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Spectrophotometric analysis of some guanidino drugs by acid-dye and charge-transfer complexation methods

ABDEL-AZIZ M. WAHBI,* MONA M. BEDAIR, SHEREEN M. GALAL and AZZA A. GAZY

Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, University of Alexandria, Alexandria, 21521, Egypt

Abstract: Two spectrophotometric methods are described for the determination of guanethidine sulphate (I), guanfacine hydrochloride (II), guanoclor sulphate (III), guanoxan sulphate (IV) and debrisoquine sulphate (V). The first method involves ion-pair formation of the selected compounds (I–V) with bromocresol purple at pH 3.8. The yellow ion pair is extracted with chloroform and the absorbance is measured at about 415 nm. The second method is based on the reaction of the basic guanidino compounds (I, III–V) with iodine in chloroform to give molecular charge-transfer complexes with maximum absorbance at 292 and 345 nm. Beer's law was obeyed for both methods and the relative standard deviations were found to be less than 2%. The apparent molar absorptivities were found to be 2.1×10^4 to 6.9×10^4 l mol^{-1} cm^{-1} using bromocresol purple and 0.7×10^4 to 2.4×10^4 l mol^{-1} cm^{-1} using iodine. The investigated drugs were assayed in tablets. The mean percentage recoveries were found to be 99.8–100.8% by the acid-dye method and around 100.4% by the charge-transfer complexation method.

Keywords: *Guanidino drugs; spectrophotometry; acid-dye method; charge-transfer complexation method; tablets.*

Introduction

An extraction–spectrophotometric method for the determination of different organic bases [1] has been described. The method is based on the reaction of basic nitrogenous compounds with an acidic dye, e.g. methyl orange or bromocresol purple to yield an ion-pair salt that may be extracted into an organic solvent such as chloroform or dichloromethane [2]. The separated ion-pair salt is determined colorimetrically. The acid-dye technique is used for the British Pharmacopoeial assay of formulations containing quaternary ammonium salts or amines, e.g. biperidine lactate injection, clonidine hydrochloride injection and tablets, neostigmine methylsulphate injection, and benzhexol hydrochloride tablets.

Charge-transfer complexation reactions have been extensively used for the determination of electron-donating basic nitrogenous compounds using an σ-acceptor (e.g. iodine) or an π-acceptor (e.g. chloranil) in organic solvents as reagents [3]. The latter reaction may also be explained by ring substitution rather than charge-transfer complexation.

Guanethidine sulphate (1), guanfacine hydrochloride (II), guanoclor sulphate (III), guanoxan sulphate (IV) and debrisoquine sulphate (V) (Fig. 1) are guanidino drugs widely used as antihypertensive agents. Several methods have been reported for their deter-

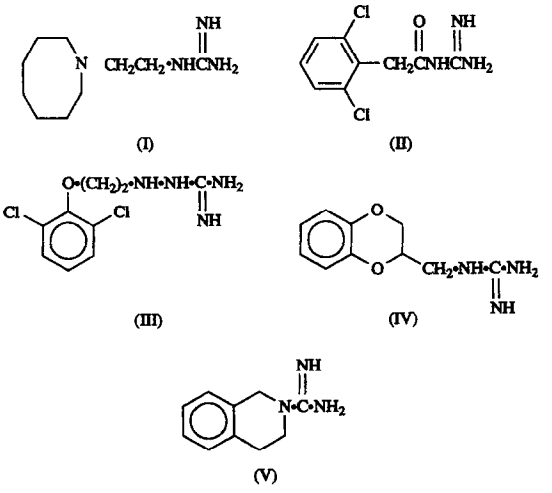

Figure 1
Chemical structure of the guanidino drugs I–V.

* Author to whom correspondence should be addressed.

mination, including spectrophotometry [4], oxidimetry [5], complexometry [6], colorimetry [7], fluorimetry [8] and high-performance liquid chromatography [9]. Recently guanethidine sulphate and guanoxan sulphate have been determined by a spectrofluorimetric method in tablets and biological fluids using the condensation reaction of the guanidino group with 9,10-phenanthraquinone [10]. The official method [11] for the assay of guanethidine sulphate is based on the colour reaction of the guanidino group with sodium nitroprusside and potassium hexacyanoferrate.

This paper introduces two spectrophotometric methods for the determination of guanidino compounds. The first is an extraction–spectrophotometric method which involves ion-pair formation with bromocresol purple at pH 3.8, followed by extraction of the ion pair with chloroform. The second is a charge-transfer complexation method based on the reaction of the selected basic drugs (I, III–V) with the σ-acceptor iodine in chloroform. The proposed spectrophotometric methods have been applied to the assay of these drugs in tablets.

Experimental

Apparatus

A Perkin–Elmer Model 550S UV–vis spectrophotometer with 1-cm quartz cuvettes and a Hitachi Model 561 recorder were used.

Materials and reagents

All reagents and solvents were of analytical grade. Authentic samples of guanethidine sulphate (Ciba-Geigy, Basle, Switzerland), guanfacine hydrochloride (Swisspharma, Egypt), guanoclor sulphate and guanoxan sulphate (Pfizer, Kent, UK) and debrisoquine sulphate (Roche, Switzerland) were kindly provided by the manufacturer and were used without further purification. Tablets containing the guanidino drugs were prepared in the laboratory. The fillers and excipients used were lactose 90, starch 7, talc 2.7 and magnesium stearate 0.3 parts. Bromocresol purple (Aldrich) 1.0 mg ml^{-1} solution in distilled water was used. McIlvaine's citric acid–phosphate buffer (pH 3.8) was prepared by mixing 65.1 ml of 0.1 M solution of citric acid monohydrate and 34.9 ml of 0.2 M solution of disodium hydrogen phosphate. Iodine solution (0.5 mg ml^{-1} in chloroform) was used.

Analysis of authentic samples

Method (A): Acid-dye method.

(1) Standard drug solution (a). Stock solutions (1.0 mg ml^{-1}) of the guanidino drugs were prepared in distilled water and further diluted with distilled water to contain 0.05 mg ml^{-1} of the analyte (standard a).

(2) Construction of calibration graphs. Aliquots of the standard (a) drug solution (0.05 mg ml^{-1}) covering the concentration ranges cited in Table 1 were transferred into 60-ml separatory funnels. To each funnel, 5-ml of buffer pH 3.8 solution and 2 ml bromocresol purple solution were added successively. The solutions were extracted by shaking for 2 min with successive quantities of 10, 5 and 5 ml chloroform. The chloroform extracts were transferred into 25-ml standard flasks and diluted to volume with chloroform. The absorbance was measured at the wavelengths (408–420 nm) specified in Table 1 against a reagent blank prepared simultaneously.

Method (B): Iodine charge-transfer method.

(1) Standard drug solution (b). An accurately weighed amount (about 100 mg) of the guanidino drugs salt was transferred into a 100-ml separatory funnel and dissolved in about 20 ml distilled water. The solution was rendered alkaline with few drops of 10% sodium hydroxide solution and extracted successively with three 25-ml portions of chloroform. Each chloroform extract was filtered through a filter paper containing a few crystals of anhydrous sodium sulphate. The chloroform extracts were collected in a 100-ml standard flask and diluted to volume with chloroform. This solution was further diluted with chloroform to give a solution containing 0.05 mg ml^{-1} of the analyte [standard (b)].

(2) Construction of calibration graphs. Aliquots of the standard (b) drug solutions within the concentration ranges stated in Table 2 were transferred into separate 10-ml standard flasks. To each flask, 4 ml iodine solution was added, mixed well and diluted to volume with chloroform. The solutions were kept in the dark for 30 min. The absorbance of the resultant complex was measured at 292 nm against a reagent blank similarly prepared.

(3) Molar ratio of reactants (Job's method). Equimolar solutions of iodine and guanidino

Table 1
Analytical parameters for the determination of five guanidino drugs using bromocresol purple

Drug	Wavelength of maximum absorption (nm)	Conc. range ($\mu g\ ml^{-1}$)	Linear regression			RSD* (%)	ϵ† ($l\ mol^{-1}\ cm^{-1}$)
			Intercept (a)	Slope (b)	Corr. coeff. (r)		
Guanethidine sulphate	408	1–4	0.0043	0.232	0.9996	1.24	6.9×10^4
Guanfacine hydrochloride	420	2–8	0.0051	0.098	0.9999	0.97	2.8×10^4
Guanoclor sulphate	410	4–12	−0.0018	0.078	0.9991	1.51	2.4×10^4
Guanoxan sulphate	415	4–10	0.0049	0.084	0.9999	0.39	2.1×10^4
Debrisoquine sulphate	415	2–10	0.0197	0.100	0.9996	1.11	4.5×10^4

*Relative standard deviation ($n = 5$).
†ϵ = Apparent molar absorptivity.

Table 2
Analytical data for the guanidine–iodine complexes in chloroform (λ_{max} = 292 nm)

Drug	Conc. range ($\mu g\ ml^{-1}$)	Linear regression: Intercept (a)	Slope (b)	Corr. coeff. (r)	RSD* (%)	ϵ† ($l\ mol^{-1}\ cm^{-1}$)
Guanethidine sulphate	2.5–12.5	0.0014	0.0812	0.9999	1.50	2.4×10^4
Guanoclor sulphate	10.0–30.0	0.0096	0.0290	0.9999	1.40	0.9×10^4
Guanoxan sulphate	10.0–30.0	−0.0024	0.0259	0.9998	1.10	0.7×10^4
Debrisoquine sulphate	5.0–20.0	0.0035	0.0409	0.9996	1.03	1.8×10^4

*Relative standard deviations (n = 5).
†ϵ = The apparent molar absorptivity.

drug base (0.5×10^{-3} M) were prepared separately in chloroform. Different volumes of the drug solution (1, 2, 3 . . . 9 ml) were mixed with iodine solution in descending order (9, 8, 7 . . . 1 ml) in 10-ml standard flasks. The flasks were left in the dark for 30 min. The absorbance was measured at 292 nm against chloroform.

Analysis of the selected guanidino drugs in tablets

For Method (A). An accurately weighed amount of the finely powdered tablets equivalent to about 100 mg of the active drug was transferred into a small conical flask and extracted with three 20-ml portions of distilled water. The combined extract was filtered into a 100-ml standard flask and diluted to volume with distilled water. A 5-ml aliquot of this solution was diluted to 100 ml with distilled water. Portions (2–3 ml) of the diluted solution (0.05 mg ml^{-1}) were transferred into 60-ml separatory funnels and the assay was continued as described under 'construction of calibration graphs' of Method (A) starting at '5-ml of buffer pH 3.8 . . .'. The amount of each drug was calculated from the corresponding calibration graph.

For Method (B). An accurately weighed amount of the finely powered tablets equivalent to 100 mg of the active drug was transferred into a 100-ml separatory funnel and dissolved as completely as possible in 20 ml distilled water. The drug base was extracted as described under 'standard drug solution' of Method (B) starting at 'The solution was rendered alkaline. . . '. A 2–3 ml volume of the prepared base solution in chloroform (0.05 mg ml^{-1}) was transferred into 10-ml standard flasks and treated as described under 'construction of calibration graphs' of Method (B) starting at the words '4 ml iodine solution was added . . .'. The concentration of each drug was calculated from the corresponding calibration graph.

Results and Discussion

Acid-dye method

The absorption curves of the chloroformic solutions of the guanidine drugs (I–V)-bromocresol purple ion-pair salts extracted from aqueous medium at pH 3.8 showed maximum absorbance at about 415 nm (Fig. 2).

The effect of the pH of the buffer solution in the range 2.2–7.0 on the absorbance reading of the drug–dye ion pair was examined. The results showed that the most efficient extraction of the ion pair with chloroform was obtained at a pH of 3.8, where maximum absorbance and high stability were achieved (Fig. 3).

The effect of the bormocresol concentration on the intensity of the colour developed at the selected wavelengths was ascertained by changing the concentration over the range 20–160 $\mu g\ ml^{-1}$. The maximum intensities were obtained by using about 80 $\mu g\ ml^{-1}$ of the bromocresol purple solution (Fig. 4).

Charge-transfer complexation method

Guanidino drugs (I, III–V), being n-electron donors, react instantaneously with the σ-acceptor iodine to give a characteristic complex. Upon reaction of the drugs with a chloroformic solution of iodine, the iodine colour fades to a pale purple or yellow colour. The absorption spectra of the products exhibit intense bands at 292 and 345 nm (Fig. 5). The product is assumed to be a molecular charge-transfer complex [12]. Guanfacine was found not to react with iodine under the same experimental conditions. This could be attrib-

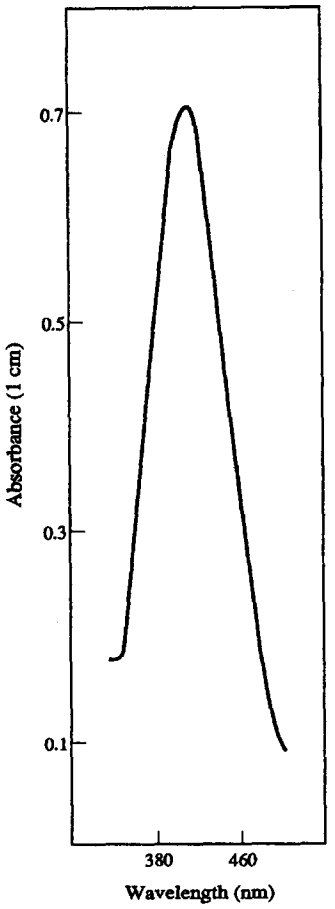

Figure 2
Asorption spectrum of the guanethidine (3.0 μg ml^{-1})–bromocresol purple ion pair in chloroform.

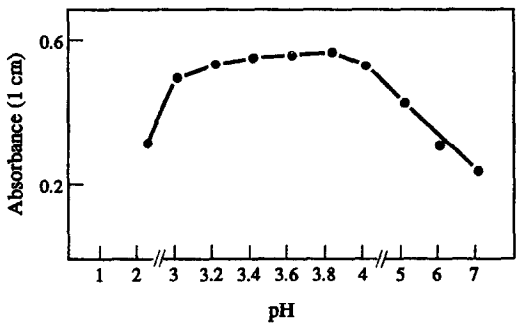

Figure 3
Effect of pH on the absorbance of guanethidine (2.5 μg ml^{-1})–bromocresol purple ion pair.

uted to the presence of a ketonic group adjacent to the guanidino group which suppressed its basicity relative to the other guanidine drugs.

The effect of iodine concentration and reaction time selected for the charge-transfer method was studied. By varying the concentration of iodine in the range 50–300 μg ml^{-1}, the optimum concentration was found to be 200 μg ml^{-1} (Fig. 6). Although the complex forms rapidly, constant absorbance readings are obtained only after the solution has stood for 30 min in the dark after which the absorbance remains constant for about 1 h. However, the time taken to reach a constant absorbance probably indicates that some other reaction such as oxidation of the amines occurs in addition to the charge-transfer complex formation.

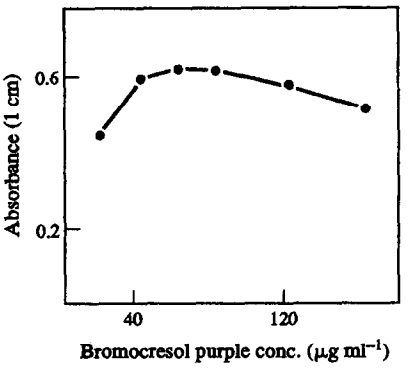

Figure 4
Influence of bromocresol purple concentration on the ion pair developed from its reaction with 2.5 μg ml^{-1} guanethidine sulphate.

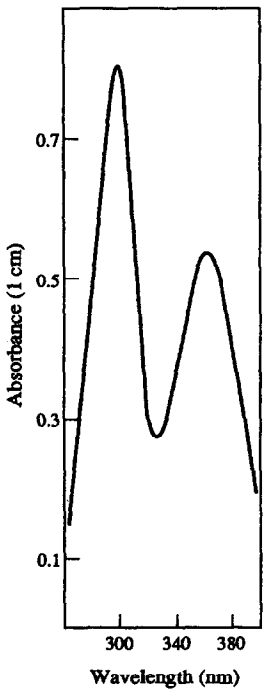

Figure 5
Absorption spectrum of the guanethidine (10 μg ml^{-1})–iodine charge-transfer complex in chloroform.

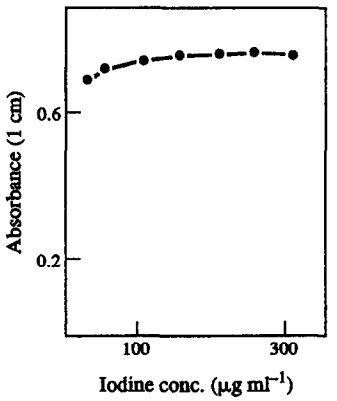

Figure 6
Influence of iodine concentration on the charge-transfer complex developed from its reaction with 10 μg ml^{-1} guanethidine sulphate.

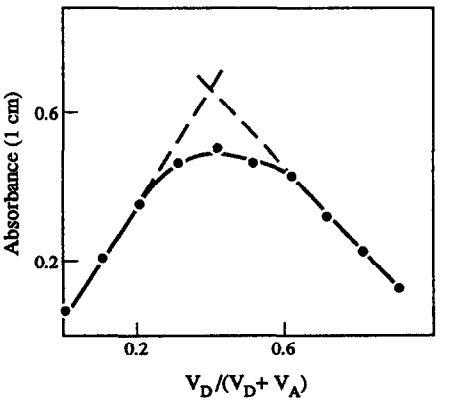

Figure 7
Job's method of continuous variation for guanethidine–iodine charge-transfer complex in chloroform (0.5×10^{-3} M). V_D = Volume of donor (guanidino drug); V_A = volume of acceptor (iodine).

Stoichiometric relationship

The molar ratio of the reactants (drug: iodine) in the charge-transfer complexes was determined by the method of continuous variations (Job's method), and found to be about 2:1 (Fig. 7). This suggests that two nitrogen atoms of the guanidine group are involved in the reaction with iodine.

Under the experimental conditions described above, calibration graphs were constructed for both methods from five data points over the concentration ranges cited in Tables 1 and 2. Regression analysis indicated a linear relationship between absorbance and concentrations (Tables 1 and 2). The correlation coefficients were between 0.9996 and 0.9999. Five replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations were found to be less than 2%, indicating reasonable repeatability of the selected methods. The apparent molar absorptivities were found to be 2.1×10^4 to 6.9×10^4 l mol^{-1} cm^{-1} using bromocresol purple and 0.7×10^4 to 2.4×10^4 l mol^{-1} cm^{-1} using iodine. The results obtained for each drug by using the two proposed methods show that the acid-dye method is more sensitive than the

Table 3
Determination of five guanidino drugs using the proposed methods and reference method [11]

Preparation	Recovery ± standard deviation* (%)		
	Acid-dye method (n = 5)	Iodine charge-transfer method (n = 5)	Reference method† (n = 6)
Guanethidine‡ sulphate	100.4 ± 1.71 t = 0.128 F = 4.45	100.4 ± 1.5 t = 0.141 F = 3.43	100.3 ± 0.81
Guanfacine§ hydrochloride	100.8 ± 1.41 t = 0.43 F = 2.62	—	100.5 ± 0.87
Guanoclor‡ sulphate	99.8 ± 0.93 t = 0.59 F = 5.1	100.3 ± 1.24 t = 0.093 F = 2.87	100.4 ± 2.1
Guanoxan‡ sulphate	100.6 ± 0.55 t = 0.99 F = 1.86	100.3 ± 1.1 t = 0.18 F = 2.15	100.2 ± 0.75
Debrisoquine‡ sulphate	100.7 ± 0.81 t = 2.11 F = 1.14	100.7 ± 1.0 t = 1.90 F = 1.73	99.7 ± 0.76

*Average of n replicates. The theoretical t-value (P = 0.05) is 2.26. The theoretical F-value (95%) is 6.26.
†The B.P. (1988) method for guanethidine sulphate tablets has been equally applied to other drugs.
‡Laboratory prepared tablets containing 10 mg for each of guanethidine sulphate, guanoclor sulphate, guanoxan sulphate and debrisoquine sulphate per tablet.
§Estulic tablets containing 5 mg guanfacine hydrochloride per tablet (Swiss-pharma, Egypt).

charge-transfer complexation method (Tables 1 and 2).

The proposed methods have been applied to the determination of several guanidino drugs in commercial or laboratory-prepared tablets. The results obtained were compared with the official colorimetric method [11] (reference method) for the determination of guanethidine sulphate which has also been similarly applied to the other investigated drugs (Table 3). The results obtained were found to be in good agreement with the proposed methods. The calculated *t*- and *F*-values did not exceed the theoretical values, indicating that there is no signifiant difference between the methods in the mean values obtained and their precision (Table 3).

The guanidino drugs investigated in this study possess either no significant absorption in the UV region (230–360 nm), e.g. guanethidine sulphate or relatively weak absorption at their wavelengths of maximum absorbance. The reported specific absorbances are: guanfacine hydrochloride, 9.8; guanoclor sulphate, 14; guanoxan sulphate, 104; and debrisoquine sulphate, 17.7. Moreover, these drugs are formulated at low dosage levels (5–10 mg per tablet). Hence the conventional spectrophotometric method cannot be applied to their determination. The problem has been overcome in this work by ion-pair formation and charge-transfer complexation methods. The high values of the apparent molar absorptivities indicate high sensitivity of the two spectrophotometric methods. The proposed methods therefore can be considered to be suitable for the quality control and routine analysis of the investigated drugs in bulk as well as in their dosage forms. Furthermore, the British Pharmacopoeial method for the assay of guanethidine sulphate has been shown to be equally applicable to the other guanidino drugs.

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