Application of energy-dispersive X-ray fluorescence spectrometry (EDX) in forensics – titanium, silicon and magnesium in the stomach contents as good indicators for ingestion of pharmaceutical tablets

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Abstract: We present a case of drug overdose in which energy-dispersive X-ray fluorescence spectrometry (EDX) was applied as a screening test. Elemental analysis by EDX identified strong peaks for titanium, silicon and magnesium in the stomach contents that seemed to be derived from pharmaceutical additives in ingested pharmaceutical tablets. Toxicological examination using liquid chromatography-tandem mass spectrometry identified toxic levels of 7-aminonitrazepam, 7-aminoflunitrazepam (metabolites of nitrazepam and flunitrazepam, respectively) and some other drugs in the blood, and also residues in the stomach. We concluded that the cause of death was poisoning due to ingestion of multiple drugs. Our results indicate that the detection of titanium, silicon and magnesium in stomach contents offers a good indicator for ingestion of tablets and that EDX provides useful information for forensic diagnosis.

Key Words: X-ray fluorescence spectrometry (EDX), toxicological screening, titanium, silicon, stomach contents.

Rapid screening, identification and quantification of drugs or chemicals is important in forensic practice. Energy-dispersive X-ray fluorescence spectrometry (EDX) can provide useful and suggestive information in forensic diagnosis, such as on the presence of mercury, arsenic, bromine, sulfur, silicon and titanium [1-6]. Here, we report the application of EDX as a first step in the toxicological screening of stomach contents in a forensic case, and discuss the utility of this modality as an indicator of drug ingestion.

CASE REPORT

A Japanese man in his thirties (height, 172 cm; weight, 58.5 kg) was found dead in his room. Subsequent investigation by the authorities revealed that the deceased had been prescribed psychotropic drugs, and empty packets were found in his room. Medico-legal autopsy revealed no injury or findings of natural disease. The left and right lungs weighed 561 g and 704 g, respectively, and both were congested. The heart weighed 291 g and contained 260 mL of blood with a small amount of coagulum, and the brain weighed 1480 g without any injury. The stomach contents comprised approximately 60 mL of greenish liquid with granules. Toxicological analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed according to the instructions from the manufacturer. Quantitation of ethanol was performed using headspace gas chromatography.

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We also applied elemental analysis by EDX to the stomach contents of the victim using an EDX system (Rayny EDX-720; Shimadzu, Kyoto, Japan) [4-6]. Operating conditions for EDX were as follows: target, Rh anode; operating voltage, 50 kV; X-ray path, vacuum; detector, Si (Li); and measurement time, 100 s. We used 100 µL of sample, dripped onto ST-30 filter paper with a paraffin circle (Shimadzu, Kyoto, Japan). The sample on filter paper was then dried at room temperature. Following drying of the filter paper, EDX analysis was performed.

**RESULTS AND DISCUSSION**

Various kinds of additives such as D-mannitol, talc, cellulose, stearates, metasilicate, and titanium dioxide are used in tablets containing pharmaceutical agents [7]. Figure 1 shows the spectra of stomach contents from the victim and a control sample using EDX. Relatively high peaks of titanium, silicon and magnesium were identified in the stomach contents of the victim. Among these, titanium is widely used as a pharmaceutical additive for coloring or shielding, in the form of titanium dioxide [6], and silicic acid compounds and magnesium compounds are also used as fluidizers in pharmaceutical products [7]. Toxicological examination revealed that the victim had ingested nitrazepam, flunitrazepam and zopiclone (Table 1). Those pharmaceutical tablets contained titanium dioxide, silicic acid compound and magnesium compounds [8-10]. The relatively high peaks of titanium, silicic acid magnesium in stomach contents therefore provided additional useful information for the dosage form as tablets.

Toxicological examination identified various kinds of psychotropic drugs, as shown in Table 1. Therapeutic, toxic and lethal ranges of each drug are also shown [11, 12]. Analytical results indicated the presence of fatal or toxic levels of 7-aminonitrazepam and 7-aminoflunitrazepam, while other drugs were within therapeutic ranges [11, 12]. Approximately 2 mg of nitrazepam and 1 mg of flunitrazepam were found in the stomach. No ethanol was detected from blood. Blood levels of the sum of nitrazepam and its metabolite 7-aminonitrazepam over 0.5 µg/mL are considered fatal [12], and relatively high concentrations of nitrazepam with flunitrazepam may cause coma and respiratory depression [11]. Based on the pathological and toxicological findings, we concluded that the cause of death was poisoning due to the combined effects of the ingested drugs.

EDX allows rapid, simultaneous multi-element analysis without any special sample preparation. Application in the field of forensic medicine has thus been widely reported, not only in the field of toxicology, but also in pathology [13-18]. In the present case, EDX rapidly provided the information that the victim

![Figure 1. The EDX spectrum of stomach contents from the present case (a; titanium, silicon and magnesium are identified) and control stomach contents (b).](image-url)

**Table 1. Concentrations in the post-mortem samples and currently established therapeutic, toxic and fatal ranges (µg/mL).**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Femoral venous blood</th>
<th>Stomach contents</th>
<th>Therapeutic range *</th>
<th>Toxic range *</th>
<th>Lethal range *</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitrazepam</td>
<td>0.038</td>
<td>32.20 (1.93)</td>
<td>0.03-0.1</td>
<td>0.2-3</td>
<td>&gt;0.5 **</td>
</tr>
<tr>
<td>7-aminonitrazepam</td>
<td>0.906</td>
<td>8.886 (0.53)</td>
<td>0.005-0.015</td>
<td>0.01-0.05</td>
<td>&gt;0.16 **</td>
</tr>
<tr>
<td>flunitrazepam</td>
<td>0.006</td>
<td>17.75 (1.07)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-aminoflunitrazepam</td>
<td>0.057</td>
<td>2.874 (0.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenobarbital</td>
<td>12.44</td>
<td>103.2 (6.19)</td>
<td>10-30</td>
<td>30-40</td>
<td>50-60</td>
</tr>
<tr>
<td>promethazine</td>
<td>0.224</td>
<td>81.80 (4.91)</td>
<td>0.05-0.4</td>
<td>1-2</td>
<td>1.8-5.4</td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>0.314</td>
<td>259.0 (15.5)</td>
<td>0.03-0.5</td>
<td>1-2</td>
<td>3-4</td>
</tr>
<tr>
<td>risperidone</td>
<td>0.006</td>
<td>0.099 (0.006)</td>
<td>0.002-0.02</td>
<td>0.12</td>
<td>1.8</td>
</tr>
<tr>
<td>zopiclone</td>
<td>0.084</td>
<td>17.05 (1.02)</td>
<td>0.01-0.05</td>
<td>0.15</td>
<td>0.6-1.8</td>
</tr>
</tbody>
</table>

* * Minimum fatal concentration includes total of flunitrazepam and 7-aminoflunitrazepam, nitrazepam and 7-aminonitrazepam [12]. Each figure in parentheses represents the total amounts of drug in the stomach (mg).
had ingested drugs in the form of tablets. Detection of titanium, silicon and magnesium in stomach contents seems to offer a good indicator of tablet ingestion. EDX offers useful information for forensic diagnosis in cases of unnatural death.

**Conflict of interest.** The authors declare there is no conflict of interest in this study.

**Ethical approval.** This article has been carried out according to Ethics in Publishing.

**References**