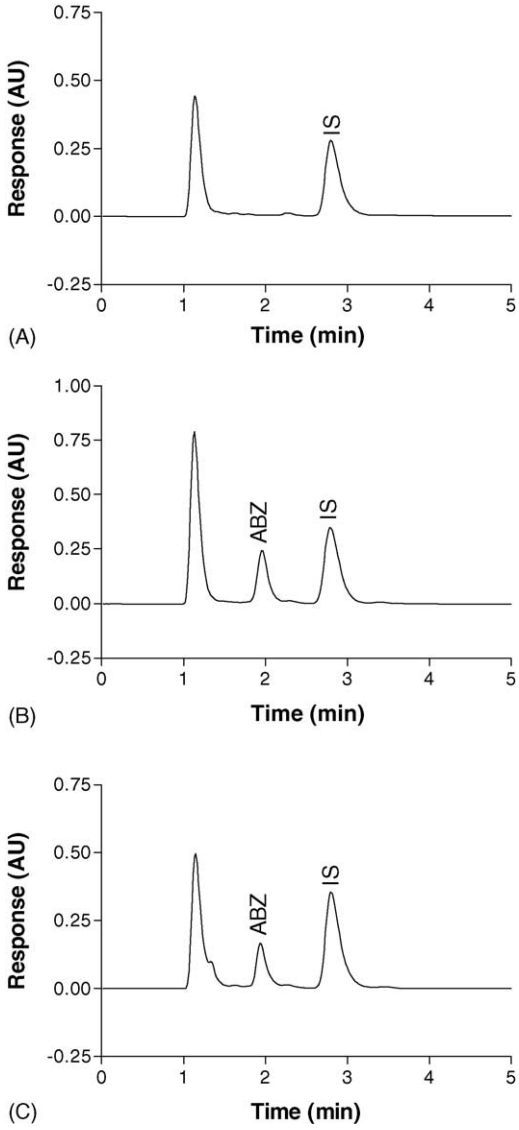

Fig. 2. Representative chromatograms of abouthiouzine (ABZ) and propranolol (internal standard, IS): A, blank plasma spiked with IS only; B, plasma extract spiked with abouthiouzine (10 μg/ml) and IS; C, plasma extract from healthy rabbit dosed with 2 mg/kg of abouthiouzine intravenously.

3.3. Sensitivity, specificity, and stability

The lower limit of quantification (LLOQ) was 0.5 μg/ml. The specificity of the method was evidenced by the lack of interfering endogenous plasma components in the chromatograms of screened plasma batches. Furthermore, none of the injected drugs interfered with the peaks of abouthiouzine or the internal standard. Stability studies showed that abouthiouzine is stable in plasma for at least 5 weeks, when kept at −20 ± 2 °C; the data are presented in Table 4.

Table 2
Intra- and inter-day precision and accuracy of abouthiouzine in human plasma

Abouthiouzine	Added concentration (μg/ml)	Measured concentration (mean ± S.D., μg/ml)	Accuracy (%)	Precision (CV%)
Intra-day	2.0	2.11 ± 0.08	105.5	4.22
$n = 6$	35.0	36.33 ± 1.18	103.8	3.26
Inter-day	2.0	2.05 ± 0.13	102.31	6.60
$n = 18$	35.0	35.82 ± 2.20	102.35	6.14

Table 3
Analytical relative recovery of abouthiouzine from human plasma

Added concentration (μg/ml)	Measured concentration (μg/ml)	Relative recovery (%)
2.0	2.01	100.5
	2.33	116.5
	2.22	111.0
	2.13	106.5
	2.18	109.0
	1.96	98.0
Mean ± S.D. (μg/ml)	2.14 ± 0.12	106.9 ± 6.2
CV%	5.8	5.8
35.0	37.26	106.5
	35.95	102.7
	35.33	100.9
	34.98	99.9
	38.10	108.9
	38.90	111.1
Mean ± S.D. (μg/ml)	36.75 ± 1.44	105.0 ± 4.14
CV%	3.93	3.94

Table 4
Stability of abouthiouzine in human plasma through five freeze (−20 ± 2 °C) thaw (room temperature) cycles during a period of 5 weeks

Added concentration (μg/ml)	Measured concentration (μg/ml)	Accuracy (%)	Precision (CV%)
2.0	2.01 ± 0.19	100.5	9.39
35.0	34.52 ± 2.99	98.6	8.67

4. Discussion

This is the first analytical assay to be developed for the determination of abouthiouzine in human plasma. The chromatographic conditions described here were arrived at after investigating several mobile and stationary phases and internal standards. The use of an isocratic mobile phase and the phenyl column yielded separation from endogenous components of the plasma and resolved peaks for both abouthiouzine and the internal standard. The blank chromatogram showed that no interferences would occur with endogenous substances. Some advantages of this assay include simplicity, reproducibility, selectivity, and rapidity of the chromatographic run (5 min). Our results have shown that abouthiouzine remains stable even after several freeze and thaw cycles, which indicates the sturdiness of the compound for prolonged analytical procedures. Inspection of the semilogarithmic plot of abouthiouzine plasma concentration versus time curve indicated that it could be described by a biexponential decline process (Fig. 3).

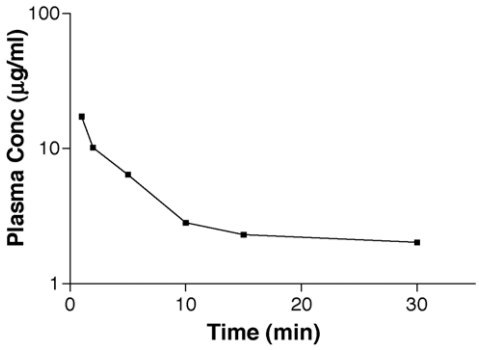

Fig. 3. Plasma–time concentration curve of abouthiouzine following I.V. administration of 2 mg/kg dose to a male vole rabbit.

Being a new drug moiety, the study of the pharmacokinetics and toxicokinetics of abouthiouzine warrants the establishment of an accurate, reproducible, selective, and specific assay capable of measuring low concentrations of the drug in biological fluids. The current method has also been successfully employed in the study of the pharmacokinetics of abouthiouzine in an animal model. This will allow for further investigation of abouthiouzine disposition in various species including humans, using different routes of administration.

References

[1] D.S. Cooper, Lancet 362 (2003) 459.
[2] E.N. Pearce, L.E. Braverman, Surg. Clin. N. Am. 84 (2004) 833.
[3] R.R. Abou-Shaaban, H.A. Al-Khamees, H.S. Abou-Auda, A.P. Simonelli, Pharm. Res. 13 (1996) 129.
[4] R.R. Abou-Shaaban, H.A. Al-Khamees, H.S. Abou-Auda, A.P. Simonelli, Saudi Pharm. J. 3 (1995) 156.
[5] A.M. Almeida, M.M. Castel-Branco, A.C. Falcao, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 774 (2002) 215.