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Amperometric determination of guanethidine sulfate in a flowing stream at the glassy carbon electrode

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Summary

A flow-injection method for the determination of guanethidine sulfate based on electrochemical oxidation at the glassy carbon electrode is presented. The amperometric method may be used to determine guanethidine sulfate in the presence of other drugs commonly found in its pharmaceutical dosage forms or administered concurrently in therapeutic situations. Using an electrode potential of +1200 mV, a calibration curve is linear in the 0.5–16 μg/ml concentration range with minimum detectability at 5 ng (S/N = 2). The method applied to the analysis of guanethidine sulfate in selected pharmaceutical dosage forms shows good accuracy and precision. Although automation was not used in this study, the method could readily be incorporated in automated systems because it employs the technique of continuous analysis in a flowing stream.

Introduction

Guanethidine sulfate is an effective antihypertensive agent that does not show central nervous system effects, such as depression, because the drug is highly polar and does not pass the blood–brain barrier easily. It has been analyzed by diverse methodology, among which are non-aqueous titrimetry (British Pharmacopoeia, 1982), colorimetry, (Tompsett et al., 1961; Tompsett, 1962; Bose and Bigayvargiva, 1964; United States Pharmacopoeia, 1980), fluorometry (Corder et al., 1973; Rahn

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and Dayton, 1969; Conn and Davis, 1959), radioimmunoassay (Loeffler and Pittman, 1979), and gas and high-performance liquid chromatography (Hengstmann et al., 1974; Pellizzari and Seltzman, 1979; Honigberg et al., 1975). The colorimetric procedures employ reagents such as sodium molybdophosphotungstate, a sodium nitroprusside–potassium ferricyanide mixture, ammonium reineckate, and Dragendorff's to form colored products with the drug. The various colors formed are used both qualitatively and quantitatively to assay for guanethidine in dosage forms, serum and urine samples with sensitivity in the microgram to milligram range. Fluorophores formed between the drug and either ninhydrin, eosin-Y, or 6 N hydrochloric acid have been used as the basis for the fluorescence procedures. The methods are used to determine drug levels in biological samples at nanogram to microgram levels. The radioimmunoassay method is sensitive to nanogram levels of guanethidine in biological samples with little or no cross-reactivity with two of its major metabolites. A disadvantage of the procedure is that the drug-specific antibody is not commercially available. The gas chromatographic procedures utilize either flame ionization or electron-capture detection with and without derivatization to detect guanethidine in the picogram to microgram range. The liquid chromatographic procedure allows the separation of various diuretic–antihypertensive mixtures containing guanethidine on an octadecylsilane column, but the method was not applied to dosage form or biological sample analysis.

Interest in this laboratory in the development of new continuous assay methods for drugs in flowing streams led us to investigate the oxidation of guanethidine at the glassy carbon electrode. There appears to be no data on the electrochemical oxidation or reduction of the drug in the literature. This laboratory has reported previously on continuous analysis in flowing streams by oxidation of drugs such as ascorbic acid (Mason et al., 1972) and methyldopa (Stewart et al., 1974) at the tubular carbon electrode and more recently on hydralazine hydrochloride (Shah and Stewart, 1983a) and isoniazid (Shah and Stewart, 1983b) at the glassy carbon electrode. The glassy carbon electrode has supplanted the tubular carbon electrode and has shown general usefulness as a sensitive tool for the determination of oxidizable drugs in flowing streams systems such as HPLC (Shoup, 1981; Koch and Kissinger, 1979; Pachla and Kissinger, 1976). These kinds of electrodes can be easily incorporated in automatic or semiautomatic systems such as would be used in dosage form analysis.

In this paper, amperometric determination of guanethidine sulfate in a flowing stream utilizing oxidation at the glassy carbon electrode is reported. The flow-injection method detects the drug in the 0.5–16 μg/ml range with good accuracy and precision. The procedure was shown applicable to the analysis of guanethidine sulfate in pharmaceutical dosage forms.

Materials and Methods

Apparatus

Cyclic voltammetry measurements were made with a Bioanalytical Systems Model

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

A Bioanalytical Systems Model TL-5A Kel F electrochemical cell and the Model CV-1B 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. Mobile phase was pumped through the cell at a fixed flow rate using a Waters Associates Model M-6000A pump (Milford, MA, U.S.A.). Samples were manually injected with a microsyringe into a Rheodyne Model 7125 Injector equipped with a 20 μl fixed loop (Cotati, CA, U.S.A.). The pump, injector, and electrochemical cell were connected via standard HPLC stainless steel tubing (0.0$^{\prime}$ $_{\prime}$ in.) and fittings. 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. An amperometric control module such as a Bioanalytical Systems Model LC-4B can also be used to set the cell potential.

Chemicals

Guanethidine sulfate and hydrochlorothiazide powders were obtained from Ciba-Geigy, Summit, NJ, U.S.A. Other drugs used in the analytical study were obtained as powders either from the innovator drug company or commercial chemical sources. All other chemicals and solvents used were obtained commercially and were utilized as received.

Preparation of guanethidine stock solution

A stock solution of guanethidine sulfate was prepared by dissolving a weighed quantity of the drug powder in 15 : 85 acetonitrile–aqueous 0.05 M monobasic sodium phosphate pH 4.5 such that the final concentration was 0.1 mg/ml. Further dilutions were made to provide guanethidine sulfate working standards in the 0.5–16 μg/ml range.

Assay procedure

A 15 : 85 mixture of acetonitrile–aqueous 0.05 M monobasic sodium phosphate is pumped through the electrochemical cell at a flow rate of 1 ml/min. Aliquots (20 μl) of guanethidine sulfate working standards (0.5–16 μg/ml) and drug samples to be analyzed are injected into the flowing stream and the current flow for each solution measured using the cell potential set at +1200 mV. Linear regression analysis of current vs concentration of working standards gave slope and intercept data, which was then used to calculate guanethidine sulfate concentrations in the 'unknown' samples.

Interference studies

The following study was performed to determine if other drugs commonly found

in dosage forms with guanethidine sulfate or administered concurrently in clinical situations interfere with the assay by altering the current flow of the drug or are oxidized at the glassy carbon electrode. Individual solutions (0.1 mg/ml) of hydrochlorothiazide, methylphenidate, prazocin, ephedrine sulfate, clonidine, chlorpromazine hydrochloride, propranolol hydrochloride, imipramine hydrochloride, α-methyldopa, and hydralazine hydrochloride were prepared in 15 : 85 acetonitrile–aqueous 0.05 M phosphate pH 4.5. Accurately pipetted aliquots of these solutions were then used to prepare various mixtures containing the individual drugs at the 2–16 μg/ml range with guanethidine concentration maintained at 4 μg/ml. Each mixture was then injected (20 μl) into the flowing stream system and the current measured at +1200 mV. The current data obtained from each mixture was then compared to that of a pure solution of guanethidine sulfate to calculate the degree of interference, if any, at the various concentration levels of the added drugs.

Analysis of solid dosage forms

Tablets containing guanethidine sulfate were powdered in a mortar and pestle and the powder quantitatively transferred to a 100 ml volumetric flask with the aid of the 15 : 85 acetonitrile–aqueous phosphate solution. The resulting solution was then diluted to the 0.5–16 μg/ml calibration range with the same solvent and a 20 μl aliquot assayed for guanethidine sulfate according to the Assay Procedure above.

In the case of tablets containing both guanethidine sulfate and hydrochlorothiazide, the tablets were powdered as described above and the powder placed in a 25 ml beaker where 10 ml of distilled water was added followed by sonication for 10 min at room temperature. The turbid solution was filtered into a 250 ml separatory funnel, extracted with 3–30 ml portions of ethyl acetate to remove any remaining hydrochlorothiazide, and the extracts discarded. The remaining aqueous solution is then diluted to the 0.5–16 μg/ml calibration range with 15 : 85 acetonitrile–aqueous phosphate and a 20 μl aliquot assayed for guanethidine content according to the Assay Procedure above.

Results and Discussion

Preliminary studies on the electrochemical oxidation of guanethidine sulfate at the glassy carbon electrode indicated that no response was obtained in such solvents as 60 : 40 Walpole acetate buffer pH 4.2–absolute methanol, 1 M acetic acid, 0.1 M perchloric acid, Michaelis phosphate buffers at pHs 5.3 and 7.2, and Britton-Robinson buffers at pHs 5.3 and 7.5. A satisfactory electrochemical response could be obtained, however, in aqueous acetonitrile mixtures containing monobasic sodium phosphate as a supporting electrolyte.

Fig. 1 shows a hydrodynamic voltammogram of guanethidine sulfate (100 μg/ml) when subjected to electrochemical oxidation at the glassy carbon electrode in 15 : 85 acetonitrile–aqueous 0.05 M monobasic sodium phosphate pH 4.5. A half-wave potential of +1000 mV was observed. A potential of +1200 mV was selected for the amperometric determination since it represented the point on the wave where

maximum drug sensitivity could be obtained. A cyclic voltammogram of guanethidine sulfate in the same solvent mix had previously indicated that the electrode process is completely irreversible since no cathodic wave similar to the anodic wave is observed in the reverse scan mode.

Using the optimum electrode potential of +1[illegible] mV, a guanethidine sulfate calibration curve was obtained in the 0.5–16 μg/ml range. Linear regression analysis of the calibration data gave typical slope, y-intercept, and correlation coefficient (r) values of 58.75, 14.32 nA, and 0.9989 (n = 20), respectively. Cell currents from drug concentrations greater than 16 μg/ml were found to deviate from the linear regression line.

To estimate the precision of the electrode response in the amperometric method, quadruplicate injections of guanethidine concentrations at 2.0, 6.0 and 12.0 μg/ml were run. Mean peak currents of 135.8 ± 1.3, 364.3 ± 1.3 and 717.5 ± 2.5 nA, respectively, were obtained. The precision of these measurements is expressed by relative standard deviations of 0.95, 0.36 and 0.35% for the 2.0, 6.0, and 12.0 μg/ml levels, respectively.

Accuracy of the procedure was assessed by assaying 2.0, 6.0 and 12.0 μg/ml guanethidine samples as 'unknowns' and calculating their concentrations using the slope and intercept data from a standard curve run concurrently. It was determined from the assay that 101.8 ± 0.4, 99.3 ± 0.4 and $99.7 \pm 0.4\%$ (n = 4) of the known amounts of drug in the 'unknown' solutions were obtained for the respective concentrations. These data indicate that the amperometric method will determine guanethidine sulfate with good accuracy.

Interference studies were performed to establish the specificity of the method for guanethidine sulfate in the presence of other drugs that are found in its combination dosage forms or are administered concurrently in therapeutic situations. The latter data was gathered with regards to using electrochemical detection of guanethidine in conjunction with a HPLC separation for assay of guanethidine levels in biological samples where other drugs might be present. As shown in Table 1, methylphenidate, prazocin, ephedrine sulfate and clonidine showed no interference with the assay method at the concentration levels studied. Hydrochlorothiazide gave no interference at the 2 μg/ml level, but showed significant interference at higher μg/ml

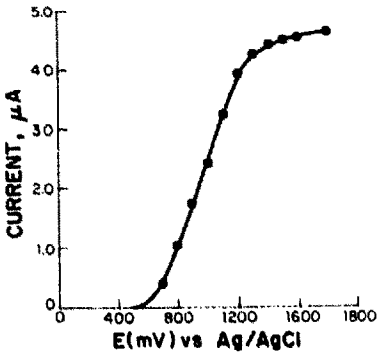

Fig. 1. Hydrodynamic voltammogram of guanethidine sulfate (100 μg/ml) in a 15:85 mixture of acetonitrile–aqueous 0.05 M monobasic sodium phosphate pH 4.5 at a flow rate of 1 ml/min.

TABLE 1
PERCENT RECOVERY OF GUANETHIDINE SULFATE IN SYNTHETIC DRUG MIXTURES

Component + guanethidine	Concentration (μg/ml) [a]		
	2	10	16
Hydrochlorothiazide	100.20 ± 0.30 [b]	137.00 ± 0.94	180.75 ± 0.61
Methylphenidate	100.17 ± 0.35	99.96 ± 0.30	100.03 ± 0.15
Prazocin	100.16 ± 0.38	100.20 ± 0.30	100.17 ± 0.24
Ephedrine sulfate	99.98 ± 0.15	100.26 ± 0.31	100.66 ± 0.42
Clonidine	100.16 ± 0.17	100.20 ± 0.30	100.66 ± 0.62
Propranolol-HCl	100.26 ± 0.31	160.56 ± 0.44	230.78 ± 0.29
Methyldopa	105.15 ± 0.32	340.53 ± 0.53	490.81 ± 0.69
Hydralazine-HCl	120.25 ± 0.50	160.35 ± 0.78	330.62 ± 0.32
Chlorpromazine-HCl	132.10 ± 1.16	220.18 ± 0.81	299.85 ± 0.98
Imipramine-HCl	140.91 ± 0.59	340.65 ± 0.26	410.50 ± 0.37

[a] Concentration of each drug in mixtures that also contained 4 μg/ml of guanethidine sulfate.
[b] Mean percent recovery ± standard deviation of guanethidine sulfate in the drug mixture. The data was based on quadruplicate determinations of each mixture.

levels. Since hydrochlorothiazide is usually present in a 2.5 : 1 concentration ratio with guanethidine in commercially available dosage forms, this study demonstrated that it should be removed from the guanethidine analytical sample prior to assay. As expected, the electroactive drugs, methyldopa, hydralazine, chlorpromazine and imipramine, all provided appreciable interference at the concentration levels studied. This would indicate that some separation technique such as HPLC prior to the detection step for guanethidine would be needed especially for guanethidine mixtures containing these latter named drugs.

Application of the method to the assay of guanethidine sulfate in commercial tablets was then studied. After sample preparation and dilution to the 0.5–16 μg/ml calibration range, the dosage form solutions were analyzed for guanethidine and the concentration of drug in each sample calculated using slope and y-intercept values generated from linear regression analysis of the guanethidine calibration data run

TABLE 2
ANALYSIS OF GUANETHIDINE SULFATE IN DOSAGE FORMS

Product component(s)	Amount of guanethidine declared per sample (mg)	Amount of guanethidine found per sample (mg) [a]	Percent recovery [a]
Guanethidine sulfate [b]	10	10.07 ± 0.13	100.74 ± 1.30
Guanethidine sulfate [c] hydrochlorothiazide	10	10.08 ± 0.08	100.75 ± 0.75

[a] Mean ± standard deviation based on quadruplicate determinations of each sample.
[b] Ismelin tablets, Ciba-Geigy, Summit, NJ U.S.A.
[c] Esimil tablets, Ciba-Geigy, Summit, NJ U.S.A. The labeled amount of hydrochlorothiazide was 25 mg.

concurrently. The results of the tablet assays shown in Table 2 indicate that guanethidine content can be conveniently determined using the amperometric method described herein with good accuracy and precision. The sensitivity of the assay based on a signal to noise ratio of two (S/N = 2) is 5.0 ng of drug.

References

Bose, B.C. and Bigayvargiva, R., Spectrophotometric method for the estimation of guanethidine. J. Pharm. Pharmacol., 16 (1964) 516–562.

British Pharmacopoeia, 1980, Addendum 1982, H.M.S.O., London (1982) p. 59.

Conn, R.B. and Davis, R.B., Green fluorescence of guanidium compounds with ninhydrin. Nature (Lond.), 183 (1959) 1053–1055.

Corder, C.N., Klaniecki T. and McDonald, R.H., Assay of bethanidine and guanethidine in plasma. Pharmacologist, 15 (1973) 194 (abstr.).

Hengstmann, J.H., Falkner, F.C., Watson, J.T. and Oates, J., Quantitative determination of guanethidine and other guanido-containing drugs in biological fluids by gas chromatography with flame ionization detection and multiple ion detection. Anal. Chem., 46 (1974) 34–39.

Honigberg, I.L., Stewart, J.T., Smith, A.P. and Hester, D.W., Liquid chromatography in pharmaceutical analysis III. Separation of diuretic–antihypertensive mixtures. J. Pharm. Sci., 64 (1975) 1201–1204.

Koch, D.D. and Kissinger, P.T., Determination of tryptophan and several of its metabolites in physiological samples by reverse-phase liquid chromatography with electrochemical detection. J. Chromatogr. Biomed. Appl., 164 (1979) 441–455.

Loeffler, L.J. and Pittman, A.W., Development of radioimmunoassay for guanethidine. J. Pharm. Sci., 68 (1979) 1419–1423.

Mason, W.D., Gardner, T.D. and Stewart, J.T., Polarographic determination of ascorbic acid by oxidation at the tubular carbon electrode. J. Pharm. Sci., 61 (1972) 1301–1303.

Pachla, L.A. and Kissinger, P.T., Determination of ascorbic acid in food stuffs, pharmaceuticals, and body fluids by liquid chromatography with electrochemical detection. Anal. Chem., 48 (1976) 364–367.

Pellizzari, E.D. and Seltzman, T.P., Two-dimensional gas–liquid chromatography electron-capture detection of guanethidine in plasma. Anal. Biochem., 96 (1979) 118–125.

Rahn, H.J. and Dayton, P.G., Studies on the metabolism of guanethidine in hypertensive patients. Biochem. Pharmacol., 18 (1969) 1809–1816.

Shah, M.H. and Stewart, J.T., Amperometric determination of hydralazine hydrochloride in a flowing stream at the glassy carbon electrode. J. Pharm. Sci., (1983a) in press.

Shah, M.H. and Stewart, J.T., Amperometric determination of isoniazid in a flowing stream at the glassy carbon electrode. Anal. Lett., (1983b) in press.

Shoup, R.E. (Ed.), Recent Reports on Liquid Chromatography with Electrochemical Detection, BAS Press, W. Lafayette, IN, 1981.

Stewart, J.T., Loo, H.C. and Mason, W.D., Determination of methyldopa in pharmaceutical dosage forms and biological fluids based on oxidation at the tubular carbon electrode. J. Pharm. Sci., 63 (1974) 954–955.

Tompsett, S.L., Detection and determination of hypotensive drugs in human serum. Acta Pharmacol. Toxicol., 19 (1962) 365–367.

Tompsett, S.L., Forshall, W. and Smith, D.C., The identification and determination of some hypotensive drugs in urine. Acta Pharmacol. Toxicol., 18 (1961) 75–79.

United States Pharmacopeia, 20th Revision, United States Pharmacopeial Convention, Rockville, MD, 1980, p. 362.