Determination of Trace Amounts of Oxalate in Renal Calculi and Related Samples by Gas Chromatography – Mass Spectrometry

J. G. March¹ / B. M. Simonet¹ / F. Grases¹ / J. A. Muñoz² / M. Valiente²

¹ Department of Chemistry, Universitat de les Illes Balears, 07071 Palma de Mallorca, Spain; E-Mail: joan.march@uib.es
² Department of Analytical Chemistry (GTS), Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Key Words
Gas chromatography – mass spectrometry
Column liquid chromatography
Cation-exchange chromatography
Oxalate in renal calculi

Summary
A GC-MS method has been developed for determination of trace amounts of oxalate in renal stones. The steps involved are: solubilisation of the oxalate in renal stones with HCl, elimination of cationic species by use of a cation exchange resin, evaporation of water, reconstitution with ethyl acetate, and formation of trimethylsilyl esters of oxalic acid with chlorotrimethylsilane. Trichloroacetic acid was used as internal standard. With appropriate sample treatment the method has also been applied to urine and food. The main analytical features of the method were: linear range 0.2–5.0 mg oxalate L⁻¹, limit of detection 0.06 mg oxalate L⁻¹ (concentrations in the solution chromatographed), coefficient of variation of the method 1.3%. Twenty-seven renal stones were analysed by the method. The average oxalic acid content of urinary calculi was 0.11 mg g⁻¹ (SD 0.08, n = 13). A larger amount of oxalate was found in struvite calculi (mean value 0.2 mg oxalic acid g⁻¹, SD = 0.13, n = 8). Hydroxyapatite stones can be classified according to their oxalate content: samples with very low oxalate content (below the LOD of the method) and samples with large amounts of oxalate (0.431 and 0.801 mg oxalic acid g⁻¹).

Introduction
Urolithiasis is a serious health problem because, depending on geographical area, between 1% and 14% of the population suffers from the disease [1]. The causes of lithiasis are, nevertheless, because of its multifactorial origin [2], often unclear. Although there is some evidence suggesting that minor components could provide information on the conditions under which calculi are formed, very few trace components of renal stones have yet been investigated [3, 4]. The determination of oxalate, as a trace component, in calculi remains unstudied. In this sense it is interesting to point out that approximately the 50% of all types of renal lithiasis are recurrent and that approximately 50% of recurrent stone formers produce different types of renal calculi. Preliminary studies on patients from Mallorca (Spain) [5] showed that approximately 27% of recurrent hydroxyapatite stone formers developed calculus of calcium oxalate, and 21% developed mixed calculus of hydroxyapatite and calcium oxalate. Similar results were obtained with recurrent uric acid stone formers – 27% developed calcium oxalate calculus and 20% developed mixed uric acid/calcium oxalate calculus. The presence of oxalate as a minor component of a calculus is, therefore, likely to provide useful information about the composition of future calculi, and so preventive therapeutic measures could be taken accordingly. Obviously, confirmation of this clinical inference requires further extensive studies. For these reasons appropriate analytical methods are required for determination of oxalate at trace levels in renal calculi.

Calcium oxalate stones are the most frequent calculi in industrialised countries (approx. 65% of cases) [6]. The amount of oxalate excreted in urine is well-known to have an important role in calcium oxalate stone formation [7]. The oxalate present in the urine is the result of two contributions – oxalate synthesised in the body from different precursors (endogenous origin) and oxalate ingested (exogenous origin) [8]. Exogenous origin is especially important for patients with gastrointestinal hyperabsorption of oxalate, because the absorbed oxalate is not metabolised and is mainly excreted in the urine [9]. Thus, knowledge of urinary oxalate concentration and its relationship with diet are relevant for diagnosis and monitoring of hyperoxalurias. The concentration of oxalate in both urine and food is, therefore, related indirectly to renal stones, and for this reason both have also been included in this study.
Figure 1. Typical chromatogram obtained from a struvite stone containing 0.198 mg oxalic acid g⁻¹ (equivalent to 0.57 mg oxalic acid L⁻¹ in the solution injected): 1 = silylated trichloroacetic acid, 2 = silylated oxalic acid.

Figure 2. Evolution of analytical signal (peak height) with silylation reaction time for reaction at (a) 20 °C and (b) 60 °C. Oxalic acid concentration in the chromatographed solution 2 mg L⁻¹. Non-reported conditions were as described in the recommended procedure.

Table 1. Effect of the solvent on the peak height of the trimethylsilyl ester of oxalic acid. Oxalic acid concentration 5 mg L⁻¹ (in the solution chromatographed).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Peak height</th>
<th>CV(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>50718</td>
<td>6.3</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>367212</td>
<td>2.7</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>329457</td>
<td>3.0</td>
</tr>
<tr>
<td>Heptane</td>
<td>342228</td>
<td>2.2</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>323282</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Three measurements.

Chromatographic techniques including high-performance liquid chromatography [10], gas chromatography [11–13], ion chromatography [14], capillary electrophoresis [15, 16], and several enzymatic determinations involving either oxalate oxidase or oxalate decarboxylase [17, 18] have been reported for analysis of oxalate. Other methods of determination of oxalate based on its enhancing effect on the oxidation of Mn(II) by periodate [19] or precipitation of calcium oxalate have been also described [20]. Because the sensitivity of gas chromatography is excellent, this technique was selected to perform this study. If oxalic acid is to be determined by gas chromatography its volatility and thermal stability must both be increased. Because the trimethylsilyl derivatives of aliphatic carboxylic acids are easy to obtain and easy to analyse by gas chromatography [21], a silylation reaction was performed.

The aim of this work was development of a method for determination of trace amounts of oxalate in renal stones which could be used by clinical chemists to establish the possible diagnostic value of these data for urolithiasic patients. Because the oxalate content of urine and food samples is related to the risk of calculi formation, determination of oxalate in such samples was included as a second objective of this work.

Experimental

Reagents

All chemicals were of analytical-reagent grade. Granular activated carbon (100 mesh), sodium oxalate, and oxalic acid were purchased from Panreac. The strong cation-exchange resin SCX (500 mg, 2.8 mL) was from Alltech. Pyridine, ethyl acetate and trichloroacetic acid were from Sigma. The derivatization chemicals, 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane, and molecular sieves, 4Å, 1.6-mm pellets, were from Aldrich.

Apparatus

Gas chromatography was performed with a Shimadzu GC-17A gas chromatograph equipped with a Shimadzu QP-5000 mass spectrometer and fitted with a 30 m x 0.25 mm i.d. SPB-20 fused-silica capillary column (Supelco); helium was used as carrier gas. The column temperature was programmed from 60 °C to 106 °C at 2.9 °C min⁻¹ and from 106 °C to 200 °C at 20 °C min⁻¹. To keep the carrier gas flow at 0.8 mL min⁻¹ the column head pressure was simultaneously increased from 40.1 to 56.2 kPa at 1 kPa min⁻¹ and then from 56.2 to 88.1 kPa at 7.2 kPa min⁻¹. The injector and the detector interface temperatures were 200 °C and 240 °C, respectively. A perfluorotributylamine standard was run every working session. Injections were performed automatically (Shimadzu AOC-17 autoinjector) in the splitless mode. The ions of fragments with m/z 63, 93, 113, 147 amu were monitored. Data processing was performed by the Class-5000 software (Shimadzu). Peak heights were used for calculation purposes.

812 Chromatographia 2003, 57, June (No. 11/12)