Bull. Pharm. Sci., Assiut University.
Vol 11, Part 1, pp 18-28 (1988).

SPECTROPHOTOMETRIC DERERMINATION OF CERTAIN PHARMACEUTICAL SECONDARY AROMATIC AMINES USING 3-METHYLBENZOTHIAZOLIN-2-ONE HYDRAZONE

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ABSTRACT

Out of 7 pharmaceutical secondary aromatic amines tested, the oxidative coupling reaction of 3-methyl-benzothiazolin-2-one hydrazone with aromatic amines in the presence of iron (III) ammonium sulphate was applied to the determination of folic acid, primaquine phosphate and lucanthone hydrochloride The wavelengths of maximum absorption were 530 nm. (for primaquine phosphate), 580 nm (for lucanthone hydrochloride) and 660 nm (for folic acid) with molar absorptivities of $4.6x10^4$, $2.3x10^4$ and $5.0x10^4$ respectively. Beer's law was obeyed in the concentration ranges of 2-10 ug ml^{-1} (for primaquine phosphate), 2-16 ug ml^{-1} (for lucanthone hydrochloride) and 1-10 ug ml^{-1} (for folic acid). The proposed method was applied for the quantitative determiantion of folic acid, primaquine phosphate and lucanthone hydrochloride in pure form as well as in tablets. Results of analyses were compared with the B.P. and U.S.P. procedures.

INTRODUCTION

Pharmacopeial methods for the assay of secondary aromatic amines usually include nitritometric[1-3] UV-spectrophotometric[4] and HPLC techniques[2].

In our laboratory, 3-methylbenzothiazolin-2-one hydrazone (MBTH) has proved to be a useful and sensitive chromogenic reagnet for the spectrophotometric determiantion of some

pharmaceutical phenols[5,6], local anaethetics having aromatic amine function[7] and sulpha drugs[8] giving stable intensely coloured products.

The present work was undertaken to investigate the ability of various pharmaceutical secondary aromatic amines to couple with MBTH in the presence of a suitable oxidant e.g. iron (III) ammonium sulphate. The possibility of utilizing this reaction for the assay of these compounds, both in pure and in some pharmaceutical preparations, was studied.

EXPERIMENTAL

Materials:

Pharmaceutically pure folic acid (Merck, W. Germany), primaquine phosphate (Imperial Chemical Industries Ltd, UK), lucanthone hydrochloride (Bayer, Switzerland), proguanil hydrochloride(ICI, UK), amodiaquine hydrochloride (Parke Davis, U.S.A.), mepacrine hydrochloride (May and Baker, UK) and chloroquine phosphate (Sigma, U.S.A.) were used as working standards.

The following commercial pharmaceutical preparations were analysed :

Folic acid tablets (Nile Co, Egypt) labelled to contain 5 mg of folic acid per tablet.

Primaquine tablets (Misr Co, Egypt) labelled to contain 15 mg of primaquine base (as Phosphate) per tablet.

Synthetic mixtures of primaquine phosphate and amodiaquine hydrochloride in the ratios 1 : 1 and 1 : 10 were also prepared and analysed.

Reagents :

1- MBTH solution, 0.35% W/V in distilled water; freshly prepared.

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2- Hydrochloric acid, 0.3 M; prepared from analytical reagent grade acid.

3- Ammonium iron (III) sulpahte solution; a 0.5 % W/V solution in 0.3 M HCl.

All solvents used were of spectroscopic grade (Merck, FRG).

Apparatus :

1- Uvidec-320 spectrophotometer (JASCO, Tokyo, Japan).

2- Thermostatically controlled water bath (mLw, GDR.)

Preparation of Sample Solutions :

Powdered Forms :

Dissolve an accurately weighed amount of the drug (about25 mg) in 0.3 M HCl and make up the volume to 25.0 ml with 0.3 M HCl. Dilute this solution with 0.3 M HCl to contain about 50 ug ml^{-1} of either folic acid or primaquine phosphate or 100 ug ml^{-1} of lucanthone hydrochloride. The solutions must be freshly prepared. They are stable for 24 hours.

Tablets and Synthetic Mixtures :

Shake an accurately weighed portion of the powdered tablets or synthetic mixture, equivalent to 25 mg of the drug, with 25 ml of 0.3 M HCl for 10 minutes (warming is necessary in case of folic acid). Filter the resulting mixture and complete the filtrate and washings to 50.0 ml with 0.3 M HCl Dilute this solution with 0.3 M HCl to contian about 50 ug ml^{-1} of folic acid or primaquine phosphate or 100 ug ml^{-1} of lucanthone hydrochloride.

Procedure :

Transfer 1.0-ml volume of the sample solution into a 10-ml volumetric flask and add 1 ml of MBTH solution and 2 ml of ammonium iron (III) sulphate solution. In case of folic acid, mix the contents well and heat for 3 to 4 minutes in a water bath at 60 ± 5°C, then cool to room temperature and dilute to volume with ethanol. In cases of lucanthone hydrochloride and primaquine phosphate, mix the contentes well and leave for 10 minutes at 20 ± 5°C, then dilute to volume with ethanol. Measure the absorbance of the solution

at 530 nm (primaquine phosphate), 580 nm (for lucanthone hydrochloride) or 660 nm (for folic acid) against a similarly treated blank, replacing the sample solution with an equal volume of 0.3 M HCl.

Calculate the concentration of the drug in the sample solution from the properly constructed calibration graph.

RESULTS AND DISCUSSION

Reaction Involved :

The reaction of MBTH with aromatic amines in the presence of an oxidant proceeds via oxidative coupling[9]. The reaction mechanism has been described in detail in previous publications[7,8].

In the present work, 7 pharmaceutical secondary aromatic amines were tested through colour formation with MBTH and ammonium iron (III) sulphate. Such amines include folic acid, primaquine phosphate, lucanthone hydrochloride, amodiaquine hydrochloride, mepacrine hydrochloride, proguanil hydrochloride and chloroquine phosphate. Among these compounds, only the first three gave coloured products with MBTH under the reaction conditions used. Absorption spectra of the resulting coloured products are shown in Fig. 1.

Negative respone of some secondary aromatic amines may be due to : (a) the absence of vacant o-or p-position to the alkylamino group in the 9-position (mepacrine hydrochloride)or (b) the formation of very stable resonant cations[10-13] (amodiaquine, chloroquine and proguanil salts).

The continuous molar variation of folic acid, primaquine phosphate or lucanthone hydrochloride and MBTH shows that the former two drugs interact with MBTH in the ratio 1:2 whereas the latter interacts in the ratio 1:1 (Fig. 2). In addition, the molar absorptivity of the coloured product from either folic

acid (5.02×10^4) or primaquine phosphat (4.63×10^4) is about two times that obtained from lucanthone hydrochloride (2.32×10^4) (Fig. 1).

These data can be easily interpreted due to the presence of one vacant o-position, available for electrophilic substitution in lucanthone and two such positions in either folic acid or primaquine phosphate.

Optimisation of Variables :

One millilitre of 0.35% MBTH solution was found to be sufficient for maximum colour intensity. Increasing reagent concentrations did not affect the colour intensity.

Several oxidizing agents were investigated e.g. ammonium iron (III) sulphate, ammonium cerium (IV) sulphate, potassium hexacyanoferrate (III), potassium dichromate, potssium bromate, potassium iodate, N-bromosuccinimide, hydrogen peroxide and bromine. Equimolar solutions of these oxidants (0.01 M) were utilized in the procedure for the three studied drugs. Iron (III) ions, giving the highest absorption intensity were used in a quantity equal to 2 ml of 0.5 % solution of ammonium iron (III) sulphate.

A development time of 10 ± 4 minutes at 20 ± 5°C is optimum for maximum absorption intensity of the products obtained for primaquine phosphate and lucanthone hydrochloride. In case of folic acid it is necessary to heat the raction mixture for 3-4 minutes in a water bath at 60 ± 5°C. The colours obtained from the three drugs are stable for at least 12 hours.

Among water, methanol, ethanol, propan-1-ol, propan-2-ol and dimethyl sulphoxide, ethanol is the best solvent for the three investigated drugs.

Quantification :

A linear correlation was found between the absorbance at λ_{max} for each chromogen and concentration in the following ranges : 1-10 ug ml^{-1} for folic acid, 2-10 ug ml^{-1} for primaquine phosphate and 2-16 ug ml^{-1} for lucanthone hydrochloride. Regression analysis of Beer's plots gave the following linear regression equations :

For folic acid: $A_{660}=0.0233 + 0.1137\ C$ ($r=0.9999$).
For primaquine phosphate : $A_{530}=0.0397 + 0.1016\ C$ ($r=0.9999$).
For lucanthone hydrochloride : $A_{580}=-0.0334+ 0.0616\ C$ ($r=0.9997$).
where C is the concentration of the respective drug in the final assay solution in ug ml^{-1}. These equations were adopted for application of the method to pure drugs and tablets.

The reproducibility of the proposed method was determined by analyzing replicate samples of series of solutions containing the following concentrations : 10, 20, 40, 60, 80, and 100 ug ml^{-1} of folic acid, 20, 40, 60, 80, and 100 ug ml^{-1} of primaquine phosphate and 20, 40, 60, 80, 120 and 160 ug ml^{-1} of lucanthone hydrochloride. The average relative standard deviations for 1-10 mcg of folic acid did not exceed 0.85 %, for 2-10 mcg of primaquine phosphate did not exceed 0.94% and for 2-16 mcg of lucanthone hydrochloride did not exceed 1.06%.

Application to Bulk Drugs, Synthetic Mixtures and Tablets :

The suggested method was applied to the quantitative determination of folic acid, primaquine phosphate and lucanthone hydrochloride in pure form, synthetic mixtures of primaquine phosphate with amodiaquine hydrochloride and in tablets of folic acid and of primaquine phosphate. Amodiaquine hydrochloride solutions did not give any dectable colours up to a concentration of 0.5 $mg.ml^{-1}$, when subjected to the assay procedure.

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The results of analysis of bulk drugs, tablets and synthetic mixtures were compared with those obtained from the B.P. 1980 method for folic acid and primaquine phosphate and with U.S.P. XVIII method for lucanthone hydrochloride. Data of Table 1 show well agreement of the results of the MBTH method for bulk drugs and tablets with those of the compendial procedures. Student's t-and F tests show no significant differences between the proposed and official methods. In addition, the interference caused by the presence of amodiaquine hydrochloride in synthetic mixtures analyzed by the B.P. procedure is completely absent in the proposed method (Table 1). Furthermore, the MBTH method has the advantages of rapidity and simplicity.

Table 1: Assay of Some Pharmaceutical Secondary Aromatic Amines with the MBTH and Official Methods.

Sample	MBTH method		Official method		t**	F**
	Recovery++ %	S.D.*	Recovery %	S.D.*		
Folic acid powder	99.7	0.75	99.9	1.63	0.249	4.72
Primaquine phosphate powder	99.9	0.84	100.1	1.12	0.319	1.78
Lucanthone hydrochloride powder	99.6	0.89	100.0	0.94	0.691	1.12
Folic acid tablets	97.8	0.81	98.0	1.58	0.252	3.80
Primaquine tablets	98.3	0.93	98.1	1.08	0.314	1.35
Primaquine phosphate+amodiaquine hydrochloride(1:1)	99.7	0.66	186.4	1.64		
Primaquine phosphate+amodiaquine hydrochloride (1:10)	100.1	0.87	495.8	1.99		

\+ B.P. 1980 methods for folic acid and primaquine phosphate and U.S.P. XVIII method for lucanthone hydrochloride.

++ Mean of Five determinations.

* S.D. = Standard deviation (n=5).

** Tabulated t for 8 degrees of freedom at P. 0.05= 2.31.

" F for (4,4) " " " " P 0.05=6.39.

Spectrophotometric Determination of Certain Pharmaceutical Secondary aromatic Amines Using 3-Methylbenzothiazolin-2-ONe HYdrazone.

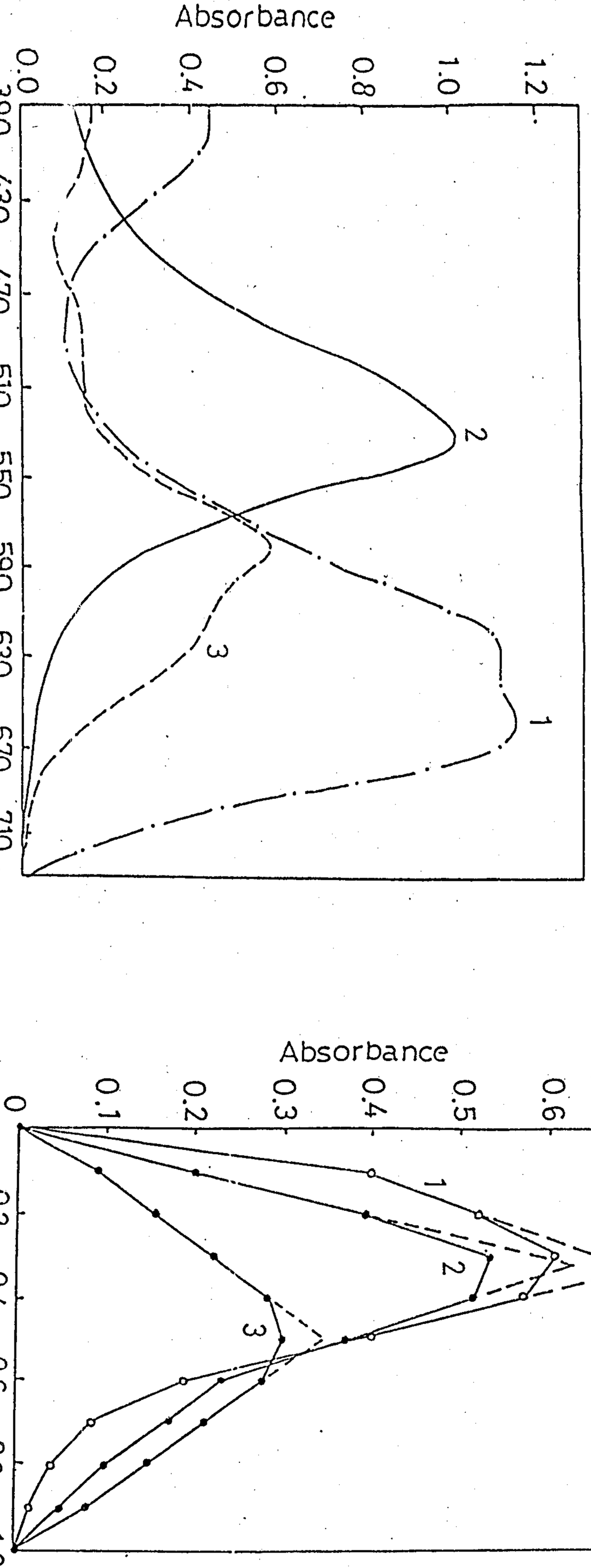

Fig. 1. Absorption spectra of the coloured products of MBTH with (1)folic acid, (2) primaquine phosphate and (3) lucanthone hydrochloride Final concentration 10 µg ml^{-1}

Fig. 2. Continuous molar variation plots obtained from solutions Folic acid (10^{-4} M), (2) Primaquine phosphate (10^{-4} M) and (3) lucanthone HCl (2×10^{-4} M) with MBTH.

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التقييم الطيفى لبعض الامينات العطرية الثانوية باستعمال ٣ ـ ميثيل بنزوثيازولين ـ ٢ ـ أون هيدرازون

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فى هذا البحث تم التوصل الى طريقة للتقدير الكمى لبعض الامينات العطرية الثانويه وهى حمض الفوليك وفوسفات البريماكين ،وهيدروكلوريد اللوكانثون . ولقد تم اختبار مجموعة أخرى من الادوية التى تحتوى على مجموعه الامين العطرية الثانوية ولكنها لم تتفاعل وهى : هيدروكلوريد البروجوانيل ، هيدروكلوريد الميباكرين ، هيدروكلوريد الامودياكين وفوسفات الكلوروكوين .

وتعتمد الطريقة على تكوين مركبات حمراء أو زرقاء أو خضراء اللون مع ٣ ـ ميثيل بنزوثيازولين ـ ٢ ـ أون هيدرازون فى وجود كبريتات الحديديك الامونيومى ، وقد وجد أن التفاعل يتم بنسبة جزيئية بين الامين والكاشف ١ : ٢ فى حالة حامض الفوليك وفوسفات البريماكين وبنسبة ١:١ فى حالة هيدروكلوريد اللوكانثون . أما أطوال الموجات التى يحدث عندها أعلى امتصاص للضوء فهى ٥٣٠ ن م (فى حالة فوسفات البريماكين) ، ٥٨٠ ن م (فى حالة هيدروكلوريد اللوكانثون) ، ٦٦٠ ن م (فى حالة حامض الفوليك) وتتراوح معاملات الامتصاص الجزيئى بين ٢٣٫٢٠٠ و ٥٠٫٢٠٠ .

وقد وجدت علاقه خطية بين درجة الامتصاص وتركيز الامين فى الحدود الاتية : ١ ـ ١٠ مكجم/مل (فى حالة حامض الفوليك) ، ٢ ـ ١٠ مكجم / مل (فى حالة فوسفات البريماكين) ، ٢٠ ـ ١٦ مكجم / مل (فى حالة هيدروكلوريد اللوكانثون) .

وقد تم تطبيق الطريقة بنجاح على بعض المستحضرات الصيدلية وقورنت النتائج مع نتائج طرق دساتير الادوية البريطانية والامريكية .

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received in 10/2/1988 - accepted in 1/10/1988