Mini Review

Drug Testing and Analysis

Received: 23 March 2009 Revised: 3 April 2009 Accepted: 3 April 2009 Published online in Wiley Interscience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.26

LC-MS: a powerful tool in workplace drug testing

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Workplace drug testing is a well-established application of forensic toxicology and it aims to reduce workplace accidents caused by affected workers. Several classes of abused substances may be involved, such as alcohol, amphetamines, cannabis, cocaine, opiates and also prescription drugs, such as benzodiazepines.

The use of alternative biological specimens such as hair, oral fluid or sweat in workplace drug testing presents several advantages over urinalysis – mainly the fact that sample collection can be performed easily without infringing on the examinee's privacy, so the subject is more likely to perform the test. However, drugs are usually present in these alternative specimens at low concentrations and the amount of sample available for analysis is small. The use of highly sensitive techniques is therefore necessary.

In fact, the successful interface of liquid chromatography with mass spectrometry (LC-MS) has brought a new light into bioanalytical and forensic sciences as it allows the detection of drugs and metabolites at concentrations that are difficult to analyse using the more commonly adopted GC-MS based techniques.

This paper will discuss the importance of LC-MS in supporting workplace drug-testing programmes. The combination of LC-MS with innovative instrumentation such as triple quadrupoles, ion traps and time-of-flight mass spectrometers will also be focused.

Keywords: alternative matrices; drugs of abuse; LC-MS; workplace drug testing

Introduction

Drug abuse is not a recent phenomenon. Indeed, drugs that promote altered psychological states and increased physical capacities have been used for hundreds or thousands of years. Nowadays, the scourge of illicit drug consumption and abuse affects all modern societies. Workplaces are inevitably affected too. Strong control measures are therefore needed to address this situation, including legislation, regulation and minimal performance requirements for analytical instruments, together with the development and implementation of treatment programmes.

Many countries have already adopted drug testing as an essential tool to assess drug exposure in a variety of fields such as clinical and forensic toxicology, crime-scene investigations and workplace drug testing.[1] The latter may be considered as an application of forensic toxicology, aiming at reducing accidents caused by impaired workers. In fact, the WHO estimates that 30% of absenteeism and workplace accidents in Costa Rica were caused by alcohol dependence, and about 8–14 million working days are lost worldwide each year due to alcohol-related problems.[2]

Drug testing can be performed in several situations, including pre-employment, where there is reasonable cause/suspicion of drug abuse, at accident or other incidents, after drug treatment, as a follow-up procedure, and on a scheduled routine basis. Random testing of safety/security-sensitive personnel in designated positions, or universal testing of all personnel on a random selection basis, can also be performed, depending on the nature of the workforce.[3] Variations in workplace drug testing policies make it necessary to establish guiding principles and develop adequate legislative measures, taking into account both the employer's and the employee's rights and obligations.

This type of drug testing programmes is not recent, however. In fact, workplace analyses have been performed in the US over the last 25 years.[3] The Substance Abuse and Mental Health Services Administration (SAMHSA), which is responsible for regulating workplace drug testing in the US, reported that an estimated 14.8 million Americans are current drug users, and 77% of these are employed.[4] This highlights the huge importance of workplace drug testing nowadays.

However, not much is known about the European situation regarding workplace drug testing, because few statistical studies are available. Workplace drug testing in Europe is mainly performed in pre-employment situations, for example in the transport, petrochemical, shipping, automobile, pharmaceutical and computer industries.[5]

In general there is no specific legislation or regulation on the matter.[6] However, in Finland written guidelines on the practical performance of drug tests do exist, from sampling to analysis and interpretation of the test results. This ensures that testing for drugs in the workplace takes into account not only good standard laboratory practices but also the integrity and protection of privacy of the tested individuals as well as their fundamental rights.[7–9] A step was taken towards the standardization of workplace drug

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testing in Europe with the formation of the European Workplace Drug Testing Society (EWDTS) in 1998.[5]

Workplace Drug Testing

Urine is traditionally the main biological sample used for testing for drugs in the workplace,[10] and it is capable of providing strong evidence of drug use or abstinence (for most drugs) for the preceding two days.[11] Several factors have contributed to this situation, namely the higher concentrations at which the drugs (and metabolites) often appear in this specimen when compared to blood, the ease of sampling and the low invasiveness of the collection procedure.

Urine has been tested for a number of drugs in workplace scenarios, including opiates,[12–18], cocaine and metabolites,[12,18–24] cannabinoids[12,21,22,24–27], and amphetamine-like stimulants.[11,26–32] It should be noted that not only illicit substances are involved in workplace accidents, as, for instance alcohol is often detected,[27,33–37] as are some prescription drugs, such as benzodiazepines.[27,34,38–42]

An important issue when dealing with this type of drug-testing programme is the establishment of cut-off values. A number of international organizations have proposed cut-offs for several drug classes and these can present small variations. These differences in the proposed concentrations become more obvious if the cut-offs proposed by European and American organizations are compared. For instance, de la Torre *et al*.[43] propose a 200 ng/mL cut-off for morphine confirmation in urine, while for SAMHSA this cut-off should be set at 2000 ng/mL, a ten-fold difference.[5] Nevertheless, these differences do not limit the usefulness of urine testing in workplace scenarios – it has been used for years as discussed above.

Sample analysis usually begins with screening assays as the expected number of negative samples is quite high.[44,45] This is normally accomplished by immunoassay techniques, usually ELISA based. However, these are generally poorly selective assays and therefore the confirmation of presumably positive samples by more selective techniques is necessary, taking into account the consequences of a positive drug-test result.[46,47] These confirmation analyses are mainly performed by GC-MS, as the desired selectivity and sensitivity are met.[46,48]

Urinalyses do present a number of disadvantages, of which the real possibility of sample adulteration or substitution[47,49] and the examinee's loss of privacy (because of the need to control the sample-collection process) are the most important. Moreover, the quite narrow window of detection for most drugs limits the assessment of long-term exposure, and it is not possible to correlate urine drug levels with impairment or blood drug concentrations. For these reasons, urine testing by itself may not be able to guarantee a drug-free workplace.[10]

To overcome the problems associated with the limitations of urinalysis, several 'alternative' biological specimens, such as hair, oral fluid and sweat, are being evaluated for their possible inclusion in workplace drug-testing programmes. These samples present a number of advantages over traditional urine samples, of which the most important is that their collection is totally non-invasive to the donor. In addition, the time window for drug detection can be increased, for instance in the case of hair, allowing the documentation of long-term drug exposure. Indeed, the possibility of covering a wide period of time, often several months or years, is an overwhelming advantage of the hair specimen in a wide range of applications, obviously including drug testing in the workplace.[10,50] However, these detection windows are generally much shorter for oral fluid than for urine, depending on the analyte and on the sensitivity of the method used.[51]

A number of papers have been published on the use of these 'alternative' biological specimens in workplace drug testing. The most detected drug classes in these samples are opiates, cocaine and metabolites, cannabinoids and amphetamine-like stimulants.[50,52–61]

Here too, the establishment of cut-off values is not an easy task. Cut-off values are still being studied for oral fluid and sweat samples, whereas they have already been proposed by the Society of Hair Testing (SoHT) and the EWDTS for the analysis of several drug classes in hair samples,[62,63] both in forensic and workplace situations.

The use of these specimens also presents some other drawbacks and these are mainly related to the low sample availability for analysis, which assumes even more importance in the case of oral fluid. The concentrations of most drugs are very small in oral fluid and the volume available for analysis is often less than 1 mL.[50] Therefore, for most applications involving these specimens, GC-MS based confirmation methods do not enable the detection of low levels of drugs. Consequently the more sensitive LC-MS(MS) appears to be a prerequisite if the desired sensitivity is to be attained.

Lc-Ms(Ms)

A dramatic increase in the capabilities of mass spectrometry (MS) has been seen in the last 20 years, and its coupling to liquid chromatographic techniques was an important event. Indeed, new and powerful technologies have enabled scientists in all analytical fields to see what they were not able to see only a few years before. Furthermore, the use of MS allowed the unambiguous identification of analytes, which is important in several analytical fields, including forensics and drug testing in the workplace.

It is not surprising, therefore, that MS-based techniques, including GC-MS, LC-MS, isotope ratio (IR)-MS and inductively coupled plasma (ICP)-MS have become routine tools, in most laboratories, to detect and quantitate small amounts of analytes in complex matrices, including biological specimens.[64,65]

Concerning LC-MS, some significant developments and improvements in instrumentation design were made in the last two decades, namely the introduction of robust and user-friendly interfaces, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Just 20 years ago, LC-MS was an exotic technique, rarely applied in routine analysis, and its upcoming widespread use could hardly have been anticipated.[66]

Single quadrupoles, triple quadrupoles and quadrupole ion-trap instruments are the most used mass analysers in routine laboratories, in the fields of forensic toxicology, therapeutic drug monitoring and doping control[64,65,67–72]. However, applications using time-of-flight (TOF) instruments, which allow accurate mass measurements and the assessment of empirical formulas of unknown molecules,[73–75] have also been published.[76]

Other advances in LC-MS technology include ultrahigh-pressure liquid chromatography (UPLC) systems. Using UPLC it is possible to analyse a large number of analytes in a single run, because particle diameters as small as 1.5 microns and operating pressures higher than 5000 psi are employed. This technology is being applied in various analytical fields, including toxicology.[64,77–79] These

appear to be promising techniques, but their possible application in workplace scenarios has not yet been described in the literature.

The advantages of LC-MS(MS) include its high specificity and sensitivity, the latter being due to an increased signal-to-noise ratio. These, in combination with short chromatographic run times and reduced sample preparation (because for most procedures there is no need for the time-consuming derivatization steps), make LC-MS(MS) the technique of choice for high-throughput confirmation of multiple substances in biological samples.[73,80] In addition, analysis of derivatized samples in GC-MS leads to a higher necessity for system maintenance, and this is important when a large number of samples is expected to be analysed.[80]

The main drawback of LC-MS(MS) is that it is susceptible to being affected by matrix constituents. This usually results in unwanted ionization suppression or enhancement phenomena,[81–83] which can vary between specimens and consequently affect the relative abundance of the mass spectrum's ions. This may reduce the accuracy of the quantitation analysis, so the documentation of matrix effects is mandatory in the development and validation of LC-MS(MS)-based analytical methods.

Matrix effects are of great importance in oral fluid testing because the sample-collection devices usually contain stabilizing salts, non-ionic surfactants for surface wetting and antibacterial agents, which are capable of interfering during analysis if a suitable clean-up method is not available.[73,82,84] Several approaches exist to evaluate the extent to which matrix effects affect analysis,[81,83,85] but these are beyond the scope of this review. To overcome this problem, the composition of the mobile phase can be adjusted in order to reduce the co-elution of matrix constituents, and matrix-matched calibrators and deuterated internal standards can be used.[73] This feature emphasizes the importance of the chromatographic step in the analysis, where good separation can reduce or eliminate these effects.[75]

Adduct formation is another commonly encountered problem in LC–MS(MS), which is due to the presence of solvent in the ionization chamber. These adducts create ions with higher *m/z* than expected, and are capable of forming bridges between ions of differing masses, thus complicating mass spectra and their interpretation.[73]

Furthermore, and unlike GC-MS, the development of searchable libraries for LC-MS(MS) is still a problem, because intensity of fragment ions differs between instruments although methods for creating reference libraries have been reported.[86–88]

It should be noticed, however, that these drawbacks do not limit the usefulness of LC-MS instruments in routine laboratory analyses or investigation and their high sensitivity is a well established fact. Furthermore, the number of papers using this type of technology is increasing, showing its high potential and wide applicability in bioanalytical sciences.

Application of Lc-Ms(Ms) in Workplace Drug Testing

Over the last few years, a large number of papers have been published on the application of LC-MS and LC-MS-MS methods to drug detection and quantitation in biological samples. However, the number of articles concerning the application of this type of technology in workplace scenarios is quite low, despite the fact that several methods developed in related fields, such as forensic toxicology, can be also applied in this situation. Papers on the application of LC-MS(MS)-based methods in workplace drug testing have been identified through references listed in known published studies. The public MedLine database PubMed was also used to identify additional studies, employing the search string 'workplace drug testing and liquid chromatography'.

The detection of abused drugs is the main issue involving the use of LC-MS for workplace drug testing, with several drug classes being determined in various matrices. Cairns *et al*.[55] have determined a number of amphetamines in hair samples of subjects that had tested positive in urine. In this study, the results obtained for drug users attending rehabilitation programmes were compared with those obtained from workplace subjects. The range of methamphetamine levels in the clinical samples did not differ from that of the workplace group, but the percentage of individuals showing higher levels of the compound was lower in the latter population. Furthermore, amphetamine concentrations varied widely in both groups, whereas only the workplace group showed positive results for methylenedioxy-methamphetamine (MDMA) and its metabolite methylenedixoy-amphetamine (MDA). Concentrations of MDMA were generally lower than those of methamphetamine, as the former is often used on a weekend and/or recreational basis rather than chronically.

The same authors conducted a similar study concerning the determination of cocaine and metabolites in hair samples.[56] They again found that the percentage of individuals showing lower levels of cocaine (below 2 ng/mg) was higher in the workplace group, while the opposite was true for high cocaine levels (above 20 ng/mg).

Techniques based on LC-MS have also shown to be useful in the determination of biomarkers of alcohol consumption, with obvious applicability in the workplace. Ethyl sulphate[36] and ethylglucuronide[80] were determined in urine samples, without the need for time-consuming derivatization procedures.

Two additional papers have been published, albeit not directly related to workplace drug testing.[71,89] In these studies, several abused drugs have been determined in preserved oral fluid using the Intercept® oral fluid collector, which is an FDA-approved sampling device widely used in the US for workplace drug testing. In one of the studies,[71] the limits of detection obtained ranged from 0.2 to 0.5 ng/mL, meeting the requirements of SAMHSA for oral fluid testing in the workplace. Table 1 summarizes the LC–MS(MS) procedures and potential applications in workplace drug testing.

One interesting paper on drug testing in the workplace was published by Kintz *et al*.,[91] but using GC-MS-MS analysis. In this paper, four cases of addiction to anaesthetics by anaesthesiologists were assessed through hair analysis.

Nevertheless, abused drugs and ethanol are not the only substances that can be tested in the workplace. Indeed, other studies have been published, including the monitoring of isocyanates in air,[92–95] the analysis of urinary N-acetyl-S-(propionamide)-cysteine as a biomarker of acrylamide exposure,[96] and the detection of perfluorooctanesulfonyl fluoride in serum.[97]

Conclusions and Perspectives

The detection of drugs in workplace scenarios is gaining importance nowadays, as a result of efforts to reduce accidents caused by drug-affected workers. Workplace drug testing can be performed in several situations, and these include pre-employment, reasonable cause/suspicion, accident or incident-related, after drug treatment or as follow-up procedures, and on a scheduled routine basis.

Table 1. Characteristics of current LC–MS(MS) procedures with potential application in workplace drug testing

Compounds	Specimen	Sample amount	Preparation	Stationary phase	Mobile phase	Detection mode	LOD; LOQ	Other information	References
AM, MA, MDMA, MDA	Hair	12 mg	Enzymatic digestion 2h pH 9.5 and LLE (–)	Keystone Scientific BETASIL C8	Acetonitrile: 0.1% formic acid in water (80 : 20)	LC-ESI- MS-MS	0.025 ng/mL; 0.025 ng/mL for AM, 0.1 ng/mL; 0.1 ng/mL for MA, 0.01 ng/mL; 0.01 ng/mL for MDMA and MDA	Previous urine screening (MA-positive) using EMIT and GC-MS Screening of hair sample with RIA	[55]
COC, BE, COET, NCOC	Hair	12 mg	Enzymatic digestion 2h pH 9.5 and SPE (Isolute SPE)	–	Acetonitrile: 0.1% formic acid in water (80 : 20)	LC-ESI- MS-MS	– ; –	Previous urine screening (COC-positive) using EMIT and GC-MS Screening of hair sample with RIA	[56]
EtG	Urine	0.1 mL	No extraction (dilution with 0.25 mL methanol)	Synergi POLAR RP	0.1% formic acid in water	LC-ESI- MS-MS	52 ng/mL; 152 ng/mL	–	[80]
EtS	Urine	0.1 mL	No extraction (addition of 0.1% formic acid)	Synergi POLAR RP	0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B)	LC-ESI- MS-MS	50 ng/mL; 110 ng/mL	Ethanol analysis in urine using HS-GC-FID and creatinine analysis	[36]
Oxazepam, desmethyl-diazepam, 3-OH-diazepam	Urine	1 mL	SPE (Isolute HCX)	Genesis Phenyl-120	0.05% formic acid in water (mobile phase A) and acetonitrile (mobile phase B)	LC-ESI-MS	– ; 20 ng/ml for all compounds	Previous screening with the Roche Abuscreen ® online Kit and confirmation in 1 case with GC-MS Creatinine analysis	[38]
O-H-LSD, LSD	Urine	10 mL for GC analysis	GC: LLE (n-butylchloride) and SPE (Clean Screen CSDAU203)	GC: HP-5	Ammonium acetate and 0.02% TEA buffer (mobile phase A) and Acetonirile (mobile phase B)	GC-EI-MS	0.4 ng/mL; 0.4 ng/mL for O-H-LSD	LC-MS and LC-MS-MS analysis for O-H-LSD detection were performed on urine samples previously positive for LSD by GC-MS	[90]

Table 1. Continued

Compounds	Specimen	Sample amount	Preparation	Stationary phase	Mobile phase	Detection mode	LOD; LOQ	Other information	References
		5 mL	LC: LLE (dichloro-metane: isopropanol (85 : 15) and SPE (Clean Screen ZSDAU020)	LC: Eclipse® XDB-C18		LC-APCI-MS	0.1 ng/mL; 0.1 ng/mL LSD with GC		
						LC-APCI- MS-MS (Ion trap)			
THC	Oral fluid	0.1 – 0. 5 mL	LLE (hexane)	XTerra MS C_{18}	1 mM ammonium formate: methanol (10 : 90)	LC-ESI- MS-MS	– ; 0.5 ng/mL (using 0.1 mL of sample) – ; 0.1 ng/mL (using 0.5 mL of sample)	Oral fluid was collected with the Intercept® collection device	[89]
AM, MA, MDMA, MDA, COC, BE, MOR, COD, MAM	Oral fluid	0.25 mL	SPE (Oasis® MCX)	XTerra MS C_{18}	10 mM ammonium bicarbonate, pH=10 (mobile phase A) and methanol (mobile phase B)	LC-ESI- MS-MS	0.2 to 0.5 ng/mL; 2 ng/mL for all compounds	Oral fluid was collected with the Intercept® collection device	[71]

Abbreviations: AM: amphetamine; BE: benzoylecgonine; COC: cocaine; COET: cocaethylene; COD: codeine; EtG: ethylglucuronide; EtS: ethyl sulfate; LSD: lysergic acid diethylamide; MA: metamphetamine; MAM: 6-acetylmorphine; MDA: 3,4-methylenedioxyamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; MOR: morphine; NCOC: norcocaine; O-H-LSD: 2-oxo-3- hydroxyl lysergic acid diethylamide; TEA: triethylamide; THC: Δ^9-tetrahydrocannabinol.
APCI: atmospheric pressure chemical ionization; EI: electron ionization; EMIT: enzyme multiplied immunoassay technique; ESI: electrospray ionization; FID: flame ionization detector; GC: gas chromatography; HS: headspace; LC: liquid chromatography; LLE: liquid-liquid extraction; LOD: limit of detection; LOQ: limit of quantitation; MS: mass spectrometry; RIA: radioimmunoassay; SPE: solid phase extraction.

While this type of analysis used to be performed in urine or blood samples, there is a growing interest in the use of alternative specimens, such as hair, oral fluid, or sweat. The use of these specimens has two major advantages: their collection is performed in a non-invasive manner (under close observation, if necessary) without infringing on the examinee's privacy; and the possibility of sample adulteration or substitution is minimal.

However, it should be taken into account that when these specimens are used to document drug exposure the concentrations will in general be low, requiring highly sensitive techniques. The addition of liquid chromatography to mass spectrometry has contributed to an increase in the sensitivity of assays and has enabled analyte detection at concentrations that were unthinkable just a few years ago. Moreover, as the results of drug testing can affect an individual's reputation, job status, or even his freedom, analytical procedures that are defensible from a forensic point of view must be used, and therefore mass spectrometric techniques are mandatory. For these reasons, these technologies are today indispensable in clinical and forensic toxicology laboratories, as well as in doping control or workplace drug testing.

Consequently, many laboratories are finally at the point where they are considering the acquisition of these capabilities, as they are becoming more accessible. Moreover, the use of TOF instruments is likely to continue to increase for the foreseeable future, mainly due to its ability to identify unknowns.

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