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SENSITIVE AND SPECIFIC PHOTOMETRIC DETERMINATION OF N-ETHYL DRUGS

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A spectrophotometric method is described for the determination of some N-ethyl drugs *via* reaction with chloranil. A mixture of a solution of the amine in benzene with the chromogenic reagent was heated on a water bath at 75-80° for one hour.

A blue colored product λ_{max} 680 nm resulted. Apparent molar absorptivities of the chrompgenic product of tertiary N-ethyl compounds ranged from 1700-5100. Under the described assay conditions N-methyl as well as quaternized N-ethyl compounds did not interfere. The amines are assayed in the range of 0.004 - 1.3 mg per ml range with an accuracy of 99- 101 % recovery and a S.D. of ± 0.74- ± 3.11% . The possible composition of the colored product is discussed.

Reaction between tertiary amines containing a flexible N-ethyl grouping and some halogenated quinones to yield colored products was reported in the course of synthesis of new compounds and study on amine oxidation[1]. However, there are no reported examples for the exploration of this reaction for the quantitative determination of N-ethyl containing compounds .

In this work, a spectrophotometric procedure is proposed for the assay of N-ethyl drugs *via* reaction with chloranil in benzene. The selectivity, sensitivity, and precision of the proposed method was determined. The scope and limitations relative to pharmaceutical preparations were explored.

EXPERIMENTAL

Equipment - Spectra and absorbance measurements were made using a single beam spectrophotometer (Spektromom 203, Mom, Budapest, Hungary).

A constant-temperature bath maintained at 75 ± 5° was utilized for accelerating color development.

Reagents and Chemicals - Chloranil was obtained commercially (E. Merk Dermstadt; GFR, Synthetic grade), and was recrystallized from benzene; m.p. 289° (subl.).

All other compounds were analytical or pharmaceutical grade obtained from various manufacturers and were purified when necessary by redistillation or recrystallization before use .

Chloranil Solution - Optimum concentration was 1 per cent w/v. The reagent (1 gm) was dissolved in warm benzene, cooled and diluted to 100 ml with benzene. This solution was stable for 4 weeks.

Amines - The amine salt solution in distilled water was treated with phosphate buffer pH 9.5 and the free base was extracted with benzene if it was of sufficient extractability. If not extractable with benzene; then with other appropriate solvent which was evaporated to dryness and the base dissolved in benzene. All of the amines tested were of sufficient solubility in benzene at the analytical concentrations utilized although not always extractable with it. The solution was quantitativey diluted to give the appropriate concentration.

Analytical procedure - In a 5 ml volumetric flask, the appropriate volume of the free base equivalent to 0.02 gm for strongly reacting and 5 mg for weakly reacting bases in benzene was placed; and 0.2 ml of chloranil solution was added. The mixture was diluted with benzene to 3 ml, the flask was stoppered and placed on a constant-temperature bath at 75 - 80° for one hour. The solution was cooled to room

temperature and made up to volume with benzene. Absorbance of this solution was measured at 680 nm in a 1 cm cell against a blank prepared in the same manner already described; but omitting the amine.

Construction of standard curves - A stock solution of the amine was prepared by dissolving 15 mg of the pure base prepared as mentioned above in 25 ml of benzene. From this solution different volumes were used for the color development according to the above procedure.

Application to formulations (Pentoxyverin syrup) - A volume of the syrup equivalent to 33.3 mg of the base calculated with regards to the labelled amount of the salt was measured, diluted with distilled water to 100 ml. The base was liborated with phosphate buffer, and the procedure was completed as mentioned before.

The recovery was further checked by the method of standard addition.

RESULTS

Intensity Variation of Color

The response of various N-ethyl compounds on interaction with chloranil under the experimental conditions mentioned are shown in Table I .

Effect of Solvent

The reaction failed to take place in aqueous solutions. Different solvents as chloroform, dioxan; and toluene gave lower yields of the blue quinone as compared with benzene.

Development of Color

Due to variation in the chemical structures of the amines; the rate of development as well as the quality of the blue color were variable; (Table I). Color development at room temperature was slow but formation of color was accelerated by heat. In all cases the color reached

maximum within 60 minutes irrespective of the variation onset of development, and was stable for at least 24 hours. Longer heating periods did not enhance or reduce the intensity of the color.

Standard Curves

The relationship between absorbance at 680 nm and concentration was found linear in the general concentration range of 0.004 - 1.3 mg per ml. In all cases Beer's law held for the system, correlation coefficient (r) = 0.938 - 0.999, (

Table I. Color intensity variation of N-ethyl drugs

Amine	Onset[a]	$\varepsilon^{*}_{680\ nm}$	$A^{1\%}_{1\ cm}$	Direct UV[b] $A^{1\%}_{1\ cm}$	Direct UV[b] λ_{max}
N-Ethyl piperidine	Rapid	13400	400	-	-
Etafedrine	Rapid	5100	126	8	257
Pentoxyverine	Rapid	4400	81	4	252
Dicycloverine	Rapid	4200	80	3	215
Oxeladine	Rapid	3300	75	7	259
Etamiphylline	Rapid	3000	58	-	-
Myrtecaine	Moderate	1700	35	-	-
Tolycaine	Moderate	390	8	66	283
Camylofine	Slow	370	7	-	-
Fencamfamine	Slow	110	2.5	9	253

(a) Color development; Rapid : within 5 minutes ; Moderate : within 25 minutes ; Slow : within 40 minutes.

(b) Direct UV band of unreacted drugs as reported[(2)], reproduced for the purpose of comparing sensitivities.

* Based on molecular weight of the chromogen under the analytical conditions.

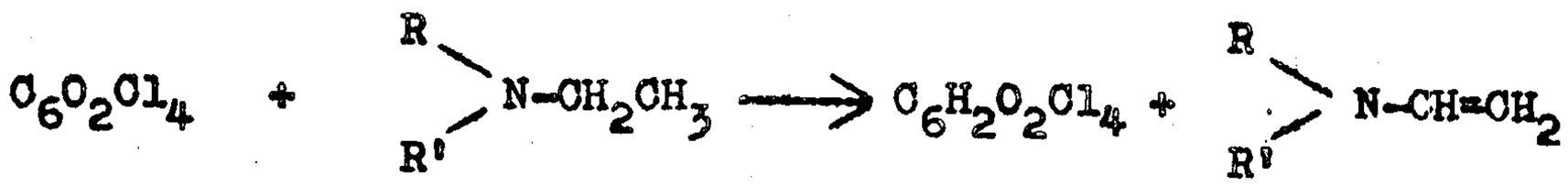
DISCUSSION

The N-alkyl functions have common occurence among pharmaceutical agents. By and large the N-methyl group has the highest abundance among these functions. However, a variety of pharmaceutical compounds contain the N-ethyl moiety, principally local anesthetics (myrtecaine, tolycaine, lidocaine, and procaine), antitussives (pentoxyverine, butethamate, and oxeladine), spasmolytics (adiphinin, bietamiverine, dioycloverine, and hexahydroadiphinin), stimulants (fencamfamine, etafedrine, and etamiphylline), and a number of other drugs (chloroquine, carbochromen, flurazepam, and phenglutarimide). These compounds may be prescribed in pharmaceutical preparations either singly or in combination with the closely related N-methyl derivatives. A case in point is a cough mixture containing N-ethyl antitussive with N-methyl antihistamine or N-methyl ephedrine analogue. This represents a potential difficulty in the analysis of such formulations because these N-alkyl analogues usually have similar solubility characteristics and close pK_a values. These two parameters are most important in determining the feasibility of separation of any two components of a mixture[3]. Furthermore, they affect the specificity of the most popular colorimetric method for tertiary amine drugs, namely, the acid dye technique. Accordingly, the two drugs are usually coanalyzed on applying this method unless a very clear separation technique is performed prior to the analysis.

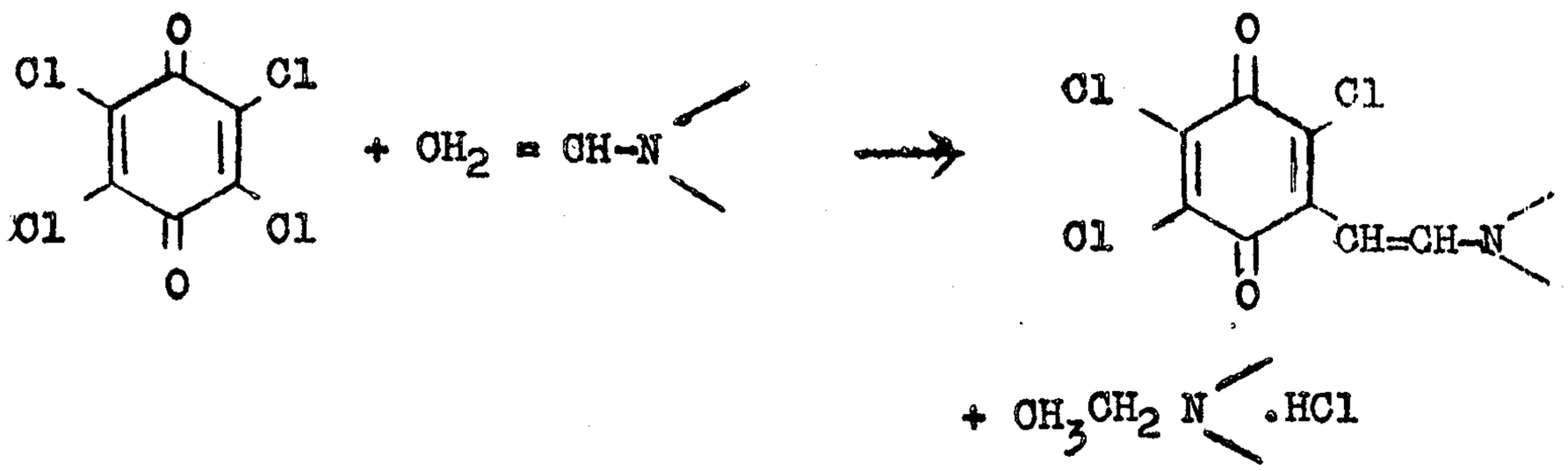
In the present work the reaction between tertiary N-ethyl compounds and chloranil has been successfully quantified so as to be used as a method for the analysis of N-ethyl drugs in presence of N-methyl analogues or quaternized N-ethyl compounds without interference from either.

Reaction Involved and Influence of Substrate Structure

In the analytical method presented chloranil is assumed to react with the flexible tertiary N-ethyl moiety of the drugs examined to yield the blue quinone through a two step reaction. The first step involves oxidation of the amine by chloranil to enamine



This is followed by condensation of the dialkylvinylamine with a second molecule of chloranil to yield the blue dialkylaminovinyl quinone.

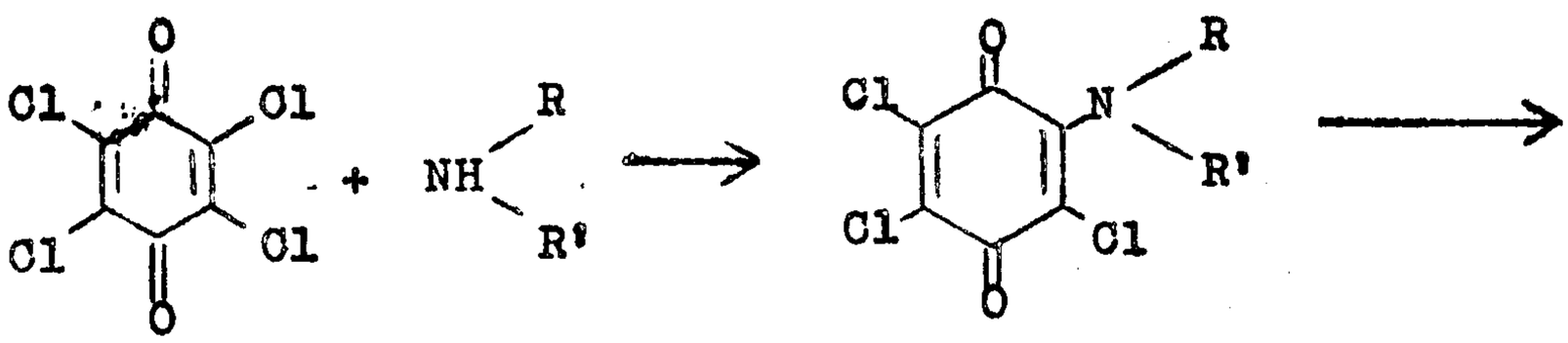


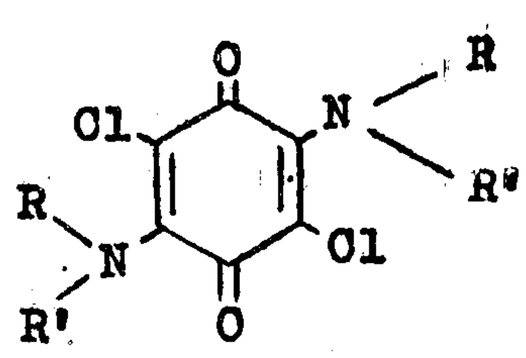
This second stage has an electronic analogy to the C-alkylation of β-dialkylaminocrotonic esters by alkyl halides[1].

Various mechanisms were suggested for the first stage which include hydride ion transfer from the amine to the quinone followed by loss of a proton[4], or a charge transfer complex of the tertiary amine with chloranil is formed followed by one electron transfer[5].

From the above equations it is obvious that a primary requisite for such a reaction to take place is the presence of the two carbon flexible chain on the tertiary nitrogen. This moiety, present in the structures of N-ethyl drugs examined is lacking from the analogeus N-methyl analogues.

On the other hand, primary and secondary amines react with chloranil to give aminoquinones which show different colors ranging from orange to violet and absorbing at different wavelengths[6-9].





Selectivity of the Analytical Reaction

Under the experimental conditions mentioned, only the N-ethyl drugs gave the blue colored chromogen. Table II illustrates some of the drugs tested and did not develop a blue color with chloranil.

Table II. Drugs which gave a negative response

Class	Example
N-Methyl	N-methylpiperidine, N-methylpipecoline, chloropyramine, phinermamine, diazepam, and clofedanol.
N-Propyl	Probenicid, and prilocaine.
Amides	Propanidid, and crotamiton.
Quaternaries	Ciclonium and valethamat bromides.

The failure of production of blue quinones on treatment of 1-methylpiperidine and 1-methylpipecoline with chloranil indicates that dehydrogenation of the piperidine ring is difficult and that the reaction requires a flexible N-ethyl group.

Quantitative Applications

The suitability of the proposed method for the determination of tertiary N-ethyl pharmaceutical amines was tested by analyzing replicate aliquots of standard solutions of various amines using the general procedure. The amount of the drug in each case was calculated with reference to the corresponding calibration curves and applying equation : $A = a + bC$ where C is concentration of the drug in the aliquot in mg per ml of final dilution, A is the absorbance, and where b and a are the slope and intercept respectively of the calibration curves calculated by the method of least square (Table_III).

The results of these replicate analyses shown in the same table reveal the suitability of the proposed method for the determination of tertiary N-ethyl pharmaceutical amines with high accuracy and precision

The procedure was also applied to analyze a simple commercial preparation of pentoxyverine citrate in the form of a cough syrup. The results (Table-IV) illustrates the applicability of the proposed method for the assay of this

N-ethyl drug with same accuracy and precision found in the analysis of the working standards.

Table III- Quantitative Determination of N-ethyl amines

Amine	a	b	r	Mean* Recovery %	SD ±
N-Ethyl piperidine	-0.041	2.24	0.998	99.89	2.74
Etafedrine	00.048	1.77	0.995	99.18	2.91
Pentoxyverine	0.325	0.39	0.938	99.91	3.11
Dicycloverine	0.344	0.15	0.945	99.73	1.56
Oxeladine	0.298	0.30	0.982	100.08	2.71
Etamiphylline	00.014	0.04	0.972	99.45	2.71
Myrtecaine	0.106	0.41	0.999	99.93	2.20
Tolycaine	0.014	0.08	0.997	101.13	2.25
Camylofine	0.014	0.39	0.999	99.77	1.38
Fencamfamine	-0.011	0.05	0.990	99.87	1.91

* Average value of at least five determinations.

Table IV- Analysis of Pentoxyverine Syrup(a)

Label claim mg/100 ml	Found* %	SD ±	Added standard mg/100 ml	Total* recovered %	SD ±
150	95.6	0.74	150	100.06	2.79

(a) Toolase syrup (CID), 7.5 mg of pentoxyverine chloride per 5 ml.

* Average of at least five determinations.

Limitations

The proposed method is fairly sensitive and selective for tertiary amines containing N-ethyl group. The procedure, however, must be considered nonspecific with regards to

degradation products containing the N-ethyl mäiety.

The free bases must be liberated from their salts and this may require an extracting solvent other than benzene which must be evaporated, and the bases are then dissolved in benzene.

Some N-ethyl drugs e.g. tolycaine gave a weak response.

CONCLUSION

The analytical method presented is essentially a microprocedure with fair sensitivity (0.004 - 1.3 mg per ml), good accuracy (99-101% recovery), and precision (SD ± 0.74-3.11%).

The method offers a relatively simple and rapid means of analysis of some pharmaceutical N-ethyl amines in admixture with primary or secondary amines. It also offered an increase in sensitivity of assay up to 50 folds relative to the natural UV bands of the drugs examined.

The reagent is available commercially or easily synthesized, and its solutions are stable at room temperature for convenient periods. The colors formed are also stable.

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استخدام القياسات الضوئية فى التقدير الدقيق الحساس للمركبات التى تحتوى على مجموعة ن ـ ثيل

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تم فى هذا البحث استحداث طريقة للتحليل الطيفى للمركبات التى تحتوى على مجموعة ن ـ الاثيل الثلاثية وذلك عن طريق تفاعل هذه المجموعة مع مادة الكلورانيل ، حيث ينتج عن ذلك التفاعل لون ازرق له درجة امتصاص قصوى عند طول موجه محدد .

وتتميز هذه النواتج بأن لها معدلات امتصاص عاليه.

وتحت الظروف التى يتم فيها التفاعل فان مركبات النيتروجين الرباعية وكذلك المركبات التى تحتوى على مجموعة ن ـ الثيل لاتتدخل ولاتتفاعل مع هذا الجوهر ، ومن المعروف ان تلك المركبات غالبا ماتوجد فى مخاليط تستخدم فى العلاج مع مركبات ن ـ الاثيل ، وباستخدام هذه الطريقة يمكن التعيين الدقيق الحساس لهذه المركبات فى الصورة النقية وفى المستحضرات الصيدلية التجارية ، كما تضمن البحث دراسة تاثير متغيرات التفاعل واقتراح التعيين الطيفى للطريقة مع شرح ميكانيكية التفاعل.