IDENTIFICATION OF ABNORMAL URINARY ORGANIC ACIDS IN INHERITED METABOLIC DISEASES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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A gas chromatography/mass spectrometry (GC/MS) coupled system has been established for the confirmatory identification of abnormal urinary organic acids in inherited metabolic diseases. Samples of patient urines were extracted with an organic solvent and trimethylsilylated (TMS). A mass spectra of gas chromatographically separated TMS derivatives can be obtained using the GC/MS coupled system with a single analytical run. Those compounds with close methylene units (*e.g.*, 4-hydroxyphenylacetaic acid and phenylpyruvic acid) in the gas chromatograph can be identified by their specific mass spectra. The results indicate that this GC/MS system is a powerful method for identifying abnormal urinary organic acids. These acids can be identified by comparison with authentic mass spectra established in our laboratories or with mass spectra files from other sources or they can be directly identified by analysis of the mass spectrum. By using this system, we were able to make positive identification of several inherited metabolic diseases found in Chinese patients, including phenylketonuria, propionic acidemia, and methylmalonic aciduria. This GC/MS system is a powerful tool for the diagnosis of inherited metabolic diseases.

In order to study inherited metabolic diseases, a gas chromatography (GC) system for the analysis of abnormal urinary organic acids has been established in our laboratories[1,2]. Because of the complexity and the wide range of organic acids present in urine, it has been difficult to analyze these urinary organic acids. The methodology of extraction and the characterization of the trimethysilylated (TMS) derivatives of urinary organic acids by GC has been applied to a small volume (mls) of urine samples.

The methylene unit of an organic acid compound can be determined by putting the treated urine sample through the gas chromatograph. By comparing the methylene unit of the unknown organic acid with the methylene units of known standards, we can identify the compound. In the past it had been demonstrated that a gas chromatograph equipped with a 10% OV-1 packed column could not separate compounds with close methylene units. Thus phenylpyruvate, an abnormal metabolite of phenylketonuria (PKU), could not be separated from 4-hydroxyphenylacetate, a normal metabolite[2]. There are other unknown compounds which can not be identified, either because standards are lacking or because they are new compounds for which standards are not yet available. Therefore, a technique with sufficient specificity to make positive

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