Review Article

RECENT APPLICATIONS OF HYPHENATED LIQUID CHROMATOGRAPHY TECHNIQUES IN FORENSIC TOXICOLOGY: A REVIEW

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ABSTRACT

Hyphenated techniques combine chromatographic and spectral methods to exploit the advantage of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra. The term forensic science covers those professions which are involved in the application of the social and physical sciences to the criminal justice system. Forensic experts are required to explain the smallest details of the methods use, to substantiate the choice of the applied techniques and to give their unbiased conclusions. All under the critical and often mistrustful gaze of the servants of the justice, as well as the general public and the media. Recent years have seen the development of powerful technologies that have provided forensic scientists with new analytical capabilities, unimaginable only a few years ago. The aim of this article is to present an overview of some of the most recent applications of hyphenated liquid chromatography to forensic analysis. Focuses on trace analysis (including chemical warfare agents, explosives and dyes Drugs of abuse in alternative matrices, trace chemicals, systemic toxicological analysis, doping agent and related compounds, therapeutic drugs of toxicological relevant, environmental poisons, Natural toxins). It is not the intention to provide an exhaustive review of the literature but rather to provide the reader with a 'flavour' of the versatility and utility of the technique within the forensic sciences.

Keywords: Hyphenated chromatography, Forensic toxicology, Analysis.

INTRODUCTION

Hyphenated techniques combine chromatographic and spectral methods to exploit the advantage of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture.[1] Spectroscopy produces selective information for identification using standards or library spectra. The term "forensic science" covers those professions which are involved in the application of the social and physical sciences to the criminal justice system. Forensic experts are required to explain the smallest details of the methods used, to substantiate the choice of the applied technique and to give their unbiased conclusions. All under the critical and often mistrustful gaze of the servants of the justice, as well as the general public and the media.[2] The final result of the work of the forensic scientist exerts a direct influence on the fate of a given individual. This burden is a most important stimulus, and one which determines the way of thinking and acting in forensic sciences. Consequently, the methods applied in forensic laboratories should assure a very high level of reliability and must be subjected to extensive quality assurance and rigid quality control programs. The legal system is based on the belief that the legal process results in justice. This has come under some question in recent years. He or she can, however, contribute to restoring faith in the judicial processes by using science and technology in the search for facts in civil, criminal and regulatory matters. The purpose of this article is to review some of the most recent applications of hyphenated liquid chromatography to forensic analysis with special focus on the following; trace analysis, the use of alternative specimens for monitoring drugs of abuse, systematic toxicological analysis and high-throughput analysis. The aim of this article is to present an overview of some of the most recent applications of hyphenated liquid chromatography to forensic analysis.

History

A couple of decades ago, Hirschfield introduce the tern "hyphenation" to refer to the on-line combination of separation technique and one or more spectroscopic detection technique. This technique developed from a marriage of a separation technique and a spectroscopic detection technique, is nowadays known as hyphenated technique. In recent years, hyphenated techniques have received ever-increasing attention as the principal means to solve complex analytical problems.[1]

AVAILABLE HYPHENATED TECHNIQUES

Double hyphenated techniques

- LC-MS
- LC-NMR
- LC-IR
- HPLC-DAD
- GC-MS
- GC-IR
- GC-FTIR
- CE-MS

Triple hyphenated techniques

- LC-API-MS
- APCI-MS-MS
- LC-ESI-MS-MS
- LC-ESI-MS
- ESI-MS-MS
- LC –MS-TSPLC-UV-NMR-MS
- LC-UV-NMR-MS-ESI
- LC-NMR-MS
- LC-DAD-API-MS
- LC –DAD-MS
- LC-PDA-MS
- LC-PDA-NMR-MS
- SPE-LC-MS
- LVI-GC-MS

GC-MS

GC-MS, which is a hyphenated technique developed from the coupling of GC and MS. Mass spectra obtained by this hyphenated technique offer more structural information based on the interpretation of fragmentation. The fragment ions with different relative abundances can be compared with library spectra. Compounds that are adequately volatile, small, and stable in high temperature in GC conditions can be easily analyzed by GC-MS. In GC-MS a sample is injected into the port of GC device vaporized, separated in the GC column, analyzed by MS detector and recorded.

LC-IR

The hyphenated technique developed from the coupling of an LC and the detection method infrared spectroscopy (IR) or (FTIR) is known as LC-IR or HPLC–IR. A useful spectroscopic technique for the identification of organic compound, because in the mid-IR region the structures of organic compounds have many absorption band that are characteristic of particular functionalities eg.-OH, -COOH.

LC-MS

LC-MS or HPLC-MS refers to the coupling of an LC with a mass spectral data. The separated sample emerging from the column can be identified on the basis of its mass spectral data. An LC-Ms combines the chemical separating power of LC with the ability of an MS to selectively detect and confirm molecular identity.

CE-MS

MS detector linked to a CE system for acquiring on-line MS data of the separated compound, the resulting combination is termed as CE-MS. CE analysis is driven by an electrical filed, performed in narrow tubes, and can result in the rapid separation of many hundreds of different compounds .Separation is achieved through channels etched on the surface of the capillary connected to an external high voltage power supply that delivers sample to ESIMS.

LC-NMR

Technological developments have allowed the direct parallel coupling of HPLC system to NMR.LC-NMR promises to be of great value in the analysis of complex mixtures of all types, particularly the analysis of natural products and drug related metabolites biofluids. The main prerequisites for on-line LC-NMR, in addition to the continuous-flow probe for recording either continuous-flow or stopped flow NMR spectra. For the high sensitivity, new RF system for multiple solvent suppression and improved dynamic range gradient elusion capability and automatic peak piking/storing capability.

APPLICATIONS

Drug of abuse

Opioids

The studies devoted to determination of opiate agonist almost exclusively the LC-API-MS method has been found. This method urine sample were extracted with Sep pak C_{18} cartridges and subjected to analysis on M3G and morphine in SIM and full scan mode.[3]

Cocaine

Cocaine and its four metabolites (BE, EME, egonine and norcocaine) were extracted from urine using SPE cartridges and determined by LC-APCI-MS in SIM mode. A applied LC-APCI-MS-MS for determination of BE in dried blood sots. The method was applied in epidemiological screening study involving newborns as confirmatory analysis after immunoassays.[3] Cocaine BE and cocaethylene were extracted from human hair samples taken from dead drug addicts and subjected to HPLC separation. Column eluent was split analyzed by ESI-MS-MS and fluorescence detection.

Cannabinoids

Determined Cannabinoids by super critical fluid chromatography coupled with APCI –MS. Hashish constituent (THC, CBD, CBN) were determined by LC-PBI-MS. The LOD ranged from 200 to 1060 ng/ml.[3]

Amphetamines

Amphetamines (Amphetamines, Methamphetamines, MDMA, MDE, and MDA.) were subjected to LC-MS investigation with TSP, ESI and APCI sources.[3]

LSD

A novel immunoassay of LSD in urine was developed and the confirmation analysis was done by ESI. The same research group determined LSD in urine after SPE by ESI with methyesergide as internal standard. N-methyl –LSD was also identified in real sample.[3]

Nicotine

A method for determination of nicotine in serum of smokers and nonsmokers by LC-APCI-MS with the daily output of 100 samples was reported.[3]

Drugs of abuse in alternative matrices

Hair

In addition to the convenience of sample collection, any drugs and metabolites incorporated into hair tend to persist much longer than in conventional specimens. Recently, hair has been used to document drug exposure in a variety of scenarios such as forensic and workplace testing, to monitor compliance to drug therapy and particularly for investigating cases of drug-facilitated crimes (DFC). The availability of standard reference materials for drugs of abuse in hair is vital and enables those laboratories performing hair analysis to check the accuracy of their methods.[4]

Hair samples have been successfully used to document cases of DFC involving a variety of drugs including; benzodiazepines and the hypnotics (zolpidem and zopiclone), methadone and buprenorphine.[5] Kintz and co-workers concluded that due to the extremely low concentrations of drugs typically encountered in hair analysis (low pg/mg) the "sensitivity of LC–MS/MS appears to be a pre-requisite to document any case involving drug-facilitated sexual assault". However, they also added the caveat that hair analysis should not simply be considered as an alternative to blood and urine testing but as a complementary technique where possible.

The importance of this was revealed in a controlled study to investigate the window of detection for lorazepam in urine, oral fluid and hair the simultaneous analysis of 26 benzodiazepines and metabolites, zolpidem and zopiclone in blood, urine and hair.[6] The method was applied to authentic samples from both clinical and forensic cases, including the analysis of hair from a woman who claimed to have been drugged and sexually abused over a period of several years. Thirty-three centimetre lengths of hair were submitted for analysis and cut into 1–3 cm sections; all segments were found to be positive for more than one benzodiazepine, indicating multiple drug exposure, with higher concentrations closer to the root. These results demonstrated the utility of hair to provide a long-term drug history.

Oral Fluids

The use of oral fluid as an alternative specimen is also increasing in popularity especially for monitoring recent drug use within the workplace, at the roadside, in prisons and to check compliance to medication. Described a method for methadone and multiple illicit drugs in addition to their metabolites in oral fluid.[7] Their method also involved PPT using acetonitrile followed by LC–MS/MS analysis. The method proved useful for determining methadone concentrations in pregnant opiate and/or cocaine addicts. Although the methods referenced above utilized oral fluid that has been collected by expectoration, it should be noted that the increased interest in oral fluid has also been accompanied by an increase in the availability of specialized collection devices; these promise a simplified, more controllable collection and sample stability. The final choice of oral fluid collection system, however, has been shown to have serious implications on Drug analysis. The Intercept is a US Food and Drugs Administration (FDA) approved sampling device that is used on a large scale in the USA for workplace drug testing and is one of the devices currently under investigation in a joint roadside study between the EU and the USA to detect driving under the influence of drugs. The collection system contains additives which can cause problems, e.g. ion suppression during LC–MS/MS analysis in the absence of a suitable cleanup method. Several groups have employed LLE (with hexane) to prepare the so-called 'preserved oral fluid' specimen prior to analysis; recently,[8] used the Salivette device to collect samples and to quantify 9-THC in oral fluid samples following SPE and LC–MS. A method was developed for the separation of the enantiomers of methadone and its metabolite EDDP in saliva. Methadone is administered therapeutically as a racemic mix, i.e. a 50:50 mix of the enantiomers. There are significant differences between the enantiomers in terms of receptor affinity, analgesic potency and pharmacokinetic profiles. Thus, therapeutic monitoring of this agent and its metabolite requires an enantioselective technique. Samples were collected using the Salivette device. Following centrifugation, analytes were separated using an immobilized 1-acid glycoprotein chiral stationary phase (AGP-CSP) in conjunction with MS detection. The optimized and validated method was applied to the analysis of samples collected from patients following a methadone maintenance program.[8]

Meconium

Drug abuse during pregnancy is a major problem and has been associated with prenatal complications and high morbidity and mortality rates of newborns. Some birth defects are thought to be related to fetal exposure to drugs. Detection of in utero drug exposure has traditionally been accomplished by urine drug testing. However, this only reflects maternal drug use over the last 3–4 days and abstinence of the mother for several days prior to delivery, may produce a negative result. Monitoring exposure through testing of alternative matrices, such as neonatal meconium and hair, offers advantages including non-invasive collection and detection earlier in gestation.[3] Meconium is the first fecal matter produced by the neonate typically within the first 5 days after birth. Since the formation of meconium starts between the 12th and 16th week of gestation and accumulates in the fetal bowel until birth, use of this specimen can extend the window of drug detection considerably, i.e. to approximately the last 20 weeks of pregnancy.[4] Have described methods for the analysis of opiates and cocaine and respective metabolites[3] and for

the analysis of amphetamine derivatives in this specimen. In both cases samples were prepared by SPE and analysis was achieved using LC–MS (three qualifying ions per compound). Sensitivity was sufficient to allow the detection of all drugs in the low nanograms per gram meconium. Another report describes the application of LC–MS/MS for the simultaneous quantification of methadone and its metabolites in Meconium after methanolic extraction followed by SPE.[3] This method represents an improvement over previous methods in terms of sensitivity and specificity and was successfully applied for the quantification of these compounds in meconium from infants whose mothers were maintained on methadone during pregnancy.

Post-mortem alternative specimens

Clearly, alternative specimens can prove invaluable for the documentation of drug use in the living person. This can also be true for post-mortem investigations. Toxicological analysis of the usual post-mortem specimens can often pose special difficulties. This may because of the decomposed nature of the specimens themselves and/or the presence of putrefactive compounds. In the absence of any suitable tissues or fluids, insects have been proposed as reliable alternate specimens and indeed have been used to identify the presence of various drugs within the cadaver. Although the involvement and contribution of the identified drugs to the actual death may be questionable, the insect tissues have, nevertheless, proved a useful sample.[4] Presented a method for the simultaneous analysis of 10 benzodiazepines in larvae and puparia of the *Calliphoravicina* (Diptera, Calliphoridae). Benzodiazepines are the most widely prescribed psychoactive active drugs in the world. However, they are frequently misused and are consequently often encountered in post-mortem analysis. Larvae were prepared by homogenization followed by precipitation using acetonitrile. Puparia were pulverized in a ball mill and then extracted by ultra sonification in methanol. All extracts were subsequently analysed using LC–MS/MS. The utility of this method was confirmed through its application to the analysis of larvae and puparia that had been reared on media spiked with a range of concentrations of nordiazepam. The concentrations were equivalent to those expected in skeletal muscle following fatal human overdoses. Both the parent drug and its metabolite oxazepam could be detected in single larvae or puparia.[2] Extended these preliminary studies to investigate the effects of different concentrations of nordiazepam on larval development and growth. Larval development can be used in the estimation of post-mortem interval. In some cases, the presence of drugs has been shown to affect development of the insect; consequently these disturbances can have serious implications on the accuracy of post-mortem interval calculations.[3]

Trace chemicals

Chemical warfare agents

Determining the use of chemical warfare agents (CWAs) in times of war or in acts of terrorism requires rapid and reliable methods. The sarin gas attacks by a Japanese cult in Matsumoto city (1994) and the Tokyo subway system (1995) represented the first cases in which a CWA was indiscriminately released against a civilian population. The latter incident resulted in the deaths of 12 people and led over 5000 to seek medical attention. Nerve agents are extremely potent organophosphorus compounds that cause biological effects by irreversibly inhibiting the enzyme acetylcholinesterase (AChE). To confirm exposure, biological samples, e.g. urine, can be analysed for the agents themselves, their metabolites or their degradation products. Nerve agents are rather volatile compounds, thus analysis by GC–MS might be considered the obvious choice. However, in an aqueous environment, these agents readily hydrolyse to produce alkyl alkylphosphonates (RMPAs), these in turn can be further hydrolysed to methyl phosphonate (MPA). LC–MS is increasingly being used for this low molecular weight, highly polar compounds whilst exploiting the benefits over GC–MS, of reduced sample handling and no requirement for derivatisation. Recently developed LC–tandem MS (LC–MS/MS) methods for the analysis of the short-lived metabolites of several CWAs including; sulfur mustard, sarin, soman, cyclohexyl methylphosphonofluridate (GF) and *O*-ethyl *S*-2-diisopropylamino ethyl methylphosphonothioate (VX) in urine. These methods were also used to determine the feasibility of using saliva as a complementary or alternative matrix to urine; this could be a particularly valuable approach to assess the exposure of young children, where collection of a urine sample on demand is often difficult. VX comprises a mixture of two enantiomers which demonstrate significant differences in the rate of AChE inhibition and overall toxicity. Thus, the ability to distinguish between them is desirable for toxicological studies and for the development of antidotes. Smith has used normal-phase LC in conjunction with MS detection for this purpose. LC–MS has also been used to investigate the longer-lived metabolites. Several groups have used LC–MS to determine the metabolites of sulfur mustard, i.e. the β-lyase metabolites in urine samples from human casualties after sulphur mustard poisoning.[3] In the case of large-scale attacks, analysis of the environment and other materials may also be required. Hancock and D'Agostino have developed a LC–ESI-MS (/MS) procedure which allows the identification of a munitions grade sample of tabun, sarin, soman, GF and the nerve agent stimulant triethyl phosphate (TEP) on manmade fibres. Although this technique uses only minimal sample preparation the same group. Have more recently experimented to omit sample preparation completely and to allow the direct analysis of TEP collected on solid-phase microextraction (SPME) fibres.[2] The biotoxin ricin originates from the seeds (castor beans)

of the *Ricinus communis* plant and is extremely toxic (human LD50 estimated at 3–30 g/kg by inhalation or ingestion, respectively). It has the unique position of being the only protein listed under the Chemical Weapons Convention and is of forensic interest due to its potential for terrorist use or as a homicide agent. Due to the high molecular weight of this compound (66 kDa) absolute structural elucidation of the intact protein is not possible using nominal mass analysis. However, several groups have used a preliminary enzymatic digestion to convert the protein into intermediate molecular weight peptides followed by LC–MS (/MS) using a hybrid quadrupole time-of-flight (QTOF) instrument. The methods were used to characterize purified ricin from several different varieties of *R. communis*and also from crude castor bean extracts.[2]

Explosive

The analysis of trace levels of explosives is critical in crime scene forensic investigations, homeland security and environmental analysis. LC–MS is a well-established technique for explosives in associated complex matrices such as post-blast residues and in environmental samples such as soil and plant material extracts.[2] Although these compounds have a low vapour pressure they tend to be heat labile and can degrade at the high temperatures typically used in GC injectors. Thus, LC–MS is particularly well-suited to the analysis of these relatively polar molecules, heat labile compounds. Many of the methods rely on the formation of cluster or adduct ions for identification. The formation of cluster ions of 1,3,5-trinitro- 1,3,5-triazacyclohexane (RDX), one of the most commonly used military explosives in both ESI and APCI.[3] Results showed that in ESI, self-decomposition of RDX did not play a role in adduct Formation; the adducts were produced from impurities present in the mobile phase at ppm levels. In contrast, with APCI, part of the RDX molecule decomposes yielding a NO2 species; this in turn clusters with other RDX molecules. More recently, Mathis and McCord presented a comprehensive method to allow the screening of a panel of high explosives including; RDX, 2,4,6,-trinitrotoluene (TNT), pentaerythritoltetranitrate (PETN), 1,3,5,7-tetramethylene-2,4,6,8- tetranitramine (HMX), nitrogycerine (NG) and ethylene glycol dinitrate (EGDN). This method was based on the *competitive* formation of adducts following infusion of the high explosives with a mixture of four anions; chloride, formate, acetate and nitrate. Information relating to the relative extent of adduct formation (based on intensity ratios) in addition to adduct stability, was used to provide a multiplexed detection scheme.[3] Anti-personnel (AP) mines are currently in place in over seventy countries and are designed to maim or kill humans. In addition to the lives that are lost, the mere suspicion that they may be present, can prevent the use of large areas which could otherwise be utilised for agriculture or social infrastructure. Removal of landmines from such areas is known as humanitarian de-mining and relies on the accurate detection of the explosive. A potentially useful approach and one which is currently under investigation, is the detection of the chemical vapors which arise from the explosives and are transported into the surrounding atmosphere. High sensitivity is required since the concentration of molecules expected to reach the gas phase is low. Sanchez *et al.* have developed a method for the sampling and identification of nitroaromatic explosives. Air was sampled at flow rates of up to 15 L/min using a holder fitted with a C18 solid-phase extraction (SPE) membrane. After sampling, trapped analytes were desorbed on-line and analysed by LC–MS/MS using an APCI interface. Storage stability studies indicated that the captured analytes were stable for 1 week or 3 weeks, when membranes were stored at room temperature or at −4◦C, respectively. The method allowed the identification and separation of most of the isomers of TNT and 2,4-dinitrotoluene (DNT); limits of detection were in the range of femtogram/L. The method is suitable for the chemical profiling of military grade explosives and is valuable for both forensic identification and for de-mining purposes.

Dyes

Textile fibres found at a crime scene can be used as chemical evidence in a wide range of crimes; dye identification and comparison can be of particular importance. Recently, have used LC–MS to enable unambiguous differentiation between structurally related textile dyes which were previously indistinguishable by UV–vis absorption profile or by microspectrophotometry. They concluded that where single stage LC–MS fails to differentiate, analysis should be extended to include LC–MS/MS of the extracted dye mixture. The groups of colour additives known as the Sudan dyes are synthetically produced azo-dyes. Their degradation products are considered to be carcinogens and teratogens. Due to this fact their use as food additives is banned in the USA and the European Union (EU). However, in some countries they are still used to enhance the colour of bell pepper and chilli powders. The discovery of a batch of chilli powder contaminated with Sudan I in February 2005 resulted in the largest product recall in British history.[3] The widespread use of this batch of chilli powder led to the withdrawal of hundreds of food products including Worcester sauce, pizza and seafood sauces. Calbiani *et al.* reported a LC–MS/MS (nominal mass/low resolution triple quadrupole) method for the simultaneous analysis of four Sudan dyes in foodstuffs. More recently, this group have used capillary LC in conjunction with high-resolution MS instrumentation to further distinguish between isobaric ions and to further increase confidence by providing elemental composition. Using exact mass in both MS and MS/MS experiments, they were able to provide unambiguous confirmation of Sudan I in authentic food samples. Pepper sprays are readily available to law-enforcement personnel and to the general public for a variety of uses including riot control and self-defence. In these cases, the presence of pepper sprays, on clothing for example, may help to determine

the facts of an incident. A common active ingredient of these sprays is capsaicin, an oily resin extracted from capsicum fruits. Some of the pepper sprays also contain a coloured dye or a UV-activated fluorescent marker to permit the localisation of the product. However, there are now a number of products on the market that do not contain such visible aids to analysis. M.J. Bogusz *et al*, have developed a method to initially visualize colourless pepper sprays on fabric and to subsequently confirm the presence of naturally occurring and synthetic capsaicinoid molecules. Visualisation was achieved by chemical derivatisation of the capsaicinoids using a diazonium salt. Identification of the capsaicinoids and their derivatives was then accomplished following methanolic extraction from the garment. Extracts were analysed within 6.5 min, using an YMC Basic column in conjunction with LC–APCI-MS detection. Work is on-going to confirm the spectra and proposed fragment ions of the derivatives via MS/MS and exact mass determination.

Doping agents and related compounds

Several contributions were developed to determination of steroid compounds. In an early paper of Sandra et al. HPLC with dual channel detection system consisting of DAD and PBI was applied to the analysis of testosterone esters. The comparison of ESI and APCI for assay of methanostenolone and its metabolites in equine urine demonstrated that APCI was better for neutral compounds and ESI for sulfate metabolites. Park et al. developed an LC-Ms-TSP screening system for 10 corticosteroids in urine .the LOD ranged from 10-50 ng/ml in their SIM mode. Steroids sulfates and glucuronides were determined in urine with ESI-MS-MS. The LOD of 20 pg on-column was achieved.

On-line coupled immunoaffinity chromatography – reversed-phase (RP) HPLC with PBI and quadrupole ion trap was applied for determination of corticosteroids (dexamethasone and flumethasone) in equine urine. the LOD's were 3-4 ng/ml. the advent of β_2 agonist as stimulating and anabolizing agents in sports was associated with development of various LC-MS detection methods, which have recently been reviewed by polettini. Few drugs these groups were determined with GC-MS, TSP and ESI. Both GC-MS and ESI gave 50-fold lower LOD than TSP.reserpine was determined in equine plasma by LC-ESI-MS-MS after SPE,with a LOD OF 0.01ng/ml. [3]

Natural toxin

42 compounds in traditional Japanese plant medicines were analyzed by HPLC with DAD and MS. 16 alkaloids fro aconitum japonicum were simultaneously determined with LC-APCI-MS. ESI-MS was applied for elucidation of structure of 18 taxanes obtained from taxus extracts. Fifty kinds of spider venoms were analyzed by HPLC-FAB-MS. Determined alkaloid colchicine in blood, plasma and urine by HPLC-ESI, with a LOD of 0.6 ng/ml.[4] α and β-amanitin were extracted from urine sample in the cases of amantia poisoning and analyzed with ESI. The LOD was 10ng/ml urine.

Environmental poisons

A published rapid LC-APCI-MS method for simultaneous analysis of methylcarbamets pesticides in serum in poisoning cases. Some problems concerned with the production of cluster ions originating from the mobile phase solvent were noticed. The most papers developed to determination of polar pesticides like organophosphates, imidazoline herbicides, and phenylurea herbicides in water sample.

CONCLUSION

The technique developed from the coupling of a separation technique and an on–line spectroscopic detection technology is known as hyphenated technique. The remarkable improvement in hyphenated analytical method over the last two decades has significantly broadened their application in the analysis of forensic toxicology. The combination of LC and MS has been used for many years. However, since the introduction of more user-friendly LC-interfaces, e.g. ESI and APCI, there has been a tremendous increase in the popularity of the technique amongst scientists from a wide variety of disciplines. LC–MS has evolved into a robust and reliable tool that also offers versatility, specificity and sensitivity. For those involved in the forensic sciences in particular, the use of LC–MS has changed considerably. Where it was once a technique that was only used very infrequently, i.e. as an alternative to GC–MS for the more 'troublesome' analytes, it is now used extensively and has proved itself invaluable especially for the analysis of highly polar, in volatile and thermolabile compounds. The analytical environment is a dynamic one. Most technological advances are driven by analytical demands; e.g. the need for faster, more accurate/robust analysis. Thankfully, continual developments in hardware and software lead to more robust, easier-to-use and more accurate instrumentation, all of which, help to establish what might initially be considered to be a 'novel' technique, and one which was only available to 'innovators', into one which is usable in a more routine setting and available to a wider group of analysts.

PERSPECTIVES AND EXPECTATIONS FOR THE FUTURE

In order to minimize the errors in a risky of prophecy, it may be reasonable to summarize the present situation concerning the application of hyphenated liquid chromatographic techniques in forensic toxicology. The recent review concerning the future of hyphenated techniques in industrial laboratory, indicated the need of sophisticated couplings, like LC-NMR-MS, LC-DAD-MS and introduction of low cost, bench-top-LC, GC and CE-MS instruments trend expected in forensic toxicology. Also the on-line combinations of all analytical steps (isolation, separation, detection, identification, quantification), together with development of commercially available LC-DAD-APCI-MS instruments, will be probably just a matter of time. An interesting, although very futuristic approach has recently been formulated by Thomson, who has observed that most molecules of interest are "happy" in

the liquid phase. Therefore, instead of forcing the ions to leave their natural environment, instruments should be developed which would able to measure the ions in solution.

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