

# Forensic Mass Spectrometry

MS allows law enforcement to detect the least of the lingering traces of crime.

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Classical forensic science is based on the concept that there can be no crime without some evidence of the criminal remaining—that every interaction leaves a trace, even if it is the minute fragment of a skin cell, a bit of oil, a microscopic shred of fiber, or the least remnant of a lingering poison in the smallest bit of blood. The evolution of forensic science has been directly in proportion to the ability of increasingly sophisticated instrumentation to discern and analyze ever-smaller samples of these relics of criminality. And it must all be done in a manner that will stand up in court, or at least provide sufficiently credible evidence to direct the course of a police investigation. It was inevitable with such demands that increasing reliance would be placed on MS as the ultimate arbiter of the physical identity of these infinitesimal fragments.

## ESI To Dye For

Imagine a victim found dead at the side of the road. Careful examination of the corpse shows the presence of a single 1-mm fiber, possibly from a carpet, present on the body. It is hard to imagine a less significant trace item, and one that could easily be argued came from crime scene contamination and not the murder site. But if technology outlined by researchers Albert Tuinman and colleagues at the department of chemistry at the University of Tennessee and Oak Ridge National Laboratory becomes routinely available, such a fiber could become the clincher in a criminal investigation.

The basal composition of the fiber is relatively uninformative in any compara-

tive study, given the infinite numbers of clothes or carpets formed out of the substrate (i.e., nylon, Orlon, cotton, etc.). But the dyestuffs used to color textile fibers are a different matter. Manufacturers have a large number of dyes to choose from, and, more importantly, they control the

comparisons can be made.

Currently, forensic fiber analysis is typically done using microspectrophotometry and thin-layer chromatography (TLC). Microspectrophotometry is most widely used because it is a nondestructive technique that evaluates the spectral fingerprint of the composite dye mixture of the fiber being tested.

Raman microspectroscopy can be used to evaluate colored fibers and has been shown capable of identifying significant spectral diversity, for example, among the wide variety of blacks available for cotton (2).

Microspectrophotometry, however, is incapable of determining the identity of the individual dye components. For that, TLC is

used—a destructive method that evaluates the dyes from solvent-extracted fibers. This is a qualitative technique that can identify the dyes present, but not their ratios; it is relatively insensitive and, according to the researchers, “may require more fiber than the forensic scientist is willing to sacrifice” (1). Promising alternatives are micro-HPLC and capillary electrophoresis.

According to Tuinman and colleagues, the obvious choice for sophisticated analysis of extremely small samples of fibers is the use of electrospray ionization mass spectrometry (ESI MS) alone or with tandem MS. Figure 1 shows the molecular structure and the ESI MS spectrum of the single dye known as no. 4 in a sample set obtained from a commercial manu-

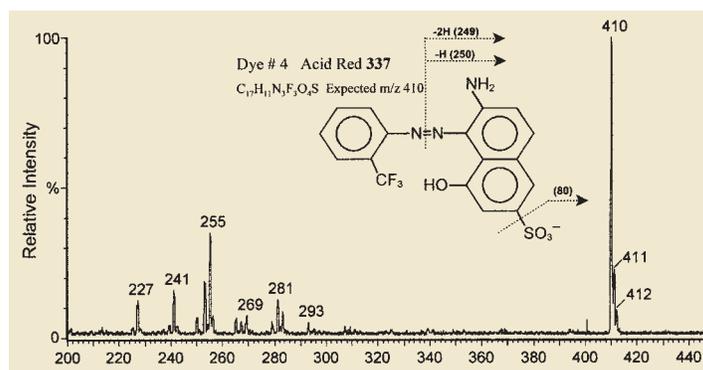


Figure 1. ESI MS of dye no. 4 (500 ppb in  $CH_3CN/H_2O$  1:1). Inset: Dye no. 4 structure and observed CID fragmentations. (Adapted with permission from Ref. 1.)

color of the final product by the ratio of the dye mixture, not generally by the use of a single appropriate dye color.

According to Tuinman et al., to determine if the two fibers from two sites (the body above, and the carpet in a reputed crime boss's study, for example) are identical, it is important that they “have the same dye components and that the ratio in which these components are present should be identical” (1). Such analysis requires a basic understanding of the modern textile-dyeing industry, which operates like a kindergarten art class, generally creating more-complex colors by making a mixture of only three dyes—a blue, a red, and a yellow (though which of a large number of possible individual blue, red, or yellow dyes are used differs between manufacturers). By fingerprinting individual dyes and having a technique that can quantitatively determine their absolute ratios in a particular fiber source, exact

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facturer of carpets. Because of the gentle nature of the ESI introduction to the mass spectrometer, the single molecular ion of each different dye can be determined in a solvent extract of a fiber colored with a dye mixture, thus allowing a positive identification of the components (see Figure 1). Individual peak heights can be compared to get the absolute ratios—thus providing the two parameters needed for comparative analysis. The required certainty to establish the absolute identity of each individual peak can be obtained from observing the tandem MS spectrum obtained using collision-induced dissociation (CID) for each putative dye molecular ion.

The researchers point to the extreme reproducibility of this method of analysis, and that it can utilize a fiber sample only 1 mm in length for complete analysis. Furthermore, they predict, with the use of micro-spray ESI MS, the length of fiber testable could easily drop to less than 0.2 mm. This could potentially make finding that small a fiber harder than analyzing it.

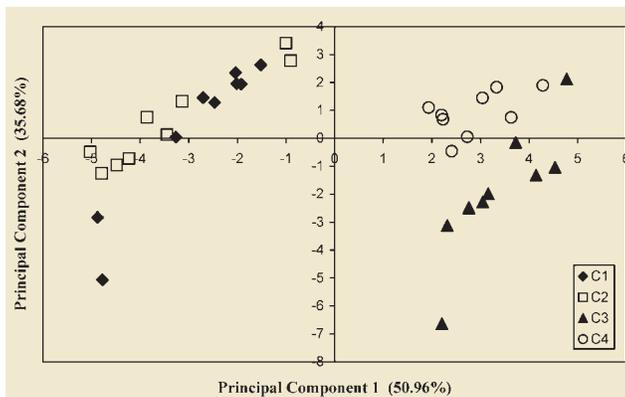
### LA-ICP and Deadly Debris

Other types of MS analysis have been adapted to other materials of interest to the forensic scientist. For example, it is often necessary to analyze fragments of metal, paint, or other materials left at a putative crime scene, be the suspected cause murder, arson, or accidental explosion. Glass fragments are particularly valuable in forensic analysis, since they are frequent habitués of crime scenes, as in breaking and entering, the violent struggles of assaults, hit and runs, fires, and explosions. Refractive index and elemental analysis are of particular utility, and the FBI has selected nine elements for discriminatory comparison of glass samples (2).

Researchers David B. Aeschliman and colleagues at Iowa State University recently reported on their use of principal-component analysis (PCA) using data obtained from laser-ablation inductively coupled plasma MS (LA-ICPMS) to identify samples of glass fragments and plain carbon steel alloys. The goal was to validate this method for possible forensic

application by determining if it could provide easy and relatively nondestructive “fingerprints” of trace-element composition that could be used for source comparison (3).

Although steel is composed primarily of iron and manganese, trace-element differences between sources are inevitable because of widespread recycling of scrap. Similarly, although glass has a limited number of common elements (silicon,



**Figure 2.** Principal-component analysis score plot for glass fragments (32-element model). (Adapted with permission from Ref. 3.)

calcium, aluminum, oxygen, etc.), it has a wide variation in trace elements, depending on source. Unlike other methods of analysis such as X-ray fluorescence or sample dissolution followed by ICP-AES (atomic emission spectroscopy) or ICPMS, LA-ICPMS is more sensitive and does not require significant destructive sampling.

In this particular study, four glass fragments were provided to the researchers by the Illinois State Police Forensic Laboratory. Using LA-ICPMS, spectra were developed, and only peaks greater than 3 times background were examined. Thirty-two different elements were monitored, from arsenic to zinc. PCA was used as a multivariate data reduction method to examine the variance patterns within the multidimensional data set generated. This allowed the production of score plots (a “fingerprint” of data clusters) that could be compared for each sample (see Figure 2). The plots easily separated the four glass samples into two groups of related samples and, furthermore, allowed even the overlapping sample data to distinguish samples within each group from one another to the 95% confidence level when Q-residual analysis was added (compar-

ing each of the other three samples to a model based on one).

The analysis was applied to steel samples of even greater similarity and, using 20 elements, the PCA plots were able to distinguish the samples. When the method was used to examine copper wire samples, it proved capable of distinguishing a separate source for each of 6 strands tested of the 65 that made up the wire. With such extreme sensitivity and utility, the researchers believe that the method should be adaptable “to measure the trace elemental composition of nearly any solid or coating, including paints, soils, pharmaceutical materials, gunshot residues, etc.”

As for the subsequent analysis method, of particular importance to forensic considerations is the fact that “the matching procedure using PCA generates its own quality control (i.e., confidence limits) and does not require subjective judgment as to which elements are most appropriate” (3).

Ultimately, the ever-increasing sensitivity of MS and the greater scope of its input devices have led researchers to a host of new and promising methods for evaluating crime scene remnants. So powerful are these techniques that the ultimate rate-limiting step to their utility may be the lack of libraries of standards for comparison. (There are no laws making every manufacturer of paints, dyes, fibers, and materials provide complete fingerprinting of all their products for forensic use.)

But as criminologists collaborate to create their own new standards and the instrumentation continues to evolve—perhaps law enforcement may one day negate the concept of a criminal escaping “without a trace,” vindicating the original vision of the founders of forensic science nearly two centuries ago (see “Pointing to Poison”, September 2004, p 43).

### References

- (1) Tuinman, A. A.; Lewis, L. A., Lewis, Sr., S. A. *Anal. Chem.* **2003**, *75*, 2753–2760.
- (2) Brettell, T. A.; Rudin, N.; Saferstein, R. *Anal. Chem.* **2003**, *75*, 2877–2890.
- (3) Aeschliman, D. B., et al. *Anal. Chem.* **2004**, *76*, 3119–3125. ♦