

While the Office of Science and Technology commissioned this review, the views are those of the authors, are independent of Government and do not constitute government policy.

## **Drug Testing**

David Cowan  
University of London

David Osselton  
Forensic Science Service

Steven Robinson  
Forensic Science Service

## **Executive summary**

This review of drug testing outlines the methods capable of being used to analyse recreational, psychiatric, cognition-enhancing or mood-altering drugs. The review discusses the techniques currently used for the analysis of psychoactive drugs and attempts to forecast methods that are likely to find a place in drug analysis within the next few years and beyond. The review attempts to predict how advances in analytical science, such as miniaturisation, linked with advances in electronics and increased instrumental sensitivity might develop over the coming years.

Psychoactive drugs are chemically and structurally diverse and encompass a vast range of chemical entities. This review therefore considers drug testing on the basis of the analytical techniques employed rather than by the chemical or pharmacological classification of individual compounds. Examples are provided of the types of drug that may be tested for using different methods. The review includes a brief survey of inexpensive techniques that are suitable for near-patient monitoring, as well as those that require more complex laboratory analysis.

The principal interest of the commissioning body is the detection of drugs in living subjects rather than the dead, and we have confined discussion to biological specimens that may be easily collected from living persons.

The relative merits and limitations of various specimen types are discussed in relation to each analytical technique.

The three most likely significant advances will almost certainly be miniaturisation, enabling point-of-care analysis, high-throughput screening using an enhanced spectrophotometric detection of antibody binding of drugs of interest, and enhanced separation techniques for the discrimination of complex mixtures.

## **Introduction**

The term 'psychoactive drug' is a loosely applied term used to describe substances that act on the central nervous system (CNS) to exert a psychological effect. There are many types of psychoactive drug which, for the purpose of this paper, are considered to fall into two categories: those that are widely used for medicinal purposes and although they have some abuse potential are largely not abused, and those that have a significant abuse potential.

The purpose of drug testing is to determine whether an individual has used any particular substance that might have affected their well-being or behaviour. The most common scenarios where testing is undertaken to detect psychoactive drug use include workplace drug testing, forensic drug testing (e.g. with drugs and driving) and compliance with treatment. Most drug testing is restricted to these areas and is not widely applied to the analysis of psychoactive drugs used in medical practice unless they pose a threat to the safety of the individual. In workplace drug testing and treatment compliance testing, the objective of the procedure is to extend the window of detection to determine whether or not the subject had been taking drugs over a period of days or possibly months prior to specimen collection. In forensic drug testing, interest is focused more on whether an individual may have been affected by a drug substance at the time of a particular event such as driving a car or at the time of an assault. In order to obtain the most appropriate information, therefore, different types of samples may be used, depending on the reason for testing.

Testing for drugs of abuse evolved in the United States during the 1960s within the armed forces and was used extensively to monitor members of the armed forces on active duty in the 1970s. All early drug testing and most testing today is based on urine samples. As technology and our understanding of how drugs behave in the body has increased, screening tests have become available that are capable of being used on a range of samples. The significant increase in the availability of psychoactive drugs in the mid- to late 1990s stimulated an increased interest in drug testing that resulted in the development of tests suitable for use within the workplace (point-of-care) as well as in the laboratory.

At the present time, testing for common drugs of abuse, whether in a clinical, forensic, sport or workplace setting, is usually undertaken as a two-step process. The first step normally involves a simple and inexpensive screening test followed by a more specific and definitive identification test (called a confirmatory analysis) that may also be quantitative. This review will outline the techniques currently in use for the screening and confirmation of commonly encountered psychoactive drugs and will then consider how advances in technology and miniaturisation might be applied over the coming years. Discussion will centre on the types of test available for the analysis of common drugs of abuse and medicinal drugs as it is not possible to predict with any certainty what new psychoactive drugs will be introduced during the forthcoming 20 or more years. It might reasonably be predicted, however, that as advances are made in the miniaturisation of electronics and microfluidics (see later), tests will be developed for near-patient use that will combine both screening and confirmation in a single test, and that the range of tests on offer will be extensive.

Most of the psychoactive drugs subject to misuse today have been used for centuries, including alcohol, opium derivatives, cannabis and cocaine, and it is envisaged that these, together with amphetamine and related compounds, will continue to be the most popular substances used for the foreseeable future. The most widely used psychoactive drugs (alcohol, heroin, morphine, cannabis and cocaine) are derived from natural sources or are easily synthesised from widely used industrial reagents in the case of synthetics such as amphetamines. These substances are easy to produce at relatively low cost and in large quantities. Until supplies become limited there is little incentive for users to switch to alternatives. Although methods for the synthesis of numerous synthetic psychoactive amines have been published (Shulgin and Shulgin 1991) few of these substances have attracted widespread use and few are regularly encountered other than amphetamine, methylamphetamine (MA), methylenedioxyamphetamine (MDA) and methylenedioxymethylamphetamine (MDMA i.e. ecstasy).

In addition to these common drugs of misuse, there are numerous psychoactive substances used medicinally that can affect performance, including the benzodiazepine anti-anxiety and sleep-inducing agents (e.g. diazepam, temazepam

and flunitrazepam), hypnotics (e.g. zopiclone, zolpidem and zaleplon) and antipsychotics and antidepressants (e.g. fluoxetine and amitriptyline).

The analytical methods employed today in drug testing for psychoactive substances are determined by the chemistry of the individual substances. As our understanding increases about how and where different drugs act in the body, drug testing may turn towards the measurement of endogenous markers within the body, for example, the monitoring of neurotransmitters and neurotransmitter binding sites rather than the drugs themselves. But for the next five to ten years at least, testing is likely to remain orientated towards the determination of the presence of psychoactive substances or their breakdown products. It is already possible to use electromagnetic scanning techniques to monitor changes to receptor binding in certain circumstances. But this technique is costly and therefore unlikely to be used routinely for drug-abuse screening.

The analytical techniques used for drug detection also depend on the chemical and physical properties of each substance or its breakdown products. Consequently, there is no universal test that can be applied to detect the presence of a drug and it is necessary to employ a range of different tests for different drugs.

### **Test matrices**

The chemistry of any particular drug and its biological breakdown products (metabolites) determines how it is distributed within the various body tissues and fluids and therefore the time-window during which it may be detected in the various body secretions and excretions. Drugs that are lipid (fat) soluble such as cannabinoids tend to accumulate in the body's fat depots and may subsequently be released over a period of days or weeks into the general circulation of the body, whereas those that are more water-soluble can be eliminated within a few hours. The time spans over which drugs can be detected in different tissues may be influenced by a number of factors. These include the state of health of an individual, presence of disease, age, the properties of the drug and its distribution within the body, the quantity of drug taken, whether drug intake involved single or chronic dosing, and the sensitivity of the analytical methods used.

Historically, urine has been collected for drug testing as it is easy to collect, may be collected in large volumes and is easy to analyse. However, as a consequence of improvements in analytical technology, blood, oral fluid, sweat and hair are now being used with increasing frequency, depending on the rationale behind the test. Urine, oral fluid, sweat and hair are favoured for use in many drug testing applications because they can be collected non-invasively by suitably trained lay people. The collection of blood, however, is regarded as invasive and requires the use of more highly trained collectors such as doctors, nurses and phlebotomists.

### **Urine**

Urine is the most widely used specimen employed for drug testing. Urine is produced in large quantities, is easy to collect and offers a wide window of detection, often facilitating the detection of drug use over a period of two to four days after the last dose was consumed. Table 1 summarises the approximate windows of detection for common drugs of abuse in urine. It should be noted that detection times can only be regarded as approximate since they are dependent on a number of factors, including the route by which the drug entered the body, the quantity taken, the frequency of use and the sensitivity and limits of detection of the assay.

Table 1: Approximate detection times for commonly used psychoactive drugs in urine  
(from Saunders and Barnes 2002)

<b>Drug</b>	<b>Approximate detection time in urine</b>
Amphetamines	1–2 days
Benzodiazepines	0.5–7 days (depends on half life)
Cannabinoids (single use)	Up to 3 days
Cannabinoids (moderate use – 4 times a week)	4 days 21 days or longer
Cannabinoids (chronic use)	
Cocaine metabolites	2–3 days
gamma-hydroxybutyrate (GHB)	0.5 day
Ketamine	2–4 days
Methadone	7–9 days (maintenance dosing)
Methamphetamine (single use)	1–3 days
Methamphetamine (heavy use)	3–5 days
Opiates	2 days
Phencyclidine (PCP)	8 days (approximately)

The advantages and disadvantages associated with urine as a matrix for drug testing are summarised in Table 2. In many ways urine is an ideal matrix for drug testing since it is relatively easy to collect and analyse and, depending on the elapsed time between drug use and specimen collection, drugs may be present in much greater quantities than in other matrices. But because of the ease with which chemicals may be added to specimens during collection that may interfere with the analysis, observed collection may be necessary to prevent specimen adulteration. Observed collection is costly, unpleasant for both the donor and collector, and is regarded as a potential infringement of personal privacy.

Table 2: Advantages and disadvantages of urine with respect to drug testing

<b>Advantages</b>	<b>Disadvantages</b>
Easy to collect	Observed collection seen as infringement of privacy
Drugs present in high concentrations relative to other specimens	Results difficult to interpret – not ideal for forensic testing
Large volumes available	Often contains metabolites and little unchanged parent drug
Provides a history of recent drug use between two days and several weeks	Easy to adulterate unless collection is observed
A wide range of drug substances can be detected	
Easy to analyse	
Inexpensive to analyse	
Suitable for workplace and compliance testing	
Untreated urine can be screened directly without the need for sample pre-treatment	
Point-of-care test kits are available for commonly abused drugs	

## **Blood**

Blood is primarily used in forensic and some areas of clinical testing and is not commonly employed when screening for drugs of abuse. The advantage of blood analysis is that it provides information about how much of the drug is present in the circulating blood and can give an indication of whether the donor of the specimen is likely to be affected by the drug at the time of collection. But blood collection requires specially trained personnel and can be costly, its collection is regarded as invasive, and current analytical methods require extensive sample pre-treatment prior to analysis, which is expensive.

The advantages and disadvantages of using blood for drug testing are summarised in Table 3. One of the potentially exciting consequences associated with the developments of miniaturisation combined with microfluidics is the possibility of being able to analyse blood without the requirement for complex and expensive sample preparation procedures.

Table 3: Advantages and disadvantages of blood drug testing.

<b>Advantages</b>	<b>Disadvantages</b>
Provides indication of recent use	Requires specially trained personnel to collect specimens
Difficult for donor to adulterate	Low concentrations of drugs present, hence relatively short window of detection
Results can be interpreted	Analysis requires specialised laboratory facilities
	Analysis is complex, therefore tends to be costly
	Current technologies not developed for point-of-care testing

The use of blood for general non-clinical drug testing may not develop owing to the issues surrounding its collection.

### **Oral fluid**

Recent advances in a technique known as lateral flow immunoassay (see later) have resulted in the development of point-of-care drug tests for the analysis of oral fluid, a term that covers fluid collected from the mouth, including saliva and other secretions, together with cellular and food debris. It was originally believed that the drugs secreted via the saliva reflected the concentration of drug present in the circulating blood and that collection of this matrix would enable results to be interpreted. More recently, it has been demonstrated that the concentrations of some drugs in oral fluid (e.g. mono-acetylmorphine, morphine, codeine, cocaine and benzoylecgonine) may be exceptionally high and would not be representative of concentrations in the circulating blood. The most likely explanation for this phenomenon is buccal absorption following smoking or snorting (inhaling the drug in powder form) since the effect is particularly noticeable in association with drugs that are smoked or snorted, such as heroin, cocaine and crack cocaine.

Oral fluid is particularly attractive as a matrix for drug testing as it is inexpensive, easy and non-intrusive to collect, requires no special facilities, is difficult to adulterate and does not require complex sample preparation prior to confirmatory testing. It can be reasonably predicted that methods for oral-fluid drug screening will predominate during the next five to ten years. The relative advantages and disadvantages associated

with oral fluid as a matrix for drug testing are summarised in Table 4. An example of a hand-held point-of-care oral-fluid test device is shown in Figure 1.

Figure 1: Hand-held point-of-care oral fluid test



Table 4: Advantages and disadvantages of oral-fluid drug testing

<b>Advantages</b>	<b>Disadvantages</b>
Easy to collect	Sample size limited – could be overcome by miniaturisation
Can be collected and tested on site with no intrusion of privacy	Drug concentrations may be low and subsequently difficult to analyse
Difficult to adulterate	Interpretation complex
Can detect a wide range of drug substances	Confirmatory analysis requires sensitive analytical facilities
Suitable for workplace, compliance and forensic testing	Cannabis derivatives and benzodiazepines do not pass readily from blood into saliva therefore potential sensitivity issues for general screening
Point-of care-test kits available for commonly abused drugs	
Indicates recent drug use for non-smoked drugs	
Nature of the matrix amenable for the development of new analytical techniques	

### **Sweat**

Many drugs are excreted in sweat and hence this matrix can be used as a source material for drug testing. Few manufacturers have devoted attention to the use of sweat as a routine specimen for drug testing, possibly because it is not easy to collect in large quantities for multiple drug screening and subsequent confirmatory analysis. Two types of test are currently available for testing drugs in sweat. One (see Figure 2)

comprises a small absorbent pad that is wiped across an area of skin such as the forehead of an individual to collect a small quantity of sweat that can be subsequently analysed by lateral flow immunoassay. The other system comprises a small absorbent patch enclosed within a tamper-evident adhesive cover that sticks to the skin rather like an Elastoplast dressing.

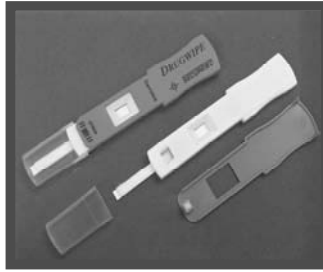


Figure 2: Point-of-care sweat testing device

The patch is applied after cleaning the skin by swabbing and left in place for a number of days, during which excreted drugs are absorbed onto the pad prior to collection. The sweat patch is subsequently sent to the laboratory for analysis. The principal use of sweat patches is for the screening of prison inmates in the US.

The major disadvantages of sweat testing are that only very small amounts of sweat are collected, thus limiting the extent of analysis with the present state of technology, and that skin can potentially become contaminated with drugs via environmental exposure prior to collection of the specimen. If a point-of-care device is used, prior swabbing of the skin to remove external contamination would also remove traces of drugs excreted in sweat. Although sweat testing is possible, these drawbacks have limited its take-up and we envisage that it will have only limited application in the future. The advantages and disadvantages associated with sweat testing are outlined in Table 5.

Table 5: Advantages and disadvantages of sweat drug testing

<b>Advantages</b>	<b>Disadvantages</b>
Non-invasive – easy to collect	Not widely used – currently available tests expensive if multidrug testing is required
Point-of-care tests available for common drugs of abuse	Small sample volumes collected – requires very sensitive and expensive analytical procedures
Sweat collection patches can be used to monitor drug use history	High possibility of external contamination with simple point-of-care tests
	Interpretation difficult

## **Hair**

The introduction of methods for the analysis of drugs in hair has developed rapidly in recent years because hair theoretically offers a non-intrusive way to monitor an individual's drug-taking history over several months or even years. Drugs enter the hair during its formation within the scalp and also by diffusion into the hair from the outside environment or via sweat and sebaceous secretions. Since drugs can enter hair following environmental exposure it is desirable that testing is designed to be able to detect drug metabolites, since these can only be formed within the body and allow the analyst to distinguish between drug use and external contamination. The advantages and disadvantages of hair for drug testing are summarised in Table 6.

Hair may be used to back up disputed urine or oral fluid tests, particularly where an individual claims not to be a regular drug user and that, for example, his or her drink was spiked without their knowledge. In these circumstances, with hair analysis it may be possible to distinguish one-time from chronic drug use. Drug binding and subsequent retention in hair may be influenced by the types of melanin present (black or blond hair) and is thus subject to potential genetic and racial bias. Hair testing is currently confined to laboratory testing and is relatively expensive. The main uses of hair testing at the present time are in child custody dispute cases where one parent may be alleging the other to be unsuitable to have custody of a child because he / she is a drug user or, in cases where a urine drug test has been disputed.

Table 6: Advantages and disadvantages of hair testing

<b>Advantages</b>	<b>Disadvantages</b>
Provides history of drug use – several months – limited mainly by length of hair available for analysis	Prone to external contamination – essential to be able to show presence of drug metabolites to exclude allegations of external contamination
Specimens stable for years	Expensive to analyse – requires specialist laboratory facilities
Wide range of drugs can be detected in hair	Not suitable for point-of-care testing
Non-invasive to collect	Need to allow hair to grow for approximately 10 days after drug use prior to collection of specimens
Particularly useful if subject is required to be tested days or weeks after last drug use	Limited interpretation
Can be used in exhumed or mummified bodies to determine drug exposure prior to death	Results may be affected by cosmetic treatments such as dying and perming
	Some indication of racial/genetic bias
	Head hair can be shaved in an attempt to foil the test

### **Analytical methods**

Drug detection techniques currently in use for the analysis of drugs in forensic, clinical, workplace and research environments are summarised in Table 7.

### **Routine techniques in use today**

Screening tests usually employ antibodies directed at a region of a test molecule, which is usually shared with other structurally related compounds. For example, the opiate drugs (heroin, morphine, codeine, dihydrocodeine) are structurally related to morphine, and an antibody raised against morphine will also react with codeine and heroin, among others. This is called cross-reactivity. A positive result in an opiate assay is therefore not specific enough to be of diagnostic value but differentiates between someone who has taken an opiate and someone who has not. An immunoassay is a flexible technique that can provide a rapid, inexpensive and convenient method to screen large numbers of samples in a variety of matrices and allow the differentiation of negative from positive specimens, thus preventing the need for further, more complicated and expensive processing or investigation.

**Table 7 Analytical techniques currently used for drug analysis in forensic, clinical and research applications in the UK**

Analytical technique <sup>1</sup>	Sensitivity	Scale of current use	Extent of likely future use	Matrix <sup>2</sup>	Suitable for point-of-care use	Applicable to a wide range of drugs
Immunoprocudures	++++	++++	++++	BUHSOP	Yes	+++
General spectrophotometry (ultraviolet, infrared, fluorescence, visible)	+	+++	++	P	Yes	++++
Raman	+ → +++	+	+++	SOP	Not yet	++
Nuclear magnetic resonance	+ → ++	+	+++	BUP	No	+++
Gas chromatography–mass spectrometry	+++	++++	+++	All	Not yet	++++
Liquid chromatography–mass spectrometry	++++	+	++++	BUHSOP	Not yet	++++
Isotope ratio mass spectrometry	+	+	++	UP	No	++
Thin-layer chromatography	+	++	++	BUOP	No	++++
Gas chromatography–nitrogen phosphorous detector	++	+++	+	BUSOP	No	++++
Gas chromatography–flame ionisation detector	+	+	+++	BBrUSOP	No	+++
Liquid chromatography–ultraviolet detector	+	++	+	BUP	No	++
Chemiluminescence	+++(+)	+	++	All	Not yet	++
Electrochemical detector	+++(+)	+	+	All	No	++
Capillary electrophoresis	+++(+)	+	+++	BUHSOP	Not yet	++++
Supercritical fluid chromatography	++	+	++	BUSOP	No	++
Time-of-flight mass spectrometry	++++	++	++++	P	Yes	++++

+ Low use/sensitivity    ++++ High use/sensitivity

Matrix: B = blood; Br = breath; H = hair; O = oral fluid; S = sweat; U = urine; P = powders and solids

<sup>1</sup> This is a generic table and does not differentiate between techniques that do or do not require sample preparation/purification

<sup>2</sup> Applicability at concentrations less than 1 part per million

The reaction between antibody and drug molecule is measured by using a label which emits a detectable signal. Labels used in immunoassay include radioisotopes (RIA), enzymes, chemiluminescent molecules, particles such as colloidal gold, latex beads, rare earth metals, and fluorescent molecules. Immunoassay formats vary from systems designed for rapid point-of-care testing of a single sample through to fully automated systems that can process thousands of samples per day.

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are the most frequently used drug-screening techniques, and are often used interchangeably to describe non-radioisotopic assays. EIA and ELISA have largely replaced RIA as the assay system of choice because radioisotopes have more health and safety constraints on their use than enzyme-based reactions.

Immunoassay is the preferred screening technique in many areas e.g. therapeutic drug monitoring, toxicological and workplace drug testing. The main difference between these applications is the degree of quantification required, and the selection of antibodies with the required cross-reactivity profiles. For example, drug-monitoring assays may require highly specific antibodies that are reactive only with a single chemical entity, whereas immunoassays used in toxicology or drug-abuse screening may be designed to be broadly cross-reactive with a whole drug group.

A further criterion for assay selection is whether the result is required immediately, or whether the collected sample can be sent to a laboratory for analysis. Most immunoassays are performed in the laboratory, either using plates with 96 or more reaction wells, allowing many samples to be analysed simultaneously, or on large autoanalysers (Figure 3), which can process many hundreds of samples per hour. Homogeneous immunoassays do not require the separation of bound and free fractions, and as a result have been automated successfully in a number of different systems. Examples of such assays include enzyme multiplied immunoassay technique (EMIT), cloned enzyme donor immunoassay (CEDIA), fluorescence polarisation immunoassay (FPIA) and kinetic interaction of microparticles in solution (KIMS).



Figure 3: Laboratory analyser

Point-of-care single-use cartridges are basically variants of a competitive ELISA assay run on a solid-phase strip rather than inside a well or tube. These tests employ a heterogeneous lateral flow mechanism using antibodies labelled with colloidal gold or other reactant particles which must be separated from the matrix before development of the result (Figures 4 and 5).

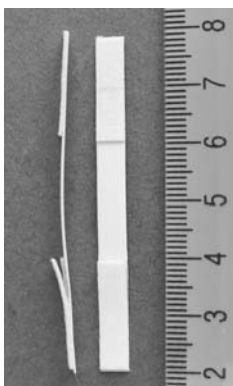


Figure 4: Lateral flow strip



Figure 5: Lateral flow strip mounted on urine collection containers

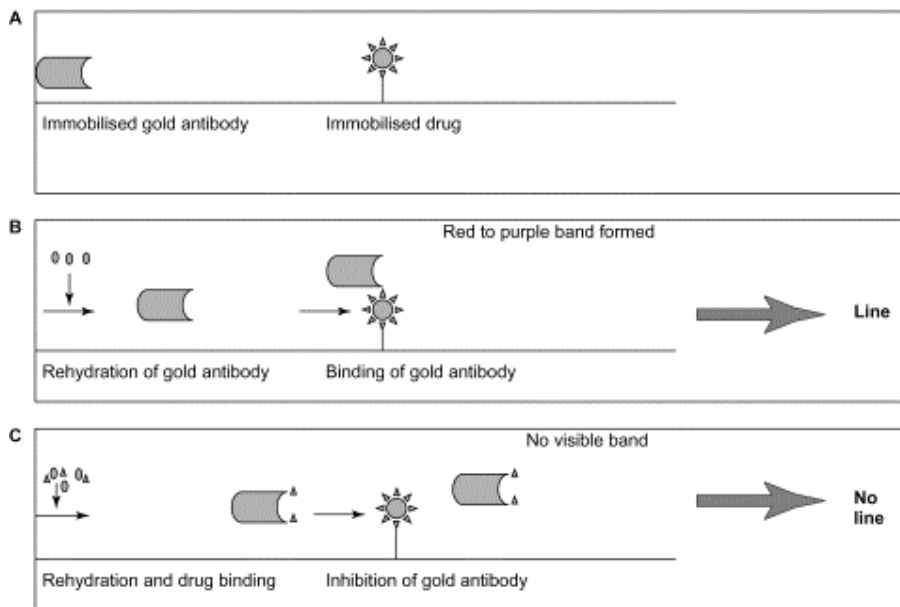


Figure 6: A competitive lateral flow immunoassay (Hand and Baldwin 2004, reproduced with permission of the Royal Pharmaceutical Society)

Figure 6 illustrates the process of ‘lateral flow’ screening for a single drug analyte. A drug-protein derivative is bound to a nitrocellulose membrane in a band at a defined position on the strip (Figure 6A). Antibody labelled with colloidal gold, coloured latex or an alternative visualisation probe is located in a pad that overlaps with the nitrocellulose membrane close to the point of the test strip where the sample is introduced. When urine, diluted oral fluid or some other liquid that might contain drugs is added to the pad, the labelled antibody migrates from the pad onto the cellulose membrane by capillary action. As the sample and rehydrated gold-labelled antibody

flow along the membrane, any drug in the sample binds to the labelled antibody and inhibits it from binding to the immobilised drug on the membrane (Figures 7B and 7C). Separation of bound and free drug continues with the movement of liquid along the membrane strip. Where a specimen contains no drug, the antibody binds to the immobilised drug forming a visible line. In specimens containing drugs, the antibody binding sites are taken up by the drug in the specimen resulting in no antibody binding to the immobilised drugs on the membrane. A positive result is therefore indicated by no coloured band being formed on the strip, whereas a line will be observed if the donor's specimen does not contain drugs. It is possible to add a number of drug-derivative bands in different positions along the length of the nitrocellulose membrane together. With suitable matching labelled antibodies this allows the analysis of multiple drugs to be undertaken simultaneously in a single test cartridge.

Most commercially available test strips are interpreted visually by the test operator. However, the criticism has been offered that visual interpretation may be subjective. A number of point-of-care test strips are now being developed by the manufacturers to be read using small portable readers based on digital imaging to facilitate more accurate and consistent results (Figure 1).

Lateral-flow immunoassay test strips based on colloidal gold-labelled antibodies are currently limited to performing up to a maximum of around five or six tests per strip. This is usually sufficient for a drug testing clinic or employer that is mainly interested in determining whether a subject has used commonly available drugs of abuse. The upconverting phosphor system developed by OraSure Technologies, however, has the potential to offer up to 15 or more tests per strip. This could in theory offer clinicians much more information, perhaps including tests to determine whether individuals were infected with HIV, hepatitis and other drug-related diseases.

The future of immunoassay-based screening tests for drugs other than those currently subject to misuse is limited by the availability of suitable antibodies, and by market forces. The cost involved in the development and production of suitable antibodies is high and unless substantial commercial sales are predicted, companies involved with the production of test kits are reluctant to risk investment. The result is that there is always a significant time lag between a new drug being encountered and the availability of an immunoassay test for the new drug. Past experience has shown that drug test manufacturers have been reluctant to develop screening tests for the European market alone as this was perceived to be too small to be commercially viable. Given the assumption, however, that a suitable commercial market is available, the next predicted development in immunoassay screening is likely to involve the miniaturisation of tests so that 20

or more assays can be performed on a microchip platform. At least one company is currently developing an automated laboratory-based microchip system capable of offering the rapid screening of several hundred urine or oral-fluid samples per day for common drugs of abuse. Within a few years, such a system could be miniaturised to facilitate portability. The limitations associated with immunoassay testing might stimulate the development of drug testing based on different technologies in the future as miniaturisation is developed further.

### **The fuel cell**

Fuel cell technology has been in use since the 1980s for measuring the amount of volatile substances, and in particular alcohol, in breath. Because fuel cells are small and relatively inexpensive, they are ideally suited for use in small portable or hand-held screening devices and have found widespread application for use by police officers at the roadside and also in workplace drug testing. It is now possible to link alcohol-detecting fuel cells to electronic locks that can only be opened when an alcohol-free specimen of breath has been supplied. Such devices have been proposed to control entry onto the bridge of a ship or to prevent drivers from being able to start their car engines if they have more than a predetermined concentration of alcohol in their breath. In principle this offers a means of preventing an intoxicated person from driving while under the influence of alcohol, but is not totally secure and has not been widely implemented at the time of writing. The use of devices as a means of harm reduction is still rather limited. Fuel cell technology is particularly suitable for the analysis of volatile solvents and is unlikely to be applicable to the analysis of non-volatile substances or solid-dose formulations.

### **Mass spectrometry (MS)**

Any positive result from a screening test must be subjected to further, more specific, confirmatory analysis to identify and quantify the substance taken. The acknowledged 'gold standard' technique for drug identification is MS, coupled either to a gas chromatograph (GC-MS) or liquid chromatograph (LC-MS) to separate the compounds in a complex mixture. The mass spectrometer is a detector that produces a signal based on the structure of the analyte itself, and so is compound-specific. There are several types of mass spectrometer, but the most common for drug analysis use the quadrupole, ion trap and time-of-flight (TOF) mass analysers.

In some clinical and toxicological drug testing laboratories, LC-MS, or more specifically its powerful sibling technique LC-MS-MS, is being used to screen and confirm medicinal drugs and drugs of abuse. The cost of these systems can be prohibitive, but high-throughput applications make it more cost-effective. Mass spectrometry in its various configurations (e.g. GC-MS, or LC-MS, or TOF) is highly versatile and can be used to analyse common drugs of abuse and most drugs used in medical practice.

### **Liquid chromatography–mass spectrometry (LC-MS)**

This is one of many hyphenated techniques that combine the separation of components in complex mixtures such as biofluids with a sensitive means of detection and identification. In this case, the separation is facilitated by a flow of liquid under pressure through a short (10–20 cm length, 2–5 mm diameter) metal column packed with a silica-based medium. The components in the mixture will ideally interact with the medium to differing degrees and so will separate spatially. As the individual components leave the column, they pass into the mass spectrometer, where controlled fragmentation occurs. Measurement of the abundance and masses of the fragments produced gives a very specific 'mass spectrum' of the compound, which is then searchable against standard reference libraries of spectra to assist in obtaining an absolute identification of the compound.

The separation in this case is usually done at room temperature, making it ideally suited to the analysis of thermally sensitive compounds. It is also suited to the analysis of large molecules (e.g. small peptides), parent drugs and their conjugates (especially glucuronides and sulphates), which can provide information on the time since ingestion or administration. One example is the simultaneous measurement of free (non-conjugated) morphine and its more water-soluble glucuronide metabolites. A further benefit of this technique is that sample pre-treatment and derivatisation are often unnecessary (unlike with GC-MS), which saves time and money.

LCMS is routinely used in toxicology, research and pharmaceutical laboratories, and many methods are available in the scientific literature for the separation, detection and quantification of many drugs and their metabolites. An enhancement of this technique, LCMS-MS, involves the addition of a second fragmentation facility and a second mass spectrometer. This can be used to generate structural information on individual fragments produced from the first fragmentation. These 'daughter' mass spectra provide additional discrimination where structurally similar compounds produce the same mass fragments. The use of MS-MS also improves sensitivity and is suitable for the analysis of a wide range of drug compounds that exert psychoactive effects. There

are published methods which can screen a single sample extract for several compounds simultaneously using LCMS-MS, demonstrating its analytical capability and value. It is likely that LCMS-MS will soon replace GC-MS as the routine laboratory analytical tool for drug analysis. Modern LC columns are now being developed to provide greater resolution, or separating power, but require higher pressures to push the liquid through the columns. It is envisaged that these higher resolution systems will become routine in the next ten years.

### **Gas chromatography–mass spectrometry (GC-MS)**

This is probably the most commonly used analytical technique in all fields of drug testing. Often referred to as the ‘gold standard’, this technique effects separation using the flow of a gas (usually helium) to carry the vaporised sample through a long (15–60 metre length, 0.2–0.5 mm diameter) column whose inner surface is coated with a silica-based medium. The entire column is held in an oven, and heat is used to effect separation of the compounds from the medium. The sample must be soluble in a volatile organic solvent to allow vaporisation upon introduction into the gas flow, and must be thermally stable to withstand the high temperatures (up to 300 C) required to achieve separation.

In order to produce satisfactory separation and resolution, it is frequently necessary to perform sample clean-up (either liquid/liquid or solid-phase extraction) and then derivatisation, the addition of a chemical ‘cap’ to cover up potentially active functional groups. This adds to the analytical time, but the result is better chromatographic separation than LC because the derivative has a higher mass and so produces more discriminatory mass spectra. The mass spectrometer is the same as that used in LCMS, but there are very different methods of sample introduction to accommodate either a high pressure flow of liquid solvent, or helium gas.

Many toxicology laboratories will use a combination of LC- and GC-MS to enable the identification and quantification of any drug or metabolite known to have been ingested, or indicated from a preliminary screening test. Instrumentation is moderately expensive but, with automated sample introduction, large cost-effective batches can be processed unattended. As with LCMS, it is possible to add further fragmentation capability to give GCMS-MS, which has the same benefits of enhanced specificity and discrimination.

## **Nuclear magnetic resonance (NMR) spectroscopy**

This technique, like mass spectrometry, measures a physical characteristic of a molecule (or more precisely, the nuclei of its component atoms), producing specific information about the structure of the molecule. The property in question is 'spin', with an energy change due to the spin of an atomic nucleus being measurable in a strong magnetic field. It is especially useful for detecting molecules containing isotopes of carbon, hydrogen, nitrogen, fluorine, silicon and phosphorus, which covers the vast majority of therapeutic and abused drugs and their metabolites. There are functional groups that do not produce NMR spectra, and so this technique is sometimes used to complement other analyses such as MS.

It is possible to couple a liquid chromatograph to the NMR instrument, allowing spectra to be obtained from complex biofluids, although low sensitivity is a problem for trace analysis. NMR spectroscopy is commonly used on pure compounds, solid dosage forms or where a high concentration of analyte is present. This instrumentation is not routinely used in analytical laboratories, but is used in the pharmaceutical industry to provide structural information capable of distinguishing polymorphic forms of the same drug.

A development of NMR technology is used in the diagnosis of disease states *in vivo*, with the aid of magnetic resonance imaging (MRI) scanning instruments. This points towards the future possibility of non-invasive drug detection using the same or similar techniques. We predict that this technique will be increasingly applied to the analysis of psychoactive drugs and their metabolites at their site of action in the body (body scanning) and to study their interactions with receptors.

## **Capillary electrophoresis (CE)**

This is purely a separation technique and must be coupled to a suitable detector to produce a measurable response. Instead of using a flow of gas or liquid to transport the sample through a separation medium, the innate electrical charge on the drug molecule is used to separate it from other molecules of differing size and charge. In classic electrophoresis, the sample is introduced into a channel, usually in a gel, and a strong electric field is applied that effects separation based on size as well as charge, small molecules travelling further and faster than heavy ones.

Traditionally, this technique has been used to separate high molecular weight molecules such as proteins and DNA fragments, but smaller molecules can also be separated in this way. In principle, any molecule which is naturally charged or can be made charged in a buffered solution can be analysed using CE. The discovery that the same principles could be applied to molecules

travelling through a narrow silica capillary, and produce more focused results, has led to considerable interest in CE for drug analysis. CE offers the ability to analyse most types of psychoactive drug and their metabolites including peptides, but suffers from the fact that it requires a very sensitive device to detect the small concentration of components submitted to this technique.

CE is seen as complementary to LC, but with the advantage of utilising smaller sample volumes (~10 µl). More analytes are detectable than in LC because negatively charged anions, positively charged cations and non-charged neutrals can be detected simultaneously. Although it is less sensitive than LC, CE lends itself to miniaturisation, and the potential for portable (or even hand-held) devices is evident. Instrument costs are similar to those for LC, but CE is much cheaper to run, with much lower solvent requirements.

Possible detectors include ultra-violet (UV) absorption, laser-induced fluorescence or even MS. CE as an analytical technique is becoming more popular, many drugs and their metabolites have been analysed by CE and the number of published methods is growing, but it has not become widespread or routine in many laboratories because of the popularity of LC.

### **Thin-layer chromatography (TLC)**

TLC is probably the oldest analytical technique still in use today, although its use is very much diminished with the advent of GC and LC techniques.

Separation is effected by the flow of solvent up a silica-coated plate standing in the solvent (or mobile phase) causing the migration of components in the same direction, much like LC only in one dimension. Once the solvent has reached the top of the plate, the run has finished and a visualisation reagent must be added to show up the position and quantity of the components.

There are no general methods for drugs as a class, but there are a large number of methods for individual drugs defined by their therapeutic or chemical categories. Most use colour development as the detection method, while some rely on induced UV fluorescence, for example, for LSD and Psilocin.

TLC methods are most effective for the low-cost analysis of a large number of samples (e.g. drug screening in biological fluids and tissues, and in herbal preparations), for example, the Toxi-lab TLC system, which is widely used in clinical toxicology laboratories for qualitative screening of tissue and fluid samples.

### **Supercritical fluid chromatography (SFC)**

This is another separation technique that must be coupled to an appropriate detector. It uses carbon dioxide at relatively high temperature and very high pressure, producing the supercritical fluid, and this is the carrier medium in the column. This technique is not widely used in drug testing or toxicology, although it offers the potential for analysing a wide range of psychoactive drugs.

### **Electrochemical detection (ECD)**

This is a very sensitive LC detector which measures the current produced by the electrolytic oxidation or reduction of analytes at the surface of an electrode. These detectors are quite sensitive (down to  $10^{-15}$  mole) and also quite selective. Two types of detector are available. The coulometric detector has a large electrode surface at which the electrochemical reaction is taken to completion. The amperometric detector has a small electrode with a low degree of conversion. Despite the difference in conversion rate, in practice these two types have approximately the same sensitivity. Samples for ECD must be electrically conductive, and this is accomplished by the addition of inert electrolytes. These detectors are difficult to maintain, and so do not lend themselves to routine analyses and are not used in high-throughput drug testing laboratories.

### **Emerging technologies**

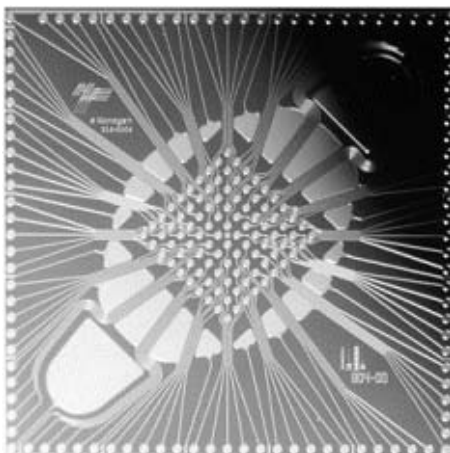
#### **Miniaturisation**

It is likely that there will be a large increase in miniature instrumentation that does not require skilled operators available for use at point of care, by the roadside, or in the office (Tudos, Besselink et al. 2001). These systems rely on a variety of physico-chemical principles that are currently being refined. A recent example uses a disposable potentiometric sensor (Gracheva, Livingstone et al. 2004). The speed of development and hence implementation of these systems will depend on the level of investment, which is relatively large since innovation in this field involves an interdisciplinary approach including physicists, analytical scientists and engineers. Commercial investment is limited because the market is insufficiently large. Typical examples of the emerging miniature devices are shown in Figures 8 and 9. The NanoChip Microelectronic Array (Figure 9) is the inner component of the NanoChip Cartridge shown in Figure 8 and is a 100-site electronically powered microarray fluidic device for DNA and RNA analysis. Nanometre-sized low-dimensional semiconductors known as quantum dots (QDs) are also being investigated for use in drug and poison detection. One recent application of this technology has been in the development of a sensitive test for cyanide.

Figure 8: The NanoChip Cartridge (Sanchez-Felix 2004. Reproduced with permission of the Royal Pharmaceutical Society.)



Figure 9: NanoChip Microelectronic Array (Sanchez-Felix 2004. Reproduced with permission of the Royal Pharmaceutical Society.)



### **Time-of-flight (TOF) mass spectrometry**

TOF mass spectrometry provides an example where mass spectrometry is already beginning to be miniaturised to provide portable testing devices for the detection of solid-dosage forms of drugs (e.g. tablets, powders and drugs on banknotes) and explosives. Advances in this field have received considerable attention as a response to the increase in drug trafficking (e.g. their use by Customs and Excise) and the potential threat from terrorism, as they can be used to screen for explosives at airports and sea terminals. TOF technology will play an increasing role in the laboratory for the analysis of a wide range of psychoactive drugs in biological fluids and tissues.

### **Isotope ratio mass spectrometry (IRMS)**

Although most psychoactive substances are foreign to the human body and their presence in body fluids is evidence of administration, some substances are virtually identical to those produced naturally in the body. Examples of these include testosterone, misused by sports competitors at least partly because of its behavioural effect, and gamma-hydroxybutyrate (GHB) allegedly used in drug-facilitated sexual assaults. Since the source of these administered 'pseudo-endogenous' substances is different from the naturally produced endogenous material, a subtle difference in their natural isotope composition is often present. The technique of IRMS has been used to detect sports cheats misusing testosterone, and work is underway to use the technique to provide evidence of GHB use. Unfortunately, the technique has relatively limited sensitivity at present, requiring about 20 ng or more at the point of analysis, but is likely to improve by at least one order of magnitude over the next ten years. The main use of this technique in the near future (two to eight years) will be to distinguish between endogenous and exogenous forms of the same substance in a biological specimen.

### **Coated microsphere technology**

This technique is suitable for the quantitative measurement of up to 100 different analytes in each sample and generally relies on immunoprocudures. Thus, any substance for which suitable antibodies have been prepared should be amenable to analysis using this technique. The principle of operation is by the use of polystyrene microspheres (5.6 micron diameter in xMAP<sup>®</sup> technology, Luminex Corporation 2001) that have been internally dyed with two spectrally distinct (red and infra-red) fluorophores in appropriate precise proportions, enabling the production of up to 100 distinct sets of microspherical beads. Each of the bead sets may be coated with the appropriate antibodies to capture the analytes of interest, with a third fluorophore acting as a reporter of antibody binding. Multiple sets of the appropriately coated beads may be placed in 96-well microtitre plates and the samples of interest added to each well. The contents of each well may then be sampled with a suitable analyser (e.g. Luminex 100). Using a system of microfluidics, the beads in the sampled microtitre plate are sequentially excited via two different wavelength laser beams to identify each microsphere (in order to determine which antibody is present) and also to detect the presence of any reporter dye captured during the assay. With suitable automation such as using the Gilson 215 Multiprobe<sup>™</sup> fluidics system, up to 32,000 wells per day have been analysed with, in theory, up to 320,000 different measurements of analytes. This technique has been proven for proteins and DNA. To date, it has not been fully explored for small molecule analysis but it has been used to measure the thyroid hormone T4

(Bellisario, Colinas et al. 2000; Bellisario, Colinas et al. 2000; Lukacs, Mordac et al. 2003; Pass 2003).

### **Chemiluminescent nitrogen detector (CLND)**

The chemiluminescent nitrogen detector (CLND) is specific for nitrogen-containing compounds, although it can also be set up to detect sulphur containing compounds. It is based on the chemiluminescent gas-phase reaction between ozone and nitric oxide which produces nitrogen dioxide formed in an electronically excited state. Relaxation of this excited state gives rise to light emission of 600–900 nm. The intensity of the emission is proportional to the mass flow of the nitrogen dioxide through the detector. The technique has been successfully coupled with gas chromatography, liquid chromatography (Taylor, Jia et al. 2002) and SFC (Shi, Taylor et al. 1997). The total nitrogen content can be determined quantitatively whatever the state of the nitrogen (except for diatomic nitrogen N<sub>2</sub>) and most drugs of abuse contain at least one nitrogen atom. Sensitivity is of the order of 5 picomolar. Since SFC shows great promise for the separation of optical isomers of many substances, it is likely to be useful for the ready distinction of certain drugs of abuse, especially amphetamine, that are marketed legally in a single isomer (dexamphetamine), whereas much of the substance sold on the black market contains both forms of the molecule. Similarly, dextromethorphan is sold in cough mixtures and is not a controlled drug whereas levorphanol is controlled under the Misuse of Drugs Act. Since levorphanol might need to be distinguished from dextrorphanol (the N-demethylated metabolite of dextromethorphan), SFC with CLND is likely to be a technique of the future.

### **Surface plasmon resonance (SPR)** (Turbadar 1959; Kretschmann and Raether 1968; Otto 1968)

This is a detection technique that allows the quantitative estimation of one or more analytes concurrently within one assay, relying on the use of suitable immunoprocures. Suitable antibodies are bound onto a thin metal (typically gold) surface that is coated onto a quartz prism. Total internal reflection occurs when light travelling in the quartz, which has a greater refractive index than at the interface, being in an aqueous solution, arrives at an angle of incidence above a critical angle. However, the light may be maximally coupled into the metal surface through the prism in such a way that, at the appropriate incident angle, the reflectivity is minimised. The incident light then feeds plasmons (the collective resonance of electrons near the surface of metal islands) (Chalmers and Griffiths 2002) in the metal and an enhanced evanescent wave is produced. Biospecific interactions occurring on the metal layer may be observed using this phenomenon since the interaction will change the solute concentration and hence its refractive

index. Thus, suitable antibodies may be bound to the metal surface and the concentration of substance bound to the antibody quantified. No tagging or chemical derivatisation is required. A linear correlation between resonance angle shift and protein surface concentration has been shown (Stenberg, Persson et al. 1991). The technique is extremely sensitive (Kooyman, Kolkman et al. 1988) and currently achieving quantitative sensitivities as low as  $2 \times 10^9$  molecules i.e. femtomoles of molecules of molecular weight of about 1,000. At least one further order of magnitude of sensitivity is anticipated. The technique can readily be adapted for multi-sample parallel analysis, for example, by using a beam spreader to focus the beam of light on a line of samples and using a charge-coupled device to measure multiple reflectivity responses simultaneously. More than 100 parallel channels could be analysed simultaneously in the near future (Plant and Silin 2004).

(<http://www.cstl.nist.gov/biotech/biomat/Projects/pharmaceuticals.html>)

### **Surface-enhanced resonance Raman spectroscopy**

Surface plasmons can also enhance Raman scattering. In contrast to surface-enhanced Raman spectroscopy, this allows the use of a smooth metal or single-crystal metal surface. Thus, in a similar manner to SPR, the interactions of biomolecules at the surface may be observed.

### **Total internal reflectance fluorescence (TIRF)**

Total internal reflection occurs when light travelling in a medium of higher refractive index such as quartz reaches an interface with a medium of lower refractive index such as an aqueous solution at an angle of incidence above a critical angle. Although the fully reflected light does not lose any net energy across the interface, an electrical field wave known as an evanescent field wave is produced that extends beyond the interface surface into the medium of lower refractive index typically to a depth of about half of the wavelength of the light (Kroger, Jung et al. 2002; Ruckstuhl, Rankl et al. 2003; Tedeschi, Domenici et al. 2003; Willard, Proll et al. 2003; Jennissen and Zumbrink 2004; Ohkawa, Okuno et al. 2004; Sapsford, Shubin et al. 2004).

The technique may be applied to fluorescent or fluorescently labelled compounds of interest. Only fluorophores adsorbed or otherwise in intimate contact with the interface will be excited and hence fluoresce, whereas those in bulk solution will not. The surface may be made biologically active, for example, with appropriate antibodies to substances of interest, so that fluorescently labelled compounds may be trapped at the surface. Since the excitation light is totally reflected away from the interface by total internal reflection, discrimination of the fluorescence signal from the excitation light is relatively effective, and so very good sensitivity and low limits of detection

are achievable. The technique is non-destructive and rapid and more than 10,000 times more sensitive than other biosensor systems based on SPR.

### **The future of drug testing**

It is predicted that new developments in analytical drug testing will be driven by commercial forces dictated by the potential market size. Although many of the technologies discussed above have the potential to be developed for drug testing, development will be dependent on market demand and manufacturers are unlikely to invest in the high cost of research and development without a clear indication for market needs. So it is not possible to predict how fast, if at all, some of the techniques discussed in this review will be developed for drug testing. It is envisaged that emphasis will be directed towards producing more specific and reliable point-of-care tests to enable employers, those involved in law enforcement and clinic-based practitioners to determine whether an individual may have used drugs.

Oral fluid offers many potential advantages over urine and is becoming a method of choice in certain areas. Technical advances should make a strong impact in developing the scope for screening drugs in oral fluid still further within the next five to ten years.

The application of pharmacogenomics and our ability to prescribe drugs based on knowledge of an individual's genetic profile could well become a reality within five years, and some of this knowledge will have applicability to interpretative toxicology. Following the completion of the human genome project, microarray tests are currently being developed that will enable genetic variations between individuals to be determined. Such tests will, for example, enable physicians to analyse genes encoding drug-metabolising enzymes of the cytochrome P450 complex.

Variations in these genes influence the metabolism of several drugs including antidepressants, psychotics, beta blockers, analgesics and some anti-cancer compounds. By enabling physicians to access information concerning an individual's genetic make-up, it should be possible to help prevent harmful drug interactions and to assure that drugs are used optimally. Near-patient testing should be developed in this area within the next five to ten years. Detailed discussion of this is outside the scope of this review and will be dealt with by other authors working within the Foresight programme.

Most drugs interact with specific sites (receptors) in the body e.g. in the brain. Receptors may be isolated to provide the basis for drug assays depending on the specific interaction that occurs

between drug and receptor. This use of receptor assays should be one of the most exciting innovations for the future. The use of DNA technology with yeast cells has enabled the production of specific receptors. For example, the use of glucocorticosteroid receptor assays has already been exploited. The significant benefit of this approach should be that it will be effective for most of the yet undiscovered psychoactive drugs. Other future techniques are likely to include the use of neural networks to deal with the complex analytical data produced by mass spectrometers and other instruments to simplify the detection of substances from a complex biological background. This approach is likely to become more important if metabolomics<sup>3</sup> is used to indicate that a psychoactive substance has been used.

### **Conclusions**

The three most likely significant advances will almost certainly be miniaturisation, enabling point-of-care analysis; high-throughput screening using enhanced spectrophotometric detection of antibody binding of drugs of interest; and enhanced separation techniques for the discrimination of complex mixtures.

The futuristic concept of a scenario where a simple probing device is placed on an area of the body may not, after all, be so far-fetched that it could not become a reality for the detection of common drugs within the next 25 years.

---

<sup>3</sup> Metabolomics is the study of the small molecules, or metabolites, contained in a human cell, tissue or organ (including fluids) and involved in primary and intermediary metabolism.

## REFERENCES

- Bellisario, R., R. Colinas, et al. (2000). Simultaneous quantitation of thyroxine (T4) and thyrotropin (TSH) from newborn dried blood-spot specimens with a multiplexed fluorescent immunoassay. *American Journal of Human Genetics* **67**(4): 1557.
- Bellisario, R., R. J. Colinas, et al. (2000). Simultaneous measurement of thyroxine and thyrotropin from newborn dried blood-spot specimens using a multiplexed fluorescent microsphere immunoassay. *Clinical Chemistry* **46**(9): 1422–1424.
- Chalmers, J. M. and P. R. Griffiths, (eds) (2002). *Handbook of Vibrational Spectroscopy*. Chichester, John Wiley & Sons Ltd.
- Gracheva, S., C. Livingstone, et al. (2004). Development of a disposable potentiometric sensor for the near patient testing of plasma thiol concentrations. *Analytical Chemistry* **76**(13): 3833–3836.
- Hand, C. and D. Baldwin (2004). Immunoassays. *Clarke's Analysis of Drugs and Poisons*. A. C. Moffat, M. D. Osselton and B. Widdop, Pharmaceutical Press. **1**: 301–312.
- Jennissen, H. P. and T. Zumbrink (2004). A novel nanolayer biosensor principle. *Biosensors & Bioelectronics* **19**(9): 987–997.
- Kooyman, R. P. H., H. Kolkman, et al. (1988). Surface-Plasmon Resonance Immunosensors: Sensitivity Considerations. *Analytica Chimica Acta* **213**(1–2): 35–45.
- Kretschmann, E. and H. Raether (1968). Radiative decay of non-radiative surface plasmons excited by light. *Zeitschrift Für Naturforschung Part A: Astrophysik Physik und Physikalische Chemie A* **23**(12): 2135–&.
- Kroger, K., A. Jung, et al. (2002). Versatile biosensor surface based on peptide nucleic acid with label free and total internal reflection fluorescence detection for quantification of endocrine disruptors. *Analytica Chimica Acta* **469**(1): 37–48.
- Lukacs, Z., C. Mordac, et al. (2003). Use of microsphere immunoassay for simplified multianalyte screening of thyrotropin and thyroxine in dried blood spots from newborns. *Clinical Chemistry* **49**(2): 335–336.
- Luminex Corporation (2001). High-throughput screening on the Luminex 100™ System: rapid, efficient, accurate. *Technical Bulletin*. **2005**: xMAP Technical Bulletins.
- Ohkawa, J., T. Okuno, et al. (2004). Single-molecule observation under isotropic evanescent wave by advanced TIRF microscopy. *Biophysical Journal* **86**(1): 601A–601A.
- Otto, A. (1968). A New Method for Exciting Nonradiant Plasma Surface Vibration. *Physica Status Solidi* **26**(2): K99–&.

- Pass, K. A. (2003). Commentary on: Use of microsphere immunoassay for simplified multianalyte screening of thyrotropin and thyroxine in dried blood spots from newborns. *Clinical Chemistry* **49**(2): 336–336.
- Plant, A. L. and V. Silin (2004). High-throughput screening of pharmaceuticals, NIST.
- Ruckstuhl, T., M. Rankl, et al. (2003). Highly sensitive biosensing using a supercritical angle fluorescence (SAF) instrument. *Biosensors & Bioelectronics* **18**(9): 1193–1199.
- Sanchez-Felix, M. (2004). Emerging Techniques. *Clarke's Analysis of Drugs and Poisons*. A. C. Moffat, M. D. Osselton and B. Widdop, Pharmaceutical Press. **1**: 550–564.
- Sapsford, K. E., Y. S. Shubin, et al. (2004). Fluorescence-based array biosensors for detection of biohazards. *Journal of Applied Microbiology* **96**(1): 47–58.
- Saunders, J. and P. Barnes (2002). *Identification and Treatment of Pharmaceutical and Illicit Drug Problems: A General Practitioners Guide*. Brisbane, ADTRU: 44.
- Shi, H., L. T. Taylor, et al. (1997). Chemiluminescence nitrogen detection for packed-column supercritical fluid chromatography with methanol modified carbon dioxide. *Journal of Chromatography A* **757**(1–2): 183–191.
- Shulgin, A. and A. Shulgin (1991). *Pihkal: A Chemical Love Story*. CA, Transform Press.
- Spiehler, V. (2004). Drugs in Saliva. *Clarke's Analysis of Drugs and Poisons*. A. C. Moffat, M. D. Osselton and B. Widdop, Pharmaceutical Press. **1**: 109–123.
- Stenberg, E., B. Persson, et al. (1991). Quantitative-determination of surface concentration of protein with surface-plasmon resonance using radiolabeled proteins. *Journal of Colloid and Interface Science* **143**(2): 513–526.
- Taylor, E. W., W. P. Jia, et al. (2002). Accelerating the drug optimization process: Identification, structure elucidation, and quantification of in vivo metabolites using stable isotopes with LC/MSn and the chemi luminescent nitrogen detector. *Analytical Chemistry* **74**(13): 3232–3238.
- Tedeschi, L., C. Domenici, et al. (2003). Antibody immobilisation on fibre optic TIRF sensors. *Biosensors & Bioelectronics* **19**(2): 85–93.
- Tudos, A. J., G. A. J. Besselink, et al. (2001). Trends in miniaturized total analysis systems for point-of-care testing in clinical chemistry. *Lab on a Chip* **1**(2): 83–95.
- Turbadar, T. (1959). Complete Absorption of Light by Thin Metal Films. *Proceedings of the Physical Society of London* **73**(469): 40–44.
- Willard, D., G. Proll, et al. (2003). New and versatile optical-immunoassay instrumentation for water monitoring. *Environmental Science and Pollution Research* **10**(3): 188–191.