

DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF 4-HYDROXY-3-METHOXY-MANDELIC ACID (VANILLYLMANDELIC ACID) IN URINE BY PAPER CHROMATOGRAPHY

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ABSTRACT

We have developed a simple and precise paper chromatographic method for the determination of 4-hydroxy-3-methoxymandelic acid (VMA) in urine. Concentrations of VMA in patients with neuroblastoma were increased in comparison to controls. The linearity was excellent in the concentration range tested. The within-assay coefficient of variation for control and patient urine was less than 2.2%. The recovery was in the range of 97.9-99.4%. Results from testing urine samples of controls and patients with neuroblastoma suggest that this method is a reliable and convenient system for quantification of VMA in urine and can be used in the mass screening of neuroblastoma in infants. Sample preparation requires minimal time and the entire procedure is completed within 5 h.

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INTRODUCTION

4-Hydroxy-3-methoxymandelic acid (VMA) is one of the major catecholamine metabolites normally excreted in urine. The determination of urinary VMA is of importance for the diagnosis of neuroblastoma, pheochromocytoma and Parkinson's disease.^{1,2} Knowledge of the excretion pattern of VMA in urine is an effective aid in the diagnosis of catecholamine secreting tumors such as pheochromocytoma, neuroblastoma and ganglioneuroma.³ Neuroblastoma is one of the few malignant tumors that excrete unambiguous markers for diagnosis. Neuroblastoma, a malignant tumor of infancy and childhood, has a relatively good prognosis, even with disseminated disease.⁴ Neuroblastoma is the most common solid tumor of childhood with an incidence of 1 in 7000 children under the age of 5 years. It arises from cells of the sympathetic nervous system and has the characteristic of secreting dopamine and its major metabolite homovanillic acid, in excess.⁵ Recently, a great deal of progress has been made concerning the use of VMA in the evaluation of psychiatric patients and VMA has been used to monitor chronic lead exposure and response to medication during the treatment of Parkinson's disease.⁶ Many procedures have been

applied to the assay of VMA including thin layer and paper chromatography,^{7,8} electrophoresis,⁹ spectrophotometry,¹⁰ gas chromatography,¹¹ gas chromatography-mass spectrometry,¹² and high-performance liquid chromatography.¹³⁻¹⁶ However, these methods are difficult to perform quantitatively for use in routine clinical chemistry laboratories.

Our aim in the present work was to separate VMA from other urinary metabolites and devise a simple, low-cost, accurate, and precise method for monitoring urinary VMA concentrations. The assay presented here is a simple procedure for quantifying VMA by paper chromatography. This method is applicable for use in routine clinical chemistry laboratories.

MATERIAL AND METHODS

Pure VMA, citrate, sodium azide, sodium sulfate, Whatman paper, formic acid, P-nitroaniline, ethyl acetate, phenolic acid, ether, ethanol, acetone and formamide were obtained from Sigma (St. Louis, Mo., U.S.A.). Other reagents were purchased from Merck (Darmstadt, F.R.G.).

Standard preparation: A stock solution (500 µg/mL of VMA) was prepared by diluting 50 mg of VMA to 100 mL

Determination of VMA Levels in Urine

roblastoma patients. After testing more urine samples, particularly those from infants suffering from neuroblastoma, we believe that this paper chromatographic method can be used effectively in the mass screening of neuroblastoma in infants.

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