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Liquid Chromatography

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Forensic science is the application of the sciences to the court of law. Consequently, forensic science and the legal system are intimately intertwined. Results obtained from the examination and analysis of forensic samples and the forensic samples themselves comprise evidence of a crime. It is the individualization of the sample, i.e. the singular association between the samples(s) and an illegal act, that is unique to forensic science. Because of the legal consequences, results require a high degree of certainty and the techniques used must be admissible in court. The Daubert rule, a 1993 decision upheld by the US Supreme Court, assigns to the judge the role of determining admissibility of scientific evidence. Among the factors considered by the judge are: (i) whether the technique has been tested and subjected to peer review; (ii) whether error rates have been defined; (iii) whether standards controlling the operation of a technique exist; and (iv) whether the technique has been widely accepted in the scientific community. Techniques used in a forensic laboratory may be applied to the investigation of a wide variety of crimes. Some examples are illegal drug use, counterfeiting, arson, tampering, fraud, poisoning, terrorism and environmental crimes. It is this diversity of cases, variety of sample matrices and the staggering number of potential analytes that necessitate a continuous evaluation of the testing that is needed to constitute proof in each situation.

Analytical requests in a forensics laboratory may be classified into four categories: (i) screen for the presence or absence of known compounds or class of compounds; (ii) screen for unspecified analytes; (iii) perform a comparative analysis; and (iv) verify and/or quantify substances in a sample. Many qualitative tests are used in forensic laboratories for their speed and ability to identify unknowns. In some cases qualitative results (identity) suffice, while in others quantification is important. Like gas chromatography (GC), high performance liquid chromatography (HPLC) can be used for both qualitative and quantitative analyses. However, 80–85% of all known compounds are not amenable to GC. The stationary and mobile-phase combinations available and the many detection modes possible make HPLC a universal separation scheme. Unlike GC, it is not limited by the volatility or thermal stability of an analyte. HPLC can analyse solutes encompassing a wide molecular weight range, from monatomic species to proteins. A range of solute hydrophobicities and polarities can be accommodated, from acidic and basic species that incorporate many drugs of abuse, pharmaceuticals, dyes and food colourings, to neutral and/or hydrophobic molecules such as pesticides and herbicides, hydrocarbons in petroleum products and carotenoids in foods. In situations where GC can determine certain compounds more readily with greater selectivity, resolution and sensitivity, HPLC offers a secondary, confirmatory method. In other cases, the use of HPLC avoids sample derivatization required by GC, and eliminates steps that could contribute to sample loss and increase analysis time. Another advance in HPLC in recent years involves the introduction of narrowbore (2.1 mm inner diameter) and microbore (1 mm inner diameter) columns. These smaller columns decrease the sample size required for injection and increase mass detection sensitivity versus the typical 4.6 mm inner diameter analytical columns.

Thin-layer chromatography (TLC) is also utilized in forensic chemistry, particularly for sample screening. Although TLC permits analysis of many samples at one time, facilitating the side-by-side comparison of suspect and authentic samples, it can suffer from a lack of resolution and from difficulties in both quantification and isolation of an individual component. Immunoassays are generally more sensitive; however, they may provide class-only determinations, may be prone to interferences and may not be available for classes such as neuroleptics and β-blockers. HPLC is useful for sample screening, comparison and quantification, and fraction collection for further analysis is more straightforward.

The UV-visible detector, and more recently the diode array detector (DAD), are the most commonly used detectors for HPLC analyses. As an alternative, one may utilize more specific devices such as fluorescence, electrochemical, chiral or mass spectrometric detectors. Because these detectors take advantage of specific molecular characteristics of the analyte(s) of interest, they are less susceptible to background interferences from sample matrix, and tend to be more sensitive. The choice of a particular detector and method depends upon the requirements of the case in hand – whether the sample is being screened for unknowns, analysed for a particular compound, compared against another sample, or quantified.

Sample Preparation

Each manipulation of the forensic sample may irreversibly alter the evidence and introduces the possibility of incomplete analyte recovery and inadvertent contamination. Therefore, sample preparation requires careful consideration and always follows a preliminary visual, and perhaps microscopic, examination. Sample preparation steps may also affect the form of the analyte, which is problematic for speciation work and subsequent toxicological evaluation. Additional complications in forensic work are the variety of sample matrices (drugs, body fluids, food, soils, etc.) and limited sample sizes (arson residues, traces of blood in a syringe, a spot on blotter paper, etc.) frequently encountered. Ideally, a portion of the sample should be reserved in case of trial to allow independent analysis. If appropriate, the sample can be homogenized. However, portions may need to be analysed separately to characterize the sample accurately. Individual samplings may also be advisable when there are visual differences within portions of a sample. Sampling in the vicinity of a visual contaminant reduces dilution with the matrix and improves detection limits.

Analytes must be in solution for determination by HPLC. Sample preparation is necessary to remove compounds such as proteins that might damage an HPLC column, as well as compounds that interfere with an analysis. Liquid–liquid extractions (LLE) are commonly used, and can be manipulated by choice of solvent, addition of salts (salting-out effect) and control of pH. For biological matrices, extractions using chloroform/2-propanol/*n*-heptane under alkaline conditions provide clean extracts with good recoveries of basic and neutral compounds. However, acidic compounds such as barbiturates and salicylates are poorly recovered (20–50%). LLE is not easily automated and can require large volumes of solvent. Solid-phase extraction (SPE) cartridges are now widely used in toxicology screens, mainly for low viscosity samples such as urine or serum. SPE has the advantages of higher efficiencies and selectivity, lower solvent volume requirements, absence of emulsions, and automation options. However, the packing materials can be irreproducible, even within batches of the same brand, resulting in variable recoveries and poor analytical reproducibility. The use of an internal standard is highly recommended for quantitative results. SPE cartridges should not be re-used due to decreased extraction performance and increased possibility of the introduction of contaminants.

General Unknowns

Screening for unknowns is a very challenging task due to the vast number of potential contaminants. Screening methods in a forensic laboratory are designed to detect the most relevant drugs and potentially hazardous chemicals. Often, screens are performed in response to a crisis such as an acute poisoning and as such require rapid response. While immunoassay techniques and TLC remain invaluable for initial screening, these methods must be supplemented by HPLC-DAD for those analytes for which the initial screen does not offer sufficient selectivity or sensitivity. Identification of a compound by HPLC-DAD is based on retention time match and spectral match, as shown in **Figure 1**.

Additionally, plotting the ratios of absorbance measurements obtained during a chromatographic analysis, taken at well-separated and characteristic wavelengths, permits evaluation of interferences and a more confident identification. For example, variation in the ratio across a peak indicates co-elution, as seen in **Figure 2**.

Since forensic screens for unknowns are often performed in biological matrices, which are inherently variable, the analysis of blanks is an additional safeguard against false positives that could be caused by

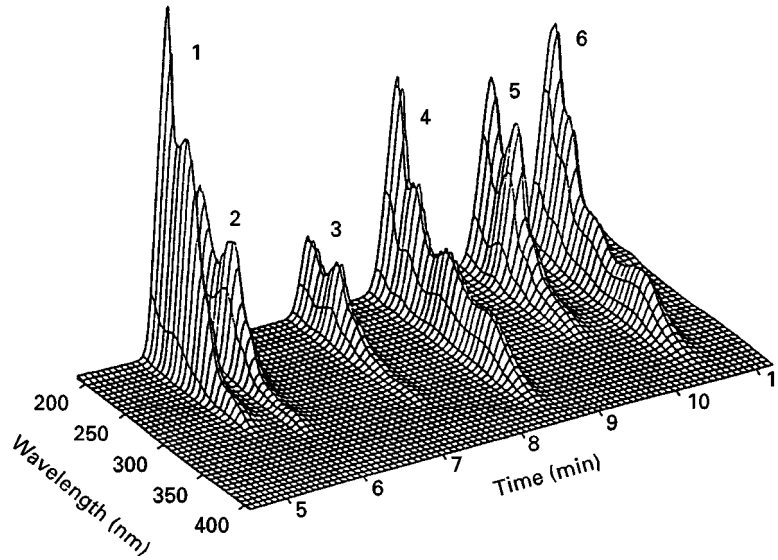

Figure 1 Spectrochromatogram showing spectral and chromatographic data for the separation of six benzodiazepines. Solutes: 1, midazolam; 2, flurazepam; 3, oxazepam; 4, nitrazepam; 5, alprazolam; 6, clonazepam. Column, 25 cm Lichrosorb RP-8; mobile phase, 35% CH_3CN in 0.05 mol L^{-1} KH_2PO_4–H_3PO_4 buffer, pH 3; flow rate, 1.5 mL min^{-1}; spectra collected between 190 and 400 nm. Reproduced from Logan (1994), with permission from Elsevier Science.

the effect of poisons on the matrix or due to putrefaction processes. Blanks should include reagents, and matrix that is contaminant-free. Screening methods also need to detect indirect indicators of the compound of interest. A screen of biological fluids should include major drug/poison metabolites such as benzoylecgonine, the primary indicator of cocaine use, which is found in urine. The addition of bleach to carbonated beverages leads to the formation of chlorate, chloride and sometimes chloroform.

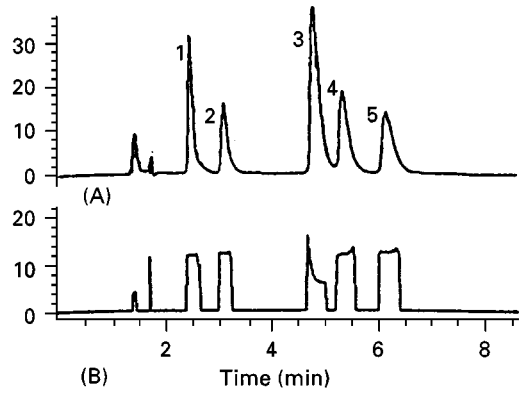

Figure 2 Use of wavelength ratioing to indicate peak impurity in the analysis of tricyclic antidepressants and metabolites by LC-photodiode array detection. (A) Chromatogram at 252 nm; (B) ratiogram 252 nm/230 nm. Asymmetric ratio of peak 3 indicates inhomogeneity and co-elution. Solutes: 1, 10-hydroxynortriptyline; 2, 10-hydroxyamitriptyline; 3, protryptyline/imipramine (co-elution); 4, nortriptyline; 5, amitriptyline. Column, 25 cm Lichrospher RP-8 (CH100); mobile phase, 40% CH_3CN in 0.05 mol L^{-1} phosphate buffer, pH 3; flow rate, 2.0 mL min^{-1}. Reproduced from Logan (1994), with permission from Elsevier Science.

Statistical toxicological analysis (STA), a general screening method for toxins in biological matrices as described by Tracqui *et al.*, often utilizes HPLC-DAD. Many laboratories have had success in creating their own databases and/or using multicomponent analysis for the identification of hundreds of substances from several classes in one run. Widespread use of HPLC-DAD for STA requires libraries that can be shared among laboratories. Unfortunately, libraries are not as common for HPLC as they are in GC work. Because the mobile phase in HPLC interacts much more strongly with analytes than the carrier gas in GC, small deviations in chromatographic conditions such as column type and batch, mobile-phase composition and pH, temperature and flow rate may affect retention time. The use of retention indices is necessary to minimize interlaboratory differences. Bogusz *et al.* proposed the use of a 1-nitroalkane index scale for toxicological screens since the C_1 to C_6 homologues are commercially available and have high UV absorbance between 200 and 220 nm. The retention times of 1-nitroalkanes are not affected by pH changes between 3.2 and 8.5, but are affected by changes in acetonitrile concentration. Because compounds are affected differently by changes in chromatographic conditions, the use of selected drugs as retention markers to correct retention indices improves accuracy and precision. One toxicological screening library that includes 900 substances is available commercially. To improve the possibility of obtaining a match, it is important to use the same chromatographic conditions as the library.

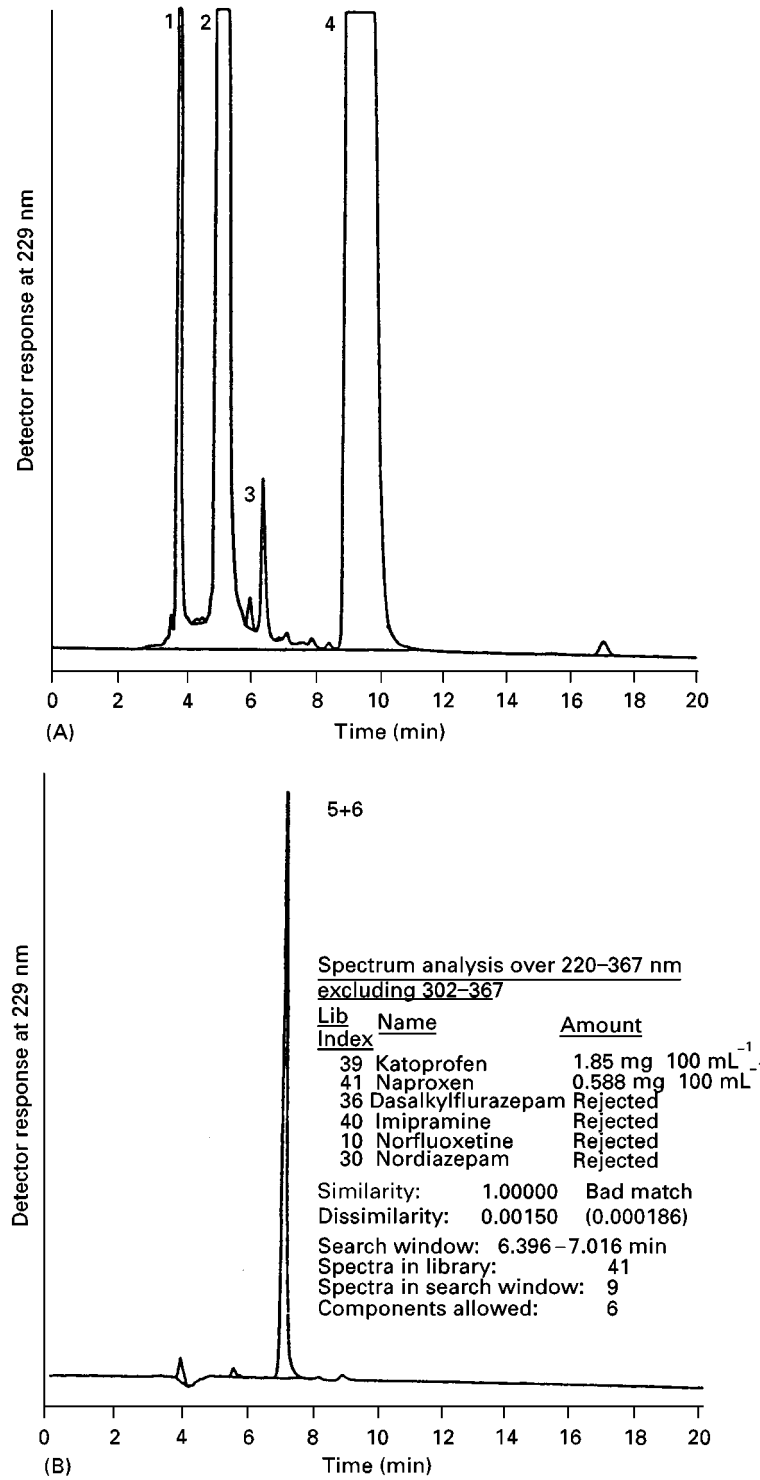

Figure 3 Chromatograms obtained from extracts of two gastric contents (A and B). Solutes; 1, zopiclone, 2, mesoridazine; 3, perphenazine; 4, thioridazine; 5 and 6, co-elution of ketoprofen and naproxen. The spectral library was developed over the wavelength range of 210–367 nm. Solute identification utilized a retention time window of $\pm$ 5% and a peak purity parameter of $\pm$ 1 nm. Columns: 250 $\times$ 4.6 mm i.d. Supelcosil LC-DP (diphenyl) and 250 $\times$ 4.0 mm. i.d. LiChrospher 100 RP-8; mobile phase, isocratic CH_3CN–0.025% (v/v) H_3PO_4–TEA buffer, pH 3.4; flow rate, 0.6 mL min^{-1}; detection, 229 nm. Reproduced from Koves (1995) with permission from Elsevier Science.

Figure 3 is an illustration of the process used to identify substances found in gastric contents. The search window needs to be set wide (20%), and peak purity should be considered. Consequently, a library search may yield as many as 10 possible matches. Spectra should be compared between a sample and

a standard run in-house for a positive identification. Even in the absence of a positive identification, the diode array spectrum can give class indications. The analysis can then be pursued by modifying the HPLC conditions or by utilizing another technique.

Analysis for a Known Analyte or Analyte Class

In contrast to screening for unknowns, sample preparation and separations can be optimized for the analysis of a known analyte or class of analytes. In order to avoid interferences and to maximize analyte recovery from the sample matrix, experimental conditions can be tailored for the class of compounds of interest. It is often necessary to determine components such as diluents, excipients, metabolites and synthesis or degradation products. **Table 1** lists examples of compounds that are analysed by HPLC in forensic laboratories. HPLC may be not be the primary method for all of these analytes but may instead be the confirmatory technique.

Analysis of drugs of abuse constitutes a major portion of forensic work. While UV may lack the necessary sensitivity, electrochemical detection offers sensitivity and selectivity for compounds such as morphine, benzodiazepines, cannabinoids, hallucinogens, fentanyl and some cyclic antidepressants. Additional compounds can be analysed using post-column photolytic derivatization followed by electrochemical detection. Sample preparation may be minimized depending on detector selection. For example, morphine can be analysed directly in poppy seed extract with electrochemical detection because it is easily oxidized at low potentials, unlike most opium alkaloids from natural products. **Figure 4** compares the sensitivity and selectivity obtained with UV, fluorescence and electrochemical detection of various alkaloids.

Table 1 Types of analytes determined by HPLC in forensic laboratories

Analgesics
Anticonvulsants
Antidiabetic drugs
Creatinine
Digitalis glycosides
Drugs of abuse (barbiturates, benzodiazepines, cannabinoids, cocaine and related compounds, LSD, opium alkaloids)
Dyes and colourings (natural and synthetic)
Ergot alkaloids
Explosives, propellants, stabilizers
Hydrocarbons (petroleum distillates, engine oils, greases)
Inks
Inorganic and organic anions (fluoride, chloride, phosphate, sulfate, azide, citrate)
Inorganic cations (sodium, potassium, calcium, ammonium)
Pesticides, herbicides, rodenticides
Pharmaceuticals
Phenothiazines
Plastics, plasticizers, polymers
Proteins
Sugars
Tricyclic antidepressants

HPLC separations can resolve lysergic acid diethylamide (LSD) from ergot alkaloids. Because LSD is typically ingested in small amounts, fluorescence detection is commonly used for its sensitivity and selectivity. Analysis of opium alkaloids by HPLC must also separate caffeine, quinine and strychnine – common additives or diluents.

Although there are more than 2000 known steroids, only a portion are controlled substances. Analysis of steroids is required in a variety of matrices, including dosage forms, oils, body fluids and tissues. Spot tests are useful for the rapid initial verification of the presence of steroids. A more specific identification is possible by either GC or HPLC with similar resolving power but different co-eluting pairs. HPLC-DAD adds the potential of distinguishing between some steroids based on differences in UV spectra.

In drug abuse cases, creatinine is analysed using ion pair reversed-phase separation and UV detection at 220 nm to determine if urine samples have been diluted. The HPLC method suffers from fewer interferences than other methods. HPLC reversed-phase or ion exchange separations of proteins for blood grouping or species identification are rapid and efficient.

Pesticides, herbicides and rodenticides may be determined by HPLC in poisoning cases. Warfarin and its metabolites have been identified in matrices such as urine and food. Carbamates are more readily analysed by HPLC with post-column derivatization than by GC because they are thermally labile.

Explosives are difficult to analyse by GC due to their thermal instability. HPLC is used for the analysis of nitroglycerin, propellants, stabilizers, plasticizers and weapon discharge residues. Inorganic explosives and explosive residues can be determined with ion chromatography. Explosives such as ammonium nitrate and residues such as chloride, chlorate, sulfide and sulfate can be determined by anion exchange with UV or conductivity detection, as illustrated in **Figure 5**.

Separations in ion chromatography (IC) are based on ion exchange, ion exclusion and reversed-phase adsorption. The use of a suppressor column to reduce the mobile-phase background chemically and increase the analyte signal permits conductivity detection of inorganic ions. There are also methods known

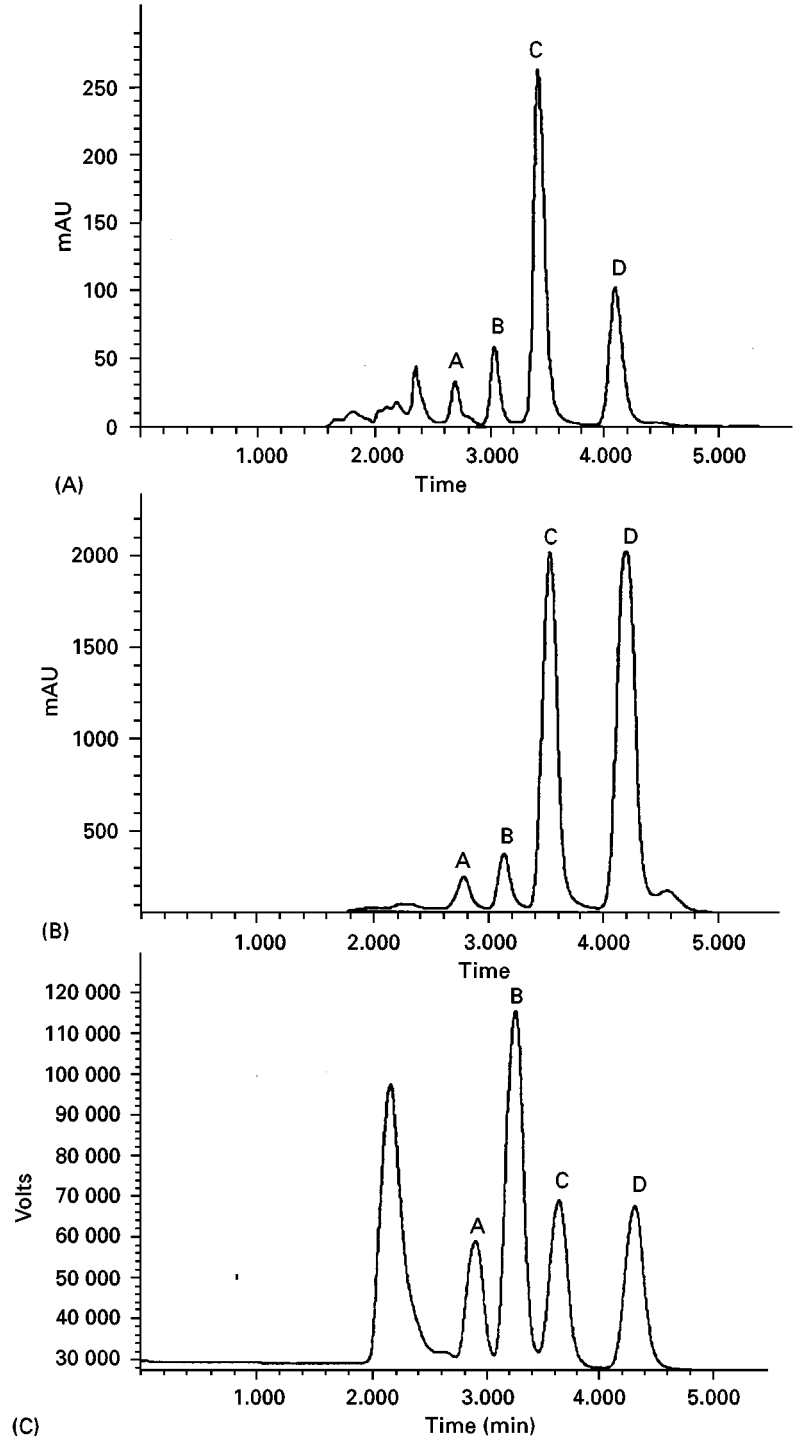

Figure 4 Comparison of detector signals. (A) UV; (B) fluorescence; (C) electrochemical. Chromatograms of orange juice samples spiked with: A, morphine; B, codeine; C, eserine; D, apomorphine. Column, 250 × 4.6 mm i.d. Interaction chemicals C_{18}; mobile phase, 55% methanol, 15 mmol L^{-1} KH_2PO_4, 3.75 mmol L^{-1} 1-octanesulfonic acid, 7.5 mmol L^{-1} KCl, adjusted to pH 4.00 with 10% H_3PO_4; flow rate, 1.0 mL min^{-1}; 30°C. Detection: UV, 254 nm; fluorescence, $\lambda_{ex} = 254$ nm, $\lambda_{em} = 408$ nm; electrochemical, 1.2 V vs. Ag/AgCl reference electrode with a glassy carbon working electrode. Reproduced from Lin (1993) with permission from Elsevier Science.

as single column which do not utilize a suppressor, but instead utilize low capacity columns and low ionic strength, low conductance mobile phases. Although conductivity detectors are the most commonly used, a variety of other detectors are available: UV-visible (direct or following post-column

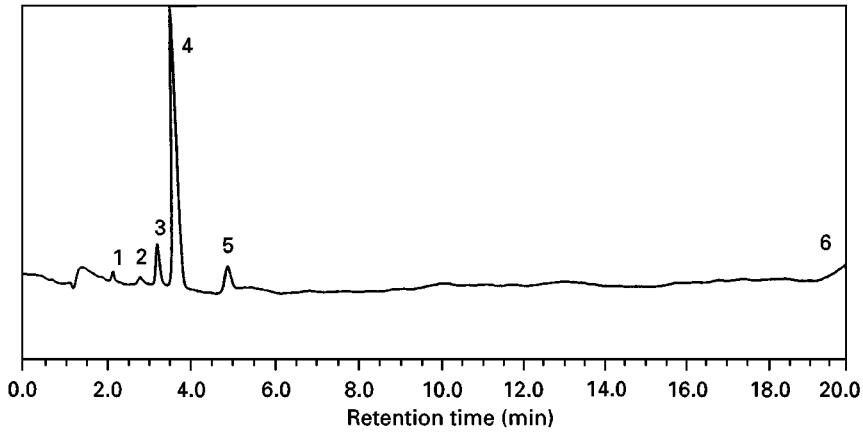

Figure 5 Analysis of residue taken from a black powder pipe bomb using ion chromatography. Solutes: 1, chloride; 2, nitrite; 3, nitrate; 4, sulfate; 5, sulfide; 6, hydrogen carbonate. Column, Vydac 302IC4.6; mobile phase 0.75 g isophthalic acid in 3 L H_2O, adjusted to pH 4.6 with 2 mol L^{-1} KOH; flow rate, 2.5 mL min^{-1}; detection, 280 nm. Adapted from Hargadon and McCord (1992) with permission from Elsevier Science.

reactions), amperometry, fluorescence and atomic spectroscopy. IC analysis of strong acids and alkalis can be important in poisoning cases. Speciation by IC of compounds such as arsenic may be important due to the higher toxicity of inorganic anions compared to the methylated forms. IC can also be used to determine counterions of drugs, as well as cleaning products and their components.

Comparison of Samples

HPLC analysis may be required to compare samples, either to differentiate one from another or to trace the source of a sample. Although retention times can be used for tentative identification of specific compounds, it is not always necessary to identify every component in the sample. Often, pattern recognition will suffice when comparing the content of a class of compounds from one sample to the next. IC has been used in the analysis of sugars present in suspect infant formulas, as seen in **Figure 6**. HPLC with refractive index detection can be used to analyse the types and amounts of sugars found as diluents in illicit street drugs, possibly linking seized evidence from several cases. **Figure 7** demonstrates an excellent separation of cannabinoids and their metabolites. Comparative analysis of cannabinoids in cannabis samples by HPLC has been shown to connect suppliers with customers.

Through the knowledge of peak area ratios among carotenoids that occur naturally in orange juice, a suspect orange juice can be analysed to determine whether carotenoids have been added to enhance the colour. Dyes extracted from fibres gathered at a crime scene and from fibres from a suspect could be profiled and compared. The profile of the subunits of haemoglobin present in a blood drop permits its source identification as human adult, human neonatal or animal.

When comparing chromatograms, the presence of extra peaks in a profile may suggest the deliberate addition of a foreign substance, or simply sample

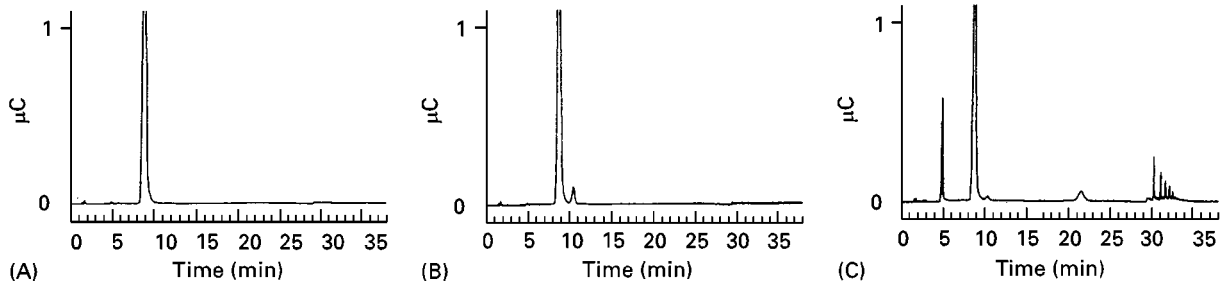

Figure 6 Comparison of sugar profiles using IC; (A) a known formula and (B,C) suspect infant formulas labelled as the known. Column, 4 × 250 mm Dionex Carbopac PA-1; mobile phase, 150 mmol L^{-1} NaOH in 0–600 mmol L^{-1} sodium acetate; flow rate, 1.0 mL min^{-1}; detection, pulsed electrochemical detection with a gold working electrode and a pH/Ag/AgCl reference electrode. Adapted from Kaine and Wolnik (1998) with permission from Elsevier Science.

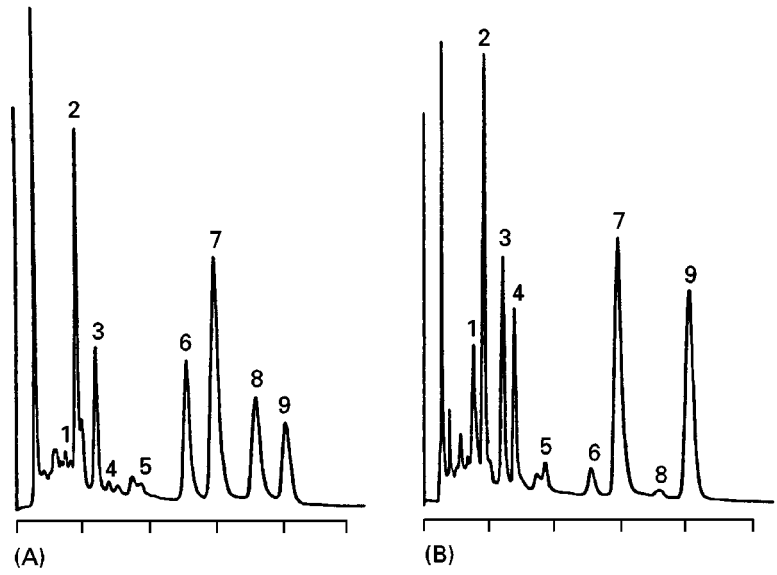

Figure 7 HPLC of cannabis resin at (A) 254 nm and 26°C, (B) 220 nm and 26°C. Solutes: 1, cannabidiol and cannabigerol (shoulder); 2, cannabidiolic acid; 3, cannabinol and cannabigerolic acid; 4, tetrahydrocannabinol; 5, cannabichromene; 6, cannabinolic acid; 7, tetrahydrocannabinolic acid; 8, cannabichromenic acid; 9, di-*n*-octyl phthalate (internal standard). Chromatographic conditions: 100 mg resin extracted with 1 mL chloroform–methanol (1:9) containing 8 g L^{-1} di-*n*-octyl phthalate; 2 μL extract injected. Column, 250 × 4.9 mm Partisil 5 C_{18}; mobile phase, 80% methanol–20% 0.02 mol L^{-1} H_2SO_4; flow rate, 2 mL min^{-1}. Reproduced from Smith and Vaughan (1976) with permission from Elsevier Science.

degradation. The lack of expected peaks may suggest that the sample is not what it claims to be. However, because many species absorb at lower wavelengths, the mobile-phase composition may affect the outcome of an analysis. The molar absorptivity of a compound may change with small changes in the mobile-phase composition, affecting quantification. A high background absorbance from the mobile phase may obscure species that are present in the sample at low concentrations. This could lead to the erroneous conclusion that there are no apparent differences between suspect and authentic samples.

Whenever the analysis of samples requires a long period of time, e.g. for a large number of samples, the variation of retention times due to chromatographic factors must be considered. An internal standard may be added to samples, or a control sample may be analysed with each set of suspect samples. For comparative analysis, there is added importance to representative sampling and replicate analyses. It is easier to state that samples are different, rather than identical. Results of comparative analyses indicating two samples appear the same might be worded as 'the samples were analytically indistinguishable using techniques x, y, z'. In some cases, the individualization of a sample can be quantified. For example, a statistical probability of a blood sample matching a specific person can be based upon the analysis of blood group and Rh antigens if the distribution of these factors among the population is known.

Verification of Analyte Identity

A high degree of certainty is required for the identification of solutes in forensic cases in order to defend the work in court. An orthogonal technique may be used for confirmation, and the use of selective detectors aids in the certainty of identification. In HPLC, diode array detectors add the ability to match spectra, while fluorescence and electrochemical detectors are more specific. Both the excitation and emission wavelengths can be selected for fluorescence detection. The oxidation potential can be adjusted to reduce interferences of some analytes determined electrochemically.

GC-mass spectrometry (GC-MS) and GC-MS-MS are widely used in forensic analysis to verify analyte identification, particularly in cases concerning illegal drugs and drugs of abuse. The retention time identifies an analyte, and the mass spectrum serves as confirmation. Until recently, the argument against the use of HPLC as the primary method in forensic analysis was the difficulty of solute confirmation. Tremendous improvements in instrumentation and interface designs have made the coupling of HPLC to a mass spectrometer straightforward, particularly with the

recent introduction of relatively inexpensive benchtop models.

The ionization methods that are available permit the analysis of a wide variety of compounds. Particle beam is effective for moderately polar compounds (certain steroids and rodenticides), operates more efficiently with narrow-bore columns, and causes fragmentation during the ionization process. Both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are amenable to gradient elution methods, thereby permitting their use in the analysis of a group of compounds. APCI is compatible with conventional columns and is used primarily to determine moderately polar analytes that are not thermally labile (clenbuterol, basic pharmaceuticals). Polar (conjugated oestrogens, proteins, peptides) and/or thermally fragile molecules (glucuronide metabolites of morphine and codeine) are analysed more effectively by ESI. ESI requires narrow- and microbore columns. In any of these methods, quantification can be performed in either the full scan mode or in the selected ion monitoring (SIM) mode. SIM offers greater sensitivity (10–100 ×), comparable to that obtained with GC-MS.

The utilization of MS-MS provides even greater specificity, further decreasing the chance of the incorrect identification of an analyte. Even if two compounds co-elute and have the same $[M + H]^+$ ion exiting the first quadrupole, it is unlikely that they would produce the same fragmentation pattern in the third quadrupole. The technique called selected reaction monitoring utilizes the known losses that occur during fragmentation of an analyte or a particular group of analytes. The method is useful when coelution of two or more compounds is suspected. Another benefit to this method is that its sensitivity is typically 10–100 times greater than that obtained in the full scan mode.

Conclusions

Numerous aspects of separation science are applicable to forensic science. Because HPLC is so versatile and can be used to determine so many different compounds, the technique is particularly well suited to the demands of a forensic laboratory. Both qualitative and quantitative information can be obtained, often with minimal sample preparation. Because only small volumes are needed for analysis, sample consumption can be minimized. Eluting fractions can be collected for further analysis – an important consideration when dealing with trace evidence. HPLC offers a cost-effective technique with the ruggedness and reliability necessary for forensic testing and consequently is widely used in forensic laboratories today.

See also: **III / Carbamate Insecticides in Foodstuff: Chromatography & Immunoassay. Clinical Diagnosis: Chromatography. Explosives:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Forensic Toxicology: Thin-Layer (Planar) Chromatography. Heroin: Liquid Chromatography and Capillary Electrophoresis. Toxicological Analysis: Liquid Chromatography. Steroids:** Gas Chromatography; Liquid Chromatography and Thin-Layer (Planar) Chromatography.

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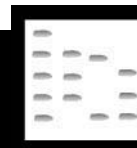
FORENSIC TOXICOLOGY: THIN-LAYER (PLANAR) CHROMATOGRAPHY



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The selection of appropriate analytical methodology for forensic toxicological investigations depends on the scope of the laboratory. Postmortem forensic toxicology investigates the cause of death, and consequently a very broad-range screening is needed to detect all potential poisons. In traffic toxicology, only such substances are relevant which may impair the driver's ability to control the vehicle. Doping control focuses on those substances that have been banned by the International Olympic Committee. Prisoners and rehabilitation clinic patients are tested for psychotropic drugs, whereas the US Mandatory Guidelines for Federal Workplace Drug Testing Programs are limited to the major drugs of abuse, cannabis, cocaine, amphetamine, opiates and phencyclidine.

Thin-layer chromatography (TLC) has found extensive use in forensic toxicology since the early 1960s when the famous book of Stahl made the technique well known. The first edition of the classic laboratory manual by AS Curry, *Poison Detection in Human Organs* from 1963 (Charles C. Thomas, Springfield, IL), still relies on paper chromatography but the second edition in 1969 utilizes TLC as a major technique for drugs. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) in the 1970s, and especially high performance liquid chromatography (HPLC) in the 1980s gradually began to replace TLC but today the planar technique is having a renaissance due to the progress in instrumentation and software. From 1990 to 1996, 26% of published TLC applications were in the field of medical, clinical and biological analysis, which also includes forensic toxicology.

The commonly recognized advantages of classical manual TLC are high throughput, low cost, easy sample preparation and versatile visual detection possibilities. Instrumental TLC extends the scope to reproducible quantitative analysis and allows the utilization of *in situ* UV spectral information for identification. The main disadvantage of TLC is low chromatographic resolution, which can be partly overcome by instrumental techniques. Another disadvantage is that quantitative calibration curves are not reproducible enough to be stored, making it necessary to co-analyse several standards along with samples on each TLC plate. Most of the substances frequently encountered in forensic toxicology can be readily analysed by TLC. These include therapeutic drugs, drugs of abuse, pesticides and naturally occurring alkaloids, which are all relatively small molecular weight organic compounds with functional groups amenable to visualization by colour reactions.

It is practical to divide the discussion of TLC in forensic toxicology into two categories, the broad-scale screening analysis and target analysis. The former approach is related to the concepts of systematic toxicological analysis or general unknown, i.e. the search for a rational qualitative analysis strategy for hundreds of potential poisons. TLC drug screening is often performed in urine or liver, where the drug concentrations are higher than in the blood. In target analysis, the aim is specifically to detect and often also to quantify a substance or a limited number of substances.

Broad-scale Screening Analysis

Chromatographic Systems

Evaluation of systems The rational selection of TLC systems for screening analysis differs from the optimization of the separation of a few-component