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Chromatography of aromatic amino acid derivatives on polyamide thin layers*

In 1964, SEGURA-CARDONA AND SOEHRING reported (in German) a thin-layer chromatographic method for separating and quantitating some catecholamine metabolites which was 100 times as sensitive as paper chromatography[1]. In 1966 an English translation of part of this paper appeared[2]. The present report describes our experience with, and modifications of, this method. Data are presented for aromatic amino acids not previously studied on polyamide and for two solvent systems not previously used with this medium.

Methods

Five 20 × 20 cm plates are prepared from 7 g of polyamid dissolved in 45 ml of methanol. Polyamide (Woelm) was used exclusively. (The Macherey-Nagel polyamide distributed by Brinkman is totally unsatisfactory.) One hundred nanograms of the substances listed in Table I were spotted, air dried, and developed in isobutanol–glacial acetic acid–cyclohexane (80:7:10). The following sprays were used: (1) diazotized *p*-nitroaniline was made as originally described[1,2]; (2) a suitable diazotized sulfanilic acid spray was made by substituting an equal amount of sulfanilic acid for the *p*-nitroaniline in solution spray (1); (3) ethylenediamine was made up as originally indicated[1,2]. There were no significant differences when the ethylenediamine was diluted with water or sodium hydroxide. The diazotization reactions were examined

TABLE I

R_F × 100 VALUES ON POLYAMIDE

Substance	*Solvent systems*		
	Isobutanol–acetic acid–cyclohexane	*Isopropanol–ammonia*	*Butanol–acetic acid*
Epinephrine	46	85	91
Norepinephrine	29	76	87
Normetanephrine	56	85	93
Metanephrine	72	88	96
Dihydroxymandelic acid	9	75	44
Dopamine	39	82	89
Vanilmandelic acid	31	80	64
3-Methoxy-4-hydroxyphenylglycol	60	81	87
Dihydroxyphenylalanine	18	—	—
Adrenochrome	36	77	32
Tyramine	74	71	100
Serotonin	38	76	92
Dihydroxyphenylacetic acid	23	85	66
Homogentisic acid	55	79	80
5-Hydroxytryptophan	20	86	91
Triiodotyrosine	15	92	85
Phenylpyruvic acid	24	82	66
Tryptophan	42	67	11
Tyrosine	48	—	12

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BLE II

:AY COMBINATIONS

bstance	*1st Ethylene-diamine*	*2nd p-Nitro-aniline*	*1st Ethylene-diamine*	*2nd Sulfanilic acid*	*1st p-Nitro-aniline*	*2nd Ethylene-diamine*	*1st Sulfanilic acid*	*2nd Ethylene-diamine*
inephrine	yellow	purple	yellow	red-orange	brown	purple	red	white
repinephrine	orange	purple	orange	red-orange	brown	pink	—	white
rmetanephrine	quench	purple	quench	orange	purple	pink	orange	quench
tanephrine	quench	purple	quench	orange	purple	pink	orange	quench
hydroxymandelic acid	orange	purple	orange	orange	brown	pink	—	white
pamine	white	purple	white	red-orange	brown	pink	orange	white
nilmandelic acid	quench	purple	quench	orange	purple	pink	orange	white
Methoxy-4-hydroxy-phenylglycol	quench	purple	quench	orange	purple	quench	orange	quench
pa	white	—	white	—	brown	pink	—	white
lrenochrome	yellow	yellow	yellow	orange	purple	quench	orange	pink
ramine	quench	—	—	—	purple	—	yellow	quench
rotonin	white	pink	light green	orange	pink	quench	red	orange
hydroxyphenylacetic acid	yellow-orange	orange	yellow	yellow	pink	pink	orange	pink
omogentisic acid	yellow	yellow	yellow-orange	yellow-orange	brown	orange	yellow-orange	yellow-orange
Hydroxytryptophan	yellow	pink	white	red	red	white	red	white
iiodotyrosine	quench	purple	quench	purple	blue	quench	purple	quench
ienylpyruvic acid	white	—	white	—	yellow	yellow	pink	white
yptophan	white	light purple	white	—	—	white	—	white
rosine	white	—	—	—	purple	—	yellow	white

in the visible range. The ethylenediamine condensations were examined under U.V. light.

Results

The $R_F \times 100$ values are shown in Table I for all the aromatic amino acid derivatives studied. The data obtained with isopropanol–ammonia (4:1) and butanol–

TABLE III

$R_F \times 100$ VALUES ON SILICA GEL

Substance	*Solvent system*		
	Isobutanol–acetic acid–cyclohexane	*Isopropanol–ammonia*	*Butanol–acetic acid*
Epinephrine	8	22	11
Norepinephrine	34	39	6
Normetanephrine	10	59	69
Metanephrine	15	47	45
Dihydroxymandelic acid	15	32	13
Dopamine	—	49	55

acetic acid–water (4:1:1) are also shown as we have found these to be valuable alternate solvent systems for proving the identity of substances and/or for performing two-dimensional TLC on polyamide for the purpose of resolving a mixture of metabolites as found in an extract of urine.

We have found the sequential use of two sprays to be a valuable adjunct in identifying the components of mixtures of unknown substances. Table II indicates the color development for each substance when studied sequentially.

Similarly, if large amounts of the substance are available they can be analyzed on pre-coated silica gel plates, (using the same solvent systems and sprays), as indicated in Table III. However, color development with the diazotized sprays will be different. With sulfanilic acid all of the substances in Table III will be orange in all solvent systems. With *p*-nitroaniline they will be red in the isopropanol–ammonia system and dark orange in the butanol–acetic acid system. In the isobutanol–cyclohexane–acetic acid system the metabases will be red and the others yellow with diazotized *p*-nitroaniline.

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Chromatography of cyclic 3′5′-adenosine monophosphate on silica gel glass microfiber sheets

In the process of devising an assay for adenyl cyclase[1], it became apparent tha, a thin-layer chromatographic system for the rapid separation of the reaction product cyclic 3′5′-adenosine phosphate (cAMP) from its precursor adenosine triphosphate (ATP) and other adenine nucleotides would be highly desirable. A satisfactory system using silica gel glass plates has been described[2], but running time is rather long, and scraping and extraction of the gel is necessary for radioactive analysis. This report is concerned with the separation of cAMP from ATP, 5′-AMP and adenosine (Ado) on silica gel glass microfiber sheets. The procedure provides for rapid development (about 25 min) and ease of counting radioactive samples by scintillation spectroscopy.

Methods and materials

Glass microfiber sheets (20 × 20 cm Gelman ITLC type SG) were spotted with