

NEW REAGENTS FOR DETECTION OF
PHENOTHIAZINE DRUGS ON THIN LAYER PLATES

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ABSTRACT

About 50 new reagents have been developed for the detection of phenothiazine drugs on TLC plates by giving different colours. Each reagent consists of an amine and an oxidant. Most of the developed reagents are very sensitive for detection of even 1 ug phenothiazine drug.

INTRODUCTION

Many reagents are available for the detection of phenothiazine drugs on paper and thin layer chromatograms. Among these reagents are sulphuric acid¹, Marquis' reagents², Dragendorff's reagent³, ceric sulphate⁴, 10% hydrogen peroxide⁵, 5% potassium permanganate⁶, 2% ferric chloride⁷, and diazotized p-nitroaniline⁸.

New reagents for detection of phenothiazine drugs on thin layer plates

The purpose of this investigation was to develop new reagents for the detection of different phenothiazine drugs. All these reagents consisted of an amine and an oxidant prepared separately. The amine may be primary or secondary (aliphatic or aromatic). The oxidants used are N-bromosuccinimide (NBS), ceric sulphate, chlorine water and potassium periodate.

EXPERIMENTAL

Materials and Reagents

Pure drug samples examined were of pharmaceutical grade, obtained as gifts from various manufacturers, and were utilized as working reference compounds without further treatment. All solvents were of analytical grade.

Precoated silica gel plates (0.25 mm) without fluorescence (Merck, Darmstadt, FRG) were used without prior activation.

Oxidant solutions: 3% w/v aqueous solution of NBS, ceric sulphate and potassium periodate. Saturated solution of chlorine in distilled water.

Amine solutions-3% v/v or w/v Methanolic solution of each amine stated in the Tables.

Developing solvents⁹: Ammonium acetate: water: methanol (3:20:100, w/v/v).

Phenothiazine Drug Solutions:

Methanolic solution of each phenothiazine drug (Tables 1-5) (1 mg/ml) was freshly prepared.

Procedure:

- a- One to five μ l of methanolic solution of each phenothiazine drugs were spotted on thin layer plate and allowed to dry. After that 5 μ l from each studied amines was overspotted on each applied spot and left to dry. Finally 5 μ l NBS was spotted on each previously mixed spot.
After air drying the produced colours were examined in day light.
This method was repeated three times but using instead of NBS, ceric sulphate or chlorine water or potassium periodate.
- b- The above procedure was repeated by using the aforementioned reagents as sprayers. This was done by spraying the amine solution upon the developed chromatogram, followed by the oxidant solution. After air drying, the developed colours were observed in day light.

RESULTS AND DISCUSSION

About 50 new reagents for detection of phenothiazine drugs on thin layer plates have been developed

New reagents for detection of phenothiazine drugs on thin layer plates

(Table 1-4). Each reagent consists of a combination of an amine (aromatic or aliphatic; primary or secondary) and an oxidant. Most of the developed reagents gave various colours with all tested phenothiazine drugs. The sensitivity for detection of these drugs was found to range between 1-5 μ g. The colour developed immediately and remained stable for at least 24 hours.

The type of oxidant plays an important role in the reaction. From 13 oxidants tested (Table 5) only four produced different colours with phenothiazine drugs in presence of the specific amine. The other oxidants gave the same colour or fail to develop any colour with all the examined drugs.

It was found that any variation in the amine or oxidant coupled reagents produced different colours with the same phenothiazine. Moreover; it was observed that the sensitivity of an oxidant in presence of the specific amine decreased in the following order NBS > **ceric sulphate** > chlorine water > potassium periodate.

Application of these reagents in the form of sprays for detection of phenothiazines on thin layer plates gave favourable results. Some developed reagents were applied successfully for the detection of phenothiazine drugs in certain pharmaceutical preparations without

prior separation.

Trials have been made to extend the application of the developed reagents for detection of intact phenothiazine drugs in biological fluids. This was done by using combination of morpholine and NBS reagents for detection of promethazine HCl in urine. The produced blue colour is identical with that obtained with reference drug applied on the same plate.

The developed reagents are simple in preparations and handling compared with the more elaborate and corrosive previously reported reagents.

Owing to the large number of the developed reagents, and the different colours produced with them when reacted with phenothiazines, it is so difficult to investigate reaction mechanisms which are not clear. However, it is suggested that phenothiazines interact with amines in presence of oxidant to form methylene blue-like dyes.

Further work on the determination of phenothiazines and the nature of products formed in the reaction with certain selected developed reagents is in progress.

New reagents for detection of phenothiazine drugs on thin layer plates

Table 1: Colours of TLC spots for phenothiazines with different amines and N-bromosuccinimide reagent.

Phenothiazines	Amines *												
	1	2	3	4	5	6	7	8	9	10	11	12	13
PMH	Bl	V	R.V	V	Bl	V	V	Bl	Bl	Bl	R.V	Gr	R.V
Ph	Pi	Pi	Pi	V	V	V	V	V	Bl	Pi	Gr.V	Gr.V	V
AMT	R	Nc	R	V	Or	V	V	V	Bl	R	Gr.Bl	Gr.Bl	R
MH	R	R	R	V	Or	V	V	V	Bl	R	Nc	Gr.Bl	R
PM	R	R	R	V	Or	V	V	V	Bl	R	Nc	Gr.Bl	R
PCPM	Nc	R	R	Or	Nc	Or	Or	Nc	Bl	R	Nc	Gr.Bl	R
CPH	Pi	Pi	Pi	V	V	V	V	Nc	R	R	Gr	V	R
LMPM	V	V	R.V	V	V	V	V	Bl.V	Bl.V	V	Gr	V	R.V
TEPM	Bl	Nc	Bl	V	Gr	V	V	Bl	Bl	R	Gr.Bl	Gr.Bl	Bl
TRH	Bl	Bl	Bl	Bl	V	V	Bl.V	V	Bl.V	Bl	Bl	V	Bl.V
TFPH	R	R	R	Or	Or	Or	Or	Or	R	R	Gr	V	R
BPM	Or	Nc	Nc	Or	Nc	Or	R	Or	Nc	R	Gr	V	Nc
FPH	Nc	Nc	Or	Or	Y	Or	Or	Or	Nc	R	Gr	V	R
TPP	Or	Nc	Nc	Or	Nc	Or	Or	Or	R	R	Gr	V	R
PC	Or	Nc	Nc	Or	Or	Or	R	Or	Nc	R	Gr	V	Nc
OMT	R	R	V	Or	Nc	R	Or	Or	R	R	Gr	V	R

Key: Amines, 1- methylamine, 2- ethanolamine, 3- glycine, 4- benzocaine, 5- p-nitroaniline,

6- m-nitroaniline, 7- p-chloroaniline, 8- p-aminobenzoic acid, 9- morpholine, 10- piperazine, 11- piperidine, 12- N-ethylamine, 13- pyridine.

Phenothiazine, PMH: promethazine HCl, PH: promazine HCl, AMT: alimemazine tartrate

MH: mepazine HCl, PM: perazine maleate, PCPM: prochlorpromazine HCl

CPH: Chlorpromazine HCl, LMPM: levomepromazine maleate, TEPM: thietilperazine maleate, TRH: thioridazine HCl, TEPH: trifluorpromazine HCl, BPM: butaperazine maleate, TRH: thioridazine HCl, TPPB: thiopropazine base, PC: pericyazine maleate, FPH: fluphenazine HCl, TTPB: thiopropazine base, PC: pericyazine maleate, OMT: oxememazine.

zine base, OMT: oxememazine.

Bl: blue, Gr: green, Nc: No colour, Or: orange, Pi: pink, R: red,

V: Violet, R.V: reddish violet, Bl.V.: bluish violet, Gr.Bl: greenish blue

Colours,

Table 2: Colours of TLC spots for phenothiazines with different amines and ceric sulphate reagent.

	Phenothiazine													
	Amines *	1	2	3	4	5	6	7	8	9	10	11	12	13
PMH	Bl	R.V	Bl	Bl.V	V	Bl.V	V	V	V	Bl	Bl	R.V	Bl.V	Bl
PH	R	R	R	V	V	V	V	V	V	Bl	R	R	Gr.V	R
AMT	R	R	R	Pi	Pi	Pi	Pi	V	R	R	R	R	R	R
MH	R	R	R	Pi	Or	Pi	Pi	V	V	R	R	R	R	R
PM	R	R	R	Pi	Or	Pi	V	V	V	Bl	R	R	R	R
PCPM	R	R	Bl	V	Or	R	V	V	R	R	R	R	Gr	R
CPH	R	R	Pi	Pi	Pi	Pi	Pi	V	V	Pi	R	R	V	Pi
LMPM	V	V	V	V	V	V	Bl	Bl	Bl	V	V	V	Gr.V	V
TEPM	Bl	R	Bl	Bl	Gr	Bl	Bl	Bl	Bl	Bl	Bl	R	Gr	Bl
TRH	Bl	Bl	R.V	V	V	Or	Bl	Bl	Bl	Bl	Bl	Bl	Gr.V	R.V
TFPH	Or	R	Or	V	Or	Or	V	V	Or	Nc	Or	Or	Gr	Or
BPM	R	Nc	R	V	R	R	V	V	V	R	R	Nc	Gr	R
FPH	Or	Nc	Bl	R.V	Or	R	V	V	R.V	Or	Or	Nc	Gr	Or
TPP	R	R	R	R.V	Or	R	R.V	R.V	V	R	R	R	Gr	R
PC	R	R	R	V	R	R	V	V	R.V	Or	R	R	Gr	Or
OMT	R	R	Bl.V	R.V	R	R.V	V	V	R.V	R	R	R	Or	R

Key: as under Table 1.

Table 3: Colours of spots for phenothiazines with different amines and chlorine water reagent

	Phenothiazine-		Amines *													
	zine		1	2	3	4	5	6	7	8	9	10	11	12	13	
PMH			R.V	Bl	R.V	Bl	V	Bl	Bl	V	Bl	Bl	Bl	Bl	R.V	R.V
PH			R	R	R	V	R.V	Bl	Bl	V	Bl	Bl	Bl	R	R.V	R
AMT			R	R	R	R	R	R	R	R	R	R	R	R	Gr	R
MH			R	R	R	R	R	R	R	R	R	R	R	R	Gr	R
PM			R	R	R	R	R	R	R	R	R	R	R	R	Gr	R
PCPM			R	R	Bl	V	Or	R	R	R	R	R	R	R	V	R
CPH			R	R	R	R	R	R	R	R	R	R	R	R	V	R
LMPM			V	V	V	V	V	V	V	V	V	V	V	V	V	V
TEPM			Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Gr	Bl	Bl
TRH			Bl	Bl	Bl	Bl.V	Bl	Bl.V	Bl	Bl	Bl	Bl	Bl	Bl.V	Bl	Bl
TFPH			R	Nc	R	Or	R	R	Or	V	R	R	R	Gr	R	R
BPM			Or	Nc	Or	V	Or	V	Or	V	V	Or	Nc	V	Nc	Nc
FPH			R	R	Bl	R	Or	Or	Or	R	R	R	R	Gr	R	R
TPP			R	R	R.V	V	R	Or	R	R.V	R	R	R	Gr	R	R
PC			Nc	Nc	Or	Or	Nc	Nc	V	V	Or	Or	Nc	Gr	Nc	Nc
OMT			R	R	Bl	V	Or	Or	R	R	R	R	R	Gr	R	R

Key: as under Table 1.

Table 4: Colour of TLC spots for phenothiazines with different amines and potassium periodate reagent.

Phenothiazine	Amines *												
	1	2	3	4	5	6	7	8	9	10	11	12	13
PMH	Bl	Nc	R	V	V	V	V	V	Bl	Nc	Nc	V	R
PH	R	Nc	R	V	R	R	V	V	Bl	Bl	Nc	V	R
AMT	R	Nc	R	R	R	R	R	R	Nc	Bl	Nc	Gr	R
MH	R	Nc	R	R	R	R	R	R	Nc	R	Nc	Gr	R
Pm	R	Nc	R	Or	R	R	R	R	Nc	R	Nc	Gr	R
PCPM	R	Nc	R	Pi	Nc	R	Or	R	Nc	Nc	Nc	Gr	R
CPH	R	Nc	R	R	Or	R	V	V	R	R	Nc	Bl	R
LMPM	R	Nc	R	V	R	V	V	V	V	R	Nc	Nc	R
TEPM	Bl	Nc	Bl	Bl	Bl	Bl	R	Bl	Nc	Bl	Nc	Gr	Bl
TRH	Bl	Nc	Bl	V	V	Bl.V	V	Bl.V	V	Nc	Nc	V	Bl
TFPH	V	V	R	Or	V	V	Or	V	Nc	Nc	Nc	Gr	Nc
BPM	V	Nc	R	Nc	Nc	Nc	Nc	Or	Nc	Nc	Nc	Gr	Nc
TPH	Nc	Nc	Nc	Nc	V	V	V	V	Nc	Nc	Nc	Gr	Nc
TPP	Nc	Nc	V	Gr	R	R	Or	R	Nc	Nc	Nc	Gr	Nc
PC	Nc	Nc	R	Nc	R	Nc	R	Nc	Nc	Nc	Nc	Gr	Nc
OMT	R	R	Bl	R	R	Nc	Or	Pi	Nc	R	R	Gr	R

Key: as under Table 1.

Table 5: Response of the studied phenothiazines to different amine and oxidant reagents.

No.	Oxidant*	Colours on TLC with all studied phenothiazine drugs
1	N-Bromosuccinimide	different colour
2	Ceric sulphate	,, ,,
3	Chlorine water	,, ,,
4	Potassium periodate	,, ,,
5	Potassium permanganate	All brown spots
6	Potassium dichromate	,, ,, yellowish green
7	Iodine solution	,, ,, dark brown
8	Ferric chloride	,, ,, red
9	Potassium ferricyanide	,, ,, grey
10	Hydrogen peroxide	no colour
11	Potassium chlorate	,, ,,
12	Potassium bromate	,, ,,
13	Potassium iodate	,, ,,

* Each oxidant was tested with all studied amines

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كواشف جديدة لادوية الفينوثيازين على رقائـق الكروماتوجرافيا
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