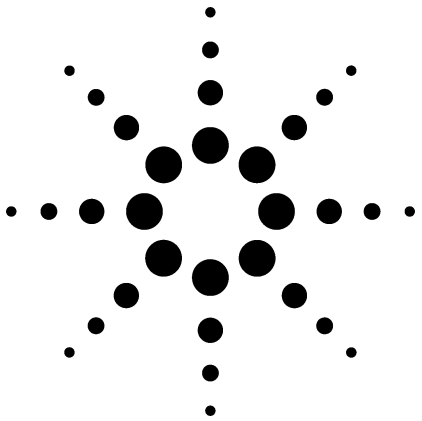
Screening Drugs of Abuse by LC/MS

Technical Overview



Forensic

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Abstract

High through-put screening of drugs of abuse is performed at St. Olav Hospital by LC/MS. Over a million analyses per year are now made. Typically done by immunoassay, this overview describes the procedures for using this highly selective and quantitative LC/MS methodology. In addition, the advantages of using LC/MS (lower cut-offs, no false positives, etc.) are discussed.

Introduction

Substance abuse is increasing in almost every country, and is associated with criminality, health hazards, accidents, and fatalities. Communities, therefore, face a significant resource investment in their efforts to reduce or stop this abuse. Most substance abuse is illegal. Symptoms and signs are unspecific, and substance abuse is difficult to identify outside the boundary of clinical criteria. This, combined with the fact that substance abuse under many circumstances leads to legal actions, makes it mandatory to be able to identify and quantify substances of abuse in biological material. Such methods were developed and are applicable for almost any possible biological matrix.

Traditionally, screening is done by immunology, which is fast and simple, but can be expensive (reagent costs), and normally determines groups of compounds, not specific analytes. Due to its lack of specificity, very often positives must be confirmed, normally by gas chromatography/mass spectrometry (GC/MS). In drug screening, immunology gives a result as “positive” or “negative”, with reference to a certain predetermined cut-off level. Cut-off values for immunoassays are fixed due to optimization of quantity, and tend to be relatively high to avoid bias from interferences. As a result, this gives a high number of false negatives that may have clinical and societal consequences.

A drug screen by liquid chromatography/mass spectrometry (LC/MS) gives a quantitative determination of specific analytes, with known accuracy and precision, within a range of concentrations from 50–100,000 ng/mL. This allows variable cut-off levels for different purposes within the calibrated range. An argument can be made that if a compound is not included in the LC/MS screen it will be missed, and that immunoassay will give a positive in that case because it is a general screen. However, confirmation will show it to be a false positive because the GC/MS confirmation is also a targeted list.

Screening by LC/MS is a new approach compared to immunoassay. LC/MS is also fast, but provides results for specific compounds, not groups. This is important, for example, where the patient is legally prescribed one benzodiazepine but may abuse a second benzodiazepine. There is no way to account for this with immunoassay; however, intake of other "nonprescribed" benzodiazepines is easily detected by LC/MS screening. A similar argument can be applied to amphetamines and other groups of drugs. As an example, LC/MS screening of amphetamines can differentiate between the following analytes: amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA or Ecstasy), methylenediox-amphetamine (MDA), and ephedrine.

LC/MS methods for "new" drugs on the street can be quickly developed, validated, and implemented into the assay within a few days. In the example of amphetamines, other related drugs such as cathinone can be easily and quickly added to the screen. This is not the case for immunoassay, where development of kits for new analytes is a challenging and time consuming procedure. LC/MS is flexible, reliable, and highly sensitive (low nanogram range). As part of its flexibility, note that systems used for other purposes, such as therapeutic drug monitoring (TDM), can also can used for drugs of abuse screening and vice versa [1]. This system flexibility and versatility is an important feature of the platform and is important both for logistics and maintenance.

LC/MS at St. Olav Hospital

This overview describes the successful use of LC/MS systems at St. Olav Hospital in routine service doing high-volume drug screens from 1998 to the present. Figure 1 shows the increase in the number of analyses performed each year during the period of 1996 to 2003. The first LC/MS was put in service in 1998 and the methodology was fully employed by 1999. The number of analyses for 2004 will approach 1,000,000. Note that because of the graph's scale, the increase in serum analyses cannot be read, but the number of serum determinations is increasing. Serum analyses are approaching 60,000 for this year. Each DOA analysis represents a determination equivalent to an immunoassay for a group of drugs (benzodiapenes, amphetamines, etc.). The actual LC/MS analysis determines specific compounds, and if charted by the compounds analyzed, the total number of analyses would be much higher. LC/MS screening is now performed as a routine service in a restricted area in compliance with national and international guidelines using quality control systems securing all aspects of sample handling, preparation, analysis, and reporting.

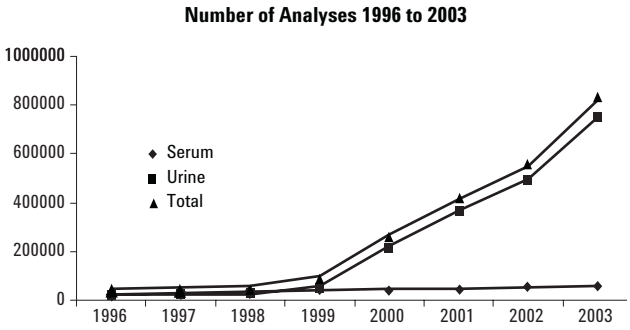

Figure 1. Number of analyses per year for drugs-of-abuse (DOA) from 1996 to 2003. Note that the first LC/MS was purchased in 1998 and LC/MS screening was fully deployed by 1999. The number of analyses represents each group of compounds equivalent to an analysis by immunoassay. In actuality, the analyses are comprised of determinations of individual drugs. The number of determinations made is much greater than indicated by this chart.

Methodology

The LC/MS platform is used for a wide variety of samples ranging from medical treatment of abuse, legal actions/negative sanctions, forensics, and clinical toxicology. Several types of these samples must be confirmed. Because GC/MS is still the accepted "gold standard" confirmation technique for legal action, this is the methodology used here. However, the use of LC/MS screening strongly reduces the number of GC/MS confirmations, a fact that saves both time and money. Comparison of GC/MS and LC/MS results show close to 100% accordance, which means no false positives. In the future, a high-throughput technology such as liquid chromatography/tandem mass spectrometry (LC/MS/MS) in full scan mode, as obtained by ion trap technology, may demonstrate the potential for performing fast confirmation. In combination with LC/MS screening, such a technique would make possible screening and confirmation in less than an hour for single samples, and within a few hours for a larger series of samples.

The systems for DOA screening and TDM use the Agilent 1100 LC/MSD quadrupoles. Presently,

24 instruments are used for these activities. All instruments (both for DOA and TDM) are equipped identically with four mobile phase constituents, using a quaternary system with methanol, acetonitrile, ammonium acetate, and formic acid. For DOA, only two columns are needed, a short C18 and a short CN. This simple strategy gives unique flexibility between instruments and very efficient backup capacity. Finally, the simplified inventory of mobile phases and columns makes fast method development easier.

Amphetamines as an Example

As an example, amphetamines are determined with a short CN column with an isocratic mobile phase (ammonium acetate and acetonitrile). Figure 2 shows amphetamine, methamphetamine, MDA, MDMA, and ephedrine with d_3-amphetamine as the internal standard (ISTD). Target ions and qualifiers are used, and the mass spectrometer is operated using electrospray ionization (ESI). The qualifier ions are obtained by collision-induced dissociation (CID) in the ion transport region of the atmospheric pressure ionization (API) interface, commonly known as "up-front or in-source CID." Note that little chromatographic separation is achieved with the fast run time. However, the single quadrupole mass spectrometer provides sufficient selectivity to separate each compound with quantitative accuracy. The qualifier ions provide additional selectivity to assure confidence in the determination of each compound. With liquid-liquid extraction of the urine samples, sufficient clean up is achieved for the analysis. Even though fast chromatography is used, there is sufficient retention for each of the analytes to be moved from the void of the column.

For complete screening of all categories of drugs of abuse, both ESI and APCI (atmospheric pressure chemical ionization) must be employed. An example of a complete group of DOA compounds best analyzed by APCI is the benzodiazepines. Some of the compounds in this category do respond well to ESI, but others do not. All do respond well to APCI. In this method, a short C18 column with gradient conditions using a mix of methanol, formic acid, and ammonium acetate, provides the best results in a relatively short time.

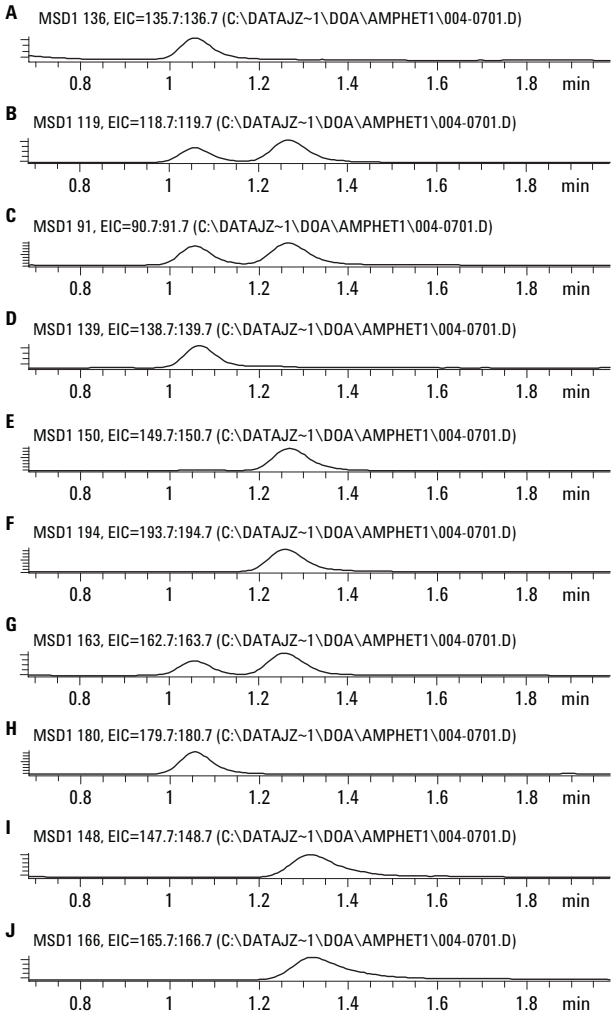

Figure 2. Selected ion monitoring (SIM) chromatograms of amphetamine screen at 100 ng/mL. The panels are A) amphetamine, B) amphetamine and methamphetamine qualifier ion, C) amphetamine and methamphetamine qualifier ion, D) ISTD, E) methamphetamine, F) MDMA, G) MDMA and MDA qualifier ion, H) MDA, I) ephedrine, and J) ephedrine qualifier ion.

Quality Assurance/Quality Control

To obtain the highest quality results, processes must be in place to assure that the instruments are running properly and that all extractions and analyses are done correctly. This assurance is provided by both internal (prepared in the laboratory) and external (obtained by sources outside the laboratory) quality control samples. Every batch of samples analyzed contains the internal quality control samples at concentrations covering the range of concern for the analytes. Table 1 shows typical results obtained for these QC samples. These QC results indicate not only the quality of the determination of each specific target compound, but their concentration as well.

Table 1. Internal QC Results for Some DOA

	QC50	QC100	QC500	QC2000
Amphetamine	48	97	517	2039
Methamphetamine	58	109	533	2049
MDMA	59	112	537	2029
MDA	50	98	517	2140
Ephedrine	56	107	512	2067
Morphine	53	105	526	1993
Codeine	53	108	511	2102
Methadone	53	104	507	2018
Benzoylecognine	59	112	503	2119
Phencyclidine (1/10)	5	10	50	204

Conclusions

The laboratory at St. Olav Hospital routinely analyzed 800,000 DOA urine samples and 30,000 TDM serum samples in 2003, using 24 LC/MS systems. This year the number is approaching 1 million analyses, taking into consideration that, for example, the amphetamine group (with five analytes) is only counted as a single analysis. This is also the case for the benzodiazepines (six analytes) as well as the opiates (four analytes). The accounting scheme is mainly for administrative reasons and for easier comparison with immunology-based laboratories. Twelve systems are set up using ESI and 12 systems using APCI and the instrument configurations are flexible enough to perform both DOA analysis and TDM analyses. The DOA screens include amphetamines, benzodiazepines, opiates, methadone, buprenorphine, PCP, cocaine and its metabolite, barbiturates, and others.

The procedures and the instrumentation briefly described here allow this laboratory to perform these analyses both in a cost-effective way and with the highest quality results possible. In addition, the laboratory uses 10 GC/MS instruments, both for confirmation and unknown compound identification. It should be emphasized that this combination of LC/MS and GC/MS instruments comprise a very strong analytical platform, especially for forensic and clinical toxicology. The laboratory performs several thousand analyses per year for these categories. Biological concentrations of specific drugs with secure identification and fast results is of great importance for the clinicians to make assessments toward the dose and clinical state of a patient. This analytical platform for these determinations requires a significant initial capital investment, but the return in both efficiency and medical quality of the results provide justifiable benefits.

Reference

1. Kolbjørn Zahlsen, Trond Aamo, and Jerry Zweigenbaum, “Therapeutic Drug Monitoring by LC/MSD - Clozapine, an Example”, Agilent Technologies, publication 5989-1267EN, www.agilent.com/chem

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