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Note

Detection of compounds with primary amino groups on thin-layer plates by dipping in a fluorescamine solution

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In a previous communication[1] we reported the detection of compounds with primary amino groups by derivatizing them at the origin of thin-layer plates. This was achieved by developing with or dipping into an acetone–hexane solution of fluorescamine. The major advantages of these techniques are simplicity and high sensitivity. However, acidic solvent systems cannot be used in the subsequent development of the plates because the fluorescamine derivatives are not stable in acidic media. Compounds first separated by thin-layer chromatography (TLC) using acidic solvent systems have been detected with fluorescamine sprays[2–5]. Unfortunately, the sensitivity of the spraying methods is only a fraction of the theoretical sensitivity because of incomplete reaction and high background. Based on our previous finding[1] that the reaction of amino compounds on silica gel plates with fluorescamine is unexpectedly slow, we have developed a dipping procedure which gives high reactivity without extraction in the case of amino acids, peptides, proteins and many biogenic amines.

EXPERIMENTAL

Aliquots (1 μl) containing $1 \cdot 10^{-9}$ mole or 5 μg of compound were spotted on silica gel 60 pre-coated plates (E. Merck, Darmstadt, G.F.R.) and air dried. Three typical solvents were employed: *n*-butanol–acetic acid–water (4:1:5), isopropanol–28% ammonia–water (7:2:3) and acetone–ethanol–water (1:4:5). The acidic solvent was removed by heating the plates for 30 min at 110°, the other solvents by heating 5 min at 110°. Detection of the compounds with fluorescamine was achieved by the procedure given in Table I.

RESULTS

While applicable to most compounds, highly organic-soluble amines, *e.g.*, β-naphthylamine cannot be detected by this dipping procedure because they are extracted by the fluorescamine solvent. Other amines such as *n*-heptylamine and *n*-octylamine could be detected with the acidic solvent system presumably because they formed acetate salts which were not extracted.

Peptides, proteins and most amines of biological interest were detected at the

TABLE I

DIPPING PROCEDURE

Step	*Treatment*
1*	Spray with a solution of 0.2 *M* sodium borate buffer (pH 9.0) or 0.2 *M* phosphate buffer (pH 8.0)**, heat for 10 min at 110°.
2	Dip in a solution of acetone–hexane (1:4) containing fluorescamine (10 mg per 100 ml) for 30 min; air dry for 1 min.
3	Spray with a solution of 10% triethanolamine in ethylene glycol monomethyl ether acetate.
4	Observe under a long-wave (366 nm) ultraviolet lamp.

* May be omitted with neutral or basic solvent systems with a slight decrease in sensitivity.

** Phosphate buffer is used for catecholamines, amino sugars and compounds with vicinal hydroxyl groups.

10-pmole level (Table II) which approximates the sensitivity previously reported using the predevelopment or predipping techniques[1]. Amino acids were detected with somewhat lower sensitivity. The method is more sensitive than the spraying methods[2–5] which require 250–500 pmoles.

TABLE II

LIMITS OF DETECTION OF SOME AMINES, AMINO ACIDS AND PEPTIDES

I = *n*-Butanol–acetic acid–water (4:1:5), upper phase; II = isopropanol–28% ammonia–water (7:2:3); III = acetone–ethanol–water (1:4:5). Figures in parentheses are $R_F \times 100$ values.

Compound	*Limit of detection (10^{-12} mole*)*					
	Solvent system					
	I		*II*		*III*	
L-Arginine·HCl	50	(4)	30	(22)	4	(2)
L-Cysteic acid	200	(0)	80	(84)	90	(84)
L-Aspartic acid	80	(4)	800	(71)	80	(81)
L-Lysine	80	(3)	30	(35)	2	(1)
L-Phenylalanine	8	(41)	20	(91)	80	(69)
Glycine	9	(7)	80	(75)	80	(56)
L-Tyrosine	2	(34)	80	(82)	80	(82)
Glutathione, oxidized	7	(0)	30	(65)	8	(81)
Bradykinin	1	(7)	1	(13)	1	(2)
Pentaglycine	80	(0)	60	(68)	7	(79)
Angiotensin I	1	(11)	7	(80)	20	(62)
Penta-L-alanine	8	(6)	7	(82)	3	(79)
Bovine serum albumin	0.02 μg	(0)	0.02 μg	(0)	0.03 μg	(3)
Cytochrome *c*	0.03 μg	(0)	0.04 μg	(3)	0.02 μg	(0)
Spermine·4 HCl	8	(0)	9	(0)	5	(0)
L-Norepinephrine·HCl	9	(1)	7	(1)	7	(3)
Tyramine·HCl	8	(50)	50	(90)	7	(6)
Histamine·2 HCl	8	(4)	80	(68)	4	(1)
Tryptamine·HCl	80	(58)	80	(95)	7	(6)
D(+)-Glucosamine·HCl	7	(7)	6	(51)	7	(7)

* Unless stated otherwise.

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