TECHNICAL NOTE

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Characterization of Acetaminophen: Molecular Microanalysis with Raman Microprobe Spectroscopy*

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ABSTRACT: The *in situ* spectroscopic identification of acetaminophen in a fatal overdose case is described. Numerous techniques have been used to analyze acetaminophen in biological fluids, however, the use of nondestructive spectroscopic techniques has not been documented. In this investigation, the demonstration of the drug material was established by using the laser Raman microprobe technique, providing an accurate identification by virtue of the drug's molecular fingerprint characteristics. Material found on the deceased was collected and placed on metal (aluminum-coated) plated slides and excited with the 514.5 nm line of an argon ion laser, which was focused to a 1 μm spot size using a high-resolution optical microscope. Spectra of acetaminophen particles with an average size of 5 to 8 μm were obtained. The Raman spectrum of this drug contains characteristic group frequencies assigned to the C=O at 1649 cm^{-1}, the N-H deformation mode at 1620 to 1612 cm^{-1}, the bendstretch mode of the H-N-C=O at 1562 cm^{-1}, the C-H bending mode at 1325 cm^{-1}, and the phenyl ring stretch at 799 cm^{-1}, respectively. The results reported here demonstrate the capability of laser Raman microprobe as a useful adjunct tool for the identification of foreign materials in forensic pathology.

KEYWORDS: forensic science, forensic pathology, forensic toxicology, acetaminophen, poisoning, Raman spectroscopy

In recent years, there has been considerable interest in the use of over the counter drugs containing acetaminophen (paracetamol, N-acetyl-p-aminophenol) because of the decreased potential for side effects as compared with aspirin. The compound has analgesic and antipyretic effects and is generally well tolerated when administered in therapeutic amounts. Acetaminophen toxicity has been well described (1), with hepatic necrosis identified as the most prevalent toxic reaction (2). The observed drug's hepatotoxicity may be enhanced by multiple factors (3,4).

Although the chromatographic analysis of acetaminophen in fluids has been widely documented, its *in situ* nondestructive molecular spectroscopic identification in forensic specimens has not been demonstrated. Recent developments in instrumentation in which a microscope and high-sensitive detectors are coupled to an infrared spectrometer or to an optical scanning instrument have greatly enhanced the sensitivity and application of spectroscopic techniques for the study of tissues in human pathology. Raman microprobe and infrared microspectroscopy have been successfully used by our group to establish the identification of foreign materials within tissues and to demonstrate the physical and chemical changes in tissues and associated cellular components (5–7). Raman microprobe in particular, has provided excellent fingerprint capabilities on the molecular differentiation of crystal deposition and mineralization events in tissues (6).

In this investigation, Raman microprobe spectroscopy has been used to establish the *in situ* identification of acetaminophen in a forensic-toxicology case, to provide structural information of the drug, and to correlate the analytical toxicologic data.

Case History

A 52-year-old woman was found dead in her undisturbed apartment with colored (violet) material that had dried on her face and neck and in her mouth (Fig. 1). Suicide notes were found though no previous suicide attempts had reportedly been made. The content of the material was not known at autopsy. On initial inspection, the material appeared corrosive because of its dark appearance on the face, though upon removal, the skin was intact. The material was not readily dissolved in distilled water and demonstrated a neutral pH. Dissection demonstrated an intact gastrointestinal tract with a bolus of the colored material found in the small intestine 80-cm distal to the ligament of Treitz. Histopathology was unremarkable; there was no necrosis of hepatocytes.

Supplemental information from the family revealed that the colored material was Pepto-Bismol (pink) boiled with Excedrin PM tablets (blue). Body fluids and postmortem tissues including liver, gastric contents, and blood were removed at autopsy and sent to the toxicology laboratory, where subsequent toxicologic studies on postmortem tissues demonstrated acetaminophen blood-level of 755 mg/L, liver 570 mg/kg, and gastric contents 4351 mg/kg; and confirmed the cause of death to be due to acute acetaminophen toxicity.

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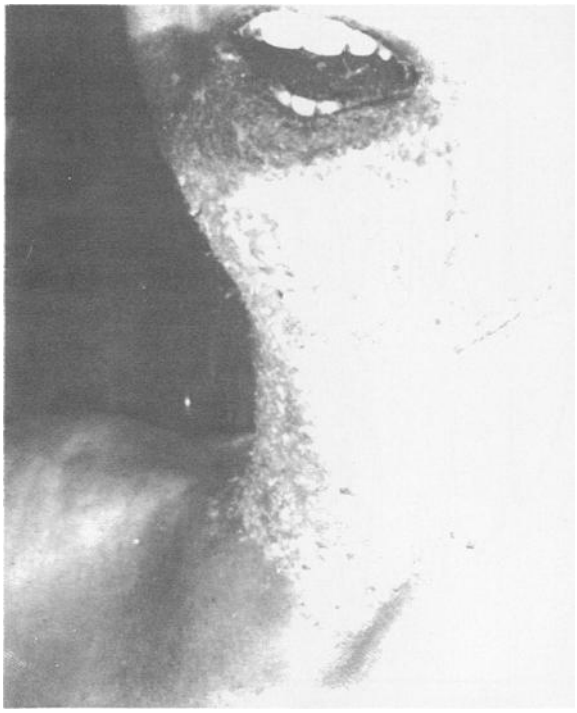

FIG. 1—*Black and white photograph demonstrating dried material in mouth and on the face and neck of the decedent. The dark material seen at corner of mouth initially raised the possibility of a corrosive agent.*

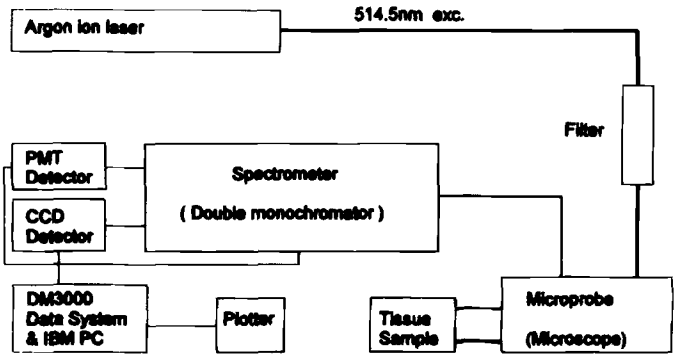

FIG. 2—*Schematic representation of the laser Raman microprobe.*

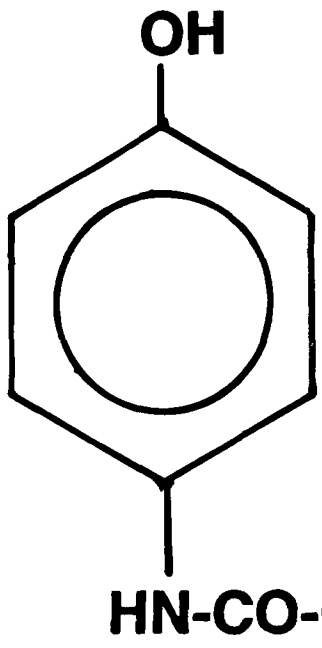

FIG. 3—*Chemical structure of acetaminophen.*

Materials and Methods

Laser Raman Microprobe

The Raman microprobe technique provides a unique *in situ* nondestructive approach for obtaining the identification of organic and inorganic materials based on the molecule's characteristic spectrum of vibrational frequencies. The Raman scattering process, although a very weak effect, describes the inelastic scattering of light by molecules; that is, the scattering process will result in a gain or loss of energy by the scattered molecules. Consequently, upon exciting a molecule with a given laser energy (of frequency $h\upsilon_0$), the energy of the scattered signal can occur at the same frequency as the incident laser beam (commonly known as Rayleigh scattering) or at frequencies that may be shifted from the incident energy by an amount smaller or greater than $h\upsilon_0$ (i.e., $h\upsilon_0 \pm \upsilon_v$, where υ_v describes the internal vibrational modes of the molecule). Frequencies shifted by $h\upsilon_0 + \upsilon_v$ are known as Stokes lines, whereas those at energies below the incident laser energy are called anti-Stokes lines. The former lines are more intense, and therefore, are used to characterized the spectrum. The spectrum obtained is displayed in the form of Raman lines or peaks, each at a frequency position indicating a shift from the laser line (x-axis) versus the intensity of the peak (y-axis). Because the Raman peaks are fingerprint features of the internal vibrations of the molecule, shifts in the natural frequency of these vibrations may reflect changes in the structural arrangement and/or chemical environment of the molecule.

Instrumentation

The Raman microprobe experiments were conducted using the system shown schematically in Fig. 2 and described elsewhere (6). Briefly, a Spex Raman spectrometer (Model 1403) equipped with holographic gratings blazed at 1800 groove/mm and interfaced to an Olympus microscope (Model BH-2) was used. An 80x objective (N.A. = 0.95) was used to focus the laser beam onto the sample. The microscope can be operated both on reflectance or transmittance illumination. The backscattered Raman signal was collected at a 180° from the incident laser light by the same objective, collimated and directed into the spectrometer to determine the frequencies or wavelengths. The microscope is equipped with a beamsplitter to reflect the laser beam onto the microscope objective, and to transmit the Raman scattered signal collected by the objective. The minimum laser spot size or spatial resolution is 1 μm as determine by the numerical aperture of the objective and the wavelength (i.e., 0.5 μm) of the incident radiation. The argon ion laser (Coherent Innova 307) operating at a wavelength of 514.5 nm was used as the illumination source. The incident laser power at the sample varies from 5 to 20 mW, with a power density (i.e., power per unit area) in the range of 5 to 50 kW/cm^2, when a laser spot diameter in the range of 4 to 20 μm was used. To avoid the thermal destruction of the sample by the laser beam, the beam was defocused providing a laser spot size of ~100 μm. To analyze the Raman signal, a liquid-nitrogen cooled charged-coupled multichannel detector (CCD) was used with an average

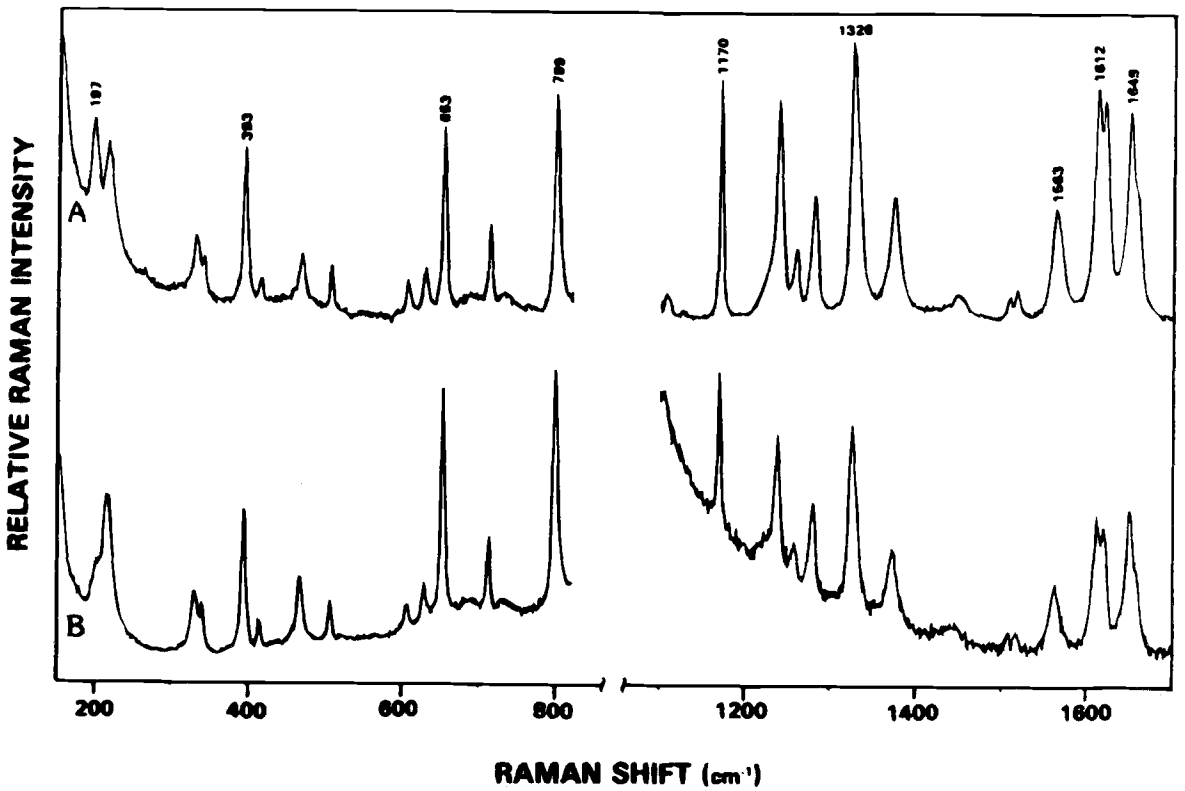

FIG. 4—*Raman microprobe spectra of acetaminophen from the forensic case (trace B) and the reference acetaminophen powder (trace A). The excitation laser line was at 514.5 nm.*

integration time of 5 s. Alternatively, a uv-vis enhanced single channel photomultiplier tube was used to collect the entire spectral region from 100 to 3500 cm^{-1}. The Raman spectrum of commercial acetaminophen was collected and analyzed in a similar fashion to serve as a control.

Results and Discussion

With the recent advent of laser sources, optical microscope, and highly sensitive detectors, the number of applications of Raman spectroscopy in analytical, medical, forensic, and research laboratories have markedly increased. Recent applications of Raman spectroscopy in forensic sciences have included the identification of trace explosives (8,9), the characterization of synthetic polymer fibers (10), and the identification of street drugs (11). All these studies have used Raman spectroscopy to provide a direct molecular fingerprint identification of the specimen unavailable by other methods such as gas chromatography or mass spectrometry.

Although subsequent toxicologic analyses using techniques such as gas chromatography and mass spectrometry confirmed the presence of acetaminophen in blood, stomach content, and liver, these analyses take a considerably longer amount of time to complete. The analysis with Raman microprobe, on the other hand, is rapid and provided identification of the material in approximately 30 min.

Raman Spectrum of Acetaminophen

The chemical structure of acetaminophen is illustrated in Fig. 3, showing the presence of a hydroxyl-substituted phenol ring and a peptide HN-C=O bond. Each of these groups is expected to exhibit characteristic Raman frequencies. The Raman microprobe spectrum of acetaminophen particles surrounded by the violet-colored material is demonstrated in Fig. 4. For comparison, the spectrum of a reference acetaminophen is also illustrated. As shown in Fig. 4A and 4B, four principal group frequencies can be identified as the C=O stretch at 1649 cm^{-1}, the −NH deformation mode 1612 cm^{-1}, the HN-C=O bend-stretch at 1562 cm^{-1}, and the phenyl ring group stretch frequency at 799 cm^{-1}. Other Raman modes in the 1350 to 1180 cm^{-1} region are due to both the N-H bending and C-H deformation vibrations. The spectra similarities and peak frequency position between the unknown particles found in the deceased and the reference materials not only provided an accurate identification of the material due to acetaminophen, but it also indicated that the structure and chemical environment of the drug remain unaltered. The current case report study was not designed to evaluate the metabolic degradation products of acetaminophen in body fluids and tissues, although this study appears worth doing because it could serve as a complementary toxicologic-chemical identification.

Summary

It has been demonstrated that the Raman microprobe technique is a powerful tool for the *in situ* nondestructive and rapid identification of micron-size particles in forensic and pathologic specimens. This technique could also be used to establish the identification of other forensic specimens including micro-fiber analysis, plastics, hair analysis, and paint chips.

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