Solid-Phase Extraction Technique for Gas-Chromatographic Profiling of Acylcarnitines

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We present a simple, new clean-up method for the gas-chromatographic profiling analysis of acylcarnitines. The use of a solid-phase, cation-exchange extraction combined with gas-chromatographic separation, based on the derivatization into acyloxy lactones by Lowes and Rose (Analyst 1990;115:511–6), allows a selective and sensitive screening for acylcarnitines in urine. As such, a quantitative approach was developed for differential evaluation of acylcarnitines for detection of inborn errors of metabolism; the evaluation is both fast and routinely applicable in any biochemical laboratory. We validate the analysis method for acylcarnitines of various chain-lengths and present examples of its application to urine samples from diseased patients. We give special attention to the medium-chain acylcarnitines because of their association with medium-chain acylCoA dehydrogenase deficiency. Finally, the quantitative nature of the analysis allows evaluation of the acylcarnitine excretion over time.

Indexing Terms: heritable disorders • screening • urine • sample preparation

L-Carnitine (3-hydroxybutyrobetaine) has a vital role in the mitochondrial β-oxidation as a carrier of fatty acyl groups across the mitochondrial membrane (1). Several other metabolic roles of L-carnitine have been elucidated, including its ability to combine with accumulating, potentially toxic acyl groups inside the mitochondrion. The resulting acylcarnitines are then passed through the mitochondrial membrane, out of the cell, to be excreted in the urine (2).

In several inborn errors of metabolism known as organic acidurias, secondary carnitine deficiency has been described as a complication. Children with defects in catabolism of fatty and branched-chain amino acids excrete acylcarnitines that are diagnostic of the particular enzyme defect involved, even in cases where the organic acid profile is nonspecific (2, 3). Additionally, clinical improvement after supplementation with L-carnitine has been noted in several patients, in each case accompanied by an important increase in renal acylcarnitine excretion (4). Consequently, a fast, selective, and routine analytical method for profiling acylcarnitines in urine would contribute to the clinical diagnosis and therapeutic follow-up of such diseases.

The highly polar, nonvolatile acylcarnitines are poor candidates for analytical methods that depend on initial sample volatilization. Several analytical approaches based either on direct mass spectrometry (fast atom bombardment mass spectrometry [FAB-MS]) or liquid chromatography have been reported (5–7). Liquid chromatography of ionic substances is generally troublesome, and most liquid-chromatographic methods lack the required resolution. In addition, HPLC analysis of acylcarnitines has a general detection problem (8). Chemical derivatization for ultraviolet detection has been done but is difficult (7, 9). Radiochemical detection, although currently the most sensitive method (10), will not detect those acylcarnitines that are not substrates for the enzyme carnitine acyltransferase (EC 2.3.1.7) (7, 8, 11). Finally, these procedures generally require an additional analysis technique for positive identification of the eluting compounds (6). Liquid chromatography with mass spectrometry, which has interesting identification capabilities, is still proving itself. The power and diagnostic usefulness of this method relies heavily on the availability of expensive, sophisticated instrumentation, and a level of expertise that very few laboratories can provide (8).

Most of our present knowledge of acylcarnitines results from FAB-MS/MS analysis. However, its advantages of speed, sensitivity, and little sample preparation are very much compromised by the limited availability of the instrumentation, and isomeric acylcarnitines are still a problem.

Gas chromatography with flame ionization detection (GC-FID) or gas chromatography–mass spectrometry (GC-MS) of volatile derivatives of acylcarnitines [as acyloxy lactones (12) or N-demethylated derivatives (13)] presents the possibility of ready implementation in any biochemical or pediatric laboratory. However, gas-chromatographic analysis with such universal detectors requires an extensive and selective sample clean-up, before the analysis. The existing sample preparation procedures, developed mainly for selective analysis methods such as FAB-MS or HPLC with single-wavelength detection, are based on sequential anion- and cation-exchange procedures or ion-exchange followed by reversed-phase extraction (5, 13, 14). The dual ion-exchange procedures are laborious and the large, aqueous elution volumes require a time-consuming lyophilization step. Here we present a fast method based on a single ion-exchange step with solid-phase cartridges. We also describe its application to the examination of

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2 Nonstandard abbreviations: FAB-MS, fast atom bombardment mass spectrometry; GC-FID, gas chromatography–flame ionization detection; GC-MS, gas chromatography–mass spectrometry; and MCAD, medium-chain acylCoA dehydrogenase deficiency.
the oxylactone derivatives of acylcarnitines (12) by GC-FID and GC-MS and to the quantitative evaluation of some clinical samples.

**Materials and Methods**

**Reagents**

All reagents and chemicals were of analytical grade and were obtained from E. Merck AG (Darmstadt, Germany) unless stated otherwise. N,N-Diisopropylethylamine (98%) and L-carnitine were from Janssen Chimica (Beerse, Belgium) and “Instant methanolic HCl” kits were purchased from Alltech Associates (Deerfield, IL). A 35 mL/L methanolic HCl solution was prepared by adding 3 mL of acetylclorex to 50 mL of ice-cold and dried methanol. Acetonitrile was dried over CaH₂ before use in the derivatization procedure.

**Column-exchange solid-phase extraction columns (PRS, Analyticchem Bond Elut), containing 500 mg of silica matrix with covalently bonded propylsulfonic acid groups (sodium form), were obtained from Varian Analytical Equipment (Harbor City, CA). We used a Varian Vac Elut SPE extraction system to facilitate and accelerate the various steps of the isolation procedure. Small in-line filters (Millex HV₂) were obtained from Waters Chromatography Division (Milford, MA).

**Standards**

All acylcarnitines were synthesized according to the method of Böhmer and Bremer (15) with use of the corresponding acetylclorex or, if unavailable, by in situ preparation from the acid and thionyl chloride. The resulting white crystalline materials were characterized by infrared spectroscopy, 1H-NMR spectrometry, FAB-MS, high-resolution FAB-MS, and B/E linked-scan tandem MS. The purity of the preparations was assessed by thin-layer chromatography (16), 1H-NMR analysis, and gas chromatography.

Stock solutions were prepared by dissolving about 250 μmol of acylcarnitine in 100 mL of methanol. From these, a standard mixture was prepared containing 17 different acylcarnitines (140 μmol/L each). A methanolic solution of undecanoyl-L-carnitine (1.4 mmol/L) was used as internal standard. All these solutions were stored in a desiccator under reduced pressure and refrigerated (−20 °C). They were freshly prepared each month to avoid deterioration.

**Samples**

Urine samples were obtained from healthy adult volunteers, normal children, and infants with various metabolic disorders. All samples were stored at −20 °C without additives. Before analysis, they were thawed and the creatinine content was determined. All procedures and experiments were in compliance with the regulations and ethical standards of our university's ethical committee and those of the Belgian government.

**Procedures**

**Solid-phase extraction.** Activate the columns by washing them consecutively with 3 mL of methanol and 3 mL of 35 mL/L methanolic HCl. Wash away the excess methanolic HCl with two 3-mL portions of doubly distilled water. Prepare the urine samples by adding 50 μL of methanolic internal standard solution to an amount of urine containing 1 mg of creatinine. Dilute the samples, if necessary, to a total volume of 5 mL with doubly distilled water and adjust the pH to 10.0–10.5 with a 100 g/L aqueous Na₂CO₃ solution. Extract this mixture with 5 mL of ethyl acetate on a rotary mixer (10 min). After centrifugation (700 × g, 2 min), discard the upper phase and adjust the pH to 2.5–3.0 with 2 mol/L HCl. Apply completely to the activated columns at slow flow rate (0.5 mL/min) and wash the columns with 3 mL of doubly distilled water twice and then with 3 mL of methanol. Dry the columns by applying full suction for 1 min. Elute the columns consecutively three times with 1 mL of a 40 mmol/L BaCl₂ solution in methanol/H₂O (75/25, by vol). Combine the eluates and evaporate under a gentle stream of nitrogen with heating at ~50 °C. Extract the residue successively with 2 mL and 1 mL of acetonitrile (10 min, mixed by using ultrasonic vibration) and combine the organic layers in a reaction vial. Evaporate to dryness under a gentle stream of nitrogen.

**Derivatization.** Redissolve the residue in 200 μL of a 7.5 mL/L solution of N,N-diisopropylethylamine in acetonitrile and heat for 30 min at 120 °C (12).

**GC-FID analysis.** We used a Hewlett-Packard (Palo Alto, CA) Model 5890 Series II gas chromatograph with helium as carrier gas at a linear velocity of about 25 cm/s. A Hewlett-Packard 3396 Series II integrator was used to determine peak areas. Injection (4 μL) was splitless with a purge-off time of 1 min, at a temperature of 200 °C; the detector temperature was maintained at 290 °C. The chromatographic column was a semi-polar DB-1701 fused silica column [30 m × 0.25 mm (i.d.), film thickness 0.25 μm] from J&W Scientific (Folsom, CA). A retention gap [2.5 m × 0.32 mm (i.d.), apolar deactivated] was mounted in front of the analytical column. The oven temperature was programmed initially from 90 to 150 °C at 30 °C/min, and then to 290 °C at 6 °C/min. The temperature ramp was started 1 min after injection; on reaching 290 °C, this temperature was maintained for 6.6 min. Relative retention indices (methylene units) (17) were calculated to identify the various peaks.

**GC-MS analysis.** We performed GC-MS to verify the identity and purity of the peaks in the acylcarnitine profiles, using a Finnigan-MAT (San Jose, CA) INCOS-500 quadrupole mass spectrometer, coupled to a Varian 3400 gas chromatograph, equipped with a J&W Scientific DB-1 fused silica column [30 m × 0.25 mm (i.d.), film thickness 0.25 μm] and a splitless injector. All GC conditions were as reported above except for the final temperature, which was maintained for only 3.4 min at 280 °C. For routine measurements we operated the instrument in the electron impact mode, with an electron energy of 70 eV and a source temperature of 150 °C. The direct-line interface was maintained at 285 °C. Repetitive scanning was used over a mass range from 50 to 450 amu, with a scan cycle time of 0.3 s. In the chemical ionization mode, methane was the reaction gas at a
source pressure of 0.19 kPa, a source temperature of 125 °C, and an emission current of 750 μA.

Analytical Variables

**Extraction recoveries.** Recoveries from urine for 17 acylcarnitines were determined by comparing the sample/internal standard peak-area ratios after extraction with the peak-area ratios for directly derivatized standard solutions. Five 0.5-mL aliquots of the standard mixture were evaporated under a stream of nitrogen after addition of 50 μL of methanolic internal standard solution. The samples were then derivatized and analyzed in triplicate by GC-FID as described above. Five additional 0.5-mL aliquots were also evaporated but without the addition of internal standard. After reconstitution of the residue in 2 mL of a pooled urine blank, the acylcarnitines were extracted together with five samples from the urine blank. This urine, pooled from neonatal urine samples, had been analyzed several times and showed no detectable acylcarnitines at the highest sensitivity setting. Additional enzymatic measurements revealed a very low total carnitine concentration (18).

Internal standard (50 μL) was added to each vial just before the evaporation of the combined acetonitrile fractions. All samples were then derivatized and analyzed in triplicate. This scheme was executed in parallel on five 0.5-mL aliquots of the standard mixture, reconstituted in doubly distilled water, and continued for four consecutive days on two samples. Response factors, recoveries, and coefficients of variation (CVs) for individual components were calculated. Reproducibilities were expressed as “within-day” and “total” (within + between) variation.

**Quantification and linear response.** Standard curves were prepared by dissolving the residues obtained after evaporation of 0, 100, 200, 350, 500, 600, and 750 μL of the methanolic standard mixture in an aliquot of pooled blank urine having a creatinine content of 1 mg. After addition of 50 μL of internal standard solution, each supplemented urine was extracted, derivatized, and analyzed by GC-FID. The peak-area ratios were calculated and subjected to a linear curve fit. For daily routine quantification we extracted with each series of unknown samples a blank urine sample, supplemented with 0.5 mL of the acylcarnitine standard mixture and internal standard, which allowed us to quantify the components of the urinary acylcarnitine profiles.

**Precision.** We evaluated the precision of the analysis for quantifying the separate acylcarnitines. Five blank urine samples supplemented with 17 acylcarnitines (each at 18.3 μmol/L (55 μmol/g of creatinine)) were analyzed on the same day, together with the samples for a calibration curve. The quantification data (based on linear regression analysis) were used to estimate the within-day reproducibility. For total reproducibility studies, three standard mixtures containing 17 acylcarnitines were prepared in urine at a concentration of 5, 16.7, and 36.7 μmol/L (respectively 15, 50, and 110 μmol/g of creatinine). By replicate analysis (n = 5) of these standard mixtures and corresponding calibration curves over a 4-week period, we determined the CVs for the calculated concentrations.

**Results and Discussion**

**Development and Optimization of the Extraction Method**

The extraction method we envisioned had to be fast, selective, and routinely applicable. For that, an extraction based on solid-phase extraction cartridges was perfectly suitable. The prepacked, modified silica sorbents allowed a reproducible and selective interaction of the acylcarnitines. Moreover, the use of a silica matrix eliminated the problem of irreversible binding of nonpolar acylcarnitines to the formerly used nonpolar polymeric resins (19). For optimal retention, the sulfonyl acid groups of the sorbent should be equilibrated with a low-selectivity counter-ion. We used a dilute solution of methanolic HCl in the column conditioning phase, which replaced the stronger sodium ions with protons. It also obviated the adsorption of an impurity onto the ion-exchange matrix that otherwise produced a large, early-eluting peak obscuring the chromatogram preceding the elution of the C3-acylcarnitines.

The preliminary preparation of the urine sample consisted of a solvent extraction at pH 10.0–10.5 to eliminate most of the basic urine constituents. We used ethyl acetate because, in our experiments with various solvents for the extraction of the post-elution residue, it showed the least overall solubility for the acylcarnitines.

Previous ion-exchange methods most commonly used an aqueous NH₄OH solution to elute the acylcarnitines from the sorbent. Elution was based on displacement of the quaternized acylcarnitines by the ammonium ion. Unfortunately, large elution volumes were necessary, given the equipotent nature of the two ion species, resulting in an inevitable and extremely time-consuming lyophilization step. Moreover, we found that the strong alkaline nature (pH 14) of such an eluent induced substantial hydrolysis of the acylcarnitine ester bond. In our method, elution was performed with 3 mL of a highly selective barium salt solution of neutral pH. This eluent completely eluted nearly all of the acylcarnitines in the first two 1-mL fractions. Only for elution of the short-chain acylcarnitines (a residual 2–3%) was a third 1-mL fraction needed. Methanol is needed in the eluent to solvate the less-polar acylcarnitines, but in a 90/10 to 60/40 (by vol) proportion, its concentration has little influence on the elution profile.

A final step removed the excess BaCl₂ as well as the remaining water, which interferes with the derivatization. The complete evaporation of the effluent and solvent extraction of the dry residue resulted in good extraction recoveries for all acylcarnitines, with adequate elimination of the salt. Only inadvertent manipulation sometimes yielded salt residues in the derivatization vial. In these rare cases we add a simple filtration, using small, in-line filters after addition of the derivatization reagent. Relative extraction recoveries of
consecutive extractions with acetonitrile (2 mL, 1 mL, 1 mL) show a nearly complete recovery (>95%) for all acylcarnitines after two extractions. We find that this extraction is simple, effective, and cost-saving.

Systematic studies of relative extraction recoveries for the whole extraction sequence and their reproducibility in urine or water samples supplemented with the standard mixture of 17 different acylcarnitines are presented in Table 1. The results are quite suitable for a multicomponent profiling analysis. Our results seem to be somewhat lower than generally reported, presumably because we included an ethyl acetate extraction step to prevent negative interference in the derivatization. The resulting FID chromatogram for a normal urine sample after extraction is illustrated in Figure 1.

Chromatographic Analysis

The derivatization methods of Lowes and Rose (12) and Huang et al. (13) both yield derivatives with good chromatographic properties. Although the latter method is preferable for analyzing dicarboxylic acid monocarnitines, we continued our experiments using the originally published acyloxyacetate derivatives. Experiments in our laboratory showed that both methods share the same disadvantages, particularly thermal degradation of the acylcarnitine derivatives into fatty acids.

To identify the different acylcarnitine homologs and evaluate the usefulness of various stationary phases, we determined methylene units. Both nonpolar and semipolar stationary phases showed high reproducibilities, with CVs well below 0.3% (n = 20). We prefer the semipolar OV-1701 column for its excellent separation characteristics and its increased retention of the acylcarnitines in comparison with the apolar stationary phases.

This increased retention allows a better separation between the reagent peak and the early-eluting acetyl-L-carnitine. Consequently, we can inject aliquots of the derivatized mixture without preliminary evaporation to eliminate the derivatization reagent. This obviates the significant loss of the volatile acetyl- and propionyl-L-carnitine oxylactones during evaporation.

Figure 2 shows the chromatogram of an extracted urine sample supplemented with the standard mixture of acylcarnitines at a concentration of 23.3 μmol/L (70 μmol/g of creatinine). Separation and individual identification of (e.g.) n-valeryl-, isovaleryl- and 2-methylbutyryl-L-carnitine and the isomers octanoyl- and valproyl-L-carnitine are quite feasible. Unfortunately, the diagnostically important separation of tiglyl- and 3-methylcrotonyl-L-carnitine is not possible with this chromatographic method.

Use of the well-established GC-MS technique extends the utility of the analysis because the combination of retention data and electron impact mass spectra can provide unequivocal identification. For elucidation of unknowns, chemical ionization supplies useful informa-

### Table 1. Relative Extraction Recoveries for 17 Acylcarnitines Added to Urine (23.3 μmol/L) and Water (14 μmol/L), and Assay Reproducibility

<table>
<thead>
<tr>
<th>Acyl-L-carnitine form</th>
<th>Extraction recovery, %*</th>
<th>CV, %b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Water</td>
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<tr>
<td>Acetyl</td>
<td>55.8</td>
<td>56.8</td>
</tr>
<tr>
<td>Propionyl</td>
<td>66.8</td>
<td>54.0</td>
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<tr>
<td>Isobutyryl</td>
<td>74.9</td>
<td>61.0</td>
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<tr>
<td>n-Butyryl</td>
<td>71.5</td>
<td>59.0</td>
</tr>
<tr>
<td>2-Methylbutyryl</td>
<td>74.7</td>
<td>68.3</td>
</tr>
<tr>
<td>Isovaleryl</td>
<td>74.8</td>
<td>67.6</td>
</tr>
<tr>
<td>n-Valeryl</td>
<td>71.0</td>
<td>64.9</td>
</tr>
<tr>
<td>Tiglyl</td>
<td>69.7</td>
<td>68.9</td>
</tr>
<tr>
<td>Hexanoyl</td>
<td>71.9</td>
<td>64.5</td>
</tr>
<tr>
<td>trans-2-Hexanoyl</td>
<td>70.6</td>
<td>67.9</td>
</tr>
<tr>
<td>Valproyl</td>
<td>84.0</td>
<td>76.8</td>
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<tr>
<td>Heptanoyl</td>
<td>72.3</td>
<td>67.1</td>
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<td>Octanoyl</td>
<td>72.5</td>
<td>66.9</td>
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<tr>
<td>trans-2-Octanoyl</td>
<td>67.5</td>
<td>67.9</td>
</tr>
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<td>Nonanoyl</td>
<td>68.8</td>
<td>68.7</td>
</tr>
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<td>Decanoyl</td>
<td>69.3</td>
<td>70.2</td>
</tr>
<tr>
<td>Lauryl</td>
<td>56.9</td>
<td>74.4</td>
</tr>
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</table>

* Five samples analyzed in triplicate injections.

b From aqueous standard solutions (n = 5).

Fig. 1. GC-FID chromatogram for a urine sample of a normal neonate Compounds identified: (1) acetyl-, (2) propionyl-, (3) isobutyryl-, (4) 2-methylbutyryl-, (5) isovaleryl-, (7) n-valeryl-, (8) tiglyl-, (9) hexanoyl-, (10) trans-2-hexanoyl-, (11) valproyl-, (12) heptanoyl-, (13) octanoyl-, (14) trans-2-octanoyl-, (15) nonanoyl-, (16) decanoyl-, (17) undecanoyl- (internal standard), and (18) lauryl-L-carnitine

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tion about molecular mass because the oxylactones of acylcarnitines have little or no molecular ion peak (12).

Method Validation

Standard curves and linearity. Calibration curves are set up in urine over a concentration interval of 0 to 23.3 μmol/L (0 to 110 μmol/g of creatinine). In urine from healthy subjects, the acylcarnitine profile can be complex, the concentration of any particular species being relatively low (20). In pathological circumstances, however, concentrations can increase sharply and are dependent on the endogenous L-carnitine status. Consequently, our calibration interval is based on the reported reference values for total esterified carnitine (14, 20). All of the calibration plots generated gave a linear response and displayed correlation coefficients > 0.996 for nearly all acylcarnitines (Table 2). For concentrations exceeding the calibration range, the response deviated from linearity, in which cases we diluted the sample and reassayed. The obtained total reproducibility permits the use of a single, coextracted standard mixture for daily routine, quantitative evaluation of the acylcarnitine profile.

Precision. Mean within-day reproducibility (CV) for the quantitative analysis (17 acylcarnitines, each at 18.3 μmol/L, in five replicate samples) was 7.5% (SD 2.1%). Mean total reproducibility (17 acylcarnitines, five complete quantitative assays over a 4-week period) was 10.5% (SD 3.1%) for the 5 μmol/L concentration, 5.7% (SD 2.7%) for 18.7 μmol/L, and 8.3% (SD 2.4%) for 36.7 μmol/L. The precision varied but met our objective of a routinely applicable, multicomponent profiling analysis. We emphasize that, for a screening analysis of particular samples, individual concentrations can be important for time-dependent evaluation, but equal importance must be given to the specific profile of the urinary acylcarnitines.

Accuracy. The accuracy of the analysis, as assessed by the analytical recovery of standards added to normal blank urine specimens, varied according to concentration. For the 17 acylcarnitines under investigation, analytical recovery was 105.8% (SD 4.7%) for the 5 μmol/L addition, 95.1% (SD 4.7%) for 16.7 μmol/L, and 92.4% (SD 5.2%) for 36.7 μmol/L. A major cause of variation was the derivatization step. As already mentioned by Lowes and Rose (12), careful control of the cyclization reaction is required. Overheating or a long reaction time liberates free acid. This effect is, in our experience, increased by the presence of impurities from the urine matrix.

Functionality and Clinical Application

The suitability of the overall analytical method for urinary acylcarnitine profiling is illustrated in Figure 3. This GC-FID chromatogram was obtained after extraction of a urine sample from a patient with medium-chain acylCoA dehydrogenase deficiency (MCAD; McKusick 22274); the patient was asymptomatic and receiving no special carnitine supplementation at the time of the urine collection. The corresponding quantitative data are summarized in Table 3. Although FID is a relatively universal detector, the extraction procedure offers the necessary specificity to allow differentiation of individual acylcarnitines. The specific marker for MCAD, octanoyl-L-carnitine, as well as several other peaks, including the internal standard, can easily be identified on the basis of their methylene units (except decanoyl-L-carnitine, for which the concentration was in this case probably too low for positive identification). This opens the prospect of a sequential approach towards acylcarnitine analysis whereby a simple, routinely applicable prescreening permits selection of those samples worth further analysis by GC-MS or even FAB-MS/MS, hence optimizing the analytical throughput.

<table>
<thead>
<tr>
<th>Acyl-L-carnitine form</th>
<th>Slope</th>
<th>Intercept</th>
<th>$S_{yx}$</th>
<th>SD slope</th>
<th>SD Intercept</th>
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<td>-4.20</td>
<td>11.66</td>
<td>0.12</td>
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<td>-2.53</td>
<td>14.96</td>
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<td>18.79</td>
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<td>21.04</td>
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</table>

*Concentration interval: 0–96.7 μmol/L.
Coextraction of a standard mixture in the urine adds a quantitative dimension to the analysis. Absolute quantification of individual acylcarnitines is difficult for many reasons, and no "gold standard" of acylcarnitine quantification is yet available (21). Our approach, however, does allow us to monitor the time-based evolution of acylcarnitine excretion. Sequential samples from a symptomatic patient with propionic aciduria (McKusick 23200) over a 1-week period showed an initially marked excretion of propionyl-L-carnitine (679 μmol/L, or 2427 μmol/L/g of creatinine), followed by a brief decrease after therapeutic action (dialysis) to 18.4 μmol/L (153 μmol/g of creatinine). Within 3 days, however, the excretion increased again to 25.7, 321, and 326 μmol/L (1029, 3576, and 4344 μmol/g of creatinine).

Figure 4 illustrates a case—an asymptomatic patient with β-ketothiolase (EC 2.3.1.16) deficiency who was receiving no carnitine supplementation—in which subsequent analysis by GC-MS is inevitable. β-Ketothiolase deficiency, a defect of the 2-methylacetoacetoy-CoA thiolase in the metabolism of isoleucine, is characterized by urinary excretion of tiglyl-L-carnitine (22). Because no functional chromatographic separation is obtainable between the biological isomers tiglyl- and 3-methylcrotonyl-L-carnitine (from catabolism of leucine), differential identification is possible only by using the differences in their mass spectra (data in preparation).

Table 3. Urinary Acylcarnitine Profile of a Patient with MCAD

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc, μmol/L (μmol/g of creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-L-carnitine</td>
<td>143 (125)</td>
</tr>
<tr>
<td>Propionyl-L-carnitine</td>
<td>32 (28)</td>
</tr>
<tr>
<td>Isobutyryl-L-carnitine</td>
<td>24 (21)</td>
</tr>
<tr>
<td>2-Methylbutyryl-L-carnitine</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Isovaleryl-L-carnitine</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Hexanoyl-L-carnitine</td>
<td>40 (36)</td>
</tr>
<tr>
<td>Heptanoyl-L-carnitine</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Octanoyl-L-carnitine</td>
<td>327 (286)</td>
</tr>
<tr>
<td>Decanoyl-L-carnitine</td>
<td>63 (55)</td>
</tr>
<tr>
<td>Nonanoyl-L-carnitine</td>
<td>21 (18)</td>
</tr>
<tr>
<td>Undecanoyl-L-carnitine</td>
<td>16 (14)</td>
</tr>
</tbody>
</table>

In conclusion, the utility of this method lies in its new approach for urinary acylcarnitine sample preparation, based on a solid-phase ion-exchange procedure. The combination of fast, selective extraction and the powerful GC-(MS) analysis technique involving the derivatization by Lowes and Rose (12) may, in our view, initiate a more widespread integration of acylcarnitine profiling analysis in the pediatric laboratories doing routine metabolic screening.

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