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can occur, at the same time or successively, to the formation and the growth of the calculus. The more frequently met chemical components are (in descending

frequency order): calcium oxalates, calcium phosphate and magnesium ammonium phosphates, uric acids and urates, various proteins, cystine. The qualitative analysis of a certain components (among the most

frequent ones) of urinary calculus represents a fir st approach to the etiological diagnosis of urinary lithiasis and the direction of the patient’s therapeutic follow-up. It is absolutely necessary to perform the test together with morphological analysis (low power stereo microscope, light microscopy) and/or with molecular and crystalline identification (X-ray diffraction, infrared spectophotometry). Because of its nature, the
calculus has to be the subject of a precise and reliable analysis. Coupled and rationally used optical and chemical methods can, in most cases, give sufficient information about the composition and the etiology of the calculus.

The presence of calculus in urine is due to the conglomeration and the crystallization of matter in supersaturation. There can be various reasons for this imbalance: nutritional, metabolical, genetical, anatomical, iatrogenical, neurological or infectious reasons. Several

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The qualitative analysis of a certain components (among the most frequent ones) of urinary calculus represents a first approach to the etiological diagnosis of urinary lithiasis and the direction of the patient’s therapeutic follow-up. It is absolutely necessary to perform the test together with morphological analysis (low power stereo microscope, light microscopy) and/or with molecular and crystalline identification (X-ray diffraction, infrared spectophotometry). Because of its nature, the calculus has to be the subject of a precise and reliable analysis. Coupled and rationally used optical and chemical methods can, in most cases, give sufficient information about the composition and the etiology of the calculus.

**PRINCIPLE** (4) (5)

This method is used for identification of main mineral components and one organical component (cystine) of urinary calculi by easy chemical tests.

**REAGENTS COMPOSITION**

<table>
<thead>
<tr>
<th>Vial</th>
<th>Composition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Hydrochloric Acid (HCl 1,65 M)</td>
<td>Corrosive, R34, S36/37/39</td>
</tr>
<tr>
<td>R2</td>
<td>Sodium Hydroxide (NaOH 6,25 M)</td>
<td>Corrosive, R35, S36/37/39</td>
</tr>
<tr>
<td>R3</td>
<td>1st Reagent for Cystine determination (NaOH, Sodium Cyanide)</td>
<td>Corrosive, Toxic, Harmful for environment</td>
</tr>
<tr>
<td>R4</td>
<td>2nd REAGENT FOR CYSTINE DETERMINATION (Sodium nitroprussiate)</td>
<td>XN: HARMFUL, R22, S21/26/37</td>
</tr>
<tr>
<td>R5</td>
<td>Reagent for Phosphates determination (Sulfuric Acid, Ammonium Molybdate, Ferric Sulfate)</td>
<td>Corrosive, R34, S36/37/39</td>
</tr>
<tr>
<td>R6</td>
<td>Reagent for Magnesium determination (NaOH, para-nitrophenylazoresorcinol)</td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>Reagent for Calcium determination (KOH, calcein)</td>
<td>X: Irritating, R34, S36/37/39</td>
</tr>
<tr>
<td>R8</td>
<td>Reagent for Ammonia determination (Potassium Iodide, Mercuric Iodide)</td>
<td>Corrosive, R36/38, S36/37/39</td>
</tr>
<tr>
<td>R9</td>
<td>Reagent for Uric Acid determination (Acetic Acid, neocurarine, Copper Sulfate)</td>
<td>X: Irritating, R36/38, S36/37/39</td>
</tr>
<tr>
<td>R10</td>
<td>Reagent for Oxalate determination (Manganese dioxide)</td>
<td>X: Harmful, R20/22, S36/37/39, S22-26-28,</td>
</tr>
</tbody>
</table>

**SPECIMEN COLLECTION AND HANDLING**

Morphological studies should be made with a whole calculus cleared out of possible impurities. Qualitative analysis and flame test should be realised on pulverised calculus using clean pestle and mortar to obtain finely ground powder.

**INTERFERENCES** (4)

- Cobalt and nickel do not interfere with the determination of the magnesium because of their weak concentration in the organism.
- Carbonate testing: if there has been effervescence during the addition of R1 revealing the presence of carbonate, add R1 drop by drop until the end of the gas evolution. Then, shake vigorously ("Vortex") for at least one minute so as to get rid of all the carbon dioxide (or failing that, bring to the boil a few seconds in a Pyrex tube and bring back at room temperature). This process is necessary to avoid a false-positive result during the determination of oxalate.

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Low power stereo microscope
2. Clean porcelain pestle and mortar
3. Metallic seeding loop
4. mg scale (weighing of the calculus powder)
5. Pipette to dispense 1 drop (approx. 50µL) of reagents and mixture M1, M2.
6. Tubes, or glass/ceramic cored plate with a white bottom
7. REAGENT 95315: stone analysis set positive and negative controls
QUALITY CONTROL

METHODS

PROCEDURE

Morphological analysis of calculus should be made before pulverizing the calculus for chemical test.

Flame test: Immerse a metallic seeding loop into distilled water, then into the calculus powder. Bring to the flame. The carbonization or the disappearance of the powder is the sign that the components are mostly of organic origin. The absence of carbonization shows that the calculus is of mineral origin.

Qualitative chemical tests: (see table below):

- **Step 1:** Weigh about 50 mg of the calculus powder, transfer into a test tube and add 10 drops of reagent R1. An effervescence shows the presence of carbonate (see § INTERFERENCES). In this case, shake vigorously for 1 minute. The remaining mixture is called M1 in the table below.
- **Preparation of the mixture M2:** Mix 50 µl of M1 and 5 ml of distilled water. Mix well and use only for the determination of carbonate (Step 5).
- **According to the step below,** dispense 1 drop (approx. 50 µl) of the mixture M1 or M2 in each cavity of a ceramic cored plate or in tubes and carry out the next tests (Step 2 to 8):

### Step 1
- **Carbonate**
  - **1 DROP**
  - **M1**

### Step 2
- **Cystine**
  - **1 DROP**
  - **M1**

### Step 3
- **Phosphate**
  - **1 DROP**
  - **M1**

### Step 4
- **Magnesium**
  - **1 DROP**
  - **M1**

### Step 5
- **Calcium**
  - **1 DROP**
  - **M1**

### Step 6
- **Ammonium**
  - **1 DROP**
  - **M1**

### Step 7
- **Uric Acid**
  - **1 DROP**
  - **M1**

### Step 8
- **Oxalate**
  - **1 DROP**
  - **M1**

**CALCULUS POWDER**
- **50 mg**
- **10 drops**
  - **(500 µL)**

**+ R1**
- **THE TIP OF A SPATULA**
- **Approximately. 60 mg**

**LET STAND FOR 5 MIN.**

**MIX**
- **LET STAND FOR 5 MIN.**

**EFFERVESCENCE**
- **visible and audible**

**RED COLOUR**
- **BLUE COLOUR**
- **BLUE PRECIPITATE**
- **YELLOW COLOUR**
- **ORANGE-BROWN PRECIPITATE**
- **YELLOW/ORANGE COLOUR**

**YELLOW COLOUR**
- **NO CHANGE IN COLOUR**
- **ABSENCE OF PRECIPITATE**
- **PURPLE COLOUR**
- **ORANGE COLOUR**
- **YELLOW COLOUR**
- **NO CHANGE IN COLOUR**

**ABSENCE OF EFFERVESCENCE**

### Notes:

1. **Negative control:** Use **REF 95315 CONTROL 3-**.
2. **Positive control:** Use **REF 95315 CONTROL 1+ and CONTROL 2+**.

**Treat as calculus powder.**

REFERENCES