Forensic Chemistry

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Key Words

seized-drug analysis, toxicology, mass spectrometry, metrology, latent fingerprints

Abstract

Forensic chemistry is unique among chemical sciences in that its research, practice, and presentation must meet the needs of both the scientific and the legal communities. As such, forensic chemistry research is applied and derivative by nature and design, and it emphasizes metrology (the science of measurement) and validation. Forensic chemistry has moved away from its analytical roots and is incorporating a broader spectrum of chemical sciences. Existing forensic practices are being revisited as the purview of forensic chemistry extends outward from drug analysis and toxicology into such diverse areas as combustion chemistry, materials science, and pattern evidence.

1. INTRODUCTION

1.1. What is Forensic Chemistry?

According to Lucas, "[f]orensic or legal chemistry may be defined as chemistry applied to the solution of certain problems that arise in connection with the administration of justice. It is chemistry exercised in the service of the law" (1, p. 2). This definition applies as well today as it did when written in 1921 (1). The contents of this first forensic chemistry textbook revealed the scope and generality of the young discipline. Chapter headings ranged from the familiar (e.g., hashish, fires and firearms, decomposition, and microscopy) to the unusual and unexpected (e.g., tobacco, string and rope, counterfeit coins, fingerprints, bloodstains, and robbery from letters and parcels). Although the specific topics of interest have since changed, the framework of forensic chemistry remains nearly the same.

Then as now, one way to discuss forensic chemistry is to examine the categories of evidence (**Table 1**). In terms of chemical specialties, forensic chemistry is often described as the application of analytical chemistry to matters of legal or judicial concern. Analytical chemistry remains the foundational discipline, but forensic chemists are increasingly using other chemical disciplines. Most notable among these are organic and medicinal chemistry and biochemistry. This review highlights instances where chemical research, broadly defined, has come to play a larger role in forensic chemistry.

Several factors distinguish forensic chemistry from other chemical disciplines. Analytical methods must be minimally destructive or nondestructive. Sample matrices vary from the predictable (e.g., blood, urine, plant matter, glass) to anything tangible. Forensic laboratories labor under high workloads, have limited resources, and must meet scientific as well as legal constraints. These considerations mean that forensic chemistry is inherently adaptive, shaping fundamental advances in science, technology, and instrumentation for forensic use. It is the ultimate applied chemical science.

1.2. Scope of this Review

The goals of this review are to describe and introduce aspects of forensic chemistry that are not typically emphasized in other sources or that may not be well known outside the forensic chemistry

Table 1 Framework of forensic chemistry

Category	Subdivision	Type of evidence
Drug analysis	Solid-dose or seized-drug analysis	Physical evidence such as pills, powders, and plant matter
	Toxicology	Blood, urine, tissue, hair, etc.
Combustion-based	Arson	Fire debris and accelerants
	Firearms and propellants	Gunshot residue, bullet lead
	Explosives	Propellant and explosive formulations, pre- and postblast samples and residue
Materials	Natural	Soil
	Mass-produced	Glass
		Paint and ink
		Fibers
		Plastics
		Paper
		Bullets

Table 2 Recent reviews

Topics	References	Notes
Context of the science	119, 120	Science, law, chemistry, and current standing
General	121–126	The biannual review in *Analytical Chemistry* covers all aspects of forensic science
Metrology	127–131	Method validation and sampling in the forensic context
Extractions	132–136	Emphasis on drug analysis and toxicology
Toxicology, general	125, 137–140	Instrumentation and preparation, tandem mass spectrometric methods
Gunshot residue and arson	141, 142	
Mass spectrometry	70, 125, 127	
Microfluidic devices	143, 144	

community. It is neither feasible nor appropriate to cover all aspects of forensic chemistry, nor is my intent to duplicate the efforts of other review authors. I attempt to illustrate what are likely to be perceived as nontraditional aspects of forensic chemistry. By necessity, many interesting and promising applications have been excluded, but I direct the interested reader to the references and to **Table 2** for further exploration. Except for historical points, the emphasis here is on work published since 2000. Because forensic chemistry by name and definition straddles the worlds of law and science, I include a brief description of the legal context.

1.3. Legal Context

Sir Alec Jeffreys, the first person to apply DNA typing in a criminal case, recently stated, "I lost my faith in the adversarial system the first time I stood up in court" due to the realization that "it all depends on the chemistry between the witness and the jury" (2, p. 67). His statement starkly illustrates the tension between the scientific and adversarial traditions, both of which influence how forensic chemists practice science and present results. Viable forensic research must address both communities. In this context, it is helpful to think of research and development in forensic chemistry as having three tiers: (*a*) At the working forensic laboratory bench, the emphasis is on metrology, process analytical chemistry, quality assurance and quality control (QA/QC), method validation, and increasing throughput. (*b*) At the opposite end of the spectrum lies fundamental research. (*c*) The middle tier involves adapting, refining, and validating casework for investigative information or parallel studies. Many techniques stall here, never finding their way into routine forensic use. Many other promising techniques hover in the middle tier for years until either instrumentation becomes affordable or the methodology proves itself superior to existing methods scientifically and in terms of cost/benefit. Tandem liquid chromatography/mass spectrometry (LC-MS) methods, highlighted in this review, are an example of this progression. With regard to admissibility of evidence, this middle tier is the crucible from which innovations in forensic chemistry emerge and where scientific reliability is established.

In the United States, admissibility of scientific evidence and testimony (i.e., the rules of evidence) vary with jurisdiction. Generally, two standards of admissibility are used: the Frye standard and the Daubert standard. The Frye standard was set forth by the Court of Appeals in the District of Columbia in 1923 in *Frye v. United States* (293 F.1013). The essence of the decision is summarized as general acceptance, meaning that the court defers to the scientific community and generally allows the admission of scientific techniques and procedures if they have won general acceptance there. *Frye* was a crucial decision, but it had little practical effect relative to scientific evidence and admissibility until after World War II.

Metrology: the science of measurement; relates to such issues as sampling, uncertainties, and units

Investigative information (forensic intelligence): scientific data gathered as part of an investigation that is used to further the process, but that is not necessarily intended for introduction into a court proceeding

Daubert hearing: hearing overseen by a judge, the purpose of which is to determine the admissibility of scientific evidence or testimony

Prosecutorial (judicial) information: scientific data gathered with the intent to admit it into evidence in a judicial proceeding; these data and associated testimony must meet the applicable admissibility standards for the jurisdiction

Drug profiling: thorough analysis of seized drug samples to gather significant amounts of forensic intelligence

As more scientific evidence became available to the courts, they increasingly called upon *Frye* as precedent. The increased volume of scientific evidence caused judges to be more involved in screening and evaluating the merit of scientific evidence and testimony in both criminal and civil cases. Partially in response to these changes, the federal government adopted the Federal Rules of Evidence in 1975 (3). Rule 702 states in part, "If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of opinion or otherwise." Rule 702 gave judges more latitude in defining what constitutes scientific and technical evidence without devaluing general acceptance and peer review.

The early 1990s were pivotal in defining current admissibility procedures. Scientifically, DNA typing had come of age and was increasingly being used in criminal cases. The obvious advantages of DNA techniques pressured courts to admit more of the novel and powerful scientific evidence than would be admitted based on *Frye* criteria alone. Simultaneously, large civil cases such as toxic tort litigation appeared. In such cases, the outcome was heavily dependent upon the scientific evidence and expert testimony admitted. These factors contributed to three landmark decisions regarding the admissibility of scientific evidence.

The key decision arose from a civil case concerning a drug named Benedictin®, which was used to control nausea in expectant mothers. The plaintiff was Jason Daubert, a child whose mother had taken the drug. The plaintiffs charged that the drug, made by Merrell Dow Pharmaceuticals, caused birth defects. The scientific challenge was to prove that the drug was directly and unambiguously to blame. The plaintiffs produced three chemical analyses that pointed out the molecular similarity of the drug to other compounds already known to cause birth defects; they also produced numerous studies indicating correlations between taking Benedictin and birth defects. The case made its way to the U.S. Supreme Court, and a ruling was handed down in *Daubert v. Merrell Dow Pharmaceuticals* (113 S. Ct. 2786; 1993). In its ruling, the Court formalized the concept of the judge as the gatekeeper for the admission of scientific evidence and provided a list of considerations to assist in doing so. One of these factors retained was general acceptance, but the Court added other considerations for judges to use at their discretion such as testability, peer review, known error rates, and existence of standards to evaluate performance. These considerations led to the creation of a new type of proceeding, known as a Daubert hearing, that judges can employ to determine the admissibility of scientific evidence. Two other cases followed, *General Electric v. Joiner* (522 US 136; 1997) and *Kumho Tire Co., Ltd. v. Carmichael* (119 S. Ct. 1167; 1999). Both decisions contributed to the expansion of expert witness testimony.

Forensic chemists produce two types of data for use by the judicial system: investigative information (forensic intelligence) and prosecutorial (judicial) information. Investigative information includes data from, for instance, drug profiling. This type of information is rarely presented in court; as such, it does not have to meet the admissibility requirements outlined above. Rather, such data are generally used to direct an investigation or to develop new information. However, prosecutorial information must meet admissibility requirements. The relationship between these two categories and admissibility is summarized in **Figure 1**. It is not unusual to see newer methods and instrumentation applied first to gather investigative data as part of the transition from research to forensic acceptance and general use.

Other factors, primarily cost and frequency of use, play a role in determining whether a new technology becomes common in forensic laboratories. For example, few forensic laboratories have nuclear magnetic resonance (NMR) instrumentation, even though the value of NMR for structural elucidation is unquestioned and numerous forensic applications of NMR have been published. The cost of the instrumentation for most labs is not justified because the devices would be used in relatively few cases and because existing instrumentation provides sufficient data to

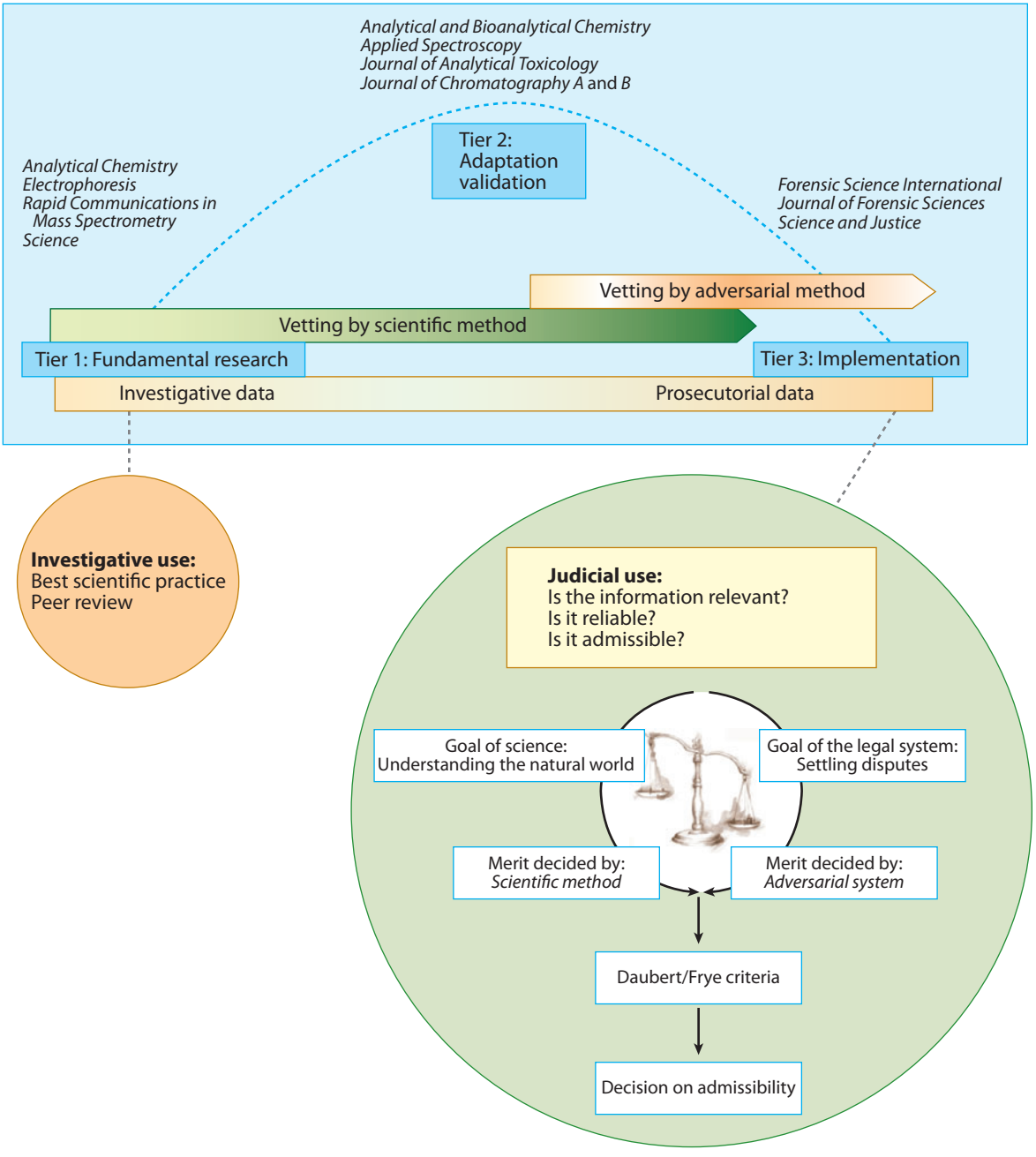

Figure 1

One way to visualize research in forensic chemistry is through a tiered system (*top*). Fundamental research and forensic application of new instrumentation can take place in working forensic labs, but they more often occur in university or national laboratory settings. Peer review is used to vet publications in journals (*lower left*). These techniques can be used to gather investigative data. In tier 2, promising research begins the transition to routine forensic application through rigorous method development, validation, and adaptation. Considerations for admissibility play a role in directing research, and parallel studies with existing methodology may be used. To produce prosecutorial (judicial) information, the methodology must be vetted through argument before a trier of fact, typically a judge (*lower right*).

answer nearly all of the pertinent forensic questions. As stated above, these questions are posed not only by science, but also by the legal system.

Controlled Substances Act (CSA): federal legislation passed in 1970 that created a unified legal framework for the control of abused drugs; established schedules of drugs classified by accepted medical use and potential for abuse

1.4. The Forensic Lifecycle: Gamma-Hydroxybutyrate

The above discussion alludes to a predictable research cycle in forensic chemistry that can be illustrated using GHB (γ-hydroxybutyrate). GHB was first utilized in the 1980s as an anesthetic and was also used by bodybuilders as a nutritional supplement. It was banned from over-the-counter sales in 1990. GHB is either synthesized from 1,4-butanediol or created by ingesting its corresponding lactone, γ-butyrolactone (GBL), which quickly metabolizes to GHB (**Figure 2**). GHB is used medically outside the United States, and it is available through diversion as well as through clandestine synthesis. During the 1990s, it was identified as a party or club drug and became notorious as a date-rape (or predator) drug. In 2000, it was added to the Controlled Substances Act (CSA) as a Schedule I drug; at the same time, GBL was added to the CSA as a List 1 controlled chemical. As shown in **Figure 3**, the number of research publications related to detection and analysis of GHB increased in the early 2000s, peaking around 2003 and declining thereafter. Such a cycle is typical in forensic drug analysis, both for seized drugs and toxicological samples relating to it and the metabolic by-products of the drugs.

Analytically, GHB is a challenging target. It is a relatively small, polar acidic molecule. Accordingly, the extraction of GHB from aqueous matrices using liquid-liquid extraction (LLE) (without derivatization) is difficult. The doses encountered in casework are usually high (percent levels in some cases), but GHB is typically spiked into alcoholic beverages and few viable presumptive and screening tests are available. Further complicating sample preparation is the interconversion of

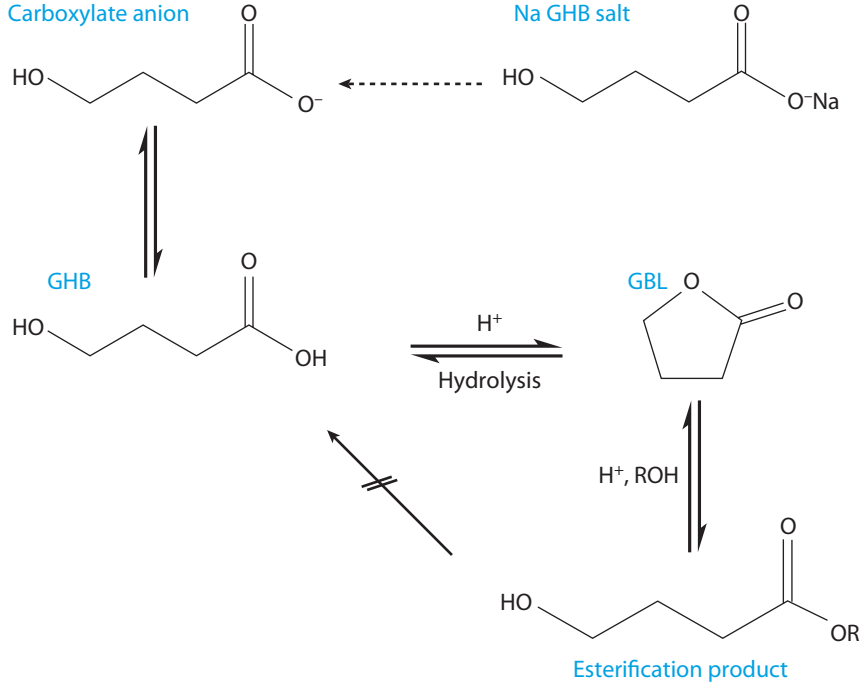

Figure 2

The structures of γ-hydroxybutyrate (GHB) and γ-butyrolactone (GBL).

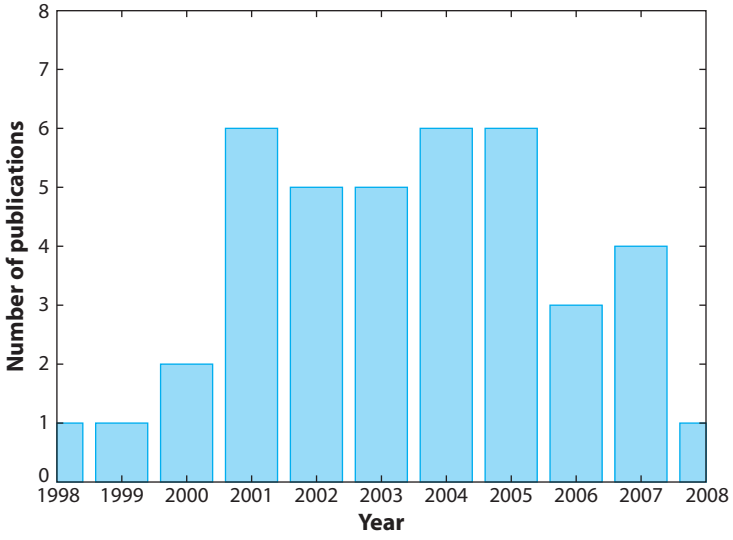

Figure 3

Trend in the number of publications relating to γ-hydroxybutyrate (GHB) and γ-butyrolactone (GBL) in the forensic context. Data are derived from the Web of Science database using key words GHB, forensic, analytical chemistry, legal medicine, reviews, and articles.

GHB to the corresponding lactone, a pH-dependent process. GHB is also subject to transesterification reactions in solutions containing ethanol (**Figure 2**) (4).

Furthermore, GHB is a natural metabolite of GABA (γ-aminobutyric acid), an inhibitory neurotransmitter, and as such is endogenous with reported concentrations of up to approximately 3 μg ml^{-1} (5). Significant intra- and interindividual concentrations have been documented (6), and generally, a cutoff level of 10 μg ml^{-1} is used for distinguishing endogenous from exogenous GHB levels. This conservative approach is not unusual for forensic thresholds. After oral ingestion of GHB, rapid onset of symptoms typically follows within 30 min, with a duration of approximately 4 h. Because of its short plasma half-life (less than 1 h), the peak concentration of the drug occurs 4 h postingestion, when a victim is probably still incapacitated. A common effect of GHB is short-term amnesia; as a result, victims may not report the assault at all, or if they do, it is often long after any toxicological evidence is detectable.

Regarding analysis of physical evidence suspected to contain GHB, research has focused on interconversion of the acid to its corresponding lactone, GBL, and on development of screening tests amenable to the complex matrix (i.e., beer, wine, or mixed drink). Interconversion is of both investigative and judicial interest given that clandestine synthesis can be performed using products containing GBL or alcohol as a starting point. Furthermore, GBL falls under varying legal definitions depending on jurisdiction; thus, the relative concentrations of GBL and alcohol in a given sample can be important (7). In pure water, equilibrium mixtures form slowly, converging at 67% GBL/33% GHB after 108 days (7). The most significant factors affecting the ratio are solution pH and storage conditions. Francesco et al. (8, 9) published a two-article series in 2006 detailing interconversion and hydrolysis, using proton NMR (^{1}H-NMR), Fourier transform infrared spectroscopy (FT-IR), and high-performance liquid chromatography (HPLC) methods to demonstrate that at pH 7 and lower, a mixture of GHB free acid, GHB anion, and GBL may be found. A report from 2004 (4) demonstrated that relative concentrations of GHB and GBL are affected by the presence of alcohol and that pH is critical in gauging the magnitude of the effect.

Because alcoholic drinks are frequently the delivery vehicles for GHB and other predator drugs, screening methods based on observed color changes are problematic. To find alternative methods, groups have examined microcrystalline test reagents in which the formation of crystals that reveal distinctive morphological characteristics (when viewed using light and polarizing light microscopy) represents a positive result (10, 11). In these studies, the test reagent consisted of dilute $AgNO_3$ plus copper or lanthanum nitrate; the authors confirmed the structure of one group of crystals via X-ray diffraction (10). Citric acid was identified as an interferent (11). FT-IR was also suggested as a possible rapid screening method (8, 12).

In a toxicological analysis, LeBeau and collaborators (13) validated a headspace method using gas chromatography (GC) with MS and flame ionization detection (FID). GC-FID is a method of particular forensic utility, given its compatibility with existing methods to determine blood alcohol concentrations. LeBeau has also published results (14) suggesting that GHB is produced in vitro and that storage conditions are critical, and other studies have described postmortem changes that affect GHB concentrations (15, 16). Finally, several alternative methods of screening and analysis have been proposed and published; these include solid-phase microextraction (17–20), ion mobility spectrometry (21–23), capillary electrophoresis (24–27), and liquid chromatography–tandem MS (LC–MS/MS) (26, 28).

The number of GHB publications continues to drop (**Figure 3**), indicating that the forensic lifecycle of GHB is nearing its end. This decrease does not imply that the use and abuse of GHB are declining dramatically. Rather, it means that adequate analytical methods have been developed to generate reliable investigation and judicial information. The needed instrumentation is affordable and cost effective. Questions remain, such as how to differentiate endogenous from exogenous GHB, but the volume of strictly forensic research is unlikely to increase given the current abuse climate. Thus, from the perspective of forensic chemistry the analytical problem is essentially solved, even if the social one is not.

2. CURRENT TRENDS AND HIGHLIGHTED RESEARCH

2.1. Metrology

Data produced by forensic chemists must meet strict legal as well as scientific criteria. As a result, all forensic chemistry research incorporates metrology. Aspects of chemical metrology such as method validation and accompanying QA/QC have become prominent in the literature and have been a key point of discussion in the forensic chemistry community, both for qualitative and quantitative methods. In no other area has the role of metrology been as visible as in solid-dose drug analysis. For analysis of solid-dose drug evidence (also referred to as seized-drug evidence), the Scientific Working Group for the Analysis of Seized Drugs and its European counterpart, the Drugs Working Group, have published recommendations for best practices for qualitative and quantitative protocols (29). Although there is no analogous working group for forensic toxicology, professional associations and accreditation boards act as umbrella organizations for QA/QC and method validation. What have traditionally been referred to as trace evidence (materials) recommendations are published by the Scientific Working Group for Materials.

2.2. Sampling

In seized-drug analysis, sampling considerations often present the greatest challenge and contribute the most to overall uncertainty. These sampling-derived uncertainties are also the most difficult uncertainties to characterize. Cases may consist of hundreds of individual exhibits

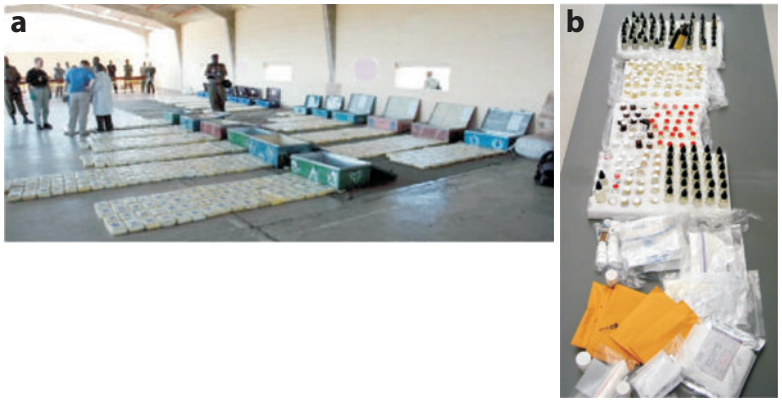

Figure 4

Exhibits of seized drugs that illustrate the importance of sampling methodology. (*a*) Seizure of suspected cocaine. Reproduced courtesy of the U.S. Drug Enforcement Agency. (*b*) Steroid seizure. Reproduced courtesy of the Oklahoma State Bureau of Investigation.

(**Figure 4**) of unknown homogeneity. There are generally two numerical measurements that can have legal significance: the total weight of the controlled substance and the purity of that substance. Total weight is used judicially to determine charges and sentencing as per state or federal guidelines (30, 31), and purity may be used judicially or for investigative purposes. Statutory thresholds must be incorporated in any sampling plan, and they often dictate how sampling and analysis should proceed. Even when qualitative identification is all that is required, sampling plans must be defensible and representative, which (as shown in **Figure 4**), is no easy task. Sampling plans must also consider sample preservation, laboratory resources, and time constraints.

Significant attention has been paid to sampling large seizures consisting of multiple exhibits such as pills, powders, and plant material. In such cases, both threshold weights and purity can be at issue. Given the time, sample, and personnel limitations of laboratories, courts have recognized that exhaustive analysis is neither feasible nor appropriate as long as a defensible sampling plan is employed (30, 31). Currently, laboratories generally use three types of methods (summarized in **Table 3**): nonstatistical, frequentist, and Bayesian. Briefly, frequentist methods rest on the assumption that in a given population of samples (N), some fixed but initially unknown proportion of the population contains a controlled substance (a positive). It is also assumed initially that the subset of positives are all positive for the same controlled substance. The quantity or purity of this substance within the population of positive samples may vary. Frequentist methods are associated with confidence intervals.

In Bayesian approaches, prior knowledge from observation and presumptive tests can be utilized to select a representative sample size. Prior knowledge can be expressed by the terms a and b (**Table 3**); the larger a is relative to b, the greater the probability that the samples are all positives. When no prior knowledge exists, it is often assumed that $a = b = 0.5$ (32).

2.3. Sample Preparation and Screening

Forensic chemists face a daunting variety of target analytes; therefore, the scope and variety of sample preparation methods available are just as large. In the area of drug analysis (solid-dose and forensic toxicology), the typical organic extraction methods are the workhorses of the laboratory. These methods include LLE and micro-LLE, solid-phase extraction (SPE), solid-phase

Table 3 Representative sampling qualitative and quantitative analysis

Basis	Description	Reference(s)	Distribution function	Description
Nonstatistical	If $n < 10$, all	145	–	–
	If $n = 10$ to 100, 10 selected at random			
	If $n > 100$, $\sqrt{N}$ selected at random			
	$n = 20 + 0.10^{*}(N–20)$	32, 146		
	Defined by the jurisdiction			
	Defined by laboratory policy			
Statistical				
Frequentist	Hypergeometric	146–149	$P(Z=z)=\frac{\binom{R}{z}\binom{N-R}{m-z}}{\binom{N}{m}}$	N = population size R = number of positives in the population $N–R$ = number of negatives in the population m = size of the subset of the population that is sampled z = number of samples in the subset that are positive P = probability of finding ($N–R$) positives in a subset of size n taken from a population of size N
	Binomial	32, 148	$P(X=x\|\theta,n)=\binom{n}{x}\theta^{x}(1-\theta)^{n-x}$	θ = proportion of positives within n n = size of the subset X = positives in population x = positives in the subset P = probability that a subset of size n contains X positives assuming that the parent population n contains a proportion of positives equal to θ
Bayesian	Beta $N > 50$	147, 148, 150	$f(\theta\|x,n,a,b) = Be(x+z,n-x+b) = \frac{\theta^{x+a+1}(1-\theta)^{n-x+b-1}}{B(x+\alpha,n-x+b)}$	Be = beta distribution a, b selected based on prior knowledge, observation, presumptive testing, etc. $f(\theta\|x,n,a,b)$ = the probability density function for the positive samples θ

microextraction, and headspace methods. In the forensic context, drugs are typically classified by schedule, physiological effect, or chemical structure, specifically the presence or absence of an ionizable center (i.e., acid or base). Approximately 95% of commercially produced drugs possess ionizable centers; 75% have basic centers (characterized by amino groups), and 20% have acidic centers (i.e., phenolic or carboxylic functionalities, or both) (33). A few have multiple ionization centers, and a small number (e.g., morphine) are amphoteric.

Basic drugs such as cocaine are seen in evidence in both free-base form (B) and salt form, typically B·HCl for cocaine (BH^+ is the ionized cation). Other common counter anions are (in approximate order of frequency) sulfate, bromide, tartrate, citrate, phosphate, acetate, iodide, nitrate, and lactate. Conversely, acidic drugs are seen in evidence either in neutral protonated form or, more commonly, in sodium- or potassium-salt form. Other counter cations are calcium, magnesium, ammonium, aluminum, zinc, piperazine, lithium, and diethylamine. There are cases in which the counter ion is of investigative, and occasionally judicial, interest.

In any extraction, the intrinsic solubility of the salt form is critical. Once dissolved, the molecule's relative solubility is a function of charge and thus of pH and proton affinities, as well as of the polarity and structure of the organic molecule. The descriptor of log P can be used to describe the aqueous solubility of drugs without ionizable centers:

$$\log \mathrm{P} = \frac{[\mathrm{A}]_{\mathrm{octanol}}}{[\mathrm{A}]_{\mathrm{aqueous}}}, \tag{1}$$

where A is the analyte molecule. The quantity log D is required when such centers exist:

$$\log \mathrm{D} = \frac{\sum [\mathrm{A}]_{\mathrm{octanol}}}{\sum [\mathrm{A}]_{\mathrm{aqueous}}}, \tag{2}$$

where the sum of all species of the analyte molecule A (ionized or unionized) is considered, typically at a temperature of 25° C. Because of pH's role in determining aqueous solubility, the log P value of drugs with ionizable centers is typically taken to mean a physiological pH of 7.4.

For toxicologists, the values of pKa, log P, and log D are essential to understanding the pharmacokinetic factors of adsorption, distribution, metabolism, and excretion. In solid-dose drug analysis, these factors dictate sample-extraction procedures based on selective partitioning across a solvent-solvent interface (LLE), a solid-liquid interface (SPE and related methods), or a liquid-gas interface (headspace), as is used for blood-alcohol determinations. Toxicological analysis may also call for additional steps including cleaving of conjugates such as glucuronides and, in some cases, formation of derivatives. Conjugates are broken using acid hydrolysis, enzymatic methods, and sometimes alkaline hydrolysis. The additional complexity of biological matrices such as blood and tissue requires additional preparation to remove proteins, fats, and other interferents, usually through a series of mixing, centrifuging, and filtering steps. Many recent advances in sample preparation emphasize rapid extractions followed by GC-MS or LC analysis; it is the relative nonspecificity of the extraction that drives additional confirmation, rather than the lack of specificity of the instrumental detection. As instrumental selectivity, sensitivity, and detection limits have improved, the sample sizes and the final volume of extracts have decreased; 100 μl is a typical final volume for many such extractions.

2.4. Instrumentation

Dramatic improvements in MS (e.g., in ionization sources, instrumentation, and detection) are significantly influencing forensic chemistry.

2.4.1. Mass spectrometry. Isotope ratio MS (IRMS) is rapidly becoming a standard tool for gathering investigative information in solid-dose drug analysis and in sports toxicology. Inorganic IRMS is used for analysis of materials such as bullet lead, and inductively coupled plasma MS is finding niche uses in materials (e.g., glass) characterization. The use of LC-MS methods has increased dramatically within the last five years, most notably with tandem-MS methods utilizing triple quadrupoles and combinations of quadrupoles, ion-trap, and time-of-flight (TOF) mass analyzers. Most recently, DESI/DART (desorption electrospray ionization/direct analysis in real time) ion sources have been adopted as evidence-screening tools for drugs and explosives. I briefly discuss each technique below.

2.4.2. Liquid chromatography–tandem mass spectrometry and variants. As in many other fields and specialties, LC coupled with various tandem-MS methods has made significant inroads into forensic chemistry, particularly in the area of forensic toxicology. Initially, LC-MS was used in other applications analogously to GC-MS and in cases where thermal decomposition or similar problems precluded the use of GC-MS. Examples of such cases include (*a*) analyses of drugs not amenable to GC such as LSD (lysergic acid diethylamide), benzodiazepines, and cannabinols (34, 35) and (*b*) ink analysis (36, 37). GC-MS analysis of GHB is typically performed with derivatization, making LC-MS an attractive alternative (26). One review (38) boldly noted that LC-MS methodology (using atmospheric-pressure chemical ionization) in the forensic context was approaching maturity in 1999 and that electrospray ionization (ESI) methods showed promise. Although this assessment was accurate at the time, it barely hinted at the explosion of forensic applications of ESI-MS methods to come.

Many applications of tandem-MS methods to forensic problems emerged around 1999 (39, 40); most reports focused on ESI techniques and on multiple-quadrupole instrumentation. Although a few reports have appeared regarding the use of tandem MS for nonbiological matrices [e.g., organic gunshot residue and ricin (41, 42)], the greatest forensic applications of tandem-MS methods are found in forensic toxicology and closely related areas (43–49). An excellent summary of pharmaceutical applications, which have much in common with forensic applications, appeared in this journal last year (50). Recently, significant attention was paid to detection of drugs and metabolites found in wastewater using tandem LC-MS methods (51–53), which combine elements of forensic toxicology with environmental forensics.

2.4.3. Exact mass screening. The evolution of high-resolution MS (particularly TOF), coupled with decreasing instrument cost, has led some laboratories to implement TOF methods for screening purposes. Exact mass methods are useful in cases in which no primary reference standards are available. This frequently occurs with synthetic by-products and metabolites. In the case of LC-TOF-MS, accurate mass data were used in conjunction with knowledge of monoisotopic masses of drug and metabolite structures to create a viable screening system (54, 55). A powerful feature of this approach is the ability to add database entries without having a chemical reference compound; all that is required is a structure and a chemical formula. This capability is useful when new drugs (sometimes referred to as designer drugs), analogs, and by-products are encountered. A Finnish research group took advantage of this capability; they reported that the combination of LC with chemiluminescence nitrogen detection and LC-TOF-MS was sufficient to identify and quantify a variety of common seized drugs with minimal false positives (56). Moreover, in toxicological applications LC-TOF-MS and FT ion cyclotron MS have been used for exact mass screening, with the latter technique achieving a mass accuracy of 3 ppm (57). Two recent reports described SPE extraction of urine followed by LC-TOF-MS (55, 58). Although few working forensic toxicology laboratories have access to FT-MS, LC-TOF-MS is becoming more

common, and it is not unreasonable to expect widespread adoption of LC-TOF-MS into forensic screening within the next 20 years.

Another new MS system that has been applied to forensic analysis is DESI/DART (59–61). DESI/DART is used principally for screening of physical evidence such as pharmaceuticals (tablets) and powders (60), as well as suspected explosives. The advantage of this type of sample introduction is that the resulting mass spectra are similar to those produced using conventional ESI sources. Additionally, analysis time is on the order of a few seconds, and detection limits are in the picogram range (61). A recent publication described the use of DESI-MS-MS to detect organic components of gunshot residue, specifically methyl and ethyl centralite (62). A similar study used atmospheric-pressure chemical ionization MS to screen banknotes and detect heroin (diacetylmorphine) (63).

2.4.4. Isotope ratio mass spectrometry. IRMS has found use in drug profiling and sourcing (64–66) and, owing primarily to the cost of the instrumentation, remains a specialized type of forensic chemical analysis. Stable-isotope analysis has also been used to characterize bullet lead (67), explosives (68), and packing tape (69). Lead was used for bullet characterization, whereas C, N, O, and H ratios were employed for the other materials. The use of IRMS in drug analysis provides an excellent example of data gathered for investigative or intelligence purposes rather than for prosecutorial reasons. Drug profiling also illustrates the use of physical and chemical data in an investigative context. For instance, if the evidence consists of pressed tablets, one can examine the physical patterns imparted by the pressing to assist in grouping samples.

Drug profiling has two purposes: (*a*) to allow for batch identification, and less frequently, (*b*) for provenance determinations of plant-based semisynthetic drugs such as cocaine or heroin. With plant-based drugs, isotopic fraction of carbon occurs via photosynthetic and climate-based phase-equilibrium processes, whereas nitrogen isotope ratios are generally attributable to soil conditions. For synthetic drugs (and other constituents, e.g., diluents) such as methamphetamine, batch characteristics are attributable to chemicals used during the manufacturing process. For batching purposes, physical properties such as color, grain-size, and particle-size distributions are often considered in conjunction with chemical characterization by GC-MS. IRMS is performed using GC-IRMS combined with bulk EA-IRMS (elemental analysis–isotope ratio mass spectrometry) (64, 65, 70, 71). Interestingly, IRMS has been reported as a possible method for distinguishing endogenous versus exogenous GHB (72), with differences attributed to metabolic effects, post-mortem conditions (if applicable), and variable synthetic routes. The same technique is used in sports toxicology, for instance in doping investigations and in discrimination of endogenous versus exogenous testosterone (73–81).

3. THE NEW INTEGRATION: EXPANDING THE SCOPE OF FORENSIC CHEMISTRY

A notable trend in forensic chemistry research is the increasing application of fundamental chemical science to diverse aspects of physical and pattern evidence. In some instances, this means examining old methodology with new scrutiny; in others, it means developing novel applications of instrumentation. The uniquely forensic science of fingerprints illustrates this trend.

The use of latent fingerprints for individual identification and for linking an individual to a crime or crime scene was arguably the first purely forensic science. Latent fingerprint residues are created by deposition of eccrine sweat, a concentrated aqueous saline solution containing significant amounts (greater than 1% w/w) of lactic acid, urea, and amino acids. Eccrine sweat is secreted by glands in the fingers, palms, and soles of the feet and lacks significant lipophilic

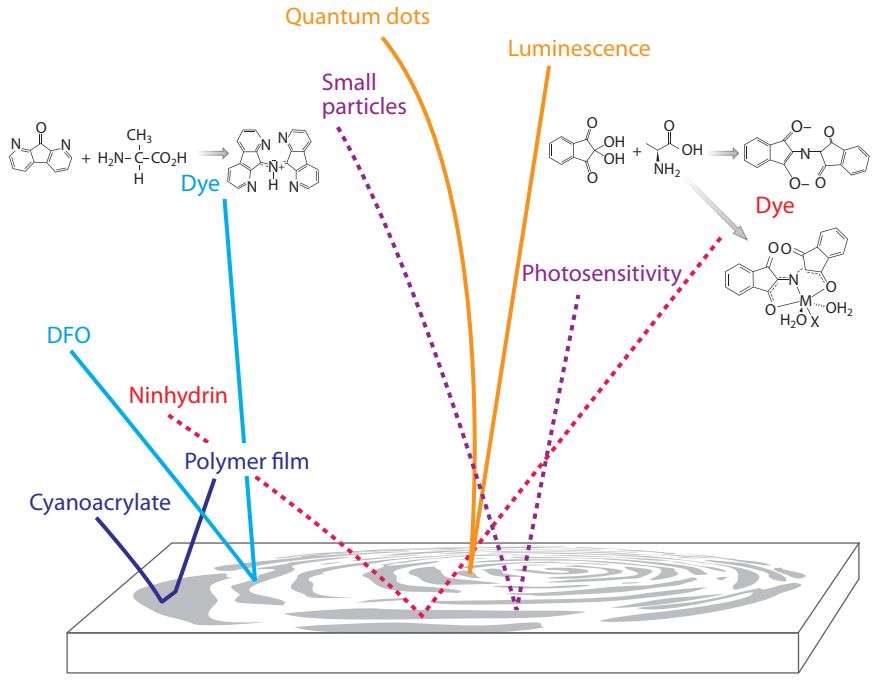

Figure 5

Chemical and instrumental methods for visualizing and developing latent fingerprints. Abbreviation: DFO, 1,8-diazafluoren-9-one.

components. Sebaceous glands, found in hair follicles, contribute fatty acids and triglycerides to latent fingerprints through secondary contact and transfer. Any of these components can be targeted with visualizing methods and via developing reagents (**Figure 5**). The fundamental goals of treating any surface are to enhance visualization, and if appropriate, to develop or fix the pattern so that clear digital images can be made. Success depends on enhancing the contrast between the substrate and the print, and it is the substrate that dictates the methodology. Substrates vary widely, with human skin, blood, paper, clothing, and metal among the most common and the most challenging.

The earliest imaging techniques (which emerged barely a century ago) were based on a triad of simple physical, chemical, and optical methods. These initially included the dusting of surfaces with powder formulations containing metal oxide or salt along with an adhesive material that allowed the powder to adhere preferentially to the latent print residues. Illumination methods, alone or in combination with powders, remain effective in many applications. Another early technique involved chemical reagents, which either stained the latent print (as in the case of iodine fuming) or formed dyes (as in the case of ninhydrin) with components of the print. Composite methods, such as the use of powders containing fluorescent compounds, were added to the toolbox in the mid-twentieth century. Although latent prints contain naturally fluorescing materials, their inherent emission is weak. Intense illumination with filtered polychromatic light coupled with viewing filters enhances detection of this fluorescence. This methodology is referred to as alternate light source technology, and it has many uses in forensic science. In laboratory settings, intense laser sources are valuable for optimizing natural fluorescence, although their utility is limited by background and substrate emissions.

The best-known chemical reagents used for latent fingerprint development are ninhydrin and cyanoacrylate. Because of their widespread use, researchers have applied their understanding of the chemical reactions and processes involved to investigate these reagents' mechanisms of action and the optimization of reagents. A significant body of research since 2000 has contributed to our understanding of reagents and developers, which in turn has driven the introduction of newer reagents and combined treatments. Of the dye-forming reagents, ninhydrin (which forms the dye complex Ruhemann's purple) is perhaps the most studied (82–87); examination of its mechanisms of action and evaluation of analogs are of principal interest (82, 83, 85, 86, 88). In 2000, Wilkinson (89) reported the results of a detailed characterization study of DFO (1,8-diazafluoren-9-one) with the amino acid alanine in a methanolic solution. Subsequently, other reports on DFO and related compounds such as 1,2-indanedione (90–93) appeared; these compounds form dyes and, with subsequent treatment with metal salts, fluorescent complexes (94–96).

Chemical imaging: combination of visual imaging with chemical or spectroscopic data to define and describe surface features and spatial relationships on a surface

Small-particulate reagents have long been used for processing latent prints. With physical developer (PD), Ag^+ ions are reduced by a ferrous/ferric redox couple to form silver metal. A related method is vacuum metal deposition (VMD), which has proven useful for supple, thin, or flexible substrates such as plastic bags and leather. In VMD, the surface is first coated with a layer of gold that penetrates into the ridges that form the latent fingerprint. Next, a layer of zinc is applied; the zinc adheres preferentially to the gold that is exposed in areas where there is no latent print residue. The result is a negative of the latent print, with zinc coating the residue-free areas. Once coated, the print can be further treated to develop the pattern. Coating the substrate can significantly diminish background luminescence and can increase the contrast between it and the print.

An exciting line of chemical research regarding latent prints pertains to nanochemistry, nanoparticles, and quantum dots (97, 98). One of the first forensic applications of nanoparticles is a process known as multimetal deposition, in which gold nanoparticles are utilized as part of PD. The gold molecules are deposited on the print residue, where they catalyze the reduction of the silver ion to metallic silver (99). An extension of this technique, first reported in 1990, is the application of quantum dots to latent fingerprint development (100, 101). Quantum dots are semiconductors on the order of 1 to 10 nm, in which electron promotion between the valence and conduction bands can result in emission of visible light. When absorption occurs and an electron is promoted, a hole is created. Depending on the crystal structure, the hole and electron can become trapped between the valence and conduction bands. When they recombine, a lower-energy photon is emitted (98). The characteristics of the luminescence also depend on the size of the dot and on the materials used; zinc and cadmium sulfides and selenides are common. Compared to organic dyes, the emission of quantum dots is typically narrower, and luminescence can be "tuned" by altering the size of the particulate (97). A study of mechanistic factors appeared in 2008 (102), as did a report of the use of SiO_2 nanoparticles doped with europium (103). Significant advantages of using quantum dots are (*a*) their ability to adhere to latent print residues by physical and chemical means, (*b*) the inherent intensity of the luminescence, and (*c*) the ability to adjust emission wavelength through particle-size adjustment (102).

The main contributions of analytical chemistry to latent print examination include chemical imaging and probing current methods to understand fundamental processes. Cyanoacrylate fuming is an example of the latter. The polymerization mechanism of cyanoacrylates is known, but it was only relatively recently that we learned why this anionic polymerization occurs preferentially on the ridges of latent fingerprints. Current research indicates that carboxylate, lactate, and alanine moieties (104), as well as water and hydroxide (105), play a role in initiation of the polymerization. Raman spectroscopy studies suggest that the preferential polymerization on the ridges and surfaces of the latent print may be attributable in part to the formation of a hydrophobic layer over the print and at the junction of the latent print and the substrate that slows or stops the polymerization

reaction. Raman spectroscopy can also detect materials (such as drugs and explosives) embedded in fingerprints (106–108).

Perhaps the most intriguing application of analytical instrumentation to latent fingerprints involves chemical mapping and chemical imaging. The potential advantages of these methods are numerous. First, often there is minimal or no sample pretreatment required (i.e., nondestructive methodology); second, visual contrast (natural or induced) between the substrate and the latent print does not dictate what methods can be used for mapping and pattern imaging. Initial research in this area focused on microspectrophotometry in the ultraviolet–visible–infrared range (109–112), with an emerging emphasis on infrared methods (109–111, 113, 114). The recent introduction of focal plane array detectors facilitates spectral mapping, which, when combined with data display tools, allows for production of friction-ridge patterns based on chemical functionality (111). Scanning electrochemical microscopy has also been used to map fingerprint patterns after deposition of metals upon the surface of the ridges (115). X-ray fluorescent mapping has also been used (116, 117), and recently, chemical imaging employing MS and a DESI interface was reported (118). Although other chemical imaging methods provide greater resolution, the advantage of the MS approach is its chemical specificity. In this report (118), images were produced by mapping exogenous compounds (in this case, cocaine) entrained in the latent print deposit, as well as by endogenous sebaceous constituents.

SUMMARY POINTS

1. Forensic chemistry is rapidly incorporating numerous chemical disciplines other than analytical chemistry.
2. Forensic chemistry generates two types of data, investigative and judicial.
3. To be admissible in court, judicial data must meet the requirements of both the scientific community (guided by the scientific method) and the legal community (guided by the adversarial method). Both communities influence forensic research and practice.
4. Forensic chemistry is adaptive and derivative by nature and design. Most forensic research emphasizes modifying existing techniques and instrumentation to meet forensic needs. Key steps in the research process are refining and validating the data to meet the needs of the legal and scientific communities.
5. Recent advances in the capability of LC-MS instrumentation, innovative ionization sources, and affordable instrumentation are driving the migration of what was once considered confirmation testing into the realm of rapid screening.
6. There is a resurgence of interest in chemical research, particularly in nontraditional areas such as pattern evidence.

FUTURE ISSUES

1. Metrology will continue to be an important research area in forensic chemistry, particularly but not exclusively in the area of solid-dose drug analysis. Whereas method validation procedures can describe instrumental and procedural uncertainties, determining sample contributions and matrix effects in defining uncertainties remains a challenge. As more types of evidence are subjected to chemical and instrumental analysis, opportunities for metrology research will grow.

2. As screening devices, chip-based analytical systems (such as microfluidic devices) appear promising for applications such as field detection and rapid screening for select analytes (e.g., drugs and explosives). It is likely that the pace of this research will increase, and it is reasonable to expect that microfluidic devices will be used in some forensic capacity within the next decade.
3. Finally, because of the adaptive nature of forensic chemistry, one way to predict the frontiers of its research is to examine this publication's contents for this year for work in quantum dots, capillary separations, HPLC, mass spectral hunting for biomarkers, miniaturization of MS, on-chip multiplexing analysis, ion microscopy, and thin-film chemical sensors. It is from these types of work that methods of forensic research will expand.

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Errata

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