Available online at www.sciencedirect.com
SCIENCE DIRECT®

Talanta 67 (2005) 975–978

Talanta

www.elsevier.com/locate/talanta

High-performance liquid chromatographic method for the simultaneous estimation of the key intermediates of duloxetine

Pankaj Soni [a], T.T. Mariappan [b], U.C. Banerjee [a,*]

[a] *Biocatalysis Laboratory, Department of Pharmaceutical Technology, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar 160062, India*
[b] *Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar 160062, India*

Received 5 January 2005; received in revised form 9 April 2005; accepted 12 April 2005
Available online 3 May 2005

Abstract

A simple reversed-phase high-performance liquid chromatographic method employing C-18 column has been developed for simultaneous analysis of three intermediates in the synthesis of *S*-duloxetine, the antidepressant drug, viz., 2-acetyl thiophene (AT), *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine (DKTP) and (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP). Good separations were achieved by employing an isocratic system using acetonitrile and 0.05 M phosphate buffer (pH 7.0) containing 0.02% diethylamine. The detection was carried out at 241 nm. The method was validated for linearity, range, accuracy and precision. The developed method was applied for monitoring the progress of chemical synthesis of DKTP from AT followed by the biocatalytic reduction of DKTP to DHTP as the disappearance of the substrate and formation of the product can be monitored simultaneously by the present method.

Keywords: Duloxetine; RP-HPLC; Asymmetric reduction; Chemical synthesis

1. Introduction

Various analogs of 3-(1-naphthoxy)-3-arylpropanamines, such as fluoxetine [1], tomoxetine [2], nisoxetine and duloxetine [3], are the most important pharmaceuticals for the treatment of psychiatric and metabolic disorders. Among these, the thiophene derivative, duloxetine, *S*-(+)-*N*-methyl-3-(1-napthalenyloxy)-3-(2-thienyl) propanamine, holds a special promise as an antidepressant drug as it is a dual inhibitor of serotonin and norepinephrine reuptake. It can also be used for stress urinary incontinence. Various features, like improved efficacy, tolerability, safety, faster recovery, fewer side effects, low affinity for neuronal receptors and dual inhibiting nature gives duloxetine an edge over other existing antidepressants, such as fluoxetine and tomoxetine [4]. Unlike other antidepressant drugs, like fluoxetine, tomoxetine etc. that are marketed as racemates, duloxetine is marketed in its *S*-enantiomeric form. In order to produce a key enantiopure intermediate for the synthesis of *S*-duloxetine, various strategies have been proposed, such as the enantioselective reduction of 3-(dimethylamino)-1-(2-thienyl) propan-1-one and with Li(ent-Chiralde^{R})$_2$ AlH$_2$ salt, the enantioselective reduction of 3-chloro-1-(2-thienyl) propan-1-one with an oxazaborolidine catalyst [5], the resolution of 3-(dimethyl amino)-1-(2-thienyl) propan-1-ol via diastereomeric salt formation [6], the resolution of 3-(methylamino)-1-(2-thienyl) propan-1-ol with (*S*)-mandelic acid [7] and through various chemoenzymatic processes using lipases [8,9]. All these chemical and chemoenzymatic processes give rise to chiral intermediates that are subsequently used for the synthesis of *S*-duloxetine. Among various intermediates, 2-acetyl thiophene (AT), *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine (DKTP) and (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP) are common to all the synthetic routes. There are various analytical methods to monitor the progress of this synthesis

---

* Corresponding author. Tel.: +91 172 2214682–7; fax: +91 172 2214692.
E-mail address: ucbanerjee@niper.ac.in (U.C. Banerjee).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.04.020

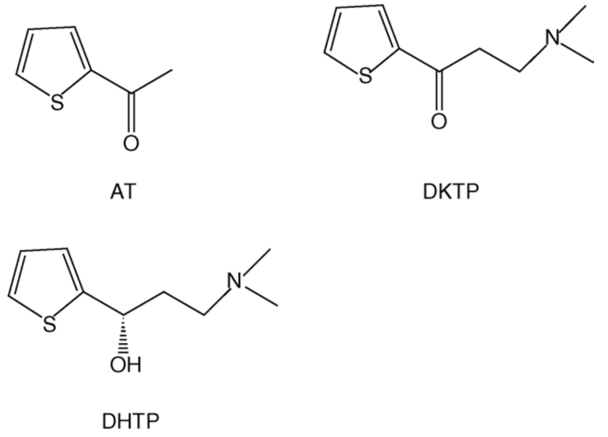

Fig. 1. Chemical structures of AT, DKTP and DHTP. *Key*: AT: 2-acetyl thiophene; DKTP: *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine; DHTP: (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine.

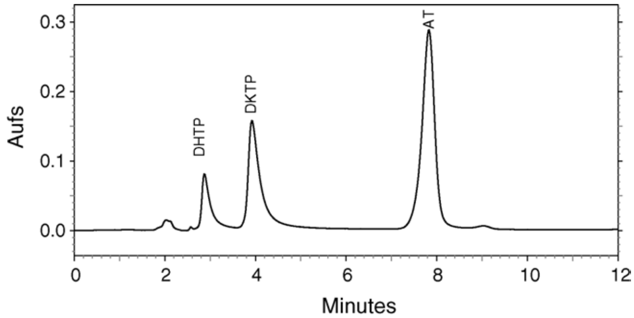

Fig. 2. Chromatogram showing the separation of 2-acetyl thiophene, *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine and (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine.

but to the best of our knowledge no HPLC method is available for the simultaneous estimation of the intermediates formed during the enzymatic process of synthesis of duloxetine.

In the present study, we report for the first time a reversed-phase HPLC method to simultaneously determine the three crucial intermediates of *S*-duloxetine, viz., 2-acetyl thiophene (AT), *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine (DKTP) and (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP) (Fig. 1) on a reversed phase C_{18} column (Fig. 2).

2. Experimental

2.1. Chemicals

AT was purchased from Lancaster Synthesis Ltd. (Lancashire, UK). DKTP was synthesized from AT according to the reported procedure [5]. DKTP was subjected to microbial reduction to obtain DHTP. HPLC grade acetonitrile and methanol was purchased from J.T. Baker (USA) and Mallinckrodt Baker Inc. (Paris, Kentucky), respectively. Buffer materials and all other chemicals were of analytical-reagent grade. Ultra-pure water was obtained from ELGA water purification unit (Elga Ltd., Bucks, UK).

2.2. Instrumentation

pH of the mobile phase was checked on a pH/ion analyser (MA 235, Mettler Toledo, Schwerzenbach, Switzerland). The HPLC system comprising of a dual piston reciprocating pump (LC-10ATVP), UV–vis dual wavelength detector (SPD-10AVP), an auto injector (SIL-10ADVP), and in-line degasser (DGU-14AM). The data was acquired using CLASS-VP software (all from Shimadzu, Kyoto, Japan). All separations were achieved on a 250 mm × 4.6 mm, 5 μM particle LichroCART RP-18 column (Merck, Germany). The mobile phase was filtered through a 0.45 μm nylon membrane and degassed before use.

2.3. Method development and validation

2.3.1. Development of method

To get a proper separation of all the three components, different mobile-phase compositions comprising of acetonitrile, methanol, water and buffer have been tried. Silanol blocking agents have also been added in the mobile phases to get sharp-peak shapes.

2.3.2. Validation

Linearity was established by triplicate injections of solutions containing the different intermediates at various concentration ranges on the same day. Limits of detection and quantification were determined by calculation of signal-to-noise ratio. Signal-to-noise ratio of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively, of the method.

Intra-day precision was established by making six injections of lowest, middle and highest concentration in the above range (25, 250 and 500 μg/ml) on the same day. These studies were also repeated on different days with different weights to determine inter-day precision. Intermediate precision was established through separation studies on a different chromatographic system by a different operator. Accuracy was evaluated by fortifying a mixture of reaction solutions with three known concentrations of the compound. The recovery of the added compound was determined.

2.4. Method application

The method was applied for monitoring the chemical synthesis as well as the biochemical reduction reactions involved in the synthesis of enantiopure DHTP. Initially the chemical synthesis of DKTP (prochiral ketone) from AT was monitored. In this reaction, AT was allowed to reflux with dimethylammonium chloride and methylamine hydrochloride in presence of paraformaldehyde. The reaction mixture was finally cooled. The crystals collected were dried and

Table 1
Regression parameters of the standard plots of AT, DKTP and DHTP

Compound	Range (μg/ml)	Slope[a]	Intercept[a]	Correlation coefficient[a]
AT	5–500	52168 ± 27, 0.518	18869 ± 10, 0.511	0.998 ± 0.006, 0.572
DKTP	20–500	2654 ± 19, 0.733	4089 ± 43, 1.048	0.998 ± 0.005, 0.556
DHTP	20–500	2560 ± 28, 1.110	2175 ± 21, 0.958	0.991 ± 0.008, 0.820

[a] Values are mean ± S.D., R.S.D. (%).

subjected to HPLC analysis apart from various other spectral analytical tools like ^{1}H NMR, IR and LC–MS.

Later on, this synthesized DKTP was subjected to microbial reduction by various soil isolates. Various soil isolates were grown for 48 h in nutrient broth medium having glucose (1%). The broth was centrifuged (7000 × *g*, 20 min), washed with sodium phosphate buffer (0.2 M, pH 7.0). In the cell suspension (1 g/6 ml of buffer), DKTP as a hydrochloride salt was added. The reaction mixture was incubated at 30 °C in an orbital shaker (200 rpm). Samples were withdrawn after regular interval of time and centrifuged to separate out the cells. The pH of the supernatant was brought to 9.0 by NaOH and then it was extracted twice with equal volume of ethyl acetate. The organic solvent was dried over anhydrous sodium sulphate and concentrated under vacuum. Then the conversion was monitored using the developed method.

3. Results and discussion

3.1. Optimization of the method

Different mobile-phase compositions containing methanol–water, methanol–buffer, acetonitrile–water and acetonitrile–buffer were tried and finally reasonable resolution was obtained in acetonitrile–sodium phosphate buffer but with band spreading. This problem was overcome by addition of diethylamine in the mobile phase.

Thus, the separation of the three intermediates was achieved by employing a simple isocratic system using acetonitrile and 0.02 M phosphate buffer (pH 7) containing 0.02% (w/v) diethylamine. Elution was carried out with a mixture of acetonitrile and buffer in the ratio of 15:85 at a constant flow rate of 1 ml/min. The analytical wavelength was 241 nm.

3.2. Linearity, limits of detection and quantification

Table 1 shows the regression parameters of the standard plots of the three compounds. It is evident that the responses for all the three compounds were strictly linear in the studied concentration range, which is evident from the R.S.D. values of slope, intercept and correlation coefficient (less than 1%). The method worked well in the range from 5 to 20 μg/ml, 20 to 50 μg/ml and 20 to 50 μg/ml for AT, DKTP and DHTP, respectively, which suggest full capacity for the quantification of each of the intermediates. The LODs are the lower side of the ranges listed in Table 1 and the LOQs are about three times the LODs.

3.3. Precision

Table 2 provides data obtained from the precision experiments. The R.S.D. values for intra- and inter-day precision were <1.0% and <1.6%, respectively, thereby indicating that the method was sufficiently precise. A similar qualitative separation of the different intermediates was obtained even on analysis on a different chromatographic system on a different day, indicating that the method has sufficient intermediate precision.

3.4. Accuracy

Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown in the data in Table 3, excellent recoveries

Table 2
Intra- and inter-day precision data[a]

Compound	Actual concentration[a] (μg/ml)	Intra-day precision[a]	Inter-day precision[a]
AT	25	24.712 ± 0.050, 0.202	24.479 ± 0.234, 0.954
	250	249.986 ± 0.287, 0.115	247.164 ± 2.356, 0.953
	500	513.735 ± 1.000, 0.195	509.900 ± 4.196, 0.823
DHTP	25	24.937 ± 0.250, 1.003	24.184 ± 0.225, 0.920
	250	251.454 ± 1.850, 0.736	246.780 ± 3.903, 1.581
	500	507.754 ± 4.061, 0.800	506.751 ± 4.481, 0.884
DKTP	25	24.305 ± 0.202, 0.835	25.191 ± 0.401, 1.593
	250	248.871 ± 1.598, 0.642	251.539 ± 0.666, 0.265
	500	512.452 ± 1.930, 0.376	504.617 ± 1.127, 0.223

[a] Values are observed concentration (μg/ml) ± S.D., R.S.D. The determinations were made three times in a same day for intra-day precision and the same experiment was repeated for three consecutive days for inter-day precision.

Table 3
Recovery studies

Intermediate	Actual concentration (μg/ml)	Observed concentration[a] (μg/ml)	Recovery (%)
AT	25	25.191 ± 0.401	100.8
	250	251.539 ± 0.666	100.6
	500	504.617 ± 1.127	100.9
DHTP	25	24.937 ± 0.250	99.7
	250	251.454 ± 1.850	100.6
	500	507.754 ± 4.061	101.5
DKTP	25	25.14 ± 0.180	100.56
	250	249.92 ± 0.371	99.96
	500	499.86 ± 0.832	99.97

[a] Values are mean ± S.D. of at least three determinations.

were made at each added concentration, despite the fact that the compound was fortified to a mixture that contained other intermediate products.

3.5. Method application

The developed method has been successfully applied for the isolation of microorganisms capable of reducing *N*,*N*-dimethyl-3-keto-(2-thienyl)-propanamine (DKTP) to (*S*)-*N*,*N*-dimethyl-3-hydroxy-(2-thienyl)-propanamine (DHTP), the key intermediate in the synthesis of *S*-duloxetine. Using this method, we have successfully isolated three potent microorganisms capable of carrying out the bioreduction of DKTP. One of them, *Candida tropicalis*, PBR-2 MTCC-5158 has been successfully used for the development of a process for the production of enantiopure DHTP. Recently, we have filed a patent application for the same [10]. This method can be used for the routine analysis of the chemo- as well as chemoenzymatic synthesis of Duloxetine. However, the developed method is unable to certify the enantiopurity of the DHTP. The R-DHTP would not be separated from its *S*-enantiomer using this method.

4. Conclusion

The developed HPLC method is simple, accurate and reproducible and offers the following advantages: It uses simple ODS column and the mobile phase consists of acetonitrile and phosphate buffer only; it separates all the three compounds AT, DKTP and DHTP. Since AT, DKTP and DHTP are the key intermediates in the synthesis of *S*-duloxetine, whether it is by chemical and chemoenzymatic methods, so this method can be used as a routine analytical tool during the synthesis of *S*-duloxetine.

References

[1] E. Klieser, E. Lehmann, K. Heinrich, Pharmacopsychiatry 28 (1995) 14.

[2] R.L. Zerbe, H. Rowe, G. Enass, D. Wong, N. Farid, L. Lemberger, J. Pharmacol. Exp. Ther. 232 (1985) 73.

[3] D.T. Wong, D.W. Robertson, F.P. Bymaster, J.H. Krushinski, L.R. Reid, Life Sci. 43 (1988) 2049.

[4] A. Kamal, G.B.R. Khanna, R. Ramu, T. Krishnaji, Tetrahedron Lett. 44 (2003) 4783.

[5] D.T. Wong, J. Frazier, G. Staten, M. Staszak, L. Weigel, Tetrahedron Lett. 31 (1990) 7101.

[6] W.H. Wheeler, F. Kuo, J. Labelled Compd. Radiopharm. 36 (1995) 213.

[7] K. Sakai, R. Sakurai, A. Yuzawa, Y. Kobayashi, K. Saigo, Tetrahedron: Asymmetry 14 (2003) 1631.

[8] R.S. Rogers, Chem. Eng. News 30 (1998) 11.

[9] L. Huiling, B.H. Hoff, T. Anthonsen, Chirality 12 (2000) 26.

[10] P. Soni, C.L. Kaul and U.C. Banerjee, Indian Patent Application no. 1573/DEL/2004 (2004).