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Use of Cellulose Thin-Layer Chromatographic Plates to Detect Methylmalonic Acid in Urine

To the Editor:

Detection of methyl malonate in urine by use of Fast Blue B (o-dianisidine, or 3,3’-dimethoxybenzidine) has been described (1). The technique is too cumbersome for use in screening. The more recent method of Coulombe et al. (2), in which urine is collected on filter paper for paper chromatography, was successfully applied to a general population for screening, but the method requires 3-4 h for the chromatographic part and 4-6 h for color development.

We present here an improved micromethod with which as little as 5 ng (0.1 μL of 50 mg/L) of methyl malonate can be detected.

Methyl malonate and Fast Blue B were purchased from Sigma Chemical Co., St. Louis, MO. A working 5 μL solution of Fast Blue B was prepared freshly each day in ethanol/water (75/25 by vol) and to 100 μL of this, 4 mL of glacial acetic acid was added. Cellulose thin-layer chromatographic plates (20 x 20 cm, Polygram cel 300, product of Machery-Mage), Germany) were obtained from Brinkmann Instruments Co., Inc., Westbury, NY 10590. The plate was cut into 5 x 5 cm pieces for routine use. Aqueous standard solutions of methyl malonate (50, 100, 150, and 500 mg/L) and 0.1-μL urine samples were applied through a 0.6-μL Microcap (disposable capillary glass tube, product of Drummond Scientific Co., distributed by Ace Scientific Supply Co., East Brunswick, NJ), 0.5 cm from the bottom. The chromatography was performed in an ordinary 200-μL glass beaker containing solvent solution (butanal/acetic acid/water, 12:3:5 by vol) to a height of 0.3 cm. The beaker was then covered with aluminum foil (3). After 30 min, when the solvent front had reached the top, the plate was removed, air-dried, sprayed lightly with a 5 g/L aqueous solution of Fast Blue B, air-dried again, and placed in a light-proof container for 30-60 min.

After this chromatography and staining, a distinct purple spot of methyl malonate (in concentrations of 50 mg/L or greater) appeared on the upper portion of the chromatogram (Rf = 0.87), with most of the other urinary components, which yield a brownish color, trailing far behind. If kept in the dark after drying, the color of the methyl malonate spot was stable for at least 4-6 h. The methyl malonate spot appears more quickly if the plate is blotted with air at about 60 °C for 5-7 min after staining. The color reaches its maximum intensity within 10-15 min, but the spot gradually becomes unidentifiable because of a rapid increase in the color of the background.

This method of detecting methyl malonate in urine with commercially available cellulose thin-layer plates is simple, rapid, and inexpensive. It requires only 0.1 μL of urine sample. No specific facility or equipment is needed. The chromatography takes 30 min, and color development 30-60 min.

The limit of detectability of methyl malonate is 50 mg/L, the same as described by Coulombe et al. (2), who recently successfully screened the entire neonatal population of Massachusetts.

We have successfully tested a urine sample from a patient with known methylmalonic acidemia, thus confirming that our method can be applied to mass screening. Possible interference by endogenous or exogenous compounds, or both, as well as the chance of false-positive and false-negative values remains to be investigated.

Auray-Blais et al. (4) described a micro-scale thin-layer chromatographic technique for urinary methyl malonate. In their method, the thin layer was prepared from slurry of silica gels or cellulose and dried overnight, and they could detect 1 μg of methyl malonate or more per applied sample. Our method can detect 5 ng (0.1 μL of a 50 mg/L sample). Thus, our method not only is more convenient, it has a higher sensitivity.

References


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Free Amino Acid Concentrations in Pericardial Fluid

To the Editor:

Pericardial fluid is considered to be a passive ultrafiltrate of plasma (1). Our review of the literature from 1976 revealed no reference to its free amino acid concentrations. We analyzed 30 samples of normal pericardial fluids from patients with no pericardial pathology or arterial or venous-collateral hypertension who were undergoing open-heart surgery.

We used single-column ion-exchange chromatography (Chromaspec, Rank-Hilger), with the following conditions. Resin: Rank-Hilger, 7 μm. Column length 50 cm; temperature 40 to 60 °C; flow = 17 mL/min; pressure 2-4 MN/m². Sample: sample injection time = 60 s; volume of sample = 200 μL. Buffer Li citrate: acid (pH = 2.2); basic (pH = 11.5). Color reagent: ninhydrin. Incubation temp. 98 °C. Absor-

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