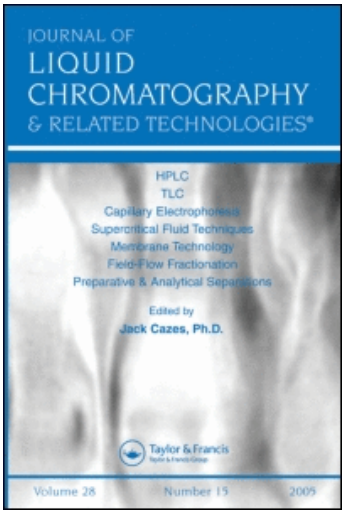

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Development of a High Performance Liquid Chromatographic Test for Impurities in 1-(2-Thienylethyl)-4-imidazoline-2-thione

C. B'Hymer[a]
[a] Chemistry Department, University of Cincinnati, Cincinnati, Ohio, USA

Online publication date: 02 March 2003

To cite this Article B'Hymer, C.(2003) 'The Development of a High Performance Liquid Chromatographic Test for Impurities in 1-(2-Thienylethyl)-4-imidazoline-2-thione', Journal of Liquid Chromatography & Related Technologies, 26: 4, 573 — 583
To link to this Article: DOI: 10.1081/JLC-120017904
URL: http://dx.doi.org/10.1081/JLC-120017904

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

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 4, pp. 573–583, 2003

The Development of a High Performance Liquid Chromatographic Test for Impurities in 1-(2-Thienylethyl)-4-imidazoline-2-thione

C. B'Hymer*

Chemistry Department, University of Cincinnati,
Cincinnati, Ohio, USA

ABSTRACT

A high performance liquid chromatographic (HPLC) procedure was devised for the determination of impurity levels in 1-(2-thienylethyl)-4-imidazoline-2-thione bulk compound. A gradient program with a mobile phase of 0.02 M sodium phosphate buffer in acetonitrile/water (apparent pH 6.9) was used with a Zorbax Rx-C8 column. The acetonitrile composition was increased linearly from 15 to 50% over a 40 min period and held at 50% for 20 min. Ultraviolet detection at 239 nm was used to quantify all components. The procedure was validated for accuracy using spiked levels (0.1–1.5% w/w) of two suspected known impurities. A three day, two column repeatability study showed consistent results with test batches of the compound.

*Correspondence: C. B'Hymer, Chemistry Department, University of Cincinnati, Mail location 0172, Cincinnati, OH 45221-0172, USA; E-mail: bhymerc@email.uc.edu.

DOI: 10.1081/JLC-120017904
1082-6076 (Print); 1520-572X (Online)

Key Words: Impurity test; Zorbax Rx-C8 column; Basic drug analysis.

INTRODUCTION

Many compounds of interest, including drugs and biologically active compounds, have basic functional groups and can present minor reversed-phase chromatographic problems when using traditional packings made with silica. Organic base compounds, analyzed on alkyl bonded silica high performance liquid chromatographic (HPLC) columns often elute in broad bands with peak tailing.[1–3] The degree of peak asymmetry varies between manufacturers of silica, and it is commonly believed that the poor chromatographic properties of basic compounds, analyzed on silica based reversed-phase columns, is due to hydrogen bonding of the ionized basic functional groups of the solute to nonbonded silanol groups on the silica support to the stationary phase.[2–5] Kohler and Kirkland[1] noted in 1987 that certain commercially prepared alkyl bonded silica materials exhibited low base adsorptivity, and they reported the production of a silica polymer exhibiting low base adsorptivity. The Zorbax Rx column, first produced by DuPont nearly 20 years ago and currently offered by Agilent Technologies, contains low base adsorptivity packing. The Zorbax Rx-C8 has been used extensively in drug analysis and reported in this role in the literature often.[3–10] Zorbax Rx-C8 packing material consists of an diisopropyl *n*-octyl ether linkage to the silanol groups of the silica support, and has been reported to have a very homogenous distribution of surface silanol groups.[5] High purity porous-silica microspheres are used for the support of this steric protected stationary phase. This results in low base adsorptivity, low peak asymmetry, and longer column life of the Zorbax Rx-C8 column.[11]

In the current reported work, a procedure was devised to quantify impurities in 1-(2-thienylethyl)-4-imidazoline-2-thione, a drug substance under study (see Fig. 1). Substituted 4-imidazoline-2-thiones and substituted imidazole-2-thiones have been known to exhibit inhibition of dopamine β-hydroxylase.[12–14] Dopamine β-hydroxylase catalyzes the conversion of dopamine to norepinephrine.[15] The possible interference with the biosynthesis of norepinephrine has been thought to be a means for treating cardiovascular disorders, such as congestive heart failure and hypertension.[12] Thus, 1-(2-thienylethyl)-4-imidazoline-2-thione may have potential use in this area of illness. As with all drug compounds, a high level of "purity" is desired. Impurity level measurement is necessary for quality monitoring of synthesized batch lots of medicinal drugs.

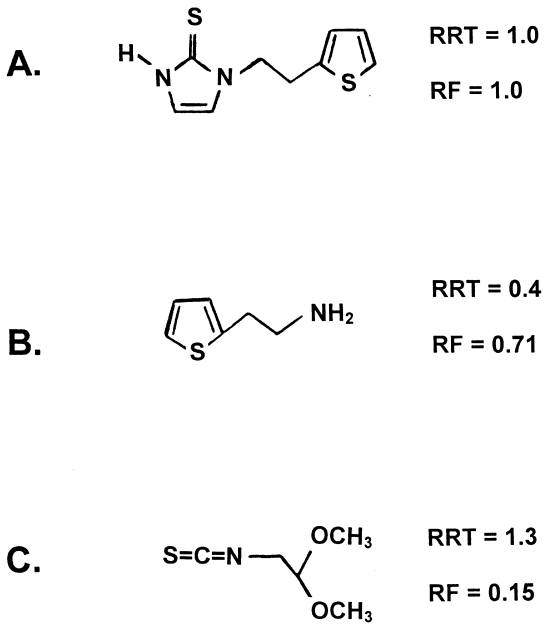

Figure 1. The structure of (A) 1-(2-thienylethyl)-4-imidazoline-2-thione, the drug substance and two of the suspected known impurities, (B) 2-(2-aminoethyl) thiophene, and (C) 1,1-dimethoxy-2-isothiocyanatoethane. The relative retention time (RRT) is of the parent drug peak and the detector response factors (RF) are also based on the drug's UV response [1-(2-thienylethyl)-4-imidazoline-2-thione = 1.0].

The compound of interest, 1-(2-thienylethyl)-4-imidazoline-2-thione, is basic in nature, as well as two of its suspected impurities from its synthesis; 2-(2-aminoethyl) thiophene and 1,1-dimethoxy-2-isothiocyanatoethane are shown in Fig. 1. Therefore, the Zorbax Rx-C8 column was ultimately used for the development of this HPLC test for impurities. A gradient mode of separation was chosen to reduce analysis time and to increase peak plate number and improve the general peak shape of the analytes. Stability of the compounds analyzed, required the choice of a near neutral pH mobile phase. The validation results for this analysis procedure, including accuracy and run-to-run reproducibility and repeatability will be discussed.

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

EXPERIMENTAL

Reagents

High purity HPLC water was provided by a Barnstead (Boston, MA) NANOpure system followed with an ultraviolet radiation treatment by a Barnstead ORGANICpure system. High performance liquid Chromatographic grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate heptahydrate were both ACS grade (Fisher Scientific, Fair Lawn, NJ). The 1-(2-thienylethyl)-4-imidazoline-2-thione, 2-(2-aminoethyl) thiophene, and 1,1-dimethoxy-2-isothiocyanatoethane were obtained "in-house."

Chromatographic Conditions and Apparatus

A Spectra-Physics (San Jose, CA) Model SP8800 liquid chromatograph equipped with a Rheodyne (Coatati, CA) Model 7010 injector valve and an Applied Biosystems (PE Biosystem, Norwalk, CT) Model 757 detector was used for all HPLC experiments. A Zorbax (MacMod Analytical, Inc., Chadds Ford, PA) Rx-C8 250 mm X 4.6 mm type column was used. Mobile phase A consisted of 15/85 acetonitrile/water (v/v) made 0.02 M in sodium phosphate buffer (2.2 g of sodium dihydrogen phosphate monohydrate and 1.1 g of disodium hydrogen phosphate heptahydrate were dissolved in each liter of mobile phase). Mobile phase B consisted of 50/50 acetonitrile/water (v/v) made 0.02 M in sodium phosphate buffer (apparent pH approximately 6.9). The gradient program was linear, starting at 100% A to 100% B during a 40 min period, and then a hold at 100% B for 20 min was used. A return ramp to mobile phase A, was followed by a 15 min equilibration time at 100% A before the next chromatographic run. The flow rate was 1.0 mL/min and detection was at 239 nm. Sample solution injection size was 20 μL. The sample solution concentration was 1.0 mg/mL prepared in mobile phase A. A reference solution at 1% concentration (a 1 to 100 dilution in mobile phase a or approximately 0.01 mg/mL) was made for each sample weight. The 1% reference solution was injected and chromatographed, then followed by the corresponding sample solution.

Spiked sample solutions containing 2-(2-aminoethyl) thiophene and 1,1-dimethoxy-2-isothiocyanatoethane were prepared at the 0.1, 0.2, 0.5, 1.0, and 1.5% (w/w) equivalent level. Response factors were determined by comparing the peak area of 0.01 mg/mL level solutions of each known component to

the same concentration of parent compound, 1-(2-thienylethyl)-4-imidazoline-2-thione.

Calculations

The peak areas for all impurity peaks and the peak area of the 1% reference peak for the drug are determined by integration. The known impurity % (w/w) were determined by the following equation:

$$\frac{(1/F) * A_i}{A_r + \Sigma A_i/100} = \%(\text{w/w})$$

for the known components in the sample, where

F = the response factor of the identified component [$F = 1.0$ for unknown components for % A/A. The response factor is 0.71 for 2-(2-aminoethyl) thiophene, and it is 0.15 for 1,1-dimethoxy-2-isothiocyanatoethane to calculate the known impurities as a % w/w].

A_i = the area of the impurity peak.

ΣA_i = the sum of the areas of all the impurity peaks in the chromatogram.

A_r = the area of the drug peak in the 1% reference chromatogram.

DISCUSSION

The separation of the two impurities of 1-(2-thienylethyl)-4-imidazoline-2-thione, as well as the low levels of unknown impurities, were easily accomplished by this procedure, as is shown in the chromatogram displayed in Figs. 2 and 3. Early chromatographic development work was done using a Spherisorb ODS-2 column; however, this column gave extreme tailing and poor response of the 2-(2-aminoethyl) thiophene, even with the use of an amine modifier in the mobile phase. The Zorbax Rx-C8 column had very little tailing and separated the known impurities from the parent compound. Peak shape of the 2-(2-aminoethyl) thiophene impurity was good using the Zorbax Rx-C8 column (see Fig. 3). A detection wavelength of 239 nm was chosen for this HPLC test method for several reasons. The λ_{max} for 1-(2-thienylethyl)-4-imidazoline-2-thione in mobile phase A was 239 nm and was close to the maxima for the two known impurities. Using a diode array UV detector and solutions prepared in mobile phase A, the maxima for the known impurities were 233 nm for 2-(2-aminoethyl) thiophene and 243 nm for 1,1-dimethoxy-2-isothiocyanatoethane. The ultraviolet spectrum of 1,1-dimethoxy-2-isothiocyanatoethane showed little absorbance at 210 nm, so lower wavelengths for detection was not reasonable for this impurity test. Also, lower wavelength

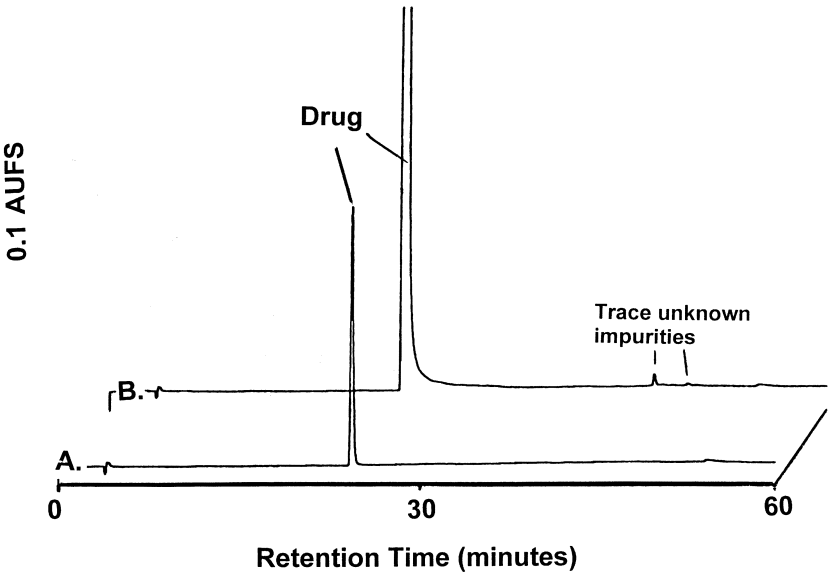

Figure 2. A chromatogram (A) of 1% reference solution and a chromatogram (B) of a sample batch lot B. The concentration of the drug in the sample solution is approximately 1.0 mg/mL, the reference solution is approximately 0.01 mg/mL. The trace unknown impurities are each less than 0.05% (A/A).

detection did not give a level chromatographic baseline during the gradient program. With all of these considerations, 239 nm was the best choice for a fixed detection wavelength for all compounds. Response factors are used for an accurate determination of the two known impurities.

The accuracy of this method was verified by chromatographing solutions of known levels of 2-(2-aminoethyl) thiophene and 1,1-dimethoxy-2-isothiocyanatoethane. Spike levels of 0.1, 0.2, 0.5, 1.0, and 1.5% (w/w) in the parent solution were run. Figure 3 displays a chromatogram of a sample solution spiked at 0.5% (w/w). The HPLC method was found to be accurate, as the data in Table 1 clearly shows. No measured level (column 4 of Table 1) of either of the two impurities was off more than 0.03% (w/w) from any actual weighed spike level (column 3 of Table 1), therefore, recovery was excellent for this HPLC procedure. The repeatability of the method was demonstrated by analyzing two batch lots of synthesized 1-(2-thienylethyl)-4-imidazoline-2-thione five times on three separate trial days using two different Zorbax Rx-C8 columns. Sample batch lot A had the largest amount

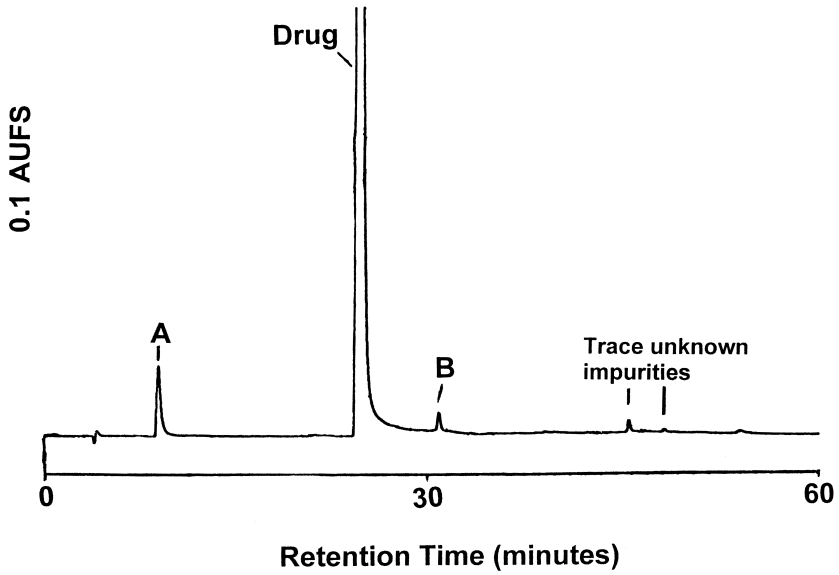

Figure 3. A spiked sample solution containing 0.5% (w/w) levels of the two known impurities; peak A is 2-(2-aminoethyl) thiophene, and peak B is 1,1-dimethoxy-2-isothiocyanatoethane. Sample batch lot B was used for the spiked recovery study owing to its low level of other impurities. The trace unknown impurities are each less than 0.05% (A/A).

of unknown impurities; this sample averaged 1.27% total impurities for the three day test. Four of the major impurity components detected in batch lot A were repeatable and the results are presented in the far right column of Table 2. Sample batch lot B was very "pure", and it measured less than 0.1% total impurities over the three day test period. Neither sample exhibited detectable quantities of either of the two known suspected impurities. The levels of individual unknown impurities were very low; identification of these impurities was beyond the scope of this study and represents possible future work. Sample batch lot B was utilized for the previously described spiking experiments, since it had such a low level of unknown impurities and would not interfere with the spiked recovery experiments. The analysis data from the two lots of drugs are shown in Table 2, and the data clearly show reasonable repeatability of the test method.

Finally, the stability of the sample solution was verified by holding a solution at room temperature for 24 hours and re-testing the solution. No

Table 1. Known impurity recovery study using spiked solutions.

Level of spike (% w/w)	Impurity	Actual weighed spike level (% w/w)	Measured level (% w/w)
0.0	2-(2-Aminoethyl) thiophene	0	0
	1,1-Dimethoxy-2-isothiocyanatoethane	0	0
0.1	2-(2-Aminoethyl) thiophene	0.10	0.09
	1,1-Dimethoxy-2-isothiocyanatoethane	0.10	0.08
0.2	2-(2-Aminoethyl) thiophene	0.20	0.19
	1,1-Dimethoxy-2-isothiocyanatoethane	0.20	0.23
0.5	2-(2-Aminoethyl) thiophene	0.50	0.51
	1,1-Dimethoxy-2-isothiocyanatoethane	0.49	0.49
1.0	2-(2-Aminoethyl) thiophene	1.00	1.05
	1,1-Dimethoxy-2-isothiocyanatoethane	0.97	0.95
1.5	2-(2-Aminoethyl) thiophene	1.47	1.52
	1,1-Dimethoxy-2-isothiocyanatoethane	1.42	1.40

appreciable increase of change in impurity peaks was noticed; thus, the sample solution appears to be reasonably stable upon short term standing.

CONCLUSIONS

An HPLC procedure to measure known impurities in 1-(2-thienylethyl)-4-imidazoline-2-thione bulk compound was developed and found to be both accurate and reproducible. A Zorbax Rx-C8 column with dimensions of 250×4.5 mm was used in a gradient system of acetonitrie/water mobile phase and 0.02 M phosphate buffer at apparent pH 6.9. The Zorbax RX-C8 easily separated the known impurities from the parent compound with little peak tailing or evidence of absorption by the basic compounds analyzed. Recovery of spiked sample solutions from 0.1 to 1.5% (w/w) of the known impurities gave accurate results. Two different Zorbax Rx-C8 columns gave reproducible results for impurity levels of two sample batch lots of the studied

Table 2. Repeatability study of method.

Sample number	Trial day	Column	Total impurities (Area %)	Four major components detected area % (RRT)[a]
		Sample batch lot A		
1	1	A	1.29	0.15 (1.2), 0.09 (1.9), 0.19 (2.0), 0.19 (2.3)
2	1	A	1.31	0.15 (1.2), 0.09 (1.9), 0.19 (2.0), 0.19 (2.3)
3	2	B	1.30	0.13 (1.3), 0.11 (1.9), 0.18 (2.0), 0.19 (2.3)
4	3	B	1.21	0.13 (1.2), 0.08 (1.9), 0.21 (2.0), 0.23 (2.3)
5	3	B	1.22	0.14 (1.2), 0.08 (1.9), 0.20 (2.0), 0.23 (2.3)
		Mean $(n=5)$	1.27	
		Sample batch lot B		
1	1	A	0.08	na
2	1	A	0.09	na
3	2	B	0.05	na
4	3	B	0.05	na
5	3	B	0.05	na
		Mean $(n=5)$	0.06	

[a]RRT is the relative retention time with the parent drug peak, 1-(2-thienylethyl)-4-imidazoline-2-thione.

Note: na, not applicable to this sample lot. Only very low level impurities were detected.

compound. One of the sample batch lots was found to have an average total impurity level of 1.3% (A/A) ($n=5$), and the other batch had a measured total impurity level of less than 0.1% (A/A) ($n=5$) using the developed HPLC procedure.

REFERENCES

1. Kohler, J.; Kirkland, J.J. Improved silica-based column packings for high-performance liquid chromatography. J. Chromatogr. **1987**, *385*, 125–150.

2. Bayer, E.; Paulus, A. Silanophilic interactions in reversed-phase high-performance liquid chromatography. J. Chromatogr. **1987**, *400*, 1–4.
3. Stadalius, M.A.; Berus, J.S.; Snyder, L.R. Reversed-phase HPLC of basic samples. LC-GC **1988**, *6* (6), 495–501.
4. Hill, D.W.; Kind, A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography. J. Liq. Chromatogr. **1993**, *16* (18), 3941–3964.
5. Hill, D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC. J. Liq. Chromatogr. **1990**, *13* (16), 3147–3175.
6. Vervoort, R.J.M.; Derksen, N.W.J.; Maris, F.A. Selection of stationary phases for the liquid chromatographic analysis of basic compounds using chemometric methods. J. Chromatogr. A **1994**, *678* (1), 1–15.
7. Bauer, J.F.; Krough S.K.; Chang, Z.L.; Wong, C.F. Determination of minor impurities in terazosin hydrochloride by high-performance liquid chromatography. J. Chromatogr. **1993**, *648* (1), 175–181.
8. Golich, T.G.; Thomsen, J.R.; Green, D.L.; Jersild, J. Determination of minor impurities of (*R*)-1acetoxymethyl-3-(7-[1-N,N-dimthylcarbanoxyl]-6-(4-fluorophenyl)-indol-3-oyl)-1H, 3H-pyrolo[1,2-c]thiozol-3-yl)pyridinium chloride by high performance liquid chromatography. J. Liq. Chromatogr. **1995**, *18* (20), 4107–4116.
9. Maniara, W.M.; Cipriano, A.; Powell, M.L. Quantitative analytical methods for the determination of a new hypertension drug, CGS 25462, and its metabolites (CGS 25659 and CGS 24592) in human plasma by high-performance liquid chromatography. J. Chromatogr. B **1998**, *706* (2), 287–294.
10. Lin, C.C.; Matuszewski, B.K.; Zagrobelny, J.; Dobrinska, M.R. Picogram determination of an avermectin analog in dog plasma by high-performance liquid chromatography with fluorescence detection. J. Liq. Chromatogr. & Rel. Technol. **1997**, *20* (3), 443–458.
11. *"Agilent Zorbax Rx-C8 data sheet"*, Part no. 880968–001 D; Agilent Technologies: Palo Alto, California, 2002; 1–2.
12. Kruse, L.I.; Kaiser, C.; DeWolf, W.E.; Frazee, J.S.; Garvey, E.; Hilbert, E.; Faulkner, W.A.; Flaim, K.E.; Sawyer, J.L.; Berkowitz, B.A. Multisubstrate inhibitors of dopamine beta-hydroxylase. Some 1-phenyl and 1-phenyl-bridged derivatives of imidazole-2-thione. J. Med. Chem. **1986**, *29*, 2465–2472.
13. Kruse, L.I.; Kaiser, C.; DeWolf, W.E.; Frazee, J.S.; Erickson, R.W.; Ezekiel, M.; Ohlstein, E.H.; Ruffolo, R.R.; Berkowitz, B.A. Substituted 1-benzylimidazole-2-thiols as potent and orally active inhibitors of dopamine beta-hydroxylase. J. Med. Chem. **1986**, *29*, 887–889.

14. Kruse, L.I.; Dewolf, W.E.; Chambers, P.A.; Goodhart, P.J. Design and kinetic characterization of multisubstrate inhibitors of dopamine beta-hydroxylase. Biochemistry **1986**, *25* (23), 7271–7278.
15. Levin, E.Y.; Levenberg, B.; Kaufman, S. J. The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. Biol. Chem. **1960**, *235*, 2080–2086.
16. Kaufman, S.; Friedman, S. Pharmacol. Rev. **1965**, *17*, 71–75.

Received September 12, 2002
Accepted October 9, 2002
Manuscript 5959