Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, Nos 3/4, pp. 555–559, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00

Short Communication

Amperometric determination of hydroxyamphetamine hydrobromide in a flowing stream at the glassy-carbon electrode

MUMTAZ H. SHAH and JAMES T. STEWART*

Department of Medicinal Chemistry, College of Pharmacy, The University of Georgia, Athens, GA 30602, USA

Keywords: *Hydroxyamphetamine hydrobromide; amperometry; electrochemistry; flow-injection analysis; oxidation; dosage form analysis; glassy-carbon electrode.*

Introduction

Hydroxyamphetamine hydrobromide is currently used as a mydriatic. It has been analysed by diverse methods which include titrimetry [1, 2], colorimetry [3, 4], fluorimetry [5], and thin-layer, gas and high-performance liquid chromatography (HPLC) [6–9]. The USP assay for the drug is a non-aqueous titration with perchloric acid [2]. The USP determination of the drug in a formulated solution involves derivatization with acetic anhydride followed by gravimetric analysis of the O,N-diacetyl derivative extracted with chloroform [10, 11].

Interest in this laboratory in the development of new assay methods for drugs in flowing streams led to an investigation of the oxidation of hydroxyamphetamine hydrobromide at the glassy-carbon electrode. There appear to be no published data on the electrochemical oxidation or reduction of the drug.

The amperometric determination of hydroxyamphetamine hydrobromide in a flowing stream utilizing oxidation at the glass-carbon electrode is reported. The flow-injection method enables the drug to be detected in the range 0.1–3.2 μg/ml with good accuracy and precision. The procedure was shown to be applicable to the analysis of hydroxyamphetamine in tablets and in a formulated solution.

Experimental

Apparatus

Cyclic voltammetry measurements were made with a cyclic voltammeter (Bioanaly-

* To whom correspondence should be addressed.

tical Systems Model CV-1B, West Lafayette, IN, USA). The three-electrode system consisted of a glassy-carbon electrode of electrode area 5 mm^2, an auxiliary platinum electrode, and a silver–silver chloride reference electrode. The voltammograms were recorded on an X–Y recorder (Houston Instruments Model HR-100, Austin, TX, USA).

An electrochemical cell (Bioanalytical Systems Model TL-5A Kel F, West Lafayette, IN, USA) and the cyclic voltammeter were used for the flowing stream analysis. The cell contained a glassy-carbon working electrode, a platinum auxiliary electrode, and a silver–silver chloride reference electrode. The mobile phase was pumped through the cell at a fixed flow rate using a pump (Waters Associates Model M-60000A, Milford, MA, USA). Samples were manually loaded into an injector (Waters Associated Model U-6K) with a 50-μl syringe (Hamilton, Reno, NV, USA). The pump, injector and electrochemical cell were connected by standard HPLC stainless steel tubing (0.009 in.) and fittings. The cell potential was set on the cyclic voltammeter using a digital voltmeter and the amperometric recordings were made at ambient temperature using a strip-chart recorder set at 1 V.

Chemicals and drug solutions

Hydroxyamphetamine hydrobromide powder (Smith Kline–Beckman Corporation, Philadelphia, PA, USA) was used in this analytical study without further purification. All other chemicals were commercially available and were utilized as received. A stock solution was prepared containing 0.1 mg/ml hydroxyamphetamine hydrobromide in acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v). Further dilutions of the solution were made to provide working standards in the range 0.1–3.2 μg/ml.

Procedure

A mixture of acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v) was pumped through the electrochemical cell at a flow rate of 1.5 ml/min. Aliquots (50 μl) of the working standards of hydroxyamphetamine hydrobromide (0.1–3.2 μg/ml) in the mobile phase were injected into the flowing stream and the current flow was measured with the cell potential set at +1600 mV. Linear regression analysis of current against concentration of each working standard gave data for the slope and intercept, which were then used to calculate the concentration of drug in an unknown sample. Calculations were performed on a programmable calculator.

The following studies were performed to determine if other drugs and preservatives that might be present in dosage forms of hydroxyamphetamine hydrobromide interfered with the assay either by altering the current flow of the drug or by being oxidized at the glassy-carbon electrode. Individual solutions (0.1 mg/ml) of phenylephrine hydrochloride, hydrocortisone and boric acid were prepared in acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v). Aliquots of these solutions were then used to prepare various mixtures containing each compound in the 0.1–1.0 μg/ml range with the concentration of hydroxyamphetamine maintained at 0.4 μg/ml. Each mixture was then injected (50 μl) into the flowing stream system and the current was measured at +1600 mV. The data obtained from each mixture were then compared to those of a pure solution of hydroxyamphetamine hydrobromide (0.4 μg/ml) to calculate the degree of any interference at the various concentrations of the added compounds.

Analysis of dosage forms

Tablets containing hydroxyamphetamine hydrobromide were sonicated in aceto-

nitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v) for 15 min. The resulting solution was filtered and diluted so that the drug concentration was 0.1–3.2 μg.

A solution formulated to represent a simulated dosage form was prepared containing 1% m/v-hydroxyamphetamine hydrobromide and 2% m/v-boric acid in distilled water. A dilution of the solution to the range 0.1–3.2 μg/ml was made with acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v).

Aliquots (50 μl) of the diluted solutions from each dosage form were then assayed for drug content by the amperometric procedure described.

Results and Discussion

Preliminary studies on the electrochemical oxidation of hydroxyamphetamine hydrobromide at the glassy-carbon electrode indicated that no anodic or cathodic responses were obtained for a 10^{-3}M solution of the drug in solvents such as: 0.2 M acetic acid and 0.2 M sodium acetate buffer (pH 4.2)–absolute methanol (60:40 v/v); 1 M acetic acid; 0.1 M perchloric acid; 0.066 M potassium dihydrogen phosphate–0.066 M disodium monohydrogen phosphate buffers at pH 5.3 and pH 7.2; and 0.04 M acetic acid–0.04 M phosphoric acid–0.04 M boric acid buffer adjusted with sodium hydroxide to give pH 5.3 or pH 7.5. A satisfactory electrochemical response was obtained, however, in aqueous acetonitrile mixtures containing lithium perchlorate as a supporting electrolyte. These data indicated that hydroxyamphetamine is difficult to oxidize in an aqueous medium and that oxidation is best achieved in a predominantly organic solution in the presence of a soluble supporting electrolyte such as lithium perchlorate.

Figure 1 shows a cyclic voltammogram of hydroxyamphetamine hydrobromide at the glassy-carbon electrode in acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v). The voltammogram indicated that the electrode process is completely irreversible, since no cathodic wave similar to the anodic wave was observed in the reversed-scan mode. In this solvent a major anodic peak at +1600 mV and a minor peak at +1050 mV were observed. Since the sensitivity at +1600 mV was approximately twice that obtained at +1050 mV, the amperometric determination was performed at +1600 mV.

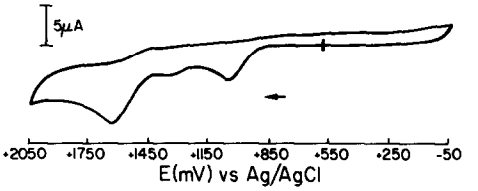

Figure 1
A cyclic voltammogram of hydroxyamphetamine hydrobromide (1 mg/ml) in acetonitrile–aqueous 0.1 M lithium perchlorate (90:10 v/v). Scan rate, 5 mV/s; area of the glassy-carbon working electrode, 5 mm^2. The current (in μA) is plotted as a function of applied e.m.f. (in mV) measured with respect to the Ag–AgCl electrode.

Using the optimum electrode potential of +1600 mV, a calibration curve for hydroxyamphetamine was obtained in the range 0.1–3.2 μg/ml (corresponding to 5–160 ng of drug). Linear regression analysis of drug concentration against current (nA) data from replicate graphs gave the regression equation (with standard error): $y = 866.1$ (± 47.2) $x + 82.57$ (± 5.03) ($n = 12$); the correlation coefficient (r) was 0.9994 ± 0.0003 ($n = 12$).

The cell current for drug concentrations greater than 3.2 μg/ml was found to deviate from linearity. The sensitivity of the assay based on a signal-to-noise ratio of 2 was 5.0 ng of drug.

To estimate the reproducibility of the electrode response in the amperometric method, triplicate injections of hydroxyamphetamine at concentrations of 0.4, 0.8, 1.2 and 2.4 μg/ml were made. Mean peak currents of 400.0 ± 1.6, 802.6 ± 1.8, 1199.7 ± 1.7 and 2401.7 ± 2.9 nA, respectively, were observed. The precision of these measurements was represented by relative standard deviations (RSD) of 0.40, 0.22, 0.14 and 0.12% at each concentration from 0.4 to 2.4 μg/ml, respectively. Between-day variation in response at the 0.8-μg/ml level gave RSD = 4.3% (n = 6).

The accuracy and precision of the procedure were assessed using recovery data from the standard solutions (0.4, 0.8, 1.2 and 2.4 μg/ml) of hydroxyamphetamine treated as unknowns, by reference to the standard curve. Recoveries of the drug were 100.6 ± 0.74%, 102.2 ± 0.51%, 99.8 ± 0.32% and 100.1 ± 0.11% (n = 3), respectively.

The method was then applied to the assay of hydroxyamphetamine hydrobromide in commercial tablets (20 mg, Smith Kline–Beckman Corporation, Philadelphia, PA, USA) and in an aqueous solution formulated to represent a simulated dosage form containing 1% drug and 2% boric acid. The concentration of drug was calculated by reference to the standard curve for hydroxyamphetamine. The recovery of drug from the tablets was 101.1 ± 2.21% (n = 4) and from the solution 100.7 ± 1.47% (n = 3) of the labelled amount of drug. No interference was noted from tablet excipients such as lactose or starch.

Other studies were performed to establish the specificity of the method for hydroxyamphetamine hydrobromide in the presence of drugs and preservatives that might be found in compound dosage forms. As shown in Table 1, only phenylephrine hydrochloride interfered appreciably with the assay at the various concentrations investigated. Interference from the drug was essentially at the same levels even when the assay was performed at the minor anodic peak (+1050 mV) for hydroxyamphetamine. Thus, if phenylephrine is present, separation of the drugs before the detection step is necessary.

Table 1
Percentage recovery of hydroxyamphetamine hydrobromide in synthetic mixtures

	Recovery* (%)		
	Concentration (μg/ml)†		
Other component of mixture	0.1	0.4	1.0
Boric acid	100.5 ± 0.53	100.9 ± 0.55	100.5 ± 0.40
Phenylephrine hydrochloride	131.8 ± 3.72	204.0 ± 4.49	337.8 ± 9.35
Hydrocortisone	99.9 ± 0.42	100.1 ± 0.06	100.1 ± 0.17

* Mean percentage recovery (±S.D.) of hydroxyamphetamine hydrobromide in the mixture. The data were based on triplicate determinations of each mixture.

† Concentration of each component in mixture that also contained 0.4 μg/ml hydroxyamphetamine hydrobromide.

References

[1] E. Varga and G. Vastagh, *Pharm. Zentralhalle* **96**, 149–152 (1957); through *Chem. Abstr.* **52**, 14965d (1958).

[2] *The United States Pharmacopeia*, 20th edn, p. 384. The United States Pharmacopeia Convention, Inc., Rockville, MD, USA (1980).

[3] J. Axelrod, *J. Pharmac. Exptl. Ther.* **110**, 315–326 (1954).

[4] M. Horioka, *Yakugaku Zasshi* **77**, 200–206 (1975); through *Chem. Abstr.* **51**, 8367c (1957).
[5] C. R. Creveling, K. Kondo and J. W. Daly, *Clin. Chem. (Winston-Salem, N.C.)* **14**, 302–309 (1968).
[6] W. W. Fike, *Anal. Chem.* **38**, 1697–1702 (1966).
[7] I. Sunshine, W. Fike and H. Landesman, *J. Forensic Sci.* **11**, 428–439 (1966).
[8] M. Donike, *J. Chromatogr.* **103**, 91–112 (1975).
[9] P. J. Cashman, J. I. Thornton and D. L. Shelman, *J. Chromatogr. Sci.* **11**, 7–9 (1973).
[10] *The United States Pharmacopeia*, 20th edn, p. 385. The United States Pharmacopeia Convention, Inc., Rockville, MD, USA (1980).
[11] L. H. Welsh and A. F. Summa, *J. Pharm. Sci.* **52**, 656–658 (1963).

[*First received for review 25 July 1983; revised manuscript received 13 January 1984*]