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Note

Specific and sensitive method for the determination of C_6—C_{10} dicarboxylic acids in serum and urine by selected ion monitoring

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The C_6—C_{10} dicarboxylic acids, adipic, suberic, and sebacic acids, have been shown in recent years to be of biological interest. Substantial amounts of adipic and suberic acids have been found in urine from patients with ketosis [1, 2], and from a patient with carnitine deficiency [3]. In the metabolic disturbances glutaric aciduria, type II [4], and dicarboxylic acidurias [5] large urinary excretions of all three acids have been detected. Small amounts have been measured in urine from patients with glycogen storage disease [6] and in normal newborn infants [7, 8].

The gas chromatographic (GC) methods employed so far in the dicarboxylic acid analyses lack both specificity and sensitivity for quantitative work in serum and in urinary concentrations below 10—20 μg/mg creatinine [7, 8].

We now report a reliable selected ion monitoring (SIM) method for the quantitation of adipic, suberic and sebacic acids in serum and urine. The method has been used for the determination of dicarboxylic acids in serum from ten normal children, aged 5—13 years and in the urine from ten normal children, aged 2.5—10 years and ten normal neonates, aged 1—4 days.

MATERIALS AND METHODS

Chemicals

Adipic acid (1,6-hexanedioic acid), suberic acid (1,8-octanedioic acid), and sebacic acid (1,10-decanedioic acid) were purchased from Koch-Light (Colnbrook, Great Britain). Diethylglutaric acid (1,5-(3,3-diethyl)-pentanedioic acid; DEGA) was obtained from EGA Chemie (Albuch, G.F.R.). The stationary phase for GC, Dexsil 300, was obtained from Analabs (North Haven, Conn.,

U.S.A.) and column support, Chromosorb W HP from Koch-Light. The silylation mixture bis-(trimethylsilyl)-trifluoroacetamide (BSTFA)—trimethylchlorosilane (TMCS) (100:1) was purchased from Pierce (Rockford, Ill., U.S.A.).

Clinical material

Urine was collected from ten full-term neonates (six males and four females), aged 1—4 days. The first morning micturition was collected from ten normal children (five males and five females), aged 2.5—9 years old. Serum was obtained from ten hospitalised children without any clinical or biological signs of a metabolic disorder. All specimens were kept at —20° until analysis.

Analytical equipment

The measurements were performed by means of an AEI MS-30 double focusing mass spectrometer equipped with a Pye Unicam 104 gas chromatograph, a 6 channel selected ion monitoring unit and a 6 channel oscillographic ultra violet recorder. The analytical conditions were as follows: The GC column was a glass coil (7 ft. × 1/4 in. I.D.) packed with 3% Dexsil 300 on Chromosorb W HP. The column temperature was programmed from 200° at 15°/min and the helium carrier flow-rate was 40 ml/min. Injection port temperature was 280°. The interface between the gas chromatograph and mass spectrometer consisted of a glass transfer line and a membrane separator at 250°. The temperature of the electron impact ion source was 220°. Ionizing and accelerating potentials were 70 eV and 4 kV, respectively.

Analytical procedure

Urine samples containing 2 mg of creatinine were mixed with water and 200 μl of a solution of 400 mg DEGA/l (internal standard) to a final volume of 10 ml. The solution was saturated with NaCl and pH was adjusted to 1 with 5 *M* HCl solution prior to four consecutive extractions with 40 ml diethyl ether. The organic phases were dried (Na_2SO_4) and the solvent was evaporated in a stream of dry nitrogen. Silylation of the remainder was performed with 400 μl BSTFA—1% TMCS at 60° for 1 h before 4 μl of the mixture were injected into the SIM system. The silylated dicarboxylic acids remained stable for at least one month when kept at —20°.

Serum samples (2 ml) were mixed with 4 ml ethanol. The precipitated protein was removed by centrifugation, then the ethanol was evaporated in a stream of nitrogen. The resulting aqueous solution of the serum dicarboxylic acids was then diluted to 10 ml with water and the extraction and silylation procedures were performed exactly as described above for the urine samples, except that the remainder was silylated with 50 μl BSTFA—TMCS mixture. The fragment ions used in the quantitative analysis of the dicarboxylic acids were in all cases $M^+ - 15$; *m/e* 275 for adipic acid, 303 for suberic acid, 317 for DEGA, and 331 for sebacic acid. The heights of the respective SIM-peaks were used as a measure of the amounts of compounds. The ratios of *m/e* 275, 303 and 331 to *m/e* 317 were calculated and compared with the standard curves (Fig. 1).

As the detector response ratios were very dependent on the conditions of the mass spectrometer, i.e. tuning and source condition, the slope of the standard

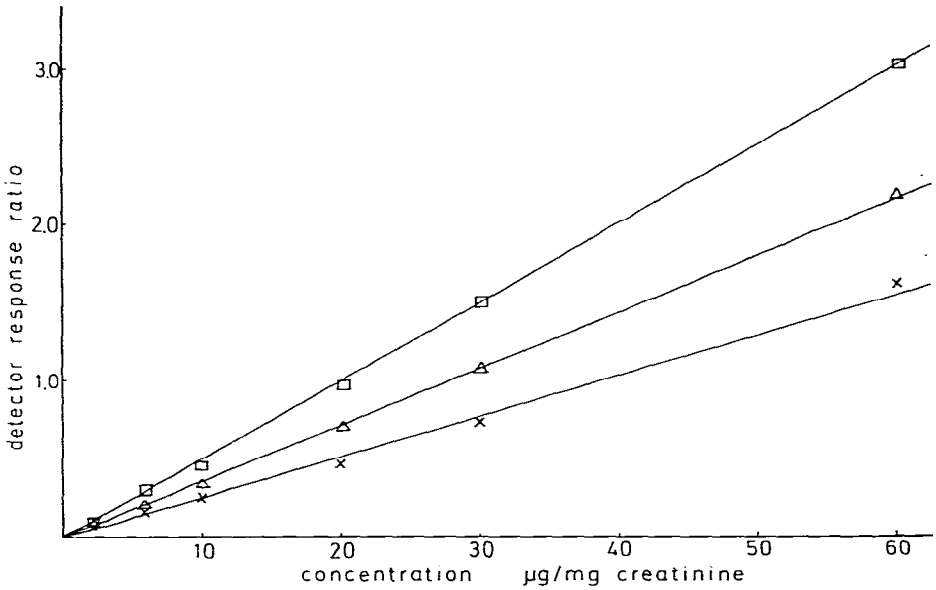

Fig. 1. Standard curves for adipic acid (x), suberic acid (△), and sebacic acid (□) in normal urine. Increase in detector response ratios (peak height of SIM peaks 275 (x), 303 (△) or 331 (□) divided by the peak height of 317) versus dicarboxylic acids added to normal urine.

curve for each acid was determined before each series of 10—20 samples. Standard solutions for urine and serum of 0 and 60 µg/mg creatinine and 0 and 50 µg/100 ml, respectively, were used in this determination of the slope. Standard solutions were prepared by enrichment of normal serum and urine samples with adipic, suberic and sebacic acids.

RESULTS AND DISCUSSION

Fig. 2A shows the SIM profiles of the ion fragments *m/e* 275, 303 and 331 from a silylated extract of a mixture of serum from a 12 year old child. The concentrations of adipic, suberic, and sebacic acids in this mixture were 4, 4 and 3 µg/100 ml, respectively. This result illustrates the high sensitivity and specificity of the present method. The specificity, expressed as the ability to differentiate between compounds with the same nominal mass, but with different atomic compositions, is about 1000 ppm, the resolution of the mass spectrometer. The interference of other compounds with the SIM peaks in the 30 investigated serum and urine samples was negligible. This is a considerable improvement compared to the GC technique with flame ionisation detector [8], where the lack of specificity resulted in a limit of precise detection for adipic acid of about 10 µg/mg creatinine. The extraction efficiency, measured as the fraction of dicarboxylic acids extracted from pure water compared to freeze drying of the same solution, was for all the dicarboxylic acids including the internal standard, above 70% at a concentration of 6 and 30 µg/2 ml solution.

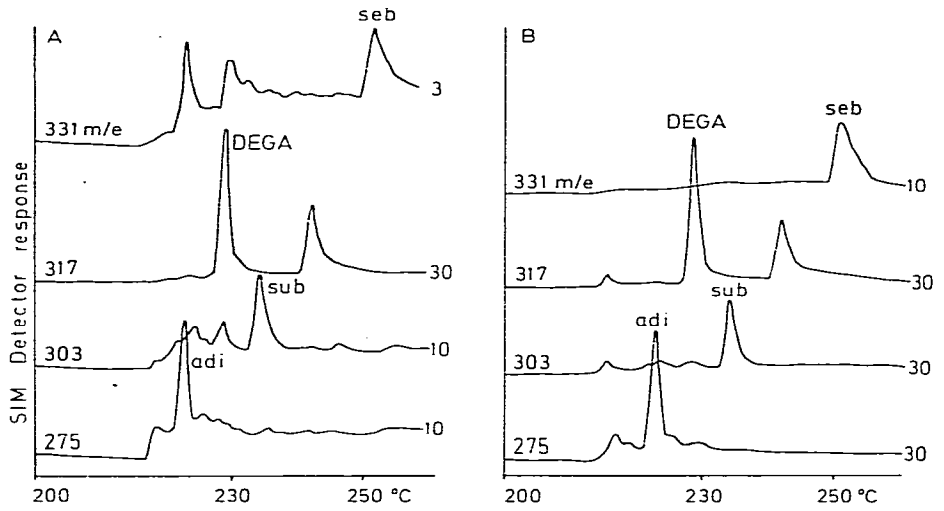

Fig. 2. Mass fragmentograms of: A, silylated extract from normal serum; B, silylated extract from normal serum enriched by 10 μg dicarboxylic acids per 100 ml serum. Peaks: adi = adipic acid; sub = suberic acid; DEGA = diethylglutaric acid (internal standard); seb = sebacic acid. The mass fragment ions are shown to the left on each trace and the relative attenuations to the right (GC conditions, see text).

Because of small variations in this extraction efficiency and also because of variation in the detector responses, it is essential for the SIM measurements to use an internal standard with a chemical structure similar to that of the compounds of investigation.

The results of the analysis of the variation between series of urines are shown in Table I. They are expressed as the standard deviation of the determinations of 10 aliquots from the same enriched urine, extracted and analysed on 10 different days. The variation coefficient of 10—15% is of the same order of magnitude as in the conventional GC method [8]. The variation within a series, i.e. the standard deviation of 10 consecutive determinations of 10 different extracts of the same urine, was 3—8% (results not shown).

The coefficient of variation for the serum analysis was 4—15%, measured at concentrations 10 and 50 μg/100 ml serum (results not shown).

The reliability of the present SIM method is shown by the results of the analysis of urine from neonates and children (Table II), and serum from children (Table III).

TABLE I

VARIATION BETWEEN SERIES OF DICARBOXYLIC ACID DETERMINATIONS

The figures represent the detector response ratios. Number of measurements = 10.

	Adipic acid		Suberic acid		Sebacic acid	
Concentration (μg/mg creatinine)	9	32	8	30	6	29
Mean ratio	0.23	0.83	0.29	1.08	0.33	1.48
Standard deviation	0.03	0.10	0.02	0.10	0.03	0.20
Variation coefficient	13%	12%	7%	9%	9%	14%

The finding of adipic, suberic, and sebacic acids in the serum and urine of all the investigated children indicates very strongly that the ω-oxidation pathway from long-chain monocarboxylic acids [9] is operative under normal physiological conditions and not only in pathological states [1—6]. The larger excretion of the dicarboxylic acids in the urine of the neonates than in that of the children (Table II) most probably reflects the high lipolytic activity and increased catabolism of fatty acids in the neonates [10].

TABLE II

EXCRETION OF ADIPIC, SUBERIC, AND SEBACIC ACIDS IN THE URINE OF NEONATES AND CHILDREN (μg/mg CREATININE)

	Adipic acid		Suberic acid		Sebacic acid	
	Range	Median	Range	Median	Range	Median
Neonates (n = 10) aged 1—4 days	2—62	15	3—19	6	0.2—12	6
Children (n = 10) aged 2.5—9 years	2—15	5	1— 9	2	0.6— 7	3

TABLE III

CONCENTRATION OF ADIPIC, SUBERIC AND SEBACIC ACIDS IN SERUM FROM CHILDREN

Concentrations in μg/100 ml; n = 10 children.

	Adipic acid		Suberic acid		Sebacic acid	
	Range	Median	Range	Median	Range	Median
Children aged 5—13 years	2—5	3	2—5	3	1—3	2

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