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Forensic Science

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CONTENTS

During the time period of this Review (in February 2009), the U.S. National Academy of Sciences published a report entitled "Strengthening Forensic Science in the United States: A Path Forward", which offers 13 recommendations.[1] Several of these recommendations discuss the importance of research and studies establishing the validity of forensic methods. We hope that this Review article will aid understanding of what work has been accomplished in recent years to demonstrate the validity of specific forensic methods.

The format selected for this survey divides coverage into three distinct areas: forensic DNA analysis, trace evidence, and drugs and poisons. Within the scope of each of the areas, key articles have been selected to describe current forensic science practices in analytical chemistry and to outline relevant forensic science research interests. In accordance with the policy of the Managing Editor, we have strived to keep this Review limited to important articles and to keep our discussions concise and meaningful.

FORENSIC DNA ANALYSIS

The literature for forensic DNA analysis has expanded rapidly in the past few years as various technologies and genetic markers have been adopted, validated, and examined in numerous populations around the world. During 2009 and 2010, more than 500 papers and a number of books were published regarding DNA markers that are applied to human identity testing. The journal *Forensic Science International: Genetics*, which focuses entirely on forensic DNA analysis, published 165 articles in the eight issues released during 2009 and 2010. Unfortunately, due to space constraints, only a selection of these articles will be highlighted below. In September 2010, Biomed Central launched a new peer-reviewed, open-access journal entitled *Investigative Genetics*, which includes coverage of forensic DNA issues. It is

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worth noting that conference proceedings are available online for important meetings in this field including the International Symposium on Human Identification (aka the "Promega meeting") (http://www.promega.com/geneticidproc/) and the International Society of Forensic Genetics (ISFG) (http://www.isfg.org) meetings. Volume 13 of *Progress in Forensic Genetics*, which contains the proceedings of the ISFG meeting held in Buenos Aires, Argentina, in September 2009, includes 250 brief articles covering current research on all aspects of forensic DNA typing.[2]

Short tandem repeat (STR) typing of autosomal markers with fluorescence-based detection is now almost universally used in forensic DNA laboratories worldwide.[3] Reduced-size STR, or miniSTR, assays are being developed for improved recovery of information from badly damaged DNA templates. Low level DNA and samples containing mixtures continue to present challenges for forensic analysts. A large portion of the literature involves reporting STR allele frequencies from various populations. However, limited space in this review prevents a summary or description of the many population studies performed in 2009 and 2010.

Single nucleotide polymorphisms (SNPs) continue to be explored as potential supplements to STR markers already in use but will probably not replace STRs in the near future. Information on uniparental lineage markers from the Y-chromosome and mitochondrial DNA continues to accumulate in the literature. These lineage markers are also widely used for human evolutionary studies and genetic genealogy. The availability of commercial kits for Y-STR amplification has enabled more widespread usage of these important male-specific markers in forensic DNA laboratories. A number of X-chromosome STRs are also being investigated. Nonhuman DNA plays a useful role in many forensic investigations. Tests have been developed for plant and animal DNA testing to associate victims or suspects to crime scenes.

National DNA databases collectively house millions of STR profiles around the world. With the demonstrated success of linking previous offenders to unsolved crimes they have committed, new legislation is expanding the number of samples that will be going into DNA databases of the future. In December 2010, the United States exceeded 9.5 million DNA profiles in the National DNA Index System of the FBI Laboratory's Combined DNA Index System (CODIS). The United Kingdom has over four million STR profiles in their national DNA database, which represents a significant portion of their active criminal population.

Automation of laboratory techniques and data interpretation with expert systems has become increasingly important with the large numbers of DNA samples that need to be examined. Forensic DNA testing also aids missing persons investigations and identification of mass disaster victims albeit with some extra issues unique to kinship and parentage analysis. At the end of the forensic DNA analysis section, we also list relevant papers on interpretation and statistical weight of DNA typing results published in 2009 and 2010 along with several general reviews of forensic DNA typing.

Collection, Characterization, Preservation, Extraction, and Quantitation of Biological Material. A special issue of *Forensic Science Review* (volume 22, number 2, July 2010) was devoted to assessment and preparation of biological specimens for DNA analysis. A retired NYPD detective shared his law enforcement perspective on the importance of a crime scene and collecting biological evidence.[4] The use of adhesive tape to recover DNA from crime scene items was explored.[5] Nylon flocked swabs improved DNA recovery from postcoital vaginal sampling.[6] Issues with expanding sexual assault evidence collection beyond 72 h were considered.[7] A study of 1000 sexual assault cases found DNA evidence in 473 of them.[8]

Measures for preventing DNA contamination of disposable plasticware and other reagents by manufacturers were outlined in an agreed upon joint statement by leading European, Australian/New Zealand, and U.S. forensic DNA organizations.[9] UV irradiation and autoclave treatments were explored,[10] and a dual cycle ethylene oxide treatment was found effective[11] for eliminating contaminating DNA from laboratory consumables. A multistrategy procedure for decontamination of PCR reagents was also described involving gamma- and UV-irradiation and treatment with a heat-labile double-strand specific DNase.[12] Room temperature storage of extracted DNA was reviewed,[13] and studies regarding the stability of DNA in a dried state were performed.[14,15]

Identification of the tissue of origin for biological stains continues to be pursued using RNA profiling[16] with reverse transcription end point PCR and real-time PCR.[17] Stable mRNA markers have been developed for blood and saliva identification,[18] saliva and semen,[19] and blood, saliva, semen, and menstrual blood.[20] MicroRNA markers have been selected by microarray screening and confirmed by quantitative RT-PCR.[21] Bacterial content of samples has been used to identify vaginal secretions[22] and saliva.[23] Salivary bacterial DNA has been used to verify expirated blood that came out of an individual's mouth rather than as a result of normal bleeding.[24] Developmental validation of a lateral flow immunochromatographic strip test for saliva has been described.[25]

A review article summarizing manual and automated extraction methods described liquid and solid-phase DNA extraction methods.[26] Additional articles in the same issue of *Forensic Science Review* report on extraction methods from human remains,[27] DNA extraction on microfluidic devices,[28] and quantitative and qualitative assessment of extracted DNA prior to genotyping.[29] Developmental validation studies of the PrepFiler chemistry were published.[30] Laser capture microdissection and its application to forensic DNA testing were reviewed.[31]

Short Tandem Repeats (STRs). A developmental validation of the PowerPlex 16 HS kit was published,[32] and forward stutter with the SGM Plus kit loci was characterized.[33] A protocol for rapid amplification of STR typing kits was described using a custom-built thermal cycler,[34] and variability with Identifiler kit parameters and mixed DNA profiles was explored.[35] Low level DNA validation challenges were described,[36] and questions concerning the validity of low copy number DNA testing were raised.[37] Validation studies with low template DNA were reported by the New York City Office of Chief Medical Examiner[38] and Orchid Cellmark.[39] The use of mass spectrometry was shown to increase the information content of some STR loci through identification of sequence differences in the STR repeat or flanking regions.[40] Several integrated microdevices for STR typing have been described,[41−43] and the design of a portable scanner for microchip capillary array electrophoresis has been reported.[44] Pentameric repeat miniSTR loci have enabled rapid analysis of forensic DNA samples on a microfluidic system.[45] Pyrosequencing has been performed on autosomal STR loci.[46] Alternative enzymes have been examined to improve forensic DNA profiles[47] and to increase PCR inhibitor tolerance.[48]

Single Nucleotide Polymorphisms (SNPs). Triallelic SNP markers were introduced to enable analysis of degraded samples and DNA mixtures.[49] A multiplex PCR coupled with a ligation assay was described to examine degraded DNA with fragment sizes less than 100 bp.[50] Validation of a 49 SNP assay was performed according to ISO 17025 requirements.[51] Automated SNP typing was completed using the Invader assay.[52,53] A genome-wide screen for individual identification SNPs led to the analysis of six SNPs in a Chinese population group.[54] Development of a SNP-based HID system was described,[55] and a set of 108 SNPs for a universal individual identification panel was identified.[56] A low-cost SNP assay using the OpenArray platform was introduced for potential forensic DNA applications.[57] A set of 38 insertion/deletion polymorphisms has been investigated for potential application in human identification.[58] A panel of 48 insertion/deletion markers was explored for their capability to assess ancestry.[59] A position paper on forensic phenotyping using externally visible characteristics discussed motivations, scientific challenges, and ethical considerations.[60] SNP testing has been used for pigmentation and biogeographical ancestry estimation on ancient skeletal remains,[61] for ancestry analysis for samples collected in connection with the Madrid bomb attack investigation,[62] for human eye color determination,[63] and in trying to predict normal pigmentation.[64]

Y-Chromosome and X-Chromosome Analysis. The use of Y-STR typing in forensic casework was reviewed.[65] Y-chromosome variation was explored in 590 males from 51 populations belonging to 8 worldwide regions using 67 Y-STRs including 49 rarely studied simple single-copy loci.[66] The Y-STR loci DYS449, DYS481, DYS612, DYS626, DYS644, and DYS710 were characterized in three South African population groups.[67] Several Y-STR loci were also analyzed using Pyrosequencing technology.[68] The mutation rates of the 17 Yfiler Y-STR markers were examined in over 1730 DNA-confirmed father−son pairs with 84 sequence-confirmed mutations found among 29 792 meiotic transfers.[69] From a set of 186 Y-STR markers, the 13 most mutable Y-STRs proved capable of distinguishing close and distantly related males.[70] The Y-chromosome deleted regions from an amelogenin negative Japanese male were reported.[71] An approach was described for interpreting Y-STR mixtures.[72] The use of X-STR loci to aid parentage casework was addressed.[73] Six closely linked X-STRs near the X-chromosome centromere were studied.[74] X-chromosomal recombination was explored with 39 X-STRs using several three-generation German pedigrees.[75]

Mitochondrial DNA (mtDNA) Typing. A sequencing strategy was described for high quality information from the entire mitochondrial genome,[76] as was the technique of base composition profiling with electrospray ionization mass spectrometry.[77] Microarray-based sequencing was performed on 93 worldwide mitochondrial genomes.[78] A strategy for Pyrosequencing of the two mtDNA control region hypervariable regions was reported.[79] Coding region single nucleotide polymorphisms proved helpful in several forensic cases.[80] Denaturing high-performance liquid chromatography was used for comparative analysis of the HV1 and HV2 regions.[81] Success rates with amplifying mtDNA from human hairs were correlated to specific melanin content.[82] The performance of mtDNA minisequencing assays was explored,[83] and a comprehensive phylogenetic tree of global human mitochondrial DNA variation was developed.[84]

Nonhuman DNA Typing Systems. A new autosomal nineplex for canine identification and parentage testing has been developed,[85] and canine STR population data was reported with a different set of dog STR loci.[86] The application of canine DNA profiling in assessing dog attacks was described.[87] Developmental validation of a canine STR kit[88] has been published along with an allele nomenclature system for the loci present in this kit.[89] Standardization of 16 bovine-specific STR loci[90] and 17 equine-specific STR loci[91] has been proposed. Informative SNPs outside of the dog mtDNA control region have been identified,[92] and an interlaboratory study was performed with dog mtDNA analysis.[93] Wildlife DNA forensic analysis was reviewed,[94] and issues with species identification using the mtDNA cytochrome oxidase I gene were discussed.[95] Species identification based on size variation of mtDNA hypervariable regions has been attempted.[96] A Cannabis STR database was developed to aid in marijuana investigations in Australia.[97] Bacterial profiling has been attempted to differentiate soil samples.[98] Analysis of oral microbial DNA has been used to identify bloodstains from blood expelled from an individual's mouth.[99]

DNA Databases, Missing Persons, and Disaster Victim Identification. Database crime to crime match rate calculations have been made,[100] and efforts have been made to model forensic DNA database performance.[101] Arguments were made for why partial matches in offender databases do not call into question the validity of random match calculations.[102] Recommendations were provided for mass fatality management of skeletal remains based on experience from the World Trade Center DNA efforts.[103] Lessons learned regarding forensic kinship DNA identification and the role of genetics professionals came from the Hurricane Katrina volunteers.[104] Human remains from World War I,[105] World War II,[106] and the Spanish Civil War from the 1930s[107] were identified using DNA testing. The final two missing Romanov children were identified,[108] and the putative remains of the famous astronomer Nicolaus Copernicus were characterized with DNA analysis.[109]

Interpretation and Statistical Weight of DNA Typing Results. The German Stain Commission[110] and an FBI mixture committee[111] offered their perspectives on DNA mixture interpretation. A method for estimating the probability of STR allele drop-out was developed.[112] Concerns regarding use of inclusion probabilities for mixture interpretation have been raised.[113] Quantitative assessment of genotype probabilities was shown to improve recovery of information from mixed DNA samples.[114]

General Reviews. Review articles have been written on future directions of forensic DNA,[115,116] trace DNA,[117] and analysis of degraded DNA.[118] Finally, several books related to forensic DNA analysis were published in 2009 and 2010.[3,119−122]

■ FORENSIC EXAMINATION OF TRACE EVIDENCE

Minute quantities of natural and man-made materials that readily transfer between objects or between people and objects is referred to as "trace evidence". The examination of glass, fiber, and paint evidence is included in this category as is the examination of debris collected from fires and explosions to detect residues of ignitable liquids and/or explosives. Trace evidence analysis of gun shot residue (GSR), building materials, inks and toners, and the chemical characterization of fingerprint residue is also often analyzed by the same scientists in the forensic laboratory and is therefore also included here. During 2009 and 2010, several reviews and more than 275 journal publications were published reporting trace evidence analyses, only a selection of which is presented below. The reviews of most interest to trace evidence examiners include a review of the field of forensic science conducted every three years by Interpol[123] and an overview of forensic science policy and practice in Australia.[124]

Reviews in the chemical analysis of explosives have included trends in the detection of peroxide-based explosives[125] and in the use of optical and fluorescence-based sensors for explosives detection.[126] Comprehensive reviews of gun shot residue (GSR)[127] and dyed textile fiber[128] analyses were also published. Reviews of the forensic application of different analytical techniques included the use of X-ray fluorescence,[129] detection of latent fingerprints on porous surfaces,[130] scanning electrochemical microscopy (SECM) for fingerprint analysis,[131] the use of stable isotopes,[132,133] hydrocarbon fingerprinting,[134] ambient ionization mass spectrometry in forensic science,[135−137] the use of ion mobility spectrometry for the detection of drugs and explosives,[138] the use of portable Raman instruments for in-field explosives detection,[139] and the use of X-ray photoelectron spectroscopy (XPS) for forensic application.[140]

Petroleum Products and Explosives. Several papers reported analytical methods for the characterization and discrimination between oil spill samples using biomarkers,[141,142] compound-specific stable isotopes,[143] hollow fiber liquid-phase microextraction coupled to a variety of chromatography and mass spectrometry systems,[144] and the use of elemental composition.[145] More traditional ignitable liquid residue (ILR) analysis studies were reported with the aid of a novel needle extraction method coupled to GC,[146] headspace single drop microextraction,[147] characterization of the biomarkers using GC-FT-IR and GC/MS,[148] the temporal changes in microbial degradation products,[149] weathering in heating-oil releases,[150] the use of differential mobility spectrometry (DMS) and comparison of DMS to GC/MS for the detection of ILR from fire debris,[151] and the sampling and recovery of ILR from a suspect's hands.[152]

A very large number of papers reporting the detection and/or forensic analysis of explosives were published in the last two years including the novel use of a variety of analytical methods including the use of isotope ratio mass spectrometry (IRMS),[153−159] Raman spectroscopy,[160−165] amperometric sensing,[166] standoff detection using hyperspectral imaging,[167] laser electrospray mass spectrometry (LEMS),[168] GC coupled to ion mobility spectrometry (IMS),[169] SPME sampling coupled to an IMS,[170,171] SPME coupled to GC/MS,[172−174] and direct IMS analysis with improved chemometric multivariate methods.[175] A low temperature plasma (LTP) ionization method was also reported[176] as was thermal desorption-single photon ionization coupled to an ion trap mass spectrometer (TD-SPI-ITMS).[177] The characterization of smokeless powders was reported using nanoelectrospray ionization coupled to MS[178] by ion chromatography (IC) MS,[179] by IC and capillary electrophoresis (CE),[180] and by SPME-GC coupled to a nitrogen phosphorus detector.[181] Nitrate ester explosives were analyzed using microchip electrophoresis;[182] C4 explosives were analyzed by Time-of-Flight Secondary Ion MS and XPS,[183] and peroxides (TATP) were analyzed using a colorimetric sensor array.[184] Canines were used as detectors for human scent identifications with postblast debris collected from improvised explosive devices,[185] and standoff detection of explosives was achieved with vibrational spectroscopy[186] and laser induced breakdown spectroscopy (LIBS).[187,188] An automated mapping procedure for fingerprints containing C4 explosives was described,[189] as well as the detection and analysis of explosives from hair[190] and from fingerprints using attenuated total reflection-Fourier transform IR,[191] infrared spectral imaging,[192] and confocal laser scanning microscopy (CLSM) coupled to an inductively coupled plasma optical emission spectrometer (ICP-OES).[193] Progress in novel sampling of explosives from hands was also reported,[194] as was the development of a new standard reference material (SRM) to simulate the handling/transfer of military explosives.[195] Multivariate analysis techniques of FT-IR data were used for the analysis of postblast residues;[196] a portable fluorescent detector was used for the detection of explosives in air,[197] and explosives analysis using morphology and composition of pyrotechnic resides was also reported.[198]

Hairs, Fibers, Glass, and Paint. Hair color determination was reported with spectroscopic analysis including the use of UV−visible microspectrophotometry,[199] digital image analysis with reflective spectrophotometry,[200] and Spectracube.[201] FT-IR-ATR was used for the examination of hair-keratin fibers,[202] and the analysis of dyed hair was reported using two-dimensional infrared correlation spectroscopy.[203] Chromatographic methods coupled to MS were used to analyze drugs and other organic compounds in hair,[204−206] and a number of papers describing the elemental analysis in hair using ICP-OES,[207] solid sampling electrothermal atomic absorption spectrometry,[208] capillary electrophoresis coupled to a chemiluminescence detector,[209] and ICPMS[210] were also reported.

A paper describing the population, transfer, and persistence of fibers on the skin of living subjects was reported,[211] as was the discrimination of blue cotton fibers[212] and the identification of artificial leather in hit-and-run cases.[213] The morphological changes in textiles fibers exposed to environmental stresses was reported using atomic force microscopic (AFM) examination;[214] the kinetics of color change in textiles and fibers due to normal use and washings was reported,[215,216] as was a procedure for identifying textile bast fibers such as flax, hemp, and jute, using microscopy[217] and the use of laser scanning confocal microscopy for the examination of hairs and textile fibers.[218] Azo and methane fiber dyes were analyzed using microextraction followed by CE-MS;[219] thermally assisted hydrolysis and methylation with pyrolysis GC/MS was used for the characterization of wood and vegetable fibers,[220] and resonance Raman and UV−visible spectroscopy was used to analyze black textile dyes.[221] UV−visible microspectrophotometry was used for general fiber characterization,[222] and elemental analysis of a single PET fiber from a trunk mat was reported using LA-ICPMS,[223] as was the analysis of white cotton fibers using LA-ICPMS[224] and LIBS.[225]

Cathodoluminescence (CL) spectroscopy was reported to be used for the discrimination of glass samples,[226] as was X-ray microanalysis,[227] LA-ICPMS of ancient glass,[228] LA-ICPMS combined with refractive index (RI) for container glass discrimination,[229] and LIBS.[230,231] Several data analysis and interpretation schemes were proposed using a Bayesian framework considering SEM-EDS data and RI data[232−235] and feature selection coupled to a Bayesian framework.[236]

Stable isotopes of white paints were determined by IRMS and used to calculate likelihood ratios;[237] Macro-ATR-FT-IR imaging of paint cross sections was reported,[238] as was the use of computer analysis of ATR-FT-IR spectra.[239] Laser desorption MS was used to analyze synthetic organic pigments in works of art,[240] as was the use of IR microspectrophotometry.[241] Fourier-transform photoacoustic infrared (PAIR) spectroscopy was used in the forensic analysis of artists' inorganic pigments,[242] and elemental analysis of automotive paint was determined by LIBS[243] and SEM-EDS.[244] Microspectrometry in the visible range was used to differentiate car paints,[245] and pyrolysis-GC/MS was used to analyze spray paints[246] and plasticizer content in polyvinyl acetate polymer (PVA) binders in paint medium.[247]

Gunshot Residue Analysis (GSR). An undergraduate laboratory experiment was developed for the analysis of GSR using

LIBS.[248] GSR collection studies using different methods was reported,[249] including a comparison study between collection by swabs or stubs.[250] The distribution and properties of GSR originating from a Luger 9 mm ammunition was also reported,[251] as was the influence of a possible contamination of the victims' clothing by gunpowder residue on the estimation of shooting distance[252] and the detection of GSR on bone fragments in a controlled experiment.[253] Volatile organic residues in 9 mm spent cartridges was used to estimate the time since discharge.[254] A study to determine the bullet entrance hole used FT-IR microscopy;[255] inorganic characterization of GSR particles was achieved using ion beam analysis,[256] and the rapid analysis of GSR was reported using microwave plasma torch-mass spectrometry (MPT-MS).[257]

Fingerprints. New reagents for latent fingerprint development have been reported including some with fluorescent dyes,[258] enhanced color intensity,[259] the use of phosphor powder,[260] the use of naphthoquinones,[261] the use of gold nanoparticles[262] and CdSe nanoparticles,[263] the use of vacuum metal deposition of ZnO,[264] the use of Swedish black powder,[265] an inexpensive natural magnetite (Fe_3O_4),[266] the use of Ninhydrin Thiohemiketals,[267] and other novel organic chemistry.[268] Two separate studies reported the variation in amino acid and lipid composition of latent fingerprints.[269,270] The development of latent fingerprints on different surfaces was also reported including those from adhesives,[271] from brass,[272] from fabrics using Au/Zn vacuum metal deposition,[273] from stainless steel surfaces,[274] and from thermal paper using a one-step ninhydrin treatment with polyvinylpyrrolidones.[275] Raman chemical imaging (RCI) was used to detect and identify explosives from contaminated fingerprints[276] and also by secondary ion mass spectrometry (SIMS).[277] A large number of publications described novel instrumental analysis methods for the detection or visualization of fingerprints including the use of IR spectral imaging,[278] near-infrared imaging,[279] an element-based sensor for curved surfaces,[280] SIMS for imaging of prints on polymeric coatings,[281] surface-enhanced Raman spectroscopy (SERS),[282] IR spectroscopy imaging coupled to multivariate analysis,[283,284] atomic force microscopy (AFM) for visualizing corrosion on brass surfaces,[285] and scanning electrochemical microscopy (SECM).[286] Chemical imaging including the use of IR and Raman was used to enhance latent fingerprint visualization,[287] as was the use of cadmium sulfide quantum dot/chitosan nanocomposites.[288] Raman spectroscopy coupled to multivariate data analysis was also used for these purposes,[289] as was the use of matrix-assisted laser desorption ionization mass spectrometry (MADLI-MS) imaging,[290] gold nanoparticle and imaging mass spectrometry,[291] the use of attenuated total reflection (ATR)-Fourier transform infrared (FT-IR) for the detection of latent prints contaminated with cosmetics,[292] the use of surface assisted laser desorption TOF-SIMS,[293] and the use of TOF-SIMS for the detection of exogenous contaminants[294] and amphetamine drugs[295] on fingerprints.

Ink and Paper Analysis. The ASTM standards for the forensic examination of inks were reviewed; improvements were proposed,[296] and the influence of storage conditions on the aging of color dye-based inkjet printing inks was reported.[297] The analysis of paper within the field of document examination was reported using digestion followed by ICPMS,[298,299] and a comparison of XRF, LA-ICPMS, and IRMS methods.[300] The sequence of intersecting gel pen and laser printed strokes was studied,[301] as was the use of quantum dots for ink jet printing of security documents and labels,[302] the characterization of toners using LA-ICP-TOF-MS,[303] and the investigation of historical documents by energy dispersive X-ray fluorescence.[304] The organic analytes in inks such as dyes were studied with a use of a variety of techniques including laser desorption ionization MS,[305] direct analysis in real time (DART) MS,[306] ESI-MS of dyes and vehicles in the ink extracted from the document surface,[307] UV/vis spectrophotometry and LDI-MS,[308] CE coupled to a diode array for detection,[309] the use of easy ambient sonic-spray ionization mass spectrometry (EASI-MS),[310] the use of pyrolysis GC/MS for the characterization of naturally and artificially aged inks and papers,[311] GC/MS and microspectrophotometry for the discrimination of pigment ink printed on white paper,[312] Raman and SERS to the analysis of synthetic dyes found in ballpoint pen inks,[313] LDI-MS for the analysis of pigmented inkjet printer inks and printed documents,[314] the use of microattenuated total reflectance FT-IR for the study of documents containing red seal inks,[315] in situ analysis of ballpoint pen inks on paper with the use of TOF-SIMS,[316] and the use of TOF-SIMS for the simultaneous analysis of organic and inorganic components from ballpoint pen inks.[317] The elemental analysis of paper and gel inks was studied using the microspectrochemical analysis techniques of LA-ICPMS and LIBS,[318] and the elemental composition of blue ballpoint pen ink was determined by LA-ICPMS,[319] as was the use of TXRF for the elemental characterization of ink samples[320] and the use of graphite furnace atomic absorption spectroscopy (GFAAS) for the determination of metals in iron gall ink.[321]

Miscellaneous Trace Evidence. A large number of publications reported on the detection and analysis of chemical and biological warfare agents (CBWAs) using chromatographic methods coupled to mass spectrometry,[322−327] including the analysis of capsicum extracts from self-defense devices,[328] carbon nanotube sensors,[329,330] direct analysis in real time (DART) coupled to MS,[331] aninon exchange SPE,[332] and nanopore sensing.[333] Light isotopes were investigated for the analysis of wood safety matches[334] and to determine geographic provenance of marijuana plants,[335] and lead isotopes were analyzed to distinguish between legal and counterfeit tobacco cigarettes.[336] Laser desorption MS was used to analyze inorganic components in costemics,[337] and the characterization of polyurethane foams was achieved using GC-FID and ICP-OES.[338]

The forensic analysis of VOCs in human saliva was reported using stir bar extraction followed by GC/MS,[339] and the VOCs from human scent evidence was carefully evaluated under different conditions and analyzed by SPME-GC/MS.[340] Acrylic and rubber-based adhesives were analyzed by ATR FT-IR,[341] and the identification and comparison of electrical tapes were reported by the same method.[342] High resolution sector field ICPMS was used for the elemental analysis of human bone and teeth after laser ablation microsampling;[343] the trace element content in rough diamonds was achieved using LA-ICPMS,[344] and the analysis of brick stones was conducted with the techniques of instrumental neutron activation analysis (INAA), XRF, and LA-ICPMS.[345] Forensic discrimination of aluminum foil was achieved using both synchrotron radiation XRF and ICP-OES,[346] and the analysis of pharmaceutical packaging was reported for the determination of Ca and Pb isotopes with the use of LA-ICPMS with a multicollector as a detector.[347]

■ DRUGS AND POISONS

During 2009 and 2010, more than 600 papers and a number of books were publishing regarding drug and poison analysis in the field of forensic drug and toxicological analysis. Unfortunately, due to space constraints, only a selection of these articles will be highlighted below. In the last two decades, the analysis of illicit drugs has undergone considerable change. The traditional drugs still dominate; however, more than 100 psychotropic substances designed to bypass controlled substance legislation have appeared and led to intoxications and fatalities.[348] John Wiley and Sons are providing a current awareness service in each issue of the journal, *Drug Testing and Analysis*.[349] It is worth noting that conference proceedings are available for Society of Forensic Toxicologists (SOFT)[350,351] and the International Association of Forensic Toxicologists (TIAFT).[352] A book entitled *Forensic Chemistry of Substance Misuse: A Guide to Drug Control* has been published.[353] Several reviews pertinent to controlled substance and forensic toxicological analysis have been published,[354,355] including reviews covering specific areas such as oral fluid,[356] hair,[357] and forensic aspects of drug-facilitated crimes.[358]

Ethanol and Volatiles. A review of the experimental research and mathematical modeling which has evaluated the pulmonary exchange processes for the determination of ethyl alcohol has been published.[359] An evidence-based survey of the elimination rates of ethanol from blood with applications in forensic casework has been reported.[360] Proficiency testing as a basis for estimating uncertainty of measurement as applied to forensic alcohol and toxicology quantitations has been described.[361] Statistical modeling of measurement errors in the gas chromatographic analyses of blood alcohol content has been reported.[362] The standards and procedures for the implementation and use of approved instruments and screening devices of the Canadian society of forensic science alcohol test committee have been presented.[363] Ethanol, acetaldehyde, methanol, and acetone have been determined in blood, vitreous humor, and urine by gas chromatography/flame ionization detection (GC/FID) with direct injection, using a capillary column.[364] A fast, sensitive, and specific method for headspace blood alcohol analysis with deuterated internal standard was developed and validated.[365] A headspace GC/FID method for the determination of ethanol and methanol in blood, urine, and saliva has been published.[366] Age and gender differences in blood-alcohol concentration in apprehended drivers in relation to the amounts of alcohol consumed has been studied.[367] The relationship between blood and urine alcohol concentrations in apprehended drivers has been studied.[368] Selected-ion flow-tube mass spectrometry (SIFT-MS) has been evaluated for the analysis of different alcohols in aqueous solutions, including blood plasma, and in particular whether the osmolality or sample volume affected vaporization.[369] Horizontal attenuated total reflectance-Fourier transform IR spectroscopy (ATR-FT-IR) has been used to determine blood alcohol concentration.[370]

Ethyl glucuronide (EtG) was detected in urine by immunoassay and confirmed by LC/MS/MS,[371,372] in hair by GC/MS,[373−377] headspace solid-phase microextraction/GC/MS/MS (HS-SPME/GC/MS/MS),[378] hydrophilic interaction LC/MS/MS (HILC/MS/MS),[379,380] and GC/negative chemical ionization tandem mass spectrometry (GC/NCIMS/MS),[381] in serum by liquid chromatography/mass spectrometry (LC/MS),[382] in vitreous humor by LC/MS,[383] and in oral fluid by ultraperformance LC/MS (UHPLC/MS).[384] EtG and ethyl sulfate (EtS) has been studied in blood.[385−387] EtG in oral fluid and both EtG and EtS in blood and urine following intense use of mouthwash and ingestion of nonalcoholic wine has been studied.[388] The combined analysis of fatty acid ethyl esters (FAEE) and EtG in hair samples has been reported.[389,390]

Studies concerning the performance characteristics of the Intoxilyzer 8000C[391] and its response to volatile solvents in vitro[392] have been reported. A controlled human subject study was conducted to analyze the possible breath-sampling differences between the standard automatic technique and three manual techniques in the Alco-Sensor IV Black Dot Model.[393] The relationship between normal body temperature, end-expired breath temperature, and blood alcohol concentration/breath alcohol concentration ratio was analyzed using an Intoxilyzer 8000 specially equipped to measure breath temperature.[394] A noninvasive, in vivo spectroscopic study has been reported for measuring tissue alcohol using near-IR spectroscopy (NIRS).[395] Bioelectronic gas sensors (biosniffers) have been developed for convenient measurement of ethanol and acetaldehyde in expired gas after drinking.[396] The kinetics of ethanol decay in mouth- and nose-exhaled breath was measured online by selected ion flow tube mass spectrometry.[397] The effect of alcohol content in energy drinks on breath alcohol testing has been assessed by GC and breath testing.[398] The effect of water-soluble OC "pepper" spray on select infrared and electrochemical breath alcohol instruments has been reported.[399] The stability of aqueous alcohol simulator solutions were studied.[400] A literature review of studies on the dermal absorption of alcohol was conducted.[401] The Federal Institute for Materials Research and Testing (BAM), Germany, has issued a series of large volume ethanol in water certified ref materials (CRMs), primarily developed for the calibration of evidential breath alcohol analyzers in Germany.[402]

A fast and simple screening procedure using SPME-GC/MS in full-scan mode for the determination of volatile organic compounds in blood has been described.[403] Blood toluene concentrations in drivers under the influence have been reported.[404] The possible misidentification of ethanol by coelution of ethyl chloride by GC/FID has been discussed.[405] A method for quantitative analysis of acetylene in blood and urine samples was investigated using cryogenic headspace GC/MS.[406] Azide in human blood and plasma samples was derivatized with propionic anhydride and determined by GC/nitrogen−phosphorus detection (GC/NPD).[407] The stability of blood concentrations of a variety of illegal and medicinal drugs that are important for forensic analyses when spiked and stored in Vacutainer or Venosafe evacuated plastic collection tubes compared to Vacutainer evacuated glass tubes has been reported.[408] A new validated method for the quantitation of the abnormal phospholipid phosphatidylethanol (PEth), a biomarker for Ethanol uptake, was developed by LC-ESI-MS/MS following miniaturized organic solvent extraction and reversed-phase chromatography with phosphatidylbutanol (PBut) as internal standard.[409] The Guidelines for European workplace drug and alcohol testing in hair has been published.[410]

Cannabionoids. A review has been published covering conventional and alternative matrixes for detection of cannabis in subjects of driving under the influence.[411] DELTA^9-tetrahydrocannabinol (THC) and its major metabolites have been determined in blood by 2-dimensional GC/MS with cryofocusing,[412−415] by semiautomated solid-phase extraction (SPE) LC/MS/MS,[416] and using a novel fluorinated SPE sorbent and fast LC/MS/MS,[417] in urine by sweeping micellar electrokinetic chromatography (sweeping-MEKC),[418] by LC/MS/MS,[419,420] by automated online SPE combined with LC/MS/MS,[421,422] and by LC/MS/MS after stir-bar sorptive extraction and liquid desorption,[423] in saliva[424] by polymer monolith microextraction (PMME) combined with GC/MS,[425] in hair by LC/MS/MS[426] and by hollow fiber liquid-phase microextraction (HF-LPME) and GC/MS/MS,[427] and in oral fluid by GC/MS with automatic SPE[428] and by 2-dimensional GC/MS.[429] The addition of small amounts of nonionic surfactant to a solution of urinary THC metabolites is a simple method to improve both the accuracy and precision of analyte concentrations, as determined by GC/MS, in such solutions by mitigating adsorptive losses during storage and handling events.[430] A previously recommended method for detecting new cannabis use with creatinine-normalized 11-nor-9-carboxy-DELTA^9-tetrahydrocannabinol (THCCOOH) urine concentrations in periodically collected specimens for treatment and workplace and judicial drug testing applications has been refined by considering the time interval between urine collections.[431] 11-Nor-DELTA^9-tetrahydrocannabinol-9-carboxylic acid Et ester (THC-COOEt) was detected in plasma and hair using GC/MS after silylation with *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide and GC/NCI/MS as well as GC/NCI/MS/MS after derivatization with pentafluoropropionyl anhydride.[432] It has been shown that tetrahydrocannabinolic acid A (THCA A) is detectable not only in blood and urine of cannabis consumers but also in THC-positive hair samples.[433]

Potency trends of THC and other cannabinoids in confiscated cannabis preparations have been reported.[434] A high performance liquid chromatography diode-array detection (HPLC/DAD) method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material has been described.[435] The differentiation of fiber and drug type Cannabis seedlings by GC/MS and chemometric tools has been reported.[436] A review has been published that highlights the existing monitoring and legislation status of synthetic cannabinoids in "Spice" products.[437] A cannabimimetic indole has been identified by LC/MS/MS and GC/MS as a new adulterant in an herbal product.[438] Several alkylaminoindoles (alkylchain C_3 to C_7) and isolated CP 47,497-C8 from "Spice Gold" were characterized by NMR and mass spectrometry methods.[439] The active "Spice" ingredient JWH-018 has been quantified in serum by LC/MS/MS[440] and in urine by GC/MS and LC/MS/MS.[441] Several synthetic cannabinoids were detected in herbal products by GC/MS and LC/MS.[442]

Morphine and Related Narcotics. The ESI mass spectrometric fragmentation pathways of heroin-related alkaloids were comprehensively elucidated with the aid of high-resolution mass spectrometry.[443] The analysis of heroin has been performed by capillary electrophoresis-mass spectrometry (CE-MS).[444] Identification and quantification of the opiates morphine and thebaine has been achieved in three poppy cultivars using FT-IR-ATR spectroscopy.[445] Trace acidic and neutral heroin impurities were isolated from basic fractions using liquid−liquid extraction and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide derivatization followed by programmed-temperature vaporization GC/MS (PTV-GC/MS).[446] Opium alkaloids in papaver plants were identified by ultrasound-assisted extraction followed by cyclodextrin-modified CE.[447] The utility of universal and specific genetic markers for opium poppy identification has been assessed.[448] Innovative methodology to transfer conventional GC/MS heroin profiling to UHPLC-MS/MS has been described.[449]

A review has been published on sample preparation and separation techniques for bioanalysis of morphine and related substances.[450] Opiates were determined in plasma using LC/ESI-MS/MS,[451,452] using SPE and quadrupole linear ion-trap LC-MS,[453] and using mixed-mode solid-phase extraction and UPLC/MS/MS,[454] in urine by reversed-phase UPLC/MS/MS,[455] by liquid−liquid extraction and cation-selective exhaustive injection and sweeping micellar electrokinetic chromatography (CSEI-sweep-MEKC),[456] by liquid−liquid extraction and GC/MS,[457] hydrolyzed with β-glucuronidase followed by LC/MS/MS,[458] by CE with fluorescence detection,[459] and by SPE and HI-LC/MS/MS,[460] and in hair by a microwave extraction and HPLC/DAD.[461] A method for simultaneous screening and quantification was developed for opioid drugs in post-mortem blood and urine by LC/MS/MS[462] and post-mortem blood and brain by reversed-phase LC/MS/MS.[463] Morphine and metabolites have been determined in hair[464] using SPE and GC/MS[465] and SPME and GC/MS.[466] Oxymorphone was detected in blood by enzyme immunoassay and confirmed by GC/MS utilizing SPE.[467] Oxycodone was determined in vitreous humor and blood using EMIT screening and GC/MS.[468] A comparison of the validity of GC/MS and LC/MS/MS analysis of urine samples for morphine, codeine, 6-acetylmorphine, and benzoylecgonine has been reported.[469]

Cocaine. A single-quantum dot-based aptameric sensor that is capable of sensing the presence of cocaine through both signal-off and signal-on modes has been developed.[470] The application of Raman spectroscopy for the in situ detection of cocaine hydrochloride in clothing impregnated with the drug has been described.[471] The effect of UV irradiation on detection of cocaine hydrochloride and crack vapors by ion mobility increment spectrometry (IMIS) and API-MS methods has been studied.[472] The pyrolysis behavior of pure cocaine base as well as the influence of various additives was studied using conditions that are relevant to the smoking of illicit cocaine by humans.[473] The structure of the hydrated gold(III) tetrachloride salt of L-ecgonine has been determined by X-ray crystallography.[474] The possible role of levamisole in illicit cocaine preparations has been discussed.[475] Aptamers, which are artificial oligonucleotides selected in vitro, have been employed to design novel biosensors (i.e., aptasensors).[476,477]

Bioanalytical methods for the determination of cocaine and metabolites in human biological samples have been reviewed.[478,479] Cocaine and its metabolites have been detected in blood using an oligosorbent based on aptamers immobilized on a solid support.[480] A HPLC method coupled to spectrofluorimetric detection has been presented for the simultaneous analysis in dried blood spots (DBS) of cocaine and metabolites.[481] Microextraction by packed sorbent (MEPS) has been evaluated for fast screening of cocaine and its metabolites in urine using a time-of-flight (TOF) mass spectrometer as the detector with direct analysis in a real-time (DART) source.[482] Cocaine and its metabolites have been detected in urine using SPE coupled with IMS,[483] CE/ESI-MS,[484] and SPE and LC/MS/MS.[485] Cocaine and its metabolites have been detected in urine and neonatal meconium using GC/MS.[486] Cocaine and its metabolites have been detected in hair,[487−489] using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry,[490,491] using LC/MS/MS,[492] using HILIC-MS/MS,[493] and using enzyme-linked immunoassay (ELISA) with confirmation by LC/MS/MS.[494] Urinary excretion of ecgonine and five other cocaine metabolites have been studied following controlled oral, intravenous, intranasal, and smoked administration of cocaine.[495]

Amphetamines. A review summarized the technical improvements in the forensic analysis for amphetamine and metabolites.[496] Amphetamines and hallucinogenic phenylethylamines have been detected using GC with vapor-phase IR spectrophotometric detection (GC/IR),[497] GC/MS and GC/IR,[498−500] and GC./surface-assisted laser desorption ionization mass spectrometry (GC/SALDI-MS).[501] Enhancements have been made in sample collection for the detection of MDMA using a novel planar SPME (PSPME) device coupled to IMS.[502]

A novel approach to the analysis of ecstasy tablets by direct mass spectrometry coupled with thermal desorption (TD) and counter-flow introduction atmospheric pressure chemical ionization (CFI-APCI) has been described.[503] Profiling of trace components present in 3,4-methylenedioxymethylampethamine (MDMA) has been done using HS-SPME.[504]

High throughput chiral analysis of urinary amphetamines by GC/MS using a short narrow-bore capillary column has been reported.[505] Amphetamines have been detected in whole blood using liquid−liquid extraction and LC/MS/MS[506] and oral fluid and urine by GC/MS.[507] GC-ion trap mass spectrometry (GC-ITMS) was used for the simultaneous measurement of MDMA and its metabolites in plasma and urine.[508] Amphetamines have been detected in urine using in situ derivatization and extractive acylation with pentafluoropropionic anhydride followed by GC/MS,[509] with an ionic liquid-based solid-phase microextraction fiber and GC/MS,[510] MAE-GC/FID,[511] online SPE and LC/MS/MS,[512] and ^{1}H NMR spectroscopy.[513]

An orthogonal array design (OAD) was applied to optimize microwave-assisted derivatization (MAD) for analysis of trace amphetamine and methamphetamine by NCI GC/MS.[514] Methamphetamine was detected in human hair using a microchip-based ELISA system (microELISA) in combination with a micropulverized extraction method,[515] GC-EI-MS after derivatization with perfluorooctanoyl chloride,[516] GC/MS coupled with a micropulverized extraction, aqueous acetylation and MEPS (MiAMi-GC/MS),[517] LC/MS/MS,[518] and GC/MS after small-volume liquid extraction and microwave derivatization.[519]

Amphetamines were detected in fingernails by GC/MS.[520] The measurement uncertainties were estimated for the determination of methamphetamine and amphetamine in human hair according to the recommendations of the EURACHEM/CITAC Guide and "Guide to the expression of uncertainty in measurement (GUM)".[521] MDMA and its metabolites were determined in sweat by SPE and GC/MS.[522] Chemical analysis of four capsules containing the controlled substance analogues 4-methylmethcathinone, 2-fluoromethamphetamine, α-phthalimidopropiophenone, and *N*-ethylcathinone has been presented.[523] The utility of hydrogen stable isotope ratio measurement by IR-MS for establishing the origin of ephedrine and pseudoephedrine was evaluated.[524,525]

Benzodiazepines. A review has been presented, focusing on sample preparation (pretreatment and extraction) and HPLC conditions for the analysis of benzodiazepines (BZPs) in biological fluids.[526] Several methods for the determination of BZPs in biological fluids have been reported using liquid−liquid and SPEs followed by LC/MS/MS,[527] GC/MS,[528] LC/MS/MS,[529,530] LC-APPI-MS,[531] SPE and HPLC-DAD,[532] and MAE and HPLC-DAD,[533] liquid−liquid extraction followed by derivatization and GC/MS,[534] liquid−liquid extraction followed by UPLC/MS/MS,[535] and immunoassay followed by LC/MS/MS.[536] The preanalytical stability of frequently abused BZDs on an oral fluid collecting device has been investigated using LC/MS/MS.[537] BZDs have been determined in vitreous humor by SPE and HPLC-DAD.[538]

The use of fingerprints as an alternative biological matrix to test for the presence of drugs and/or their metabolites is a novel area of research in analytical toxicology. The detection and quantification of lorazepam and its 3-O-glucuronide in fingerprint deposits has been performed by LC-MS/MS.[539] The reductive transformation of oxazepam to nordiazepam has been reported during enzymatic hydrolysis.[540] A GC/MS method with a capillary column was developed for the simultaneous determination of underivatized BZPs and ketamine from drinks by extraction with chloroform/isopropanol 1:1 (v/v).[541]

δ-Hydroxybutyrate. A new screening method for detecting δ-hydroxybutyric acid (GHB) in drink matrixes using a DART ion source coupled to a time-of-flight mass spectrometer (TOFMS) was validated and compared with the current screening methodology.[542] GHB has been determined in serum and urine by headspace solid-phase dynamic extraction combined with GC-PCIMS[543] and in urine by GC/MS,[544] GC/MS after derivatization,[545] and LC/MS/MS.[546] GHB has been determined on dried whole blood samples using GC/MS.[547] GHB was determined in hair using liquid−liquid extraction followed by LC/MS/MS with APCI in the negative-ion mode, multiple reaction monitoring, and deuterated internal standard.[548] A method has been developed to determine the carbon isotopes content of δ-butyrolactone (GBL) by means of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS).[549] A colorimetric sensor array based on complexes of fluorescent dyes with organic capsules (cucurbiturils) has been designed for the detection of GHB.[550]

Miscellaneous Drugs and Procedures. A review of the literature from the past decade has been published with chromatographic and spectroscopic data for bioanalytical methods for the determination and quantification of club drugs including sample preparation techniques and validation data.[551] Fentanyl was identified in human blood and urine using SPE and ESI-LC/MS/MS[552] and in decomposed human tissue and bone tissue by liquid−liquid extraction and ELISA.[553] Fluoromethcathinone was identified by GC/MS and ^{1}H, ^{13}C, and ^{19}F NMR as well as FT-IR.[554] 4-Bromo-2,5-dimethoxybenzylpiperazine was identified by GC/MS, GC/MS/MS, two-dimensional NMR spectroscopy, UV spectroscopy, and LC/MS/MS using ESI.[555] A method describing the determination of cyanide in blood by HS-GC with electron capture detection (GC/ECD) was reported.[556] A simple, sensitive, and reproducible LC/MS/MS method has been developed and validated for the quantification of the antipsychotic drug olanzapine in whole blood using dibenzepine as internal standard.[557]

Carisoprodol and meprobamate were determined in whole blood using SPE and GC/MS with benzylcarbamate and meprobamate-d_7 as internal standards.[558] An UPLC-MS/MS method was validated for the confirmation and quantitation of LSD and structurally related compounds in blood and urine.[559] The differentiation of the regioisomeric 2-, 3-, and 4-trifluoromethylphenylpiperazines (TFMPP) by GC-IRD and GC/MS has been reported.[560]

A LCMS method was developed and validated for the simultaneous determination of buprenorphine (BUP) and metabolites in human umbilical cord.[561] TLC and GC/MS were used to analyze *Salvia divinorum* plant material for salvinorin A.[562] Tricyclic antidepressants were determined in human hair by microwave-assisted hydrolysis and microwave-assisted extraction (MAH-MAE) followed by HPLC-DAD.[563] HF-LPME coupled with HPLC was used to simultaneously determine Aconitum alkaloids.[564] An LC/MS method was developed for benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP), constituents of "party pills" or "legal herbal highs", and their metabolites in human blood plasma.[565] A basic liquid−liquid extraction followed by pentafluoropropionic anhydride (PFPA) derivatization and full scan (m/z 42−550) GC/MS was used to screen urine samples for 33 designer drugs.[566] A simple and sensitive procedure, using *p*-tolylpiperazine as internal standard, has been developed and validated for the qualitative and quantitative analysis of 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), and

1-(4-methoxyphenyl)piperazine (MeOPP) in hair.[567] A method has been developed and validated using LC/MS/MS for quantification of methadone and its main metabolite in developmental larvae.[568] A recent review provides an overview of analysis methodologies on detection and characterization of 40 *N,N*-dialkylated tryptamines.[569] A fast screening procedure for the determination ketamine and metabolites in urine samples has been developed that uses LC/MS/MS.[570] A method for the determination of xenobiotics in human specimens has been published that uses SPE with molecularly imprinted polymers (MIPs) before instrumental analysis.[571] In recent years, a new class of designer drugs has appeared on the drugs of abuse market in many countries, namely, the so-called beta-keto designer drugs such as mephedrone, butylone, and methylone. A study identified the metabolites of mephedrone in human urine using GC/MS.[572] Identification and quantitation of ketamine in biological matrixes has been performed using SPE and GC/MS.[573] Phencyclidine was determined in urine and plasma by liquid−liquid extraction and GC/MS with an internal standard.[574] A review has been published that discusses current bioanalytical techniques used for the verification of cyanide exposure, common problems associated with the analysis of cyanide and its biological breakdown products, and briefly addresses the metabolism and toxicokinetics of cyanide and its breakdown products in biological systems.[575]

BIOGRAPHIES

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