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Spectrophotometric determination of some MAO inhibitors using 7,7,8,8-tetracyanoquinodimethane and iodine monochloride

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Abstract: Two simple and sensitive spectrophotometric methods are described for the assay of three MAO inhibitors: isocarboxazid, tranylcypromine sulphate and iproniazid phosphate. The first method is based on the formation of a highly coloured stable radical anion between the drug as an n-donor and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as a π-electron acceptor. Beer's law is obeyed in the concentration range 0.2–3, 0.2–4 and 0.5–4 $\mu g\ ml^{-1}$ for isocarboxazid, tranylcypromine sulphate and iproniazid phosphate, respectively. The second method involves the use of iodine monochloride (ICl) as an σ-acceptor. It was found that ICl reacts quantitatively only with isocarboxazid and tranylcypromine sulphate; with iproniazid phosphate results were very poor. Beer's law is obeyed in the concentration range 2–20 $\mu g\ ml^{-1}$ for both drugs. The optimum experimental parameters for colour production in each case were determined. The percentage recoveries obtained were in accordance with those obtained by the official methods. The proposed methods are characterized by high sensitivity.

Keywords: *Spectrophotometry; isocarboxazid; tranylcypromine sulphate; iproniazid phosphate; TCNQ; ICl.*

Introduction

Isocarboxazid, tranylcypromine sulphate and iproniazid phosphate are potent inhibitors of the monoamine oxidase enzyme, and are used as antidepressants in the treatment of psychiatric disorders.

Numerous analytical procedures have been reported for the determination of isocarboxazid in pure form and in pharmaceutical preparations; examples are titrimetric [1, 2], polarographic [3] and spectrophotometric methods [4, 5]. The USP XXII [6] specifies a nitrosometric titration method, with potentiometric detection of the endpoint, for analysis of the pure drug; for the tablets, a spectrophotometric method using ammonium molybdate is described.

Iproniazid phosphate has been determined spectrophotometrically with tetrazolium [4], molybdic acid [7], thiobarbituric acid [8] and ammonium vanadate [9].

For tranylcypromine sulphate the British Pharmacopoeia (BP) [10] recommends a non-aqueous titration method for analysis of the raw material and a spectrophotometric method for the dosage forms. Relatively few other methods have been described for its determination but gas chromatography (GC) [11, 12], gas–liquid chromatography (GLC) [13], NMR [14] and enzymatic [15] methods were reported for its evaluation in bulk biological fluids.

7,7,8,8-Tetracyanoquinodimethane (TCNQ) has been used as a chromogenic reagent for the determination of some alkaloids [16], procaine [17], imidazoline derivatives [18], and recently for the determination of gliclazide and tolazamide [19]. A similar use of iodine monochloride (ICl) in this field has not been reported.

The present paper describes the use of TCNQ and ICl for the sensitive and rapid spectrophotometric determination of isocarboxazid, tranylcypromine sulphate and iproniazid phosphate, either in pure form or in dosage forms.

Experimental

Apparatus

A Perkin–Elmer 550 S spectrophotometer with matched 10-mm quartz cells was used.

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Materials

Pure drug samples were kindly supplied by Hoffmann–La Roche Co. (Basel, Switzerland) for isocarboxazid and iproniazid phosphate, and Kahira Pharmaceutical Co. (Cairo, Egypt) for tranylcypromine sulphate. Dosage forms containing these drugs were obtained from commercial sources.

Reagents

All the reagents used were of analytical-reagent grade. ICl (Aldrich Chemical Co. Ltd, UK) as a 1×10^{-2} M solution in dioxane and TCNQ (Merck, FRG) as a 0.3% (w/v) solution in acetonitrile were used as chromogenic reagents.

Preparation of sample solutions

Weigh accurately 100 mg of the drug in the form of a salt (tranylcypromine sulphate or iproniazid phosphate), transfer into a 60-ml separator, dissolve in a small volume of distilled water and make alkaline with ammonia solution. Extract the liberated base with five 15-ml portions of chloroform. Combine the chloroformic extracts, dry with anhydrous sodium sulphate for 5 min and filter through dry filter-paper into a 100-ml volumetric flask; rinse the sodium sulphate and the filter with chloroform and dilute the combined filtrates and washings to 100 ml with chloroform.

For preparation of a working solution in a solvent other than chloroform, an appropriate volume of solution corresponding to the required concentration is evaporated to dryness with a stream of nitrogen, and the residue is dissolved in the desired solvent (dioxane or acetonitrile).

1×10^{-4} M, 2×10^{-4} M and 3×10^{-5} M solutions of tranylcypromine and iproniazid are prepared as described above. For isocarboxazid, a solution containing 1 mg ml^{-1} is prepared in the required solvent; 1.5×10^{-4} M and 1.66×10^{-4} M solutions are prepared by dilution.

Construction of calibration curves

TCNQ method. Transfer by pipette aliquots of a solution of the drug in acetonitrile (in the concentration range cited in Table 1) into separate 10-ml volumetric flasks. Add 2 ml of TCNQ solution (for isocarboxazid) or 3 ml of TCNQ solution (for tranylcypromine sulphate and iproniazid phosphate). Dilute to 10 ml with acetonitrile, and allow to stand at room temperature for the specified time (Table 1). Measure the absorbance at 843 nm against a reagent blank prepared simultaneously.

ICl method. Transfer by pipette aliquots of a solution of the drug in dioxane (in the concentration range cited in Table 1) into separate 10-ml volumetric flasks. Add 2 ml of ICl solution (for isocarboxazid) or 1 ml of ICl solution (for tranylcypromine sulphate). Heat in a water-bath at 40°C for 15 min (for isocarboxazid) or 90°C for 5 min (for tranylcypromine sulphate). Dilute to 10 ml with dioxane. Measure the absorbance at 280 nm (for tranylcypromine sulphate) or 290 nm (for isocarboxazid) against a reagent blank prepared simultaneously.

Analysis of the tablets

Parnate tablets (tranylcypromine sulphate) and Marslid tablets (iproniazid phosphate). Weigh and powder 20 tablets. Transfer an accurately weighed amount of the powder equivalent to about 50 mg of the drug into a 60-ml separator, dissolve in 10 ml of water, make alkaline with ammonia, and proceed as described previously beginning with "Extract the liberated base . . .". Transfer an aliquot of the chloroformic extract (in the concentration range cited in Table 1) into a 10-ml volumetric

Table 1
Performance data for the spectrophotometric determination of the studied drugs with TCNQ

	TCNQ method				
Compound	Working concentration range ($\mu g\ ml^{-1}$)	Volume of TCNQ solution (ml)	Development time (min)	Regression equation	Correlation coefficient*
Isocarboxazid	0.2–3.0	2	10	$A = 0.2929c + 0.0003$	0.9999
Iproniazid phosphate	0.5–4.0	3	35	$A = 0.1527c - 0.0018$	0.9986
Tranylcypromine sulphate	0.2–4.0	3	20	$A = 0.2182c + 0.0023$	0.9998

*Based on eight separate determinations.
c = concentration in $\mu g\ ml^{-1}$.
A = absorbance at λ_{max}.

flask. Evaporate to dryness using a stream of nitrogen, dissolve in the appropriate solvent, and proceed as described under the heading "Construction of calibration curves" using the TCNQ and ICl methods. Calculate the nominal content from the corresponding calibration graph or regression equation.

Marplan tablets (isocarboxazid). Weigh and powder 20 tablets. Transfer an accurately weighed amount of the powder equivalent to 50 mg of isocarboxazid into a small conical flask. Extract with 4 × 20-ml portions of dioxane (ICl method) or acetonitrile (TCNQ method). Transfer into a 100-ml volumetric flask and dilute to 100 ml with the same solvent. Proceed as described under "Construction of calibration curves" using the TCNQ and ICl methods. Calculate the nominal content from the corresponding calibration graph or regression equation.

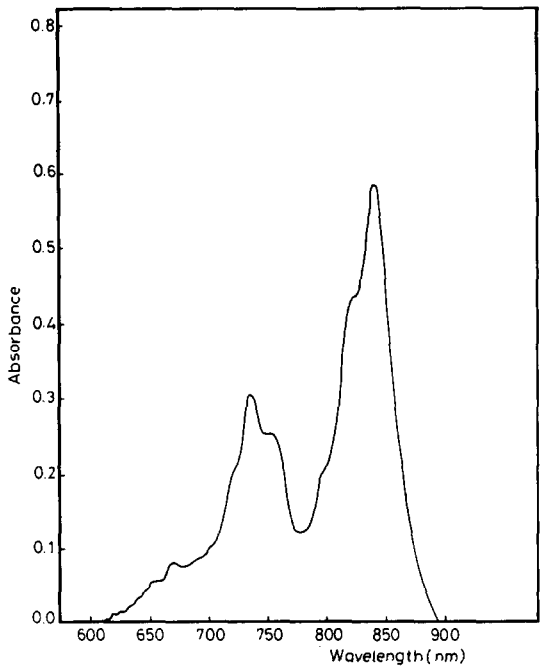

Figure 1
Absorption spectrum of isocarboxazid–TCNQ reaction product (2 μg ml^{-1}).

Results and Discussion

Structurally, isocarboxazid, iproniazid phosphate and tranylcypromine sulphate contain amino-functional groups and hence act as n-electron donors (Lewis bases). They react with TCNQ as a π-electron acceptor (Lewis acid) to give a bluish-green chromogen that exhibits strong absorption maxima at 843, 825, 762 and 742 nm (major), and at 730, 684 and 668 nm (minor) (Fig. 1). These bands may be attributed to the formation of the TCNQ radical anion which is formed by the complete transfer of n-electrons from the donor moiety to the acceptor moiety in a polar medium [20] (Scheme 1).

$$\underset{\text{Donor}}{\ddot{D}} + \underset{\text{Acceptor}}{A} \rightarrow [\ddot{D}^{\frown}\!\!\searrow\!\!\rightarrow A] \rightarrow D^{\cdot+} + \underset{\text{Radical anion}}{A^{\cdot-}}$$

Scheme 1

The absorption spectrum of the reaction product with isocarboxazid as a model example is shown in Fig. 1.

The spectrophotometric properties of the coloured species as well as the different parameters affecting the colour development were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of the reagent, nature of the solvent, reaction time and stability. For isocarboxazid, maximum absorbance is attained using 2 ml of 0.3% TCNQ solution. In contrast, for tranylcypromine sulphate and iproniazid phosphate, maximum absorbance is attained when 3 ml of 0.3% TCNQ solution is used. Acetonitrile is considered to be an ideal solvent for the colour reaction as it offers excellent solvent capacity for TCNQ and gives the highest yield of the radical [20].

Reaction time is determined by following the colour development at different time intervals at room temperature. Maximum absorption is attained after 10 min (for isocarboxazid), 20 min (for tranylcypromine sulphate) or 35 min (for iproniazid phosphate). The colour remains stable for more than 1 h (Fig. 2).

Under the experimental conditions described, standard calibration curves for isocarboxazid, iproniazid phosphate and tranylcypromine sulphate were constructed by plotting absorbance versus concentration. Con-

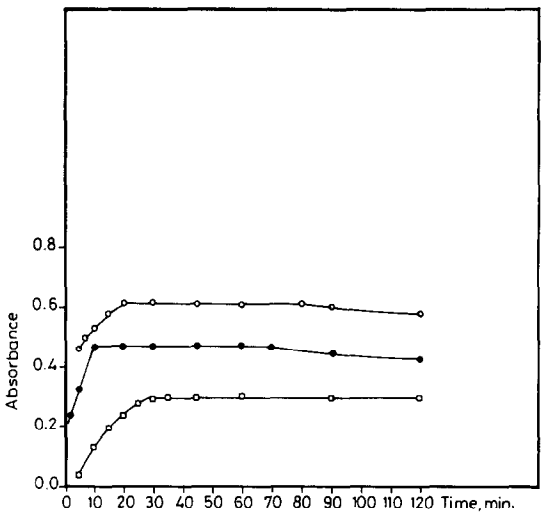

Figure 2
Stability of: ●, isocarboxazid–TCNQ reaction product (1.6 μg ml^{-1}); □, iproniazid–TCNQ reaction product (2 μg ml^{-1}); ○, tranylcypromine–TCNQ reaction product (2.8 μg ml^{-1}).

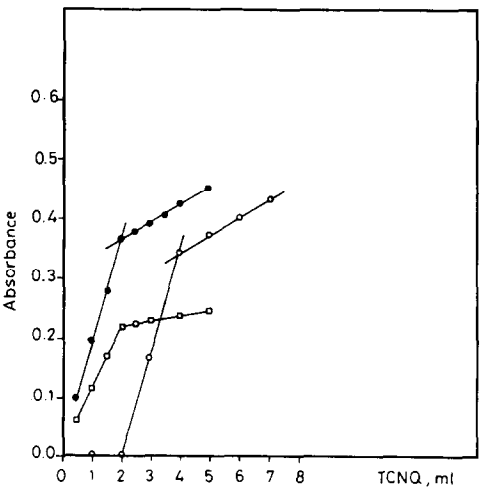

Figure 3
Molar ratio plot of: ●, isocarboxazid–TCNQ reaction product 1.5 × 10^{-4} M (2 ml); □, iproniazid–TCNQ reaction product 3 × 10^{-5} M (2 ml); ○, tranylcypromine–TCNQ reaction product 1 × 10^{-4} M (2 ml).

formity with Beer's law was evident in the concentration range of the final dilution: 0.2–3.0, 0.5–4.0 and 0.2–4.0 μg ml^{-1}, respectively.

Regression equations, derived using the least-squares method [21], indicate that the values of the intercepts are small (Table 1) for all the compounds studied.

The stoichiometry of the reaction was studied by the molar ratio method [18]. The ratios are 1:1, 1:1 and 1:2 (donor–acceptor) for isocarboxazid, iproniazid phosphate and tranylcypromine sulphate, respectively (Fig. 3); the apparent molar absorptivities are 6.8 × 10^4, 4.2 × 10^4 and 8.0 × 10^4 l mol^{-1} cm^{-1}, respectively.

The absorption spectrum of ICl in dioxane shows absorption maxima at 258 nm (major) and 448 nm (minor). Reaction with isocarboxazid or tranylcypromine sulphate (n-donor) results in a bathochromic shift to 290 or 280 nm, respectively (Fig. 4). This shift is due to the formation of a charge-transfer complex between ICl as σ-acceptor and these drugs. Attempts were made to determine iproniazid phosphate using ICl but the results were not quantitative.

The following parameters were studied in order to select the most suitable procedure for the quantitative application.

Solvent

The spectra of the complexes formed be-

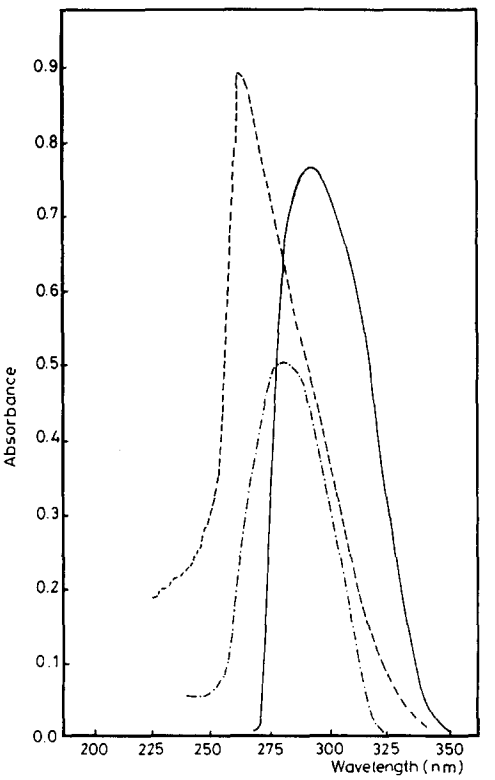

Figure 4
Absorption spectra of: ——, isocarboxazid–ICl complex (15 μg ml^{-1}); – · – ·, tranylcypromine–ICl complex (11 μg ml^{-1}); – – –, ICl in dioxane.

tween ICl and the studied drugs were recorded individually in chloroform, carbon tetrachloride, methylene chloride, dichloroethane, dioxane and cyclohexane. Dioxane was found to be the solvent of choice for the formation of the complex because of the greater molar absorptivity of the complex.

Effect of temperature on the formation of the complex

Experiments in which the reaction mixture was heated at different temperatures for different time periods shows that complete reaction is achieved by heating at 40°C for 15 min (for isocarboxazid) or 90°C for 5 min (for tranylcypromine sulphate).

The stability of the formed complexes, 2 days for tranylcypromine sulphate and 1 h for isocarboxazid, enables replicate determination to be achieved with good reproducibility.

Reagent concentration

Figure 5 indicates that 1 ml of 1×10^{-2} M ICl in dioxane is sufficient for maximum absorbance for tranylcypromine sulphate; for isocarboxazid, 2 ml of ICl solution is needed.

Molar ratio of the reactants

The stoichiometry of the reaction was studied by the molar ratio method [22], and it was found that the ratios are 1:1 and 1:2 (donor–acceptor) for isocarboxazid and tranylcypromine sulphate, respectively (Fig. 6).

Conformity with Beer's law

The absorbance of the formed complexes conforms with Beer's law in the concentration range of 2–20 μg ml^{-1} for both drugs.

The linearity was shown by the regression equation derived using the least-squares method [21], and the corresponding correlation coefficient for the studied compounds determined by the proposed method (Table 2).

The apparent molar absorptivities are 1.2×10^4 and 1.6×10^4 l mol^{-1} cm^{-1} for isocarbox-

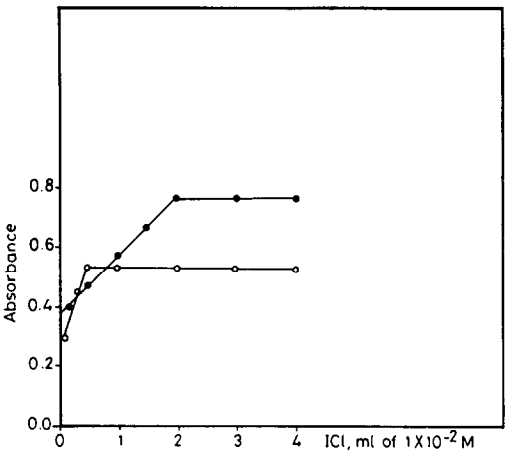

Figure 5
Effect of the volume of ICl (1×10^{-2} M) on the absorbance of: ●, isocarboxazid–ICl complex (15 μg ml^{-1}); ○, tranylcypromine–ICl complex (12 μg ml^{-1}).

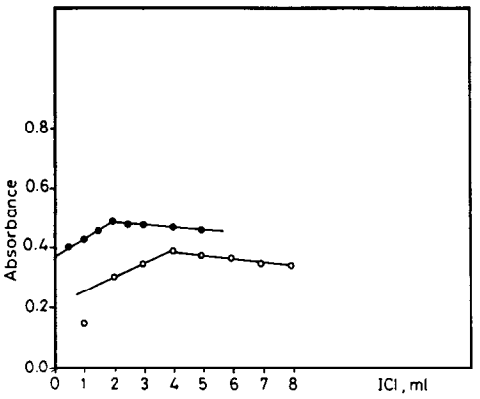

Figure 6
Molar ratio plot of: ●, isocarboxazid–ICl complex 1.66×10^{-4} M (2 ml); ○, tranylcypromine–ICl complex 2.00×10^{-4} M (2 ml).

Table 2
Performance data for the spectrophotometric determination of the studied drugs with ICl

	ICl method				
Compound	Working concentration range (μg ml^{-1})	Volume of ICl solution (ml)	Development time (min)	Regression equation	Correlation coefficient*
Isocarboxazid	2–20	2	15 at 40°C	$A = 0.0513c + 0.0024$	0.9999
Iproniazid phosphate	—	—	—	—	—
Tranylcypromine sulphate	2–20	1	5 at 90°C	$A = 0.0440c + 0.0007$	0.9999

*Based on eight separate determinations.
c = concentration in μg ml^{-1}.
A = absorbance at λ_{max}.

azid and tranylcypromine sulphate, respectively.

The appreciable values of molar absorptivities and the stability of the products in both methods permit the successful application of the proposed methods to the determination of these compounds, either in pure form (Table 3) or in their dosage forms (Table 4). These results were compared with those obtained by the official methods [6]. The proposed methods are shown to be precise and reproducible.

Table 3
Determination of pure drugs by the TCNQ and ICl methods

Compound	TCNQ method, % found	ICl method, % found	Official method [6],* % found
Isocarboxazid	98.9	101.3	101.8
	102.1	99.0	100.6
	100.9	101.9	100.2
	99.4	100.9	
	100.0	98.1	
Mean ±SD	100.3 ± 1.27	100.2 ± 1.62	100.9 ± 0.83
Tranylcypromine sulphate	97.8	98.6	101.0
	101.4	100.6	100.2
	100.6	99.4	100.4
	99.2	100.8	
	100.0	100.9	
Mean ±SD	99.8 ± 1.38	100.1 ± 1.01	100.5 ± 0.42
Iproniazid phosphate	99.2	—	99.9*
	100.9	—	100.1*
	101.3	—	100.9*
	100.8	—	
	100.0	—	
Mean ±SD	100.4 ± 0.84		100.3 ± 0.53*

*Analysed by the nitrosometric titration method as for isocarboxazid [6].

Table 4
Analysis of dosage forms of the studied drugs by the TCNQ and ICl methods

Preparation	TENQ method, % found	ICl method, % found	Official method [6], % found
Marplan tablets*	96.9	98.1	97.3
(10 mg isocarboxazid)	99.0	99.1	98.2
	98.4	98.7	98.8
	99.3	99.0	
Mean ±SD	98.4 ± 1.07	98.7 ± 0.45	98.1 ± 0.75
Parnate tablets†	106.5	105.7	104.2
(10 mg tranylcypromine sulphate)	105.7	105.1	105.2
	104.8	106.3	106.3
	105.9	104.5	
Mean ±SD	105.7 ± 0.70	105.4 ± 0.77	105.2 ± 1.05
Marsilid tablets*	100.8	—	98.6‡
(25 mg iproniazid phosphate)	98.1	—	101.2‡
	98.9	—	100.3‡
	101.6	—	
Mean ±SD	99.9 ± 1.63		100.0 ± 1.32‡

*Products of Hoffmann–La Roche Co. (Basel, Switzerland).
†Product of SK & F.
‡Analysed by the method described for Marplan tablets [6].

Statistical analysis [21] of the results by the TCNQ, ICl and official methods [6] using the Student's (*t*-test) and the variance ratio (*F*-test) shows no significant difference between the performance of the three methods in respect of accuracy and precision.

Conclusions

TCNQ and ICl are sensitive chromogenic reagents for the determination of the studied drugs in pure form and in tablets. The molar absorptivity of tranylcypromine sulphate increased from 532.2 to 1.6×10^4 and 8.0×10^4 l mol^{-1} cm^{-1}, respectively, by the ICl and TCNQ methods. The suggested methods are rapid, simple and suitable for routine analysis in control laboratories. Compared with the official method described for tranylcypromine sulphate, the proposed method is more sensitive and less subject to interference. For isocarboxazid, the proposed method, especially that with TCNQ, is time saving, less laborious and more sensitive.

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