ISSN : 0974-7419 Volume 13 Issue 9

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 9(1) 2010 [332-336]

Thin layer chromatographic separation and identification of certain phenothiazines

S.Thangadurai*, M.Suresh Kannan, A.Dhanalakshmi

Post Graduate Studies and Research Department of Chemistry, Raja Doraisingam Government Arts College, Sivagangai – 630 561. Tamil Nadu, (INDIA)

E-mail : drstdurai@gmail.com

ABSTRACT

Phenothiazines have been reported to be rather often the cause of acute intoxications, either in overdoses or in associations with other medicines and / or alcohol. These drugs are frequently encountered in the field of forensic toxicology because of their relatively narrow safe ranges of therapeutic doses. In this paper we have described the thin layer chromatographic separation of four of the phenothiazine drugs (prochlorperazine, promethazine, chlorpromazine, trifluroperazine) as such and also to assess their degradation products after an "in situ" acid hydrolysis on the chromatoplate. Our aim was to develop a simple, rapid and efficient method for separation of these phenothiazine drugs. Using pre-coated silica gel G UV_{254} as stationary phase and selecting ten different mobile we succeeded in the separation of the studied phenothiazines. Each phenothiazine can be separated from the others by using an appropriate mobile phase. Any of the phenothiazines can be identified by combining the results obtained with different mobile phases.

KEYWORDS

Phenothiazines;
Thin layer chromatography;
Identification, detection;
Acid hydrolysis.

INTRODUCTION

The Phenothiazine group of drugs is a very effective major tranquilizer. It played a major role in changing the pattern of treatment, and the " life style" of the psychiatric patient. Much less restraint or detention is now required; and this permits considerably more treatment on an outpatient basis with excellent results. Since so many millions of doses are now prescribed, it should be no surprise that they are found in so many homes and that frequently questions of overdose or side reactions occur.

Toxicological data on lethal poisonings of humans by phenothiazine drugs are scarce. Since the toxicity of e.g. chlorpromazine in rats, mice and guina pigs is comparable to that of certain barbiturate, one could expect a high incidence of suicides by the phenothiazine type of drugs. These compounds are quite commonly used by patients suffering from depressions and similar mental disorders in which suicides are relatively frequent. The widespread use of these drugs also contributes to cases of accidental poisoning, especially in children.

Phenothiazine drugs are widely used[1] as tranquilizers, antihistaminics, antiemetics and anticholinergic. Phenothiazines have been reported[2] to be rather often the cause of acute intoxications, either in overdoses or

in associations with other medicines and / or alcohol. The drugs are frequently encountered[3] in the field of forensic toxicology because of their relatively narrow safe ranges of therapeutic doses. Overdosage with promethazine preparations is not very common, with fatal poisonings generally resulting from the additive effects of the drug in the presence of other central nervous system (CNS) depressants.

Bonnichsen *et al*.[4] have reported the concentration and distribution of phenothiazine drugs in the body fluids and tissues of sixty six autopsy cases are reported. The cases are presented in three groups: (i) those in which poisoning by a phenothiazine drug was the sole cause of death, (ii) cases with "mixed poisoning" by phenothiazine derivatives and other drugs or ethanol, and (iii) instances of death by physical means where phenothiazine drugs were found but had not directly caused death. From the data presented it seems that the level of this kind of drugs in the liver can be of value for evaluating the severity of intoxication. Rasulev *et al*.[5] have investigated the post-mortems (stomach, intestine, kidney and urine) for the chlorpromazine poisoning, using methods of surface ionization-mass spectrometry (SI/MS) as well as chemical, optical, TLC and GC/MS. Cimbura[6] had briefly discussed the methods of analysis of phenothiazine drugs in biological material and the main advantages and limitations of the methods have also been reviewed. Steinbrecher[7] have studied collaboratively with eight laboratories for the identification of phenothiazine derivative drugs by a TLC method. Each collaborator was examined twenty phenothiazine drugs. Steinbrecher[8] had studied on the thin-layer chromatographic identification of twenty phenothiazine bases official in the U.S.P. XX. On the basis of four chemically different solvent systems and the color developed with a spray reagent, all phenothiazines could be distinguished from each other. For greater precision, relative R_f values have been used. He has also evaluated these solvent systems, the relative efficiencies of various brands of commercially prepared thin-layer plates. Wieter *et al*.[9] have studied the TLC separation of twelve drugs from three pharmaceutical groups: phenothiazines and tri and tetracyclic antidepressants. Jain *et al*.[10] were also presented two newly developed, TLC solvent systems to separate and identify commonly used phenothiazines in the presence of other drugs in urine specimens. Temerdashev *et al*.[11] have proposed the chromatographic behavior of phenothiazine derivatives. The effect of interactions of different types on the value of their chromatographic retention is estimated; the relation of the obtained coefficients of equations for the substances under study is analyzed. The obtained empirical dependences are used to calculate the R_f values. Since the beginning, planar chromatography had a wide application in the pharmaceutical field. Nowadays, in addition to qualitative analysis, TLC is also used for quantitative purposes. However, it is not used in quantitative analysis[12], in spite of its precision, accuracy, and reliability reached as an instrumental technique. It provides a quick, economical, and reliable method for rapid screening of pharmaceuticals. In this study our aim was to develop a useful rapid and sensitive TLC method for identification and separation of four of the phenothiazine drugs (prochlorperazine, promethazine, chlorpromazine, trifluroperazine), and also to assess their degradation after an "in situ" acid hydrolysis on the chromatoplate. The detection by TLC without prior hydrolysis was therefore also tried. The present work was undertaken in order to use unimpergnated, thin layers of Silica gel G UV_{254} as an adsorbent and to develop economical and convenient methods for the separation and detection of phenothiazine drugs.

EXPERIMENTAL

Materials

Silica gel G UV_{254} precoated commercial plates (20×20 cm, 0.25 mm thickness) were supplied by Merck (Darmstadt, Germany). A 10 µL Hamilton syringe calibrated at 0.1 µL intervals was used.

Substances were applied to the plates with the aid of 10 µL Hamilton syringe; spots were visualized using a spray gun.

Reagents and chemicals

Phenothiazine drugs prochlorperazine, promethazine, chlorpromazine and trifluroperazine, drugs were of pharmaceutical grade. All of the solvents and reagents used were of analytical reagent grade. Deionized water was used to prepare all solutions. Freshly prepared so-

lutions were always employed.

PREPARATION OF REAGENTS

Dragendorff's reagent

Stock solution: Dissolve 1.7 g of basic bismuth nitrate and 20 g of tartaric acid in 80 mL of deionized water; dissolve 16 g of potassium iodide in 40 mL of deionized water; mix equal volumes of these two solutions. This solution is stable for several months when stored in a refrigerator.

*Spray solution:*Mix 1 part of stock solution with 10 parts by volume of 1.33 mol/l aqueous tartaric acid solution.

Iodine methanol solution: One gram of iodine is dissolved in 125 mL of methanol.

Sulphuric acid (10%) : 10 mL of sulphuric acid dissolved in 90 mL of distilled water

Sample preparation

A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 10 mg of phenothiazine drugs were taken and dissolved in 10 ml of methanol and sonicated for five minutes. About 20 mL of methanol was added and sonicated for further 5 minutes. The mixture was mixed well for 2 minutes and transferred to a 100 mL volumetric flask through a Whatmann No. 40 Filter paper. The residue was washed thrice with methanol and the combined filtrate was made up to the mark with methanol. The sample solution thus prepared was diluted with methanol to get the solutions containing different concentrations of phenothiazine drugs. These solutions were stored in well closed vessels and direct contact with light was avoided.

Procedure

The plates were divided into strips 1.5 cm wide and solute spots were applied using a calibrated Hamilton syringe. Then, 2-10 µL each of the drug solutions were spotted on the TLC plates as separate spots. The plates were developed in a closed glass sintered chamber containing developing solvents (listed in TABLE 1), having 30 minutes prior saturation at 25-30º C temperature. The solvent in the chamber was allowed to reach the lower edge of the adsorbent, though the spot points were not allowed to be immersed. The cover was put in place, and the system was maintained until the solvent ascended to a point 10 to 15 cm above the initial spots; this usually required about 30 to 90 minutes. After a plate had been run, the plate was removed from the developing chamber and dried in air. Non-destructive procedure, such as the use of ultraviolet light (both 254 nm and 356 nm) was then used for the localization of separated spots. After that plates were sprayed by two different methods using chromogenic reagents; (i) dragendorff's reagent (ii) iodine/methanol reagent and (iii) sulphuric acid (10%) for the identification of phenothiazine drugs. The R_f values for the separated drugs were recorded. All experiments were carried out at room temperature.

TABLE 1: Developing solvent system for Phenothiazine drugs.

S.No	System	Developing solvent	Composition[a] (v/v)
1.	I	Chloroform: methanol	50: 50
2.	II[b]	Methanol: ammonium hydroxide	100: 2.5
3.	III	Chloroform: acetone	90:10
4.	IV	Ethyl acetate	100
5.	V	Ethyl acetate: methanol: aq.ammonia	70: 20: 10
6.	VI	Chloroform: toluene: methanol	40:50:10
7.	VII	Chloroform: ethanol: aq.ammonia	75:15:5
8.	VIII	Cyclohexane: toluene: dimethylamine	75:15:10
9.	IX	Hexane: ethyl acetate: methanol: aq.ammonia	50:50:10:0.3
10.	X	Hexane (for hydrolyzed drug)	100

[a]The developing solvents are expressed as parts, not percentages; [b]Developer containing volatile materials such as NH_4OH should be prepared just prior to use and not; stored for future.NH_4OH has been prepared as 1.5 mL aq.NH_3 in 3 mL of distilled water.

Procedure for in situ hydrolysis

The amount of 0.5 µL of each phenothiazine drugs were spotted on the chromatoplate. Then 0.5 µL dilute sulphuric acid (10%) was placed over each spot; thereafter the plate was covered with a glass plate and kept for 15 minutes in an oven at 120º C. The plate was

cooled at room temperature and on each spot 0.5 µL concentrated ammonia solution (25%) was placed. The spots were dried by heating at 120ºC for 5 minutes. The aforementioned procedure has been followed for this experiment.

RESULTS AND DISCUSSION

Silica gel G UV_{254} was used as an adsorbent. The use of adsorbents containing fluorescent inorganic pigments is advisable for the detection of substances which absorb in the UV-region. The great advantage of this method is that the separated materials can be detected without being chemically modified[13] in any reaction. There is virtually no difference in the performance between fluorescent and non-fluorescent plates, though the former has the advantage of visibility of a spot under ultraviolet light.

The developing solvent systems are given in TABLE 1. The R_f values in various solvents and the colours developed at each stage for phenothiazine drugs are given in TABLE 2. Solvent systems I to X were proposed for phenothiazines drugs and visualizations of the separate drugs were performed by both short and long wavelength UV (254 nm & 356 nm) and also sprayed with Dragendorff's reagent, iodine in methanol and followed by 10% sulphuric acid. For all of the drugs the Dragendorff's spray gave orange spot in solvent systems II, IV and VI. In the Iodine/methanol spray all of the drugs gave brown spot in solvent systems IV, V, VI. In the 10% sulphuric acid spray red and reddish orange color spots were appeared in solvent systems I to VI.

It has been observed that out of the ten solvent systems for phenothiazine drugs, a minimum of three solvent systems is sufficient for separation and identification. Whereas in the case of in situ hydrolysis for the phenothiazines in the solvent system hexane (X) good reliable separation has been achieved the spots were also identified using the aforementioned spray reagents.

The results obtained are compared with the studied solvent system are given in TABLE 3. Several sol-

TABLE 2 : The R_f values of certain Phenothiazine drugs on silica gel G UV_{254}

Drug	System I					System II					System III				
	R_f	Colour in				R_f	Colour in				R_f	Colour in			
		UV	DDR	I_2/CH_3OH	H_2SO_4		UV	DDR	I_2/CH_3OH	H_2SO_4		UV	DDR	I_2/CH_3OH	H_2SO_4
Prochlorperazine	0.49	NCD	NCD	NCD	Red	9.2	Red	Red	NCD	Red	0.7	NCD	NCD	NCD	Purple
Promethazine	0.5	NCD	NCD	NCD	Orange	9.4	Orange	Orange	NCD	Orange	5.2 0.6)	NCD	NCD	NCD	Purple
Chlorpromazine	0.49	NCD	NCD	NCD	Red	0	NCD	NCD	NCD	NCD	0	NCD	NCD	NCD	NCD
Trifluroperazine	0.55	NCD	NCD	NCD	Orange	9.3	Orange	Orange	NCD	Orange	0	NCD	NCD	NCD	NCD

Drug	System IV					System V					System VI				
	R_f	Colour in				R_f	Colour in				R_f	Colour in			
		UV	DDR	I_2/CH_3OH	H_2SO_4		UV	DDR	I_2/CH_3OH	H_2SO_4		UV	DDR	I_2/CH_3OH	H_2SO_4
Prochlorperazine	0.2	NCD	Orange	Brown	Red	0	NCD	NCD	NCD	NCD	3.1(3.5)	Brown	Orange	Brown	Orange
Promethazine	4.7(9.2)	Purple	Orange	NCD	Orange	0	NCD	NCD	NCD	NCD	2.2	Brown	Orange	Brown	Orange
Chlorpromazine	0	NCD	NCD	NCD	NCD	0	NCD	NCD	NCD	NCD	0	NCD	NCD	NCD	NCD
Trifluroperazine	0.6	Purple	Orange	Brown	Orange	9.8	Orange	NCD	Brown	Brown	3.3(3.2)	Brown	Orange	Brown	Orange

UV : Ultraviolet light in 254nm & 356nm ; DDR: Dragendorff's reagent; NCD: No Colour Development ;

TABLE 3 : Comparison of R_f values of Phenothiazine drugs

Phenothiazines	R_f values									
	I	II	III	IV	V	VI	VII	VIII	IX	X* (After acid hydrolysis)
Prochlorperazine	0.49	9.2	0.7	0.2	0	3.5	0	0	0	0.9 (0.8)
Promethazine	0.5	9.3	0.6 (5.2)	4.7 (9.2)	0	2.2	8	0	0	4.9
Chlorpromazine	0.49	0	0	0	0	0	0	0	0	0
Trifluroperazine	0.55	9.3	0	0.6	9.8	3.1	0	0	0	0.7 (0.8)

vent systems can be successfully used for separation of the compounds investigated, but it has been observed that the most successful system is ethylacetate, and in this case R_f values are very reliable. In the same manner when we observe after acid hydrolysis of the phenothiazines in the solvent system hexane, the R_f values are very much reliable than earlier systems. The solvent systems studied in this work were not reported earlier.

CONCLUSIONS

We consider that it is convenient to check the identity of phenothiazines by the use of three or four chromatographic systems. This method is fast and reliable as four or even more system can run simultaneously at the same time and many samples can be put on the same plate. Separation of phenothiazines using TLC can be solved using the intact molecules or its degradation products obtained after in situ acid hydrolysis, and are providing to be a very useful method in the preliminary of these drugs. The chromatographic systems presented in this paper permit an easy and rapid identification of a wide range of phenothiazines currently in use.

Concerning the results, it is apparent that TLC is the method of choice to confirm the presence of phenothiazine drugs. Thus, this method is well suited to analyze these phenothiazine drugs. The separation and simultaneous identification of four phenothiazine drugs by TLC was achieved in this work, which has not been previously reported. The purpose of this investigation was to report on the separation and identification of four types of phenothiazine drugs using various solvent systems that would rapidly separate these drugs. Hence, this approach could also be applied to identify the phenothiazine drugs in drug abuse cases in forensic science laboratories as well as in pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors are very grateful to Dr. M.Panneerselvam, Principal, Raja Doraisingam Government Arts College, Government of Tamil Nadu, Sivagangai and Dr.A.Cyril, Head of the Department, Post Graduate Studies & Research Department of Chemistry, Raja Doraisingam Govt.Arts College, Sivagangai - 630 561 for providing constant encouragement and necessary facilities.

REFERENCES

[1] J.J.Lewis; An introduction to pharmacology, 3rd Edition, Churchill Livingstone, Edinnburgh, **(1965)**.
[2] L.Blendea, D.Balalau, D.L.Baconi, M.Ilie, M.Ionica, D.Blendea; Timisoara Medical Journal, **55(5)**, 156 **(2005)**.
[3] A.Ruri, A.Tetsuya, K.Takeshi, H.Hideki, N.Hiroshi; Forensic Toxical., **25**, 8 (**2007)**.
[4] R.Bonnichsen, P.Geertinger, A.C.Maehly; Journal of Legal medicine, **67(3)**, 158 **(1970)**.
[5] U.K.Rasulev, U.Khasanov, T.K.Islamov, M.M.Shakhitov, B.Eshmuratov; Problems of Forensic Sciences, **43**, 231 **(2000)**.
[6] G.Cimbura; J.Chromatographic Science **10(5)**, 287 **(1972).**
[7] K.Steinbrecher; J.Assoc. Off Anal Chem., **69(6)**, 1030 **(1986)**.
[8] K.Steinbrecher; J.Chromatogr., **260(2)**, 463 **(1983)**.
[9] I.Wiater, K.Madej, A.Parczewski, M.Ka³a; Microchimica Acta, **129(1, 2)**, 121 **(1998)**.
[10] N.C.Jain, W.J.Leung, R.D Budd, T.C.Sneath; The American journal of medical technology, **41(9)**, 322 **(1975)**.
[11] Z.Temerdashev, N.Kiseleva, R.Klishchenko, A.Udalov; Journal of Analytical Chemistry, **61(1)**, 2 **(2006)**.
[12] B.Fried, J.Sherma; Thin layer chromatography, 4th Edition, Marcel Dekker Inc, New York, **(1999)**.
[13] E.Stahl, Thin Layer Chromatography, George Allen & Unwin Ltd., London, **(1969)**.