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SENSITIVE AND SPECIFIC DETECTION OF TRYPTOPHAN, TRYPTAMINE AND N-TERMINAL TRYPTOPHAN PEPTIDES ON THIN-LAYER PLATES USING A UNIQUE FLUOROGENIC REACTION WITH FLUORESCAMINE

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SUMMARY

Two sensitive and specific methods have been developed for the detection of tryptophan, tryptamine, N-terminal tryptophan peptides and other 3-(2-aminoethyl)-indoles on thin-layer plates. The reaction is based on the unique fluorescence of the fluorescamine derivatives after treatment with perchloric acid (PCA). In method I, compounds are separated on plates and reacted by dipping the plates in acetone–*n*-hexane (1:4) containing fluorescamine. In method II, compounds are first derivatized with fluorescamine at the origin of the plates by dipping in the solution and then separated. When sprayed with 40% PCA, the fluorescamine derivatives appear as yellow-orange fluorescent spots. Certain 3,4-dihydroxyphenylethylamines and 3-methoxy-4-hydroxyphenylethylamines that have a free amino group in the side-chain also react, but they give a bluish fluorescence. Depending on the compounds, amounts of 1–800 pmole could be detected; most could be detected below the 100-pmole level.

INTRODUCTION

Indoleamines such as tryptophan and tryptamine have been detected colorimetrically with ninhydrin[1–3] and other reagents[4], including *p*-dimethylaminobenzaldehyde (Ehrlich's reagent), *p*-dimethylaminocinnamaldehyde, cinnamaldehyde, xanthydrol, diazotized derivatives of sulphanilic acid, *p*-nitroaniline, benzidine and ethyl-α-naphthylamine, all of which are non-specific and require microgram amounts of the compounds. More sensitive fluorometric methods have been reported which employ the Procházka reagent[5–8], gaseous formaldehyde[7–16], *o*-phthalaldehyde[11,17] and glyoxylic acid vapour[18]. The formaldehyde methods are non-specific, as catecholamines also fluoresce under the same conditions[6,7,9,11–15]. The Procházka reagent

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(formaldehyde in hydrochloric acid) has been used for the detection of indoleacetic acid, tryptamine, tryptophan and peptides containing amino-terminal tryptophan[5,19]. Recently, glyoxylic acid vapour has been reported to be more sensitive than gaseous formaldehyde for the detection of indoleamines[18]; however, it is equally non-specific. Also non-specific is *o*-phthalaldehyde, which gives similarly fluorescent products with such compounds as histamine[20,21], histidine[22–24], N-terminal histidine peptides[24–26], arginine[27], agmatine[27], spermidine[28,29], glutathione[30,31] and thiols[32].

Recently, we reported that fluorescamine derivatives of certain indoleamines are uniquely fluorescent in strong acid[33]. In this paper, we report on the specific and sensitive detection of these indoleamines on thin-layer plates.

EXPERIMENTAL

Chemicals and solvents

The following were obtained commercially: fluorescamine (Pierce, Rockford, Ill., U.S.A.), 70% perchloric acid (PCA), boric acid (Fisher Scientific, Fair Lawn, N.J., U.S.A.), sodium hydroxide pellets and sulphuric acid (Mallinckrodt, St. Louis, Mo., U.S.A.).

The test compounds were dissolved in 0.2 *M* sodium borate buffer (pH 9.0) to make 10 m*M* stock solutions. They were purchased from the Sigma (St. Louis, Mo., U.S.A.), Nutritional Biochemical (Cleveland, Ohio, U.S.A.), Schwartz/Mann (Orangeburg, N.Y., U.S.A.), Fox Chemical (Los Angeles, Calif., U.S.A.) and Cyclo Chemical (Los Angeles, Calif., U.S.A.). Acetone, *n*-butanol, benzene, *n*-hexane, methanol, ethyl acetate, dioxane (all distilled-in-glass) were purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.).

Materials

The following pre-coated glass chromatoplates (20 × 20 cm, 0.25 mm layer, without fluorescent indicator) were used without any treatment: silica gel 60 thin-layer chromatographic (TLC) plates (E. Merck, Darmstadt, G.F.R.) and silica gel Q5 plates (Quantum Industries, Fairfield, N.J., U.S.A.). Samples were spotted with volumetric micro-pipettes (Microcaps, Drummond, Broomall, Pa., U.S.A.). Ascending chromatography was performed at ambient temperature (*ca.* 20°) in glass tanks. All spraying procedures were carried out with an aerosol spray kit (Quixpray Kit; Pierce).

Solvent systems for separation of underivatized compounds

(A) *n*-Butanol–acetic acid–water (5:2:3).
(B) *n*-Butanol–acetic acid–water (4:1:5, upper phase).
(C) Chloroform–isopropanol–water (2:7:1).
(D) Ethyl acetate–*n*-propanol–water (5:4:1).

Solvent systems for separation of fluorescamine derivatives

(E) Dioxane–triethanolamine–methanol (6:1:1).
(F) Chloroform–isopropanol–water (2:8:1).
(G) Ethanol–chloroform–28% ammonia solution–water (5:2:1:1).
(H) Ethyl acetate–*n*-hexane–methanol–water (60:20:25:10).

Recommended procedure for TLC of tryptophan and related compounds

A 1-μl aliquot of sample buffered with 0.2 *M* sodium borate (pH 9.0) was applied with the micro-pipette 1.5 cm from the lower edge of a plate, air-dried and analysed by one of the two methods described below.

(a) Method I: detection after chromatography of native compounds. The separated compounds are derivatized with fluorescamine by modification of the dipping method[34]. The plates are dried at 110° for 5 min, sprayed with 0.2 *M* sodium borate buffer (pH 9.0), re-dried at 110° for 5 min and dipped for 30 min in acetone–*n*-hexane (1:4) containing fluorescamine (10 mg per 100 ml). The plates are then sprayed with 40% PCA for 5 sec* and fluorescence is observed within a few minutes under a long-wave (366 nm) ultraviolet (UV) lamp. Plates developed with acidic media are sprayed with 0.5 *M* sodium borate buffer (pH 9.0) and sprayed with 40% PCA for about 10 sec.

(b) Method II: detection after chromatography of fluorescamine derivatives. The compounds are derivatized at the origin[35] by dipping the lower 2 cm of the plate in the fluorescamine solution for 15 min. The plate is dried without heating and then developed with the appropriate solvent. Air-dried plates are sprayed with 40% PCA for 5 sec*.

Spot test for reactivity of compounds

An aliquot (1 μl) of the stock solution (1 m*M*) was spotted on a silica gel 60 plate, derivatized by the pre-dipping method[35] and detected by spraying with 40% PCA as described above.

Determination of sensitivity

A 1-μl aliquot of various concentrations of buffered sample was applied to a silica gel 60 plate, which was developed with solvent C for method I and solvent H for method II.

RESULTS

Effect of PCA concentration on fluorescence

Various derivatized amines behave differently when the spots are sprayed with increasing concentrations of PCA (Table I). The bluish green fluorescence of many fluorescamine derivatives of primary amines is partially quenched by 10% or 25% PCA and completely quenched by 40% PCA. On the other hand, the fluorescence intensity of indoleamines, catecholamines and their 3-O-methyl derivatives was not reduced. In several instances the colour changed from bluish green to yellow or yellowish green (indoleamines) or to blue (catecholamines and their 3-O-methyl derivatives). Similar results were obtained with 55% and 70% PCA but the optimal concentration is 40% (Table II). Catecholamines and their 3-O-methyl derivatives are easily distinguished from tryptophan and related compounds by their different colours. It should be noted that N(side-chain)-substituted indoleamines such as N-methyltryptamine, N,N-dimethyltryptamine, L-prolyl-L-tryptophan and melatonin

* The spraying time should be changed depending upon the plate size to obtain maximal fluorescence.

TABLE I

EFFECT OF CONCENTRATION OF PCA SPRAY ON THE FLUORESCENCE INTENSITY OF VARIOUS COMPOUNDS SPOTTED ON SILICA GEL PLATES

A 1-μl aliquot of a 1 m*M* stock solution in 0.2 *M* sodium borate buffer (pH 9.0) was spotted on a silica gel 60 plate, air-dried and dipped for 30 min in acetone-*n*-hexane (1:4) containing fluorescamine (10 mg per 100 ml). After it had dried, the plate was sprayed for 5 sec with various concentrations of PCA. The colour and intensity of fluorescence are designated as follows: BG, bluish green; LBG, light bluish green; Y, yellow; YG, yellowish green; FY, faint yellow; G, green; GB, greenish blue; LB, light blue; −, negative; ±, trace to negative; tr, trace; +, weak; ++, intermediate; +++, strong; ++++, extremely strong..

Fluorescence observed within a few minutes after the spray (A); fluorescence observed 15 min after the spray (B).

Compound	*PCA concentration (%)*									
	0: A	*10*		*25*		*40*		*55 and 70*	
		A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
L-Histidine	+++(BG)	+(BG)	tr	+(BG)	−	−	−	−	−
Histamine·2HCl	+++(LBG)	±	tr	−	−	−	−	−	−
L-His-L-Leu	+++(LBG)	++(BG)	tr	+(BG)	−	−	−	−	−
L-Tryptophan	++(BG)	++++(Y)	++(Y)	++++(Y)	tr	++++(Y)	++(Y)	++++(YG)	++++(YG)
Tryptamine·HCl	+++(BG)	++(YG)	++(YG)	+++(YG)	++(Y)	++++(YG)	+++(YG)	++++(G)	++++(YG)
L-Trp-Gly	+++(BG)	++++(Y)	++(Y)	++++(Y)	tr	++++(Y)	+(Y)	++++(YG)	++++(YG)
Glycine	+++(BG)	±	−	−	−	−	−	−	−
L-Tyrosine	+++(BG)	±	−	−	−	−	−	−	−
L-Arginine·HCl	+++(LBG)	+(BG)	tr	−	−	−	−	−	−
D(+)-Glucosamine·HCl	tr*	−	−	−	−	−	−	−	−
Tyramine·HCl	+++(LBG)	+(Y)	tr	−	−	−	−	−	−
Spermine·4HCl	++(BG)	+(BG)	tr	−	−	−	−	−	−
DL-Octopamine·HCl	+++(BG)	+(BG)	tr	−	−	−	−	−	−
3-Methoxytyramine·HCl	+++(LBG)	+(BG)	tr	−	−	++(B)	+++(LB)	+++(LB)	+++(GB)
Dopamine·HCl	++(BG)	+(Y)	++(Y)	tr	++(LBG)	++(B)	++(GB)	+++(BG)	+++(GB)
DL-Normetanephrine·HCl	+++(LBG)	+(BG)	tr	−	++(YG)	++(G)	++(B)	+++(B)	+++(B)
L-Norepinephrine·HCl	++(BG)	+(BG)	tr	tr	++(YG)	++(B)	+++(B)	+++(B)	+++(B)
L-DOPA	+(BG)	+(BG)	tr	tr	++(YG)	++(BG)	++(BG)	+++(BG)	+++(BG)
DL-Metanephrine·HCl	−	−	−	−	−	−	−	−	−
DL-Epinephrine·HCl	−	−	−	−	−	−	−	−	−
L-Phenylalanine	+++(LBG)	+(BG)	tr	−	−	−	−	−	−

* Due to chelation of hydroxyl groups with borate.

TABLE II

EFFECT OF CONCENTRATION OF PCA SPRAY ON THE FLUORESCENCE INTENSITY OF FLUORESCAMINE-TREATED INDOLEAMINES AND PHENYLETHYLAMINES AFTER TLC

A 1-μl aliquot of a 1 m*M* stock solution in 0.2 *M* sodium borate buffer (pH 9.0) was spotted on a silica gel 60 plate, air-dried and dipped for 15 min in the fluorescamine solution. After it had dried, the plate was developed with solvent H, air-dried and sprayed for 5 sec with various concentrations of PCA. The intensity of fluorescence is designated as follows: —, negative; tr, trace; +, weak; ++, intermediate; +++, strong. Fluorescence within a few minutes (A); fluorescence in 30–60 min (B).

Compound	*PCA concentration (%)*							
	25		*40*		*55*		*70*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
L-Trp-L-Ala	+++	+++	+++	+++	++	+++	++	+++
L-Tryptophan	+++	+++	+++	+++	+++	+++	++	+++
Tryptamine·HCl	+++	+++	+++	+++	+++	+++	+++	+++
5-Hydroxy-DL-tryptophan	+*	++	+*	+++	+	+++	+	+++
5-Methoxy-DL-tryptophan	++	+++	++	+++	++	+++	++	+++
5-Hydroxytryptamine oxalate salt	++	—	++	—	++	—	++	—
5-Methoxytryptamine·HCl	+++	+++	+++	+++	+++	+++	+++	+++
N-Methyltryptamine	+*	+	+*	+	+	+++	++	+++
N,N-Dimethyltryptamine	+*	+	+*	+	+	+++	++	+++
L-Pro-L-Trp	+*	+	+*	+	+	+++	++	+++
L-Leu-L-Trp	—	—	—	—	—	—	++	++
N-Acetyl-5-methoxytryptamine (melatonin)	+*	tr	tr	tr	+	+	+	+
N-Acetyl-L-tryptophan	—	—	—	+	tr	+	+	+++
3-Methoxytyramine·HCl	—	—	++*	++	+++	+++	+++	+++
Dopamine·HCl	tr	+	++	++	++	++	+++	+++
DL-Normetanephrine·HCl	—	—	—	—	++	+++	++	+++
L-Norepinephrine·HCl	—	—	+++*	+	+	+	+	++
L-DOPA	—	—	+	+	+	++	++	++
DL-Metanephrine·HCl	—	—	—	—	—	—	—	—
DL-Epinephrine	—	—	—	—	—	—	—	—

* Fluorescence not detectable within a few minutes but detectable in 5–10 min.

also gave a yellowish fluorescence within a few minutes if sprayed with 55% or higher concentrations of PCA.

Specificity of the fluorogenic reaction

As summarized in Table III, only a few classes of fluorescamine derivatives fluoresced after spraying with 40% PCA. The positive compounds which gave any fluorescence in amounts of less than 1 nmole included tryptophan, tryptamine, amide

TABLE III

SPECIFICITY OF THE 40% PCA SPRAY REAGENT TOWARDS VARIOUS AROMATIC ETHYLAMINES

A 1-μl aliquot of 1 m*M* stock solution, equivalent to 1 nmole, was spotted on silica gel 60 plates and derivatized with fluorescamine by dipping the plates in the fluorescamine solution for 30 min. After spraying the 40% PCA solution for 5 sec, the plates were observed for fluorescence with a long-wave UV lamp in the dark. The colour of fluorescence of tryptophan and related compounds observed within a few minutes was usually yellow to yellowish green unless specified as follows: OR, orange; G, green; B, blue; BG, bluish green; GB, greenish blue; LB, light blue; DYG, dark yellowish green.

Positive compounds	*Fluorescence*	*Negative compounds*
Tryptophan and its derivatives		*Tryptophan derivatives*
L-Tryptophan		N-Acetyl-L-tryptophan
DL-Tryptophanamide·HCl		N-Acetyl-L-tryptophanamide
L-Tryptophan hydroxamate	OR	N-CBZ-L-tryptophan
DL-Tryptophan methyl ester·HCl		N-CBZ-L-tryptophan-*p*-nitrophenyl ester
DL-Tryptophan ethyl ester·HCl		Tryptophol
DL-Tryptophan butyl ester·HCl		5-Hydroxytryptophol
DL-Tryptophan octyl ester·HCl		5-Methoxytryptophol
L-Tryptophan benzyl ester·HCl		L-Pro–L-Trp
4-Methyl-DL-tryptophan		L-Val–L-Trp
5-Methyl-DL-tryptophan	OR	L-Leu–L-Trp
6-Methyl-DL-tryptophan		L-Phe–L-Trp acetate·0.5 H_2O
7-Methyl-DL-tryptophan		Gly–L-Trp
DL-α-Methyltryptophan	OR*	L-Ala–L-Trp
DL-4-Fluorotryptophan		Gly–L-Trp–Gly
DL-5-Fluorotryptophan	OR	L-Lys–L-Trp–L-Lys
DL-6-Fluorotryptophan	G	L-Leu–L-Trp–L-Leu
5-Hydroxy-DL-tryptophan	DYG	Gly–Gly–L-Trp
5-Methoxy-DL-tryptophan	OR	
DL-5-Benzyloxytryptophan	OR	*Tryptamine derivatives*
DL-6-Benzyloxytryptophan	OR	N-Methyltryptamine
DL-7-Benzyloxytryptophan	OR	N,N-Dimethyltryptamine
L-Trp–L-Ala		N-Acetyl-5-methoxytryptamine
L-Trp–β-Ala		(melatonin)
L-Trp–Gly		6-Hydroxymelatonin
L-Trp–L-Glu		N-Acetyl-5-hydroxytryptamine
L-Trp–L-Ile		(N-acetylserotonin)
L-Trp–L-Leu		
L-Trp–α-L-Lys		*Indole and its derivatives*
L-Trp–L-Phe		Indole
L-Trp–L-Trp		2-Methylindole
L-Trp–L-Tyr		3-Methylindole

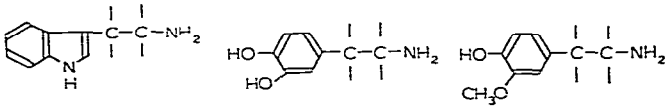
(Continued on p. 159)

TABLE III (*continued*)

Positive compounds	*Fluorescence*	*Negative compounds*
L-Trp–L-Val		5-Methylindole
L-Trp–Gly–Gly		7-Methylindole
L-Trp–L-Met–L-Asp–L-Phe·NH_2·HCl		5-Hydroxyindole
		5-Hydroxyindole-3-acetic acid dicyclohexylammonium salt
Tryptamine and its derivatives		Indole-3-acetic acid
Tryptamine·HCl		3-Indoleacetamide
5-Methyltryptamine·HCl		3-Indoleacetonitrile
7-Methyltryptamine		3-Indolepyruvic acid
5-Fluorotryptamine·HCl		3-Indoleacetone
6-Fluorotryptamine·HCl		DL-3-Indolelactic acid
5-Hydroxytryptamine oxalate salt	OR	5-Methoxy-3-indoleacetic acid
6-Hydroxytryptamine creatinine sulphate complex		Indoxyl-β-D-glucoside
5-Methoxytryptamine·HCl	OR	Indoxyl sulphate potassium salt
5-Benzyloxytryptamine·HCl	OR	Indomethacin
		Isatin
Catecholamine and its 3-O-methylated derivatives		*Tryptophan metabolites*
L-Norepinephrine·HCl	B	Quinolinic acid
L-DOPA	BG	Picolinic acid
Dopamine·HCl	BG	Xanthurenic acid
DL-Normetanephrine·HCl	B	DL-Kynurenine sulphate
3-Methoxytyramine·HCl	LB	
		Catecholamine and its derivatives
Tryptophan metabolites		DL-Epinephrine
Kynurenic acid·0.5 H_2O	B**	DL-Metanephrine·HCl
3-Hydroxyanthranilic acid	B**	Tyramine·HCl
		DL-Octopamine·HCl

* Delayed development of fluorescence in 5–10 min.
** Native fluorescence.

and esters of tryptophan, tryptophan hydroxamate, tryptophans and tryptamines with methyl-, fluoro-, benzyloxy-, hydroxy- or methoxysubstituent(s) in the indole ring, and N-terminal tryptophan peptides. Of the tryptophan metabolites tested, kynurenic acid and 3-hydroxyanthranilic acid also gave the blue fluorescence. The following indole derivatives gave no fluorescence at the 1-nmole level: N(side-chain)-substituted tryptophans and tryptamines, simpler indoles having no nitrogen in the side-chain and peptides containing tryptophan at the C-terminus or in the peptide chain. All other classes of compounds, including polyamines, amino acids, peptides, amino sugars and alkylamines, did not fluoresce. Some catecholamines and their 3-O-methylated derivatives gave fairly intense fluorescence, including dopamine (bluish green), L-β-3,4-dihydroxyphenylalanine(L-DOPA)(bluish green), norepinephrine (blue), normetanephrine (blue) and 3-methoxytyramine (light blue). However, other phenylethylamines, such as epinephrine, metanephrine, tyramine, octopamine, tyrosine and phenylalanine, were negative. Thus, the structural requirements for fluorescence are a 3-(2-aminoethyl)indole, a 3,4-dihydroxyphenylethylamine or a 3-methoxy-4-hydroxyphenylethylamine, all of which have a free $-NH_2$ group in the side-chain:



TLC of underivatized compounds followed by derivatization with fluorescamine and detection with 40% PCA spray (method I)

The derivatization of tryptophan derivatives on chromatograms is performed by the modified dipping method[34]. Generally, fluorescamine derivatives of tryptophans and N-terminal tryptophan peptides gave an orange fluorescence while those of tryptamines gave a yellowish green or bluish green fluorescence. After the 40% PCA spray, all changed to a characteristic yellowish fluorescence, which was more intense. The use of 0.5 *M* instead of 0.2 *M* borate buffer prior to the fluorescamine staining gave more reproducible results when the plates were developed with acidic solvents. When present in large amounts, *e.g.*, 1 nmol, reactive compounds are detectable a few minutes after the plate is dipped in the fluorescamine solution. However, prolonged reaction with fluorescamine apparently does not cause the loss of fluorescence of either the fluorescamine derivatives or the PCA-induced fluorophores. The R_F values of the underivatized tryptophan and related compounds obtained with four solvent systems are given in Table IV.

TLC of fluorescamine derivatives followed by detection with 40% PCA spray (method II)

All of the positive compounds listed in Table III are successfully derivatized with fluorescamine at the origin of TLC plates by the modified pre-dipping method[35]. Neither the underivatized compounds nor fluorescamine derivatives are displaced or extracted by the fluorescamine solution. Although a quantitative study was not performed, the derivatization seems to be complete within 15 min. After TLC, most of the positive compounds can be detected at the 1-nmole level as their fluorescamine derivatives. However, the fluorescence is significantly intensified by the 40% PCA spray. The R_F values of the fluorescamine derivatives with four solvent systems are given in Table V. Usually, fluorescamine derivatives prepared by the modified pre-dipping method[35] give a single fluorescent spot. However, if the plates are dipped for more than 15 min, additional intense yellow fluorescent spots with higher R_F values appear after spraying with 40% PCA.

Sensitivities of methods I and II

As summarized in Table VI, the detection limit in methods I and II varied from 2 to 800 pmole and 0.8 to 800 pmole, respectively. 5-Hydroxy, 6-hydroxy- and 5-methoxy derivatives of tryptophan and tryptamine were oxidized in the borate buffer, forming coloured products, and gave weaker spots than the other indoleamines.

DISCUSSION

Fluorescamine derivatives of 3-(2-aminoethyl)indoles, certain 3,4-dihydroxyphenylethylamines and 3-methoxy-4-hydroxyphenylethylamines uniquely fluoresce on

TABLE IV

R_F VALUES OF FREE COMPOUNDS

Compounds (1 nmole each) dissolved in 0.2 *M* sodium borate buffer (pH 9.0) were spotted on silica gel 60 plates, developed and derivatized with fluorescamine by using the dipping method as described under Experimental.

Abbreviations: t, trace; m, main.

Compound	*R_F × 100 value in solvent system*			
	A	*B*	*C*	*D*
L-Tryptophan	56	38	46	33
DL-Tryptophanamide·HCl	60	42	30	24
L-Tryptophan hydroxamate	55	33	5	31
DL-Tryptophan methyl ester·HCl	57	38	46	32
DL-Tryptophan ethyl ester·HCl	57	38	46	33
DL-Tryptophan butyl ester·HCl	57	38	45	33
DL-Tryptophan octyl ester·HCl	57	38	45	31
L-Tryptophan benzyl ester·HCl	57	38	46	33
4-Methyl-DL-tryptophan	60	42	49	35
5-Methyl-DL-tryptophan	60	43	49	36
6-Methyl-DL-tryptophan	60	42	48	35
7-Methyl-DL-tryptophan	59	41	47	37
DL-5-Benzyloxytryptophan	65	48	53	44
DL-6-Benzyloxytryptophan	64,70[t]	48[m],46,40[t]	52	44,39,0[t]
DL-α-Methyltryptophan	61	44	48	40
DL-4-Fluorotryptophan	58	39	45	35
DL-5-Fluorotryptophan	59	41	46	38
DL-6-Fluorotryptophan	60	42	45	36
L-Trp–L-Ala	65	44	43	23
L-Trp–β-Ala	62	30	31	13
L-Trp–Gly	54	32	35,41[t]	17
L-Trp–L-Glu	51,55		26,22[t]	7,0
L-Trp–L-Ile	76	69	56,60[t]	45,52[t]
L-Trp–L-Leu	76	66	55,60[t]	40,47[t]
L-Trp–α-L-Lys	46[m],47,51	12[m],15,7	2	0
L-Trp–L-Phe	72	59	55	42
L-Trp–L-Trp	74	62	54	42
L-Trp–L-Tyr	69	56	52	43
L-Trp–L-Val	73[m],69	62	49	36,42[t]
L-Trp–Gly–Gly	49	22	25	8
L-Trp–L-Met–L-Asp–L-Phe·NH_2·HCl	67[m],64[t],54[t]	47	50,30,34[t]	40,33[t],25[t],16[t],8[t]
Tryptamine·HCl	67	52	9	8
5-Methyltryptamine·HCl	69	55	10	8
7-Methyltryptamine	68	54	10	9
5-Benzyloxytryptamine·HCl	74	60	12	10
5-Fluorotryptamine·HCl	69	55	9	9
6-Fluorotryptamine·HCl	70	56	20	12
6-Hydroxytryptamine creatinine sulphate complex			53	0

TLC plates sprayed with 40% PCA. Indoleamines give yellowish spots and the phenylethylamines bluish spots. Should the need arise for prior separation, catecholamines can be selectively adsorbed on an alumina column[36] or destroyed by heating

TABLE V

R_F VALUES OF FLUORESCAMINE DERIVATIVES

Compounds (1 nmole each) dissolved in 0.2 *M* sodium borate buffer (pH 9.0) were spotted on TLC plates, directly derivatized with fluorescamine by the pre-dipping method as described under Experimental, and chromatographed.

E, F, H: Silica gel 60 plates; G: silica gel Q5 plates.

Compound	*R_F × 100 value in solvent system**			
	E	*F*	*G*	*H*
L-Tryptophan	33 (83)	39 (78)	66	56 (68)
DL-Tryptophanamide·HCl	(87)	76 (94)	83	68 (88)
L-Tryptophan hydroxamate	(85)	40	65	(71)
DL-Tryptophan methyl ester·HCl	32 (84)	40 (77)	66	53 (68)
DL-Tryptophan ethyl ester·HCl	31 (83)	40 (77)	66	54 (68)
DL-Tryptophan butyl ester·HCl	33 (84)	40 (77)	66	53 (68)
DL-Tryptophan octyl ester·HCl	32	40 (78)	65	53 (68)
L-Tryptophan benzyl ester·HCl	32 (84)	42 (79)	63	52 (70)
4-Methyl-DL-tryptophan	34 (85)	40 (78, 82)	63	56 (70)
5-Methyl-DL-tryptophan	34 (85)	40 (79)	64	55 (70)
6-Methyl-DL-tryptophan	35 (85)	40 (78)	64	57 (70)
7-Methyl-DL-tryptophan	34 (85)	40 (78)	63	56 (70)
DL-5-Benzyloxytryptophan	51 (86)	40 (80)	67	66 (71)
DL-6-Benzyloxytryptophan	51 (87)	39 (79)	68	65 (71)
DL-7-Benzyloxytryptophan			66	66 (71)
DL-α-Methyltryptophan	30 (76)	39 (82)	64	52
DL-4-Fluorotryptophan	30 (85)	39 (79)	62	53 (70)
DL-5-Fluorotryptophan	34 (84)	39 (77)	63	55 (70)
DL-6-Fluorotryptophan	34 (84)	39 (77)	63	57 (70)
L-Trp–L-Ala	35 (81)	36 (74)	70	37 (68)
L-Trp–β-Ala	29 (85)	38 (83)	70	43 (70)
L-Trp–Gly	31 (68)	26 (60)	69	31 (66)
L-Trp–L-Glu	6 (66)	20 (38)	56	26
L-Trp–L-Ile	59 (88)	73 (84)	75	65 (70)
L-Trp–L-Leu	(88)	75 (86)	75	62 (70)
L-Trp–α-L-Lys	13, 30 (80)	12, 38	64, 67	30, 48 (65)
L-Trp–L-Phe	(85)	39 (80)	73	61 (69)
L-Trp–L-Tyr	(86)	39 (82)	70	57 (68)
L-Trp–L-Val	(86)	40 (82)	72	55 (69)
L-Trp–Gly–Gly	32 (72)	22, 40	65	31
L-Trp–L-Met–L-Asp–L-Phe·NH_2·HCl	18 (81)	39, 43 (72)	66, 77	31, 53 (68)
Tryptamine·HCl	36 (72, 79, 83)	86 (94)	87	73 (94)
5-Methyltryptamine·HCl	87	87 (95)	88	74 (94)
7-Methyltryptamine	88	86 (94)	87	73 (94)
5-Benzyloxytryptamine·HCl	87	87 (94)	89	75 (94)
5-Fluorotryptamine·HCl	89	85 (94)	85	74 (93)
6-Fluorotryptamine·HCl	88	86 (94)	86	74 (93)
6-Hydroxytryptamine creatinine sulphate complex	19	39, 83	62	30

* The R_F × 100 values of spots which could not be detected after TLC under long-wave UV light and detected by the successive 40% PCA spray are shown in parentheses.

TABLE VI

COMPARISON OF SENSITIVITIES OF PROPOSED METHODS

The reactive compounds were spotted on silica gel 60 plates and treated as described under Experimental. The final fluorescence was developed by spraying with 40% PCA. Method I, with solvent C, method II with solvent H.

Compound	*Limit of detection (pmole)*	
	Method I	*Method II*
L-Tryptophan	2	15
DL-Tryptophanamide·HCl	8	15
L-Tryptophan hydroxamate	80	60
DL-Tryptophan methyl ester·HCl	2	20
DL-Tryptophan ethyl ester·HCl	2	20
DL-Tryptophan butyl ester·HCl	2	20
DL-Tryptophan octyl ester·HCl	2	20
L-Tryptophan benzyl ester·HCl	2	15
4-Methyl-DL-tryptophan	20	15
5-Methyl-DL-tryptophan	20	80
6-Methyl-DL-tryptophan	2	15
7-Methyl-DL-tryptophan	2	15
DL-5-Benzyloxytryptophan	50	80
DL-6-Benzyloxytryptophan	50	20
DL-7-Benzyloxytryptophan	50	70
DL-α-Methyltryptophan	800	800
DL-4-Fluorotryptophan	2	9
DL-5-Fluorotryptophan	50	9
DL-6-Fluorotryptophan	3	9
L-Trp–L-Ala	50	80
L-Trp–β-Ala	50	20
L-Trp–Gly	9	20
L-Trp–L-Glu	50	80
L-Trp–L-Ile	50	20
L-Trp–L-Leu	50	30
L-Trp–α-L-Lys	5	40
L-Trp–L-Phe	5	20
L-Trp–L-Trp	7	
L-Trp–L-Tyr	50	30
L-Trp–L-Val	50	30
L-Trp–Gly–Gly	8	20
L-Trp–L-Met–L-Asp–L-Phe·NH_2·HCl	50	40
Tryptamine·HCl	3	1
5-Methyltryptamine·HCl	5	4
7-Methyltryptamine	9	4
5-Benzyloxytryptamine·HCl	4	3
5-Fluorotryptamine·HCl	3	3
6-Fluorotryptamine·HCl	4	0.8
5-Hydroxy-DL-tryptophan	250	250
5-Methoxy-DL-tryptophan	350	100
5-Hydroxytryptamine oxalate salt	400	200
5-Methoxytryptamine·HCl	400	100
6-Hydroxytryptamine creatinine sulphate complex	700	700
Kynurenic acid·0.5 H_2O	5*	5*
3-Hydroxyanthranilic acid	>1000	200*

* Native fluorescence.

the sample in a base. However, the 3-O-methylated catecholamines would not be removed by either treatment.

Unnecessarily long reaction of the indoleamines with fluorescamine on the TLC plates can lead to the generation of by-products derived from the indoleamines. This does not occur in solution because the excess of fluorescamine is rapidly destroyed. Samples reacted in solution and then spotted and analysed gave only one spot after spraying.

We previously reported that 3-substituted indoles uniquely fluoresce on silica gel plates after simply spraying with PCA[37]. Using 70% PCA, approximately 100 pmole of N-substituted tryptophans, tryptamines and peptides containing tryptophan could be detected. These indoles do not fluoresce in the 40% PCA used in the present study, which involves fluorescamine derivatives of 3-(2-aminoethyl)indoles. We also reported that fluorescamine derivatives of histidine, histamine, peptides with amino-terminal histidine and other 2-(4-imidazolyl)ethylamines uniquely fluoresce after heating in strong acid[38,39]. It should be noted that they also do not interfere in the present method because the plates are not heated.

With the present method, as little as 1 pmole of 3-(2-aminoethyl)indole can be detected on TLC plates, making it much more sensitive than previous methods[5–17]. The specific detection of catecholamines and their 3-O-methylated derivatives by the present method will be reported elsewhere[40].

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