A METHOD FOR THE THIN-LAYER CHROMATOGRAPHY OF ANALGESIC DRUGS AND RELATED COMPOUNDS IN NON-AQUEOUS SYSTEMS

JOHN E. EMMERSON AND ROBERT C. ANDERSON
The Lilly Toxicology Laboratories, Eli Lilly and Company, Greenfield, Ind. (U.S.A.)
(Received March 5th, 1964)
(Modified July 24th, 1964)

Thin-layer chromatography has been found to be eminently suited for the separation and characterization of alkaloids and other basic drugs. The extensive work in this area has been reviewed by WALDI AND GÄNSHIRT[1]. During the course of studies on the metabolism of *d*-propoxyphene, thin-layer chromatography was investigated as a possible means of separating urinary metabolites. The problems which were encountered in these studies led to the development of the method that is described in the present report.

It is essential in identification procedures involving thin-layer chromatography that the unknown be compared directly with authentic compounds. In the class of drugs under consideration, a preliminary step is often necessary to convert all samples to a common chemical form, *i.e.* either the free base or a salt. The known compounds are most often available as hydrochloride or sulfate salts, while the unknown in question is generally isolated as the free base. In the method described below, drugs may be spotted for thin-layer chromatography either in the form of the base or the salt. A single solvent is used to develop the plates in an atmosphere saturated with ammonia. Alkaloids and related synthetic compounds travel as bases regardless of whether the base or a salt of the chemical has been applied to the plate. The results are compared to those obtained on alkaline silica gel layers in the absence of ammonia.

EXPERIMENTAL

The preparative procedures that are given below generally follow the techniques that were developed by STAHL[2–5].

(*A*) *Preparation of thin-layer plates*

Glass plates (200 × 200 mm) were coated with a 250 μ layer of Silica Gel G (E. Merck, Germany) by the use of a Desaga/Brinkmann applicator (Model S II). The initial slurry was prepared by vigorously shaking 30 g of the adsorbant with 60 ml of de-ionized water. In the preparation of alkaline adsorption layers 60 ml of a 0.5 *N* LiOH solution was used instead of de-ionized water. After the silica plates had dried at room temperature, they were placed in an oven for 1 h at 110°. The plates were stored in a desiccant cabinet and were always used within the first 4 h after activation of the layer.

The compounds were spotted at 1 cm intervals in a line parallel to and 1.5 cm from the bottom of the plate. Only the center portion of the plate was used. A horizon-

tal line was drawn through the silica layer at a distance of 10 cm from the origin to establish a pre-determined solvent front.

(*B*) *Application of drugs*

Three natural and seven synthetic drugs were selected for chromatography. A sufficient amount of each compound, as the available salt, was dissolved in absolute methanol to give a concentration of 5 μg/μl. A micropipette was used to spot 25 μg of the salt of each compound at the origin. The diameter of each spot was limited to 0.5 cm.

(*C*) *Preparation of developing chamber*

Small glass tanks (215 × 215 × 115 mm) were lined with filter paper, and 110 ml of the developing solvent was poured into each chamber. At this time, care was taken to wet the filter paper liners. The internal atmosphere was allowed to equilibrate for 1 h. Twenty minutes before the chromatographic plate was to be introduced, a 30 ml beaker containing 10 ml of ammonia (28 %) was placed in one corner of the chamber. Benzene, ethyl ether and other volatile solvents readily condense on top of the evaporating ammonia solution and effectively trap the ammonia vapors. It is essential, therefore, that the tank be used shortly after the ammonia beaker is introduced. In the present study, the developing chambers were freshly prepared for each plate. Ammonia was not used when the LiOH–silica gel plates were studied.

(*D*) *Development of the thin-layer plates*

All plates were developed in an ascending manner. When the solvent reached the 10 cm mark the plate was removed. The time of solvent ascent was recorded. During the experiments the room temperature was 24 ± 0.5°. The wet plates were dried under a stream of air until the ammonia odor was gone. An iodoplatinate spray reagent[6] was used to locate the spots. The distance from the origin to the center of the spot was used in the calculation of the R_F value.

RESULTS AND DISCUSSION

In preliminary studies the salts of 10 analgesic drugs were applied to silica gel plates for chromatography. It was noted that acidic or alkaline solvent systems were required to obtain movement of the spots. Neutral organic solvents were not effective. In order to facilitate the chromatography of organic bases it has been suggested that an inorganic alkali be incorporated into the adsorption layer[5,7,8]. This modification creates an alkaline environment which increases the mobility of basic drugs by enhancing their solubility in organic solvents and by decreasing the adsorption activity of the silica layer. In this manner the desired mobility can be obtained without the addition of aqueous alkaline components to the solvent system. Amines have also been employed in the thin-layer chromatography of organic bases. WALDI *et al.*[9] have used diethylamine in a series of non-aqueous solvents to systematically analyze 54 alkaloids. The amine was incorporated in a concentration of 10 % as an integral part of the developing solvent.

The present report concerns another method for the introduction of alkalinity in adsorption chromatography without the use of aqueous solvents. If the develop-

TABLE I

THIN-LAYER CHROMATOGRAPHY OF ANALGESIC DRUGS IN AN AMMONIA ATMOSPHERE

	$R_F \times 100$** *in solvent**:													
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*Mean R_F value*
d-Propoxyphene HCl	0	13	34	48	49	71	69	72	76	69	68	80	83	56.3
Cocaine HCl	0	7	17	39	51	75	72	52	68	66	63	78	79	51.3
dl-Methadone HCl	0	11	27	42	37	58	55	67	73	61	63	79	77	50.0
Anileridine di-HCl	0	0	4	8	20	37	40	27	61	61	59	77	82	36 6
Meperidine HCl	0	4	6	14	17	32	43	23	40	54	54	61	76	32.6
Ethoheptazine citrate	0	3	5	12	13	24	33	21	34	43	44	58	67	27.5
d-Methorphan HBr	0	4	4	11	11	20	32	16	24	44	45	47	64	24.8
Codeine SO_4	0	0	0	0	2	5	8	1	8	22	23	30	61	12.3
Morphine SO_4	0	0	0	0	0	0	0	0	2	9	10	12	60	7.2
Normorphine HCl	0	0	0	0	0	0	0	0	0	6	6	5	37	4.2
Mean R_F value	0	4.2	9.7	17.4	20.0	32.2	35.2	27.9	38.6	43.5	43.5	52.7	68.6	
Time for 10 cm rise (min)	17	61	31	31	39	28	36	24	30	142	129	22	39	

* Solvents: (1) petroleum ether (30–60°); (2) carbon tetrachloride; (3) isopropyl ether; (4) benzene; (5) ethylene dichloride; (6) methylene chloride; (7) chloroform; (8) ethyl ether; (9) ethyl acetate; (10) *n*-butyl alcohol; (11) isopropyl alcohol; (12) acetone; (13) methyl alcohol.

** Each figure represents the mean of 3 or more runs.

ment chamber is first saturated with ammonia vapor, single organic solvents may be used for the chromatography of organic bases and their salts. The results from this method are compared with those obtained on alkaline adsorption layers.

Table I shows the R_F values of 10 drugs in 13 organic solvents in the presence of ammonia vapor. The corresponding data for the modified adsorption layers are given in Table II. Examination of these data shows that excellent separations can be obtained without the use of water or complex solvent systems.

When the salt of a basic drug is spotted for chromatography, it is assumed that in subsequent exposure to the ammonia atmosphere the compound is converted to the free base. That this conversion occurs was verified in chromatographic studies where both the salt and the base of the 10 drugs were applied to the same plate in pairs. The resulting spots for each pair were identical in appearance and R_F value. It should be noted, however, that the initial application of the drug in the form of the base is not sufficient for movement in organic solvents. In order to effect chromatography the spots must be continually exposed to an alkaline environment during the rise of the solvent front.

The use of an ammonia development chamber eliminates the need for specially prepared plates. After development in one direction an unmodified silica gel layer can be regenerated by activation. It is then ready for use with a second solvent in two-dimensional chromatography.

The rows and columns in Tables I and II were summed and the mean R_F values calculated. Then according to the mean values, the solvents were listed in order of elutive power and the drugs in order of mobility. This presentation allows a better evaluation of the interaction of drug solubility and solvent polarity upon the R_F values.

The drugs exhibited a greater overall mobility in the ammonia atmosphere. Six or more of the compounds moved in all of the solvents with the exception of petroleum ether. In contrast, the alkaline adsorption layer was not suitable for use with solvents less polar than ethyl ether. No movement was obtained unless solvents with more elutive power were employed. The best chromatograms resulted from the use of benzene or dichloromethane in conjunction with the ammonia chamber. These two solvents gave good resolution of the test substances, and spots which were compact and well-defined. In none of the experiments, however, was streaking or diffusion a problem. By selection of the proper solvent any two of the ten drugs in this study can be separated.

In our laboratory the ammonia chamber is being employed in initial chromatographic studies on new compounds. A solubility (mobility) profile is first established using single organic solvents. If the mobility characteristics of the individual compounds are known, the proper system for chromatographic resolution can usually be devised even though the chemical may be encountered as a component of a complex mixture of drugs or metabolites. The ammonia method should also be valuable in toxicological analysis.

SUMMARY

A thin-layer chromatographic procedure is described which is applicable to natural and synthetic alkaloidal drugs. Neutral organic solvents are used to develop

TABLE II

THIN-LAYER CHROMATOGRAPHY OF ANALGESIC DRUGS ON AN ALKALINE SILICA GEL LAYER

	$R_F \times 100$** in solvent*:													
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*Mean R_F value*
d-Propoxyphene HCl	0	0	10	0	0	0	0	46	42	41	43	64	74	24.6
Cocaine HCl	0	0	4	0	0	0	0	29	38	27	36	69	73	21.2
dl-Methadone HCl	0	0	4	0	0	0	0	22	24	18	25	48	68	16.1
Anileridine di-HCl	0	0	0	0	0	0	0	19	37	39	39	62	72	20.6
Meperidine HCl	0	0	0	0	0	0	0	6	7	10	15	19	66	9.5
Ethoheptazine citrate	0	0	0	0	0	0	0	4	4	5	8	12	58	7.0
d-Methorphan HBr	0	0	0	0	0	0	0	3	3	8	9	7	54	6.5
Codeine SO_4	0	0	0	0	0	0	0	0	2	6	7	9	55	6.1
Morphine SO_4	0	0	0	0	0	0	0	0	0	4	4	4	62	5.7
Normorphine HCl	0	0	0	0	0	0	0	0	0	3	3	0	45	3.9
Mean R_F value	0	0	1.8	0	0	0	0	12.9	15.7	16.1	18.9	29.4	62.7	
Time for 10 cm rise (min)	13	30	21	25	25	18	22	16	21	110	96	14	29	

* See footnotes Table I.
** See footnotes Table I.

silica gel plates in an atmosphere of ammonia vapor. Identical results are given upon development of the plate when either the free base or a salt form is spotted for chromatography. The procedure is shown to offer more convenience and versatility than an existing method in which alkaline silica gel layers are used.

REFERENCES

1 D. WALDI AND H. GÄNSHIRT, in E. STAHL (Editor), *Dünnschicht Chromatographie, ein Laboratoriumshandbuch*, Springer-Verlag, Berlin, 1962, pp. 287–300, 315–344.
2 E. STAHL, *Pharmazie*, 11 (1956) 633.
3 E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
4 E. STAHL, *Parfuem. Kosmetik*, 39 (1958) 564.
5 E. STAHL, *Arch. Pharm.*, 292 (1959) 411.
6 I. SMITH, *Chromatographic and Electrophoretic Techniques*, Vol. I, Interscience, New York, 1960, p. 396.
7 E. NÜRNBERG, *Arch. Pharm.*, 292 (1959) 610.
8 K. TEICHERT, E. MUTSCHLER AND H. ROCHELMEYER, *Deut. Apotheker-Ztg.*, 100 (1960) 477.
9 D. WALDI, K. SCHNACKERZ AND F. MUNTER, *J. Chromatog.*, 6 (1961) 61.