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Note

Detection of catecholamines and metabolites by fluorescence on thin-layer chromatograms

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Severals methods have been reported for the detection and analysis of catecholamines and their metabolites in plasma[1–4], urine[5–8], and tissue[9–11] samples.

Spectrophotometric[12,13], fluorometric[14–16], as well as radioenzymatic[17–19] assay, gas chromatography with flame ionization[20,21], electron capture[22,23], or coupled with mass spectrometry[24,25] have been applied in the analysis of catecholamines and their metabolites, but high-performance liquid chromatography (HPLC) has acquired special importance[26–29]. Several workers have proposed pre-column derivatization using fluorescamine[30–32], dansyl chloride[33–35] and *o*-phthalaldehyde. Trihydroxyindole[37,38] and borate[39] have also been used as post-column reagents.

Fluorescence and thin-layer chromatography (TLC) have also been applied to the detection of catecholamines and their metabolites[40] as well as in other fields such as pesticides[41–48].

This paper describes a procedure involving the production of fluorescence on thin-layer chromatograms by heat treatment alone and also by first spraying the plates with borate solutions of various pH and concentrations prior to heating at an optimum temperature for a definite period of time. The ultimate goal is to develop analytical methods suitable for the quantitative determination of catecholamines by a combination of TLC and fluorometry.

EXPERIMENTAL

Chemicals and apparatus

Dopamine, homovanillic acid (HVA), l-norepinephrine hydrochloride, 3-hydroxy-4-methoxymandelic acid (VMA) were obtained from Aldrich (Milwaukee, WI, U.S.A.). DL-Metanephrine hydrochloride, 4-hydroxy-3-methoxyphenylglycol, DL-normetanephrine and (±) epinephrine were purchased from Sigma (St. Louis, MO, U.S.A.). Sodium borate decahydrate, reagent grade (J. T. Baker, Phillipsburg, NJ, U.S.A.) and silica gel 60 H (Terochem, Toronto, Canada) were used as received. All solvents were purified grade (Canlab Supplies, Ste-Foy, Canada). For visual observation of the fluorescence, a Blak-Ray long-wave ultraviolet lamp (Ultra-violet Products, San Gabriel, CA, U.S.A.) was used.

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TABLE I

VISUAL LIMITS OF DETECTION OF CATECHOLAMINES AND METABOLITES

Borate 0.15 *M*, pH 8.

Amine	*Temp. (°C)*	*Heating period (min)*	*Detection limit (μg)*
Epinephrine	100	45	0.002
Dopamine	200	45	0.002
4-Hydroxy-3-methoxy-phenylglycol	200	60	0.02
Metanephrine	200	45	0.02
Normetanephrine	200	45	0.02
VMA	200	30	0.02
HVA	200	30	0.02
Norepinephrine	200	20	0.004

General procedure

Standard solutions of 500 ppm (w/v) were prepared in methanol. This did not apply to epinephrine which was dissolved in 49.5 ml of acetone and 0.5 ml of concentrated hydrochloric acid. Dilution series were made with methanol. Borate solutions were prepared in distilled water and the pH adjusted with concentrated hydrochloric acid or sodium hydroxide solution.

TLC plates (20 × 20 cm) were coated 250 μm thick with a Desaga applicator from a suspension of 30 g of silica gel in 80 ml of distilled water. The plate was divided into eight squares and the samples were applied with a Hamilton microsyringe. The entire plate was then subjected to the desired treatment, *i.e.* sprayed with borate solution and/or heated in an oven at various temperatures: 50, 75, 100, 200 and 250°C for varying periods of time: 10, 20, 30, 45, 50 and 120 min, in order to establish the optimum conditions for maximum visual fluorescence.

RESULTS AND DISCUSSION

The best detection limits of all catecholamines and derivatives tested as well as the appropriate reaction conditions are presented in Table I.

TABLE II

VISUAL LIMITS OF DETECTION AT ROOM TEMPERATURE

N.D. = not detected.

Amine	*No spray*		*Sprayed with borate (0.15 M, pH 8)*	
	Heating period (min)	*Detection limit (μg)*	*Heating period (min)*	*Detection limit (μg)*
Epinephrine	60	0.03	30	0.003
Norepinephrine	N.D.		60	0.01

For epinephrine, dopamine and norepinephrine the detection limits are 2, 2 and 4 ng, respectively, but for the metabolites about ten times higher.

In general, the detection limit is better at higher temperatures but above 200°C (*e.g.*, 250°C) the glass plates have a tendency to break on cooling. From our results it seems that neither the variation of pH (8–10.2) of the borate solutions nor the length of the heating period has much influence on the detection limit.

The influence of borate on the detection limit of epinephrine and norepinephrine is more important at room temperature (Table II) where the results are better with borate than without it.

The concentration of borate does not improve the detection limit but the heating period can be shorter at a concentration of 0.15 *M*.

Of the amines investigated, norepinepkrine, dopamine and epinephrine gave the best results (2–4 ng) and it should be possible to develop an analytical method suitable for their quantitative determination by fluorescence on thin-layer chromatograms.

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