Current trends in modern pharmaceutical analysis for drug discovery

Hwee-Ling Koh, Wai-Ping Yau, Pei-Shi Ong and Akhil Hegde

Traditionally, pharmaceutical analysis referred to the chemical analysis of drug molecules. However, over the years, modern pharmaceutical analysis has evolved beyond this to encompass combination techniques, high-throughput technologies, chemometrics, microdosing studies, miniaturization and nanotechnology. These analytical advances are now being employed in all stages of drug discovery and the focus of this review will be on how these technologies are being employed within this process. With new, improved and evolving technologies, as well as new applications for existing technology, the search for new drugs for the prevention and treatment of human diseases continues.

Hwee-Ling Koh*
Wai-Ping Yau
Pei-Shi Ong
Akhil Hegde
Department of Pharmacy
Faculty of Science
National University of Singapore
18 Science Drive 4
117543 Singapore
*e-mail: phakohhl@nus.edu.sg

▼ In this new millennium, the pharmaceutical industry faces new opportunities created by the completion of the Human Genome Project and the increased emphasis on genomics and proteomics in drug discovery. Pathology might now be elucidated by understanding processes at the protein level [1].

Technological advances have focused drug discovery effort towards the search for drugs directed at molecular targets or pathways believed to have a causal role in the disease [2]. Pharmacotherapy is undergoing a paradigm shift as genomics evolves into an important component of diagnosis and treatment. The ability to analyze the entire genome has also led to the identification of more potential drug targets. Small protein drugs are fast becoming an important and rapidly growing segment of the prescription drug market. In addition, recombinant protein drugs are becoming an even more important resource for the physician.

Currently, drug discovery efforts are being revolutionized by high-throughput technologies, combinatorial chemistry, genomics, proteomics, informatics and miniaturization. Successful drug discovery and development depends upon close interactions between various disciplines with inputs from biotechnology, biomedical engineering, proteomics and genomics *inter alia*. Today, pharmaceutical analysis is employed throughout the whole drug discovery and development process. It is used to provide accurate and precise data, not only supporting drug discovery and development but also post-market surveillance. This review illustrates some important developments in the field of modern pharmaceutical analysis. Figure 1 shows the applied fields of modern pharmaceutical analysis in drug discovery.

Current trends in modern pharmaceutical analysis

Combination techniques

In the past ten years, demands on analytical support for drug discovery have intensified. As a result, new technology is continually evolving to meet these challenges. In addition, the use of more established methodologies is being enhanced by incremental improvements in technology and protocol. Hyphenation (combination) of analytical techniques [3,4] is one such approach adopted by modern pharmaceutical analysts in meeting the needs of today's industry.

Combination of techniques was first successfully accomplished with gas chromatography–MS (GC–MS) in the 1960s. By combining various techniques, the modern pharmaceutical analyst hopes to achieve the goal of pooling the virtues of each technique to establish purity and identity. Frequently this also permits the analysis of smaller sample volumes more quickly and provides more information

1359-6446/03/$ – see front matter PII: S1359-6446(03)02846-0

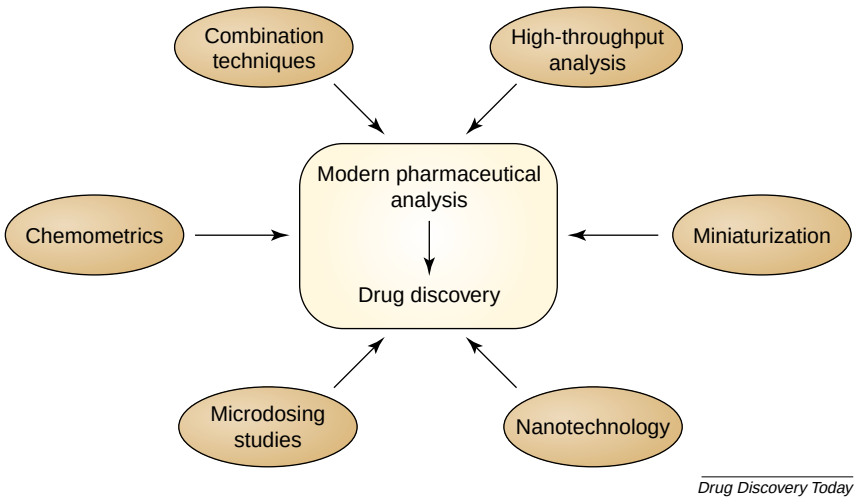

Figure 1. Applied fields of modern pharmaceutical analysis in drug discovery.

content [3]. Combining disparate techniques is frequently hampered by instrumental limitations. For MS, some of these limitations can be overcome by advances in ionization technology, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) [5] and/or the development of new probes. Box 1 summarizes some of the instruments that have been successfully combined.

Currently, MS combined with different chromatographic methods, provides some of the most powerful techniques available for pharmaceutical analysis. With its capability of speed, sensitivity and high-throughput, MS has evolved to be a mainstay of drug discovery [5]. In particular, LC–MS has been one of the most prominent and valuable techniques for the analysis of pharmaceuticals [6] and has been applied to drug metabolism studies (both *in vitro* and *in vivo*), high-throughput analysis of drugs and metabolites, analysis and identification of impurities and degradation products in pharmaceuticals, and analysis of chiral impurities [6].

Over the past 15 years, MS has resulted in remarkable progress in the fields of biomedical and biological research, in particular drug discovery and proteomics [1]. The ability to apply MS to these areas has been brought about principally by the development of ESI and MALDI. These two soft ionization methods have enabled the ionization of large, polar and thermolabile biomolecules (including proteins, peptides, nucleic acids and highly polar drug metabolites). This has been invaluable for protein and peptide analysis and drug metabolism and disposition studies during drug development [1]. In addition to advances in ionization techniques made over the past two decades, new linear or 2D-quadrupole ion-trap technology has proven to be another highly significant advancement in MS [7,8]. Compared with 3D ion-trap, 2D-quadrupole ion-trap has the advantage of improved trapping efficiency and increased ion capacity [7]. In addition, all the standard triple-quadrupole MS scan modes (e.g. precursor ion, neutral loss scans, multiple reaction product monitoring) are also available [8]. 2D-quadrupole ion-trap MS is a potentially promising analytical tool for metabolism studies and proteomics in today's drug discovery process.

Combined HPLC–NMR spectroscopy is another rapidly growing technology, enabling the rapid and detailed structural characterization of complex mixtures [9]. HPLC–NMR, as well as HPLC–NMR integrated with MS (HPLC–NMR–MS), have been applied to drug discovery, especially in the separation and structural elucidation of drug impurities, reaction mixtures, degradation products, *in vitro* and *in vivo* metabolites, and combinatorial library samples [9]. NMR coupled with other analytical techniques has the intrinsic advantage of providing structural and dynamic details derived from NMR, as well as the high resolution and sensitivity provided by the other coupled techniques [10,11].

High-throughput analysis

High-throughput analysis (HTA) is methodology aimed at the rapid analysis of large numbers of compounds. This field has been expedited by the requirement to provide analytical support for multiple drug targets emerging from the field of molecular biology, human genetics and functional genomics. Further drivers for development have been in the support for the analysis of large compound libraries arising from parallel and combinatorial chemistry, as well as economic pressure to reduce time-to-market for new drug candidates [12]. The ability to characterize and analyze large numbers of compounds in a high-throughput mode has thus become an integral component of modern drug discovery over the past decade.

HTA is having an increasingly important role in early stage drug development, providing qualitative and quantitative characterization of compound libraries and bioanalytical support for preclinical and clinical ADME studies [12]. This facilitates early elimination of unsuitable compounds [13–15], which could reduce the attrition rates of candidate drugs later in clinical development, hopefully

reducing development costs – currently estimated at US$800 million [16] – and time.

HTA techniques currently support three main areas: structural, purity and quantitative determination [17]. The current trend is to continually interpose automated techniques, in particular combination techniques, for HTA applications. Table 1 [17,18] summarizes some of the current analytical techniques being applied in the pharmaceutical industry for HTA.

Box 1. Examples of combination analytical techniques

GC–MS	HPLC–NMR
LC–MS	CE–NMR
LC/LC–TSP/MS/MS	LC–NMR–MS
HPLC–ESI–MS	GC–ECD/ICP–MS
HPLC–ICP–MS	HPIC–ICP–SFMS
CE–MS	CE–ICP–SFMS
CE–ESI–MS	CE–ICP–MS

Note: TSP and ESI are ionization techniques and are not separate techniques that can function on their own.

Abbreviations: CE, capillary electrophoresis; ECD, electron capture detector; ESI, electrospray ionization; ICP, inductively coupled plasma; HPIC, high-performance ion chromatography; SFMS, sector field mass spectrometry; TSP, thermospray.

Structural analysis

MS is currently the method of choice for compound characterization because of its selectivity, sensitivity, resolution, sample throughput and capability of sample identification and structure elucidation [12,18,19]. Some hyphenated MS techniques can be applied in a high-throughput mode. The success of MS in high-throughput analysis lies in its capability to easily and selectively separate target molecules from a complicated mixture, based on mass, without an extensive sample preparation procedure [1].

A multichannel device with an array of 96 electrospray tips for high-throughput ESI–MS gives a potential throughput of up to 720 samples per hour (5 s per sample) [19]. Flow injection analysis–MS (FIA–MS) with an eight-probe autosampler enables the characterization of combinatorial libraries in a single 96-well microtiter plate in 5 min [20]. In FIA systems, sample plugs are delivered by a liquid stream to an external detector (e.g. MS) for measurement [18]. In combination with MS, FIA can be used for structural analysis [17,18]. High throughput and ease of automation renders it one of the most useful MS-based techniques for characterization of compound libraries [1,20]. An automated MALDI–Fourier transform–MS (MALDI–FT–MS) can analyze 20 samples from a combinatorial library in one hour [21]. Comparisons between LC–ESI–MS/MS and MALDI–TOF–MS have been reported [22,23]. Combined LC–ESI–MS/MS analysis with MALDI–TOF–MS/MS analysis has been advocated [23,24].

Although data interpretation is more time-consuming compared to MS [17], NMR is also being developed as a key high-throughput technology by the use of automation and computerization for sample-changing, spectral parameter and field homogeneity optimization, as well as data collection and processing [18]. In the pharmaceutical industry, high-throughput NMR-based screening is emerging as a useful tool for high-throughput structural characterization of protein–ligand interactions, aiding the identification of compounds that bind to specific protein targets [11,25,26]. Direct injection–NMR (DI–NMR) enables the analysis of 88 combinatorial library samples stored in 96-well microtitre plates in 4–8 h [27]. Both FIA and DI analysis are capable of high throughput and automation. In FIA, a plug of sample is transported into the detector by a liquid stream, while DI analysis delivers a continuous sample

Table 1. Summary of high-throughput analytical techniques

Analytical technique	Relative throughput[a]	Structural analysis*	Purity analysis*	Quantitative analysis*
FIA/DI–MS	+++	+	+	+
MALDI–FT–MS	+	++	N/A	N/A
DI–NMR	++	+++	++	N/A
HPLC–UV	++	N/A	++	N/A
HPLC–UV/MS	++	+	++	N/A
HPLC–ELSD	++	N/A	++	++
HPLC–NMR	+	+++	++	+
FIA–CLND	+++	N/A	N/A	+
HPLC–CLND	++	N/A	N/A	+++
SFC–UV	++	N/A	++	N/A
SFC–MS	++	+	++	N/A
ESI–FT–ICR–MS	+	++	N/A	N/A

[a]An indication of relative throughput and applicability for structural, purity and quantitative analysis of the analytical techniques (+++ = highest).
Abbreviations: CLND, chemiluminescent nitrogen detection; DI, direct injection; ELSD, evaporative light-scattering detection; FIA, flow injection analysis; FT, Fourier Tansform; ICR, ion cyclotron resonance; MALDI, matrix-assisted laser desorption/ionization; SFC, supercritical fluid chromatography.
Adapted and reproduced with permission from references [17] and [18].

flow to the detector. In addition to combination with NMR, DI can also be coupled to MS for structural analysis [18].

The coupling of HPLC and NMR, as mentioned in the previous section, is one of the most powerful methods for structural elucidation. However, compared with other analytical techniques for compound characterization, NMR suffers the drawback of limited relative throughput [18]. Nevertheless, technological advances – such as the use of higher magnetic fields, digital signal processing and new probe design [18] – and further developments in HPLC–NMR techniques make it a potentially key tool for high-throughput structural characterization of compounds.

Purity analysis

HPLC is the key technique for determining purity and is capable of high throughput status via reduction in cycle times and development of generic analytical methods [17]. HPLC–MS is one of the most powerful high-throughput purity analysis methods available [6,12] and has been used in the analysis of chiral impurities present in diastereomeric peptide drugs [28].

In recent years, the application of gradient-packed column supercritical fluid chromatography (pcSFC) has been used for high-throughput purity analysis; analysis speed and speed of system recovery to initial conditions are up to 10-times faster than HPLC [17,29,30]. In SFC, the lower viscosity of the mobile phase (due to the use of supercritical fluids like CO_2) enables higher flow rates and thus faster analysis times [18]. For example, an ultra-fast analysis of dextromethorphan samples in a 96-well plate was achieved by pcSFC–MS/MS in ~10 min [31].

Quantitative analysis

HPLC–evaporative light scattering detection (ELSD) is an attractive quantitation tool that can be applied in a high-throughput mode [17]. ELSD is sensitive to the mass of an analyte, rather than its absorbance or ionization efficiency [18]. The advantage is that a more uniform response is obtained from small-molecule libraries with ELSD compared with UV absorbance, because the extinction coefficient of compounds within the library can vary widely [18]. However, ELSD suffers from the limitation of not being able to detect volatile, low melting-point compounds because detection depends on the determination of the mass of material remaining after solvent evaporation [17,18].

As mentioned previously, in addition to DI–NMR being used as a tool for structural analysis, it can also be applied to quantitative analysis through the use of appropriate internal standards. In the absence of suitable standards, chemiluminescent nitrogen detection (CLND) can be applied to FIA (FIA–CLND) or HPLC (HPLC–CLND) to provide accurate quantitation in a high-throughput mode [17]. The CLND is a nitrogen-selective, nitrogen-sensitive linear detector responsive and is related to the number of nitrogen atoms in the sample [17,18]. It is particularly well suited for quantitating combinatorial chemistry libraries because >90% of all compounds of interest contain at least one nitrogen atom [18]. FIA-CLND gives good linearity but it is limited by the presence of nitrogen-containing impurities and solvents that contribute to the 'total' nitrogen recorded [17]. HPLC–CLND, by contrast, is reported to be comparatively superior because nitrogen-free solvent systems can be used [17].

Chemometrics

The advent of combinatorial chemistry and high-throughput applications in drug discovery has dictated the development of computational methods that are capable of efficiently identifying molecules with acceptable biopharmaceutical properties early in the drug development process [32–34]. Thus, the chemometric approach for analysis has been adopted by the pharmaceutical industry. Chemometrics is a data analytical methodology based on multivariate mathematical modelling and analysis of all data (including both chemical and biological data) collectively [35,36]. Although chemometrics started in the 1970s, there is a renewed interest in this area for pharmaceutical analysis. In fact, the Food and Drug Administration (FDA; http://www.fda.gov) Subcommittee on Process Analytical Technologies (PAT) is actively involved in addressing the needs and requirements of the pharmaceutical industry on the use of chemometrics [36].

Chemometrics is currently being applied in processes of computer-aided drug discovery, such as chemoinformatics [34,36] and chemo-bioinformatics [37]. The use of chemometric techniques, mainly principal component analysis (PCA) and projections to latent structures (PLS), proves valuable in early-stage preclinical research as a fast computational and analytical tool for screening the increasing numbers of potential candidate drugs. Many studies in recent years have been conducted to develop chemometric models to aid in the accelerated drug discovery process (listed in Table 2 [32,33,38–40]).

With the increasing amount of data generated, the analysis of large datasets with numerous variables and observations by the methods of early chemometrics (mainly PCA and PLS) is becoming increasingly difficult [41]. Recently, extensions of PCA and PLS, known as Quilt-PCA and Quilt-PLS, have been proposed for the modelling and analysis of such huge datasets [41]. With the evolution of these new extended analytical approaches, chemometrics is likely to have a greater and more challenging role in the field of

Table 2. Some chemometric models developed for pharmaceutical analysis in drug research and development

Chemometric model developed	Applications of model	Refs
Structure–property model of drug transport processes	For screening and detection of compounds with potential drug transport problems	[32,33]
Structure-property model for membrane partitioning of oligopeptides	For development of peptidic drugs	[38]
Quantitative model for blood–brain barrier (BBB) permeation based on 3D molecular structure of drugs	For prediction of BBB permeability of drug candidates	[39]
Computational model for oral drug absorption	For screening drug candidates for potential problems in oral absorption	[40]

pharmaceutical analysis, with respect to the handling of multivariate data and larger datasets in the drug discovery process.

Recent ADME advances in drug discovery and development

Pharmacokinetics (PK) is one of the most crucial areas in evaluating whether promising clinical candidates can be developed into marketable drugs. So far, poor PK has accounted for up to 40% attrition in late stage drug development, which has in turn had substantial effect on R&D costs [15,42,43]. This is one of the major areas that accounts for the costly and high late-stage failure rates in drug development, therefore, the working paradigm in the pharmaceutical industry today has shifted to conducting PK investigations at a much earlier stage in drug discovery [42].

In addition to the application of chemometrics in early drug development to aid in the acceleration of screening potential drug candidates with acceptable biopharmaceutical properties, *in silico* technology has emerged as a new and promising tool for early ADME–Tox investigations [42]. Commercial ADME–Tox software is available to screen compounds in early stage drug development, however, more work needs to be performed to refine current *in silico* models because these predictive models are based on limited PK and toxicity datasets. Nevertheless, *in silico* approaches to ADME–Tox prediction for early drug development appear promising; it has been estimated that by 2006, 10% of pharmaceutical R&D expenditure will be on computer simulation and modelling, rising to 20% by 2016 [42]. Automated and high-throughput assays will go hand in hand with *in silico* predictions.

Another exciting novel innovation for ADME studies in early clinical drug development is human microdosing using two ultrasensitive analytical techniques, accelerator MS (AMS) and positron emission tomography (PET) [43]. In AMS, direct detection of radionuclides from trace-enriched ^{14}C-labelled drugs affords sensitivity in the attomole to zeptomole range (10^{-18}–10^{-21} mole). PET is an imaging technique that monitors the distribution of radiotracers (e.g. ^{11}C, ^{18}F) in the body, often for biochemical and metabolic studies. Human microdosing studies are also known as trace-dose human ADME screening studies and might be considered as 'human Phase 0' trials conducted by the administration of microdoses of novel drug candidates isotopically labelled for detection by AMS or PET [43]. A microdose is one-hundredth of the proposed pharmacological dose determined from animal and/or *in vitro* models, or a dose up to 100 μg, whichever is the smallest. Such human microdosing could facilitate early determination of PK and pharmacodynamic properties of investigational drug candidates [35]. The ability to conduct human microdosing studies of developmental drugs relies on the ultra-sensitivity of both AMS and PET in measuring drug concentrations in the low μg range. The microdose levels are often below the limits of detection of other analytical techniques, such as LC–MS [43]. These two sensitive analytical techniques, as well as NMR [44], hold great promise in early PK and pharmacodynamic studies in drug discovery and development. A workshop organized in November 2001 by Volunteers in Research and Testing (at Aston University, Birmingham, UK) concluded that early volunteer studies using microdoses should be introduced into the drug development process in a way that does not compromise volunteer safety or the scientific quality of the resulting safety data [44]. In a small human Phase I study involving AMS, nCi doses of a ^{14}C-labelled farnesyl transferase inhibitor were administered to four healthy male subjects [45]. The administered radioactive doses were at least 1000-times lower than that used for conventional radioactive studies. More information on single microdose studies can be found in a position paper on non-clinical safety studies to support clinical trials with a single microdose prepared by the Committee for Proprietary Medicinal Products (CPMP) under the European Agency for the Evaluation of Medicinal

Products (EMEA) (http:// www.emea.eu.int/pdfs/human/swp/259902en.pdf).

Miniaturization

The concept of shrinking a laboratory and cramming it onto a silicon chip the size of a postage stamp was conceived about a decade ago [46]. Over the intervening years, the field of miniaturization technology – or microfluidics – has made tremendous progress and has grown explosively. Similar trends can be observed in the pharmaceutical industry where the interest in miniaturization has been fuelled by the needs in the areas of high-throughput drug discovery and point-of-care testing [47]. Miniaturization of analytical and bioanalytical processes has become an important area in research today with particular focus on laboratory-on-a-chip technology [46,48,49].

Advantages of microminiature analytical systems include a reduction in manufacturing costs, ease of transport and shipping, and minimal space requirements in the laboratory. These microscale devices offer the possibilities of high-density testing and integration of multiple steps in complex analytical procedures [47,48]. Their diversity of application, sub-microliter consumption of reagents and sample, portability and ease of manipulation have made them all the more attractive.

Presently, high-density arrays of microreaction wells are gaining popularity in pharmaceutical analysis and drug discovery. Chemical analyses of samples from 96-well microtitre plates have been reported [27,31]. The 96-well format for microplates is currently being replaced by higher density microplates with up to 20,000 wells per plate [47], for HTS. It will be of interest to see whether the throughput for analysis can match that achieved for screening in years to come.

Another microminiature platform that could have potential for pharmaceutical analysis is the microchip [47,49]. These devices contain a range of microfluidic elements, such as microchannels and microchambers, designed for specific analytical tasks. A typical microchip measures ~1.5 cm × 1.5 cm and has a thickness of a few millimetres. The microchannels enable intra-chip transfer of fluid or electrophoretic separations and also function as posts and dams for separation and isolation. The microchambers of a chip are where reactions and assays can be performed. Some of the analytical techniques that can support the microformat for analyte detection include MS and GC [49].

Applications of microchips in pharmaceutical analysis have included on-chip separation of amphetamine and related compounds achirally or chirally using electrokinetic capillary chromatography [50]; analysis of active constituents from pharmaceutical preparations using 8-channel plastic microfluidic chips coupled with ESI–MS [51]; screening for barbiturates using microchips coupled to ESI–MS [52] and colorimetric determination of catecholamines in commercial pharmaceutical preparations following on-chip metaperiodate oxidation [53]. Catecholamine neurotransmitters (e.g. dopamine and L-dopa) can be detected using quartz microfluidic chips at sub-μg ml^{-1} levels at a throughput of >200 samples per hour using autosamplers [54].

The concept of Miniaturized-Total Analysis Systems (μ-TAS) [55,56] is being realized by advances in sample preparation, detection and data management. Here, all the component stages of a complete analysis including sample pre-treatment, chemical reactions, analytical separations, analyte detection, product isolation and data analysis are integrated and automated into a miniaturized format that would be particularly useful in today's high-throughput drug discovery process [55,56]. As the technology is developed further, the next challenge in miniaturization will be the development of nanotechnology and the fabrication of the nanochip.

Nanotechnology

In the broadest sense, nanotechnology is defined as products, processes and systems operating at nanometric tolerance with dimensions of less than ~1,000 nanometers [57]. The nanoworld is also associated with objects with dimensional limits of 100–300 nm [58]. The US National Science Foundation projected that the total worldwide market size for nanotechnology will reach >US$1 trillion annually by 2015 (http://www.wtec.org/loyola/nano/societalimpact/nanosi.pdf). In drug discovery, advances in nanotechnology are accelerating the identification and evaluation of new drug entities. Currently, it is being employed in various fields of pharmaceutical analysis, as summarized in Table 3 [59–62].

New analytical tools using nanostructures that are sensitive enough to detect individual molecules have been developed. Individual chemical species can now be detected and manipulated using nanodevices, nanoprobes and nanobiosensors. Integration of functional aspects of biological and non-biological systems has resulted in the development of specialized systems such as nanobiosensors. Enzymes, antibodies, receptors and also their molecular imprints, have been used as recognition elements for analytes in some of these biosensors [63]. Single-walled fullerene carbon nanotubes (SWNTs) have been used as probe tips for scanning probe microscopy imaging with molecular-level resolution [64].

An important pharmaceutical application of nanotechnology is in the use of nanotubes for the extraction and chiral separation of drug analytes [64]. Nanotubes can be

Table 3. Selected applications of nanotechnology

Applications	Comments	Refs
Single cell analysis	Qualitative analysis, identification of new ligands, analysis of minute volumes of analytes in drug discovery processes	[59]
Micromachined devices for analysis of drug molecules	Nanostructures, created by molecular imprinting with specific interaction sites and specific shape to recognize host molecules; used as biosensors, antibody, enzyme or receptor mimics in potential assays for drugs such as cortisol, theophylline, diazepam, morphine, (S)-propranolol	[60,61]
Hyphenated technology	Nanoscale LC–MS in the detection of DNA adducts	[59]
Miscellaneous	Chiral separation of drugs using bio-nanotube membranes with specific enantiomer recognition agent attached	[62]
	Sample stacking to improve the detection limits to low attomole levels	[59]

used to selectively sequester lipophilic drugs and analytes to separate them from aqueous solutions [64]. Enantioseparation of drugs can be achieved using antibody-based bio-nanotubes [62] as antibodies can have selectivity for single enantiomers [64]. Nanotubes are also used in stochastic sensors to detect analytes, such as metal ions and small organic molecules [65].

Microfluidic devices have automated and scaled-down separation and chemical reactions of analytes as discussed in the previous sections. It has been possible to introduce attoliters (10^{-18} l) of drug samples into separation capillaries for analysis [59]. Zeptomole (10^{-21} mol) detection limits for proteins can be achieved even through picoliter (10^{-12} l) sample volumes [59].

Nanomachines and nano-objects are fast becoming a new tool in the arsenal of pharmaceutical analysts. In the near future, these nanodevices could become commonplace in pharmaceutical analysis and drug discovery processes. Nanotechnology will have an increasingly important role in the development of commercial analytical and preparative tools. Nanodevices that are being realized or envisaged as biotechnological tools for the future include biosensors at nano-levels, nanosamplers, cell orienters, nanoanalyzers [58], nanoarrays and nanofluidics. In the years to come, nanotechnology is likely to lead to many exciting biomedical and biotechnological applications, including pharmaceutical analysis.

Concluding remarks and future prospects

During the past decade, there has been a focus on improving throughput for accelerated drug discovery by increasing instrument use and generating more data per system per unit time. The increase in data generation via high-throughput techniques made automated data processing and information management essential. Despite the acceleration in data processing that is brought about by advances in laboratory information management systems (LIMSs), further developments are still required to keep up with the ever-increasing amount of data generated. The application of nanotechnology in the fields of genomics and proteomics is likely to change the face of pharmaceutical research and development in drug discovery, accelerating advances in biomedicine.

As the pharmaceutical industry embraces new approaches, R&D costs might rise in the short term [16,66] as a result of the unprecedented number of novel drug targets. The cost of analysis could also escalate at certain points in the development timeline. Only time and experience will tell whether the new technologies and advances have delivered their promise of potential benefits. [2,67].

As researchers combine available analytical tools, develop new technologies and find new applications for existing technologies, the search for new drugs and vaccines continues. The cooperation of industry players, regulators, researchers, healthcare professionals, patients and the public is necessary to achieve a win–win situation for all.

Acknowledgement

We thank the referees and editor for their constructive and helpful comments.

References

1 Mano, N. and Goto, J. (2003) Biomedical and biological mass spectrometry. *Anal. Sci.* 19, 3–14
2 Gershell, L.J. and Atkins, J.H. (2003) A brief history of novel drug discovery technologies. *Nat. Rev. Drug Discov.* 2, 321–327
3 Sweedler, J.V. (2002) The continued evolution of hyphenated instruments. Anal. Bioanal. Chem. 373, 321–322
4 Albert, K. *et al.* (2002) Hyphenated techniques. *Anal. Bioanal. Chem.* 372, 25–26
5 Glish, G.L. and Vachet, R.W. (2003) The basics of mass spectrometry in the twenty-first century. *Nat. Rev. Drug Discov.* 2, 140–150
6 Lim, C-K. and Lord, G. (2002) Current developments in LC-MS for pharmaceutical analysis. *Biol. Pharm. Bull.* 25, 547–557
7 Schwartz, J.C. *et al.* (2002) A two-dimensional quadrupole ion trap mass spectrometer. *J. Am. Soc. Mass Spectrom.* 13, 659–669

8 Hager, J.W. (2002) A new linear ion trap mass spectrometer. *Rapid Commun. Mass Spectrom.* 16, 512–526
9 Peng, S.X. (2000) Hyphenated HPLC-NMR and its applications in drug discovery. *Biomed. Chromatogr.* 14, 430–441
10 Pochapsky, S.S. and Pochapsky, T.C. (2001) Nuclear magnetic resonance as a tool in drug discovery, metabolism and disposition. *Curr. Top. Med. Chem.* 1, 427–441
11 Pellecchia, M. *et al.* (2002) NMR in drug discovery. *Nat. Rev. Drug Discov.* 1, 211–219
12 Kyranos, J.N. *et al.* (2001) High-throughput high-performance liquid chromatography/mass spectrometry for modern drug discovery. *Curr. Opin. Biotechnol.* 12, 105–111
13 Avdeef, A. and Testa, B. (2002) Physicochemical profiling in drug research: a brief survey of the state-of-the-art of experimental techniques. *Cell. Mol. Life Sci.* 59, 1681–1689
14 Wenlock, M.C. *et al.* (2003) A comparison of physicochemical property profiles of development and marketed oral drugs. *J. Med. Chem.* 46, 1250–1256
15 Roberts, S.A. (2003) Drug metabolism and pharmacokinetics in drug discovery. *Curr. Opin. Drug Discov. Devel.* 6, 66–80
16 DiMasi, J.A. *et al.* (2003) The price of innovation: new estimates of drug development costs. *J. Health Econ.* 22, 151–185
17 Hughes, I. and Hunter, D. (2001) Techniques for analysis and purification in high-throughput chemistry. *Curr. Opin. Chem. Biol.* 5, 243–247
18 Kyranos, J.N. *et al.* (2001) High-throughput techniques for compound characterization and purification. *Curr. Opin. Drug Discov. Dev.* 4, 719–728
19 Liu, H. *et al.* (2000) Development of multichannel devices with an array of electrospray tips for high-throughput mass spectrometry. *Anal. Chem.* 72, 3303–3310
20 Wang, T. *et al.* (1998) A new ultra-high throughput method for characterizing combinatorial libraries incorporating a multiple probe autosampler coupled with flow injection mass spectrometry analysis. *Rapid Commun. Mass Spectrom.* 12, 1123–1129
21 Tutko, D.C. *et al.* (1998) Sequential mass spectrometry and MS^n analysis of combinatorial libraries by using automated matrix-assisted laser desorption/ionization Fourier transform mass spectrometry. *Rapid Commun. Mass Spectrom.* 12, 335–338
22 Escoubas, P. *et al.* (1999) A comparison of matrix assisted laser desorption/ionization time –of –flight and liquid chromatography electrospray ionization mass spectrometry methods for the analysis of crude tarantula venoms in the *Pterinochilus* group. *Rapid Commun. Mass Spectrom.* 13, 1861–1868
23 Person, M.D. *et al.* (2003) Comparative identification of prostanoid inducible proteins by LC-ESI-MS/MS and MALDI-TOF Mass Spectrometry. *Chem. Res. Toxicol.* 16, 757–767
24 Heller, M. *et al.* (2003) Trypsin catalyzed ^{16}O –to-^{18}O exchange for comparative proteomics: Tandem mass spectrometry comparison using MALDI-TOF, ESI-QTOF, and ESI-Ion trap mass spectrometers. *J. Am. Soc. Mass Spectrom.* 14, 704–718
25 Hajduk, P.J. and Burns, D.J. (2002) Integration of NMR and high-throughput screening. *Comb. Chem. High Throughput Screen.* 5, 613–621
26 Sem, D.S. and Pellecchia, M. (2001) NMR in the acceleration of drug discovery. *Curr. Opin. Drug Discov. Devel.* 4, 479–492
27 Keifer, P.A. *et al.* (2000) Direct-injection NMR (DI-NMR): A flow NMR technique for the analysis of combinatorial chemistry libraries. *J. Comb. Chem.* 2, 151–171
28 Ermer, J. and Vogel, M. (2000) Applications of hyphenated LC-MS techniques in pharmaceutical analysis. *Biomed. Chromatogr.* 14, 373–383
29 Berger, T.A. and Wilson, W.H. (2000) High speed screening of combinatorial libraries by gradient packed- column supercritical fluid chromatography. *J. Biochem. Biophys. Methods* 43, 77–85
30 Ventura, M.C. *et al.* (1999) Packed column supercritical fluid chromatography/mass spectrometry for high throughput analysis. *Anal. Chem.* 71, 2410–2416
31 Hoke, S.H. *et al.* (2001) Increasing bioanalytical throughput using pcSFC-MS/MS: 10 minutes per 96 well plate. *Anal. Chem.* 73, 3083–3088
32 Norinder, U. and Osterberg, T. (2001) Theoretical calculation and prediction of drug transport processes using simple parameters and partial least squares projections to latent structures (PLS) statistics. The use of electrotopological state indices. *J. Pharm. Sci.* 90, 1076–1085
33 Osterberg, T. and Norinder, U. (2001) Prediction of drug transport processes using simple parameters and PLS statistics. The use of ACD/logP and ACD/ChemSketch descriptors. *Eur. J. Pharm. Sci.* 12, 327–337
34 Blake, J.F. (2000) Chemoinformatics - predicting the physicochemical properties of 'drug-like' molecules. *Curr. Opin. Biotechnol.* 11, 104–107
35 Wold, S. (1991) Chemometrics, why, what and where to next? *J. Pharm. Biomed. Anal.* 9, 589–596
36 Lavine, B.K. and Workman, J. (2002) Chemometrics. *Anal. Chem.* 74, 2763–2770
37 Hansch, C. *et al.* (2002) Chem-bioinformatics: comparative QSAR at the interface between chemistry and biology. *Chem. Rev.* 102, 783–812
38 Alifrangis, L.H. *et al.* (2000) Structure-property model for membrane partitioning of oligopeptides. *J. Med. Chem.* 43, 103–113
39 Crivori, P. *et al.* (2000) Predicting blood-brain barrier permeation from three-dimensional molecular structure. *J. Med. Chem.* 43, 2204–2216
40 Oprea, T.I. and Gottfries, J. (1999) Toward minimalistic modeling of oral drug absorption. *J. Mol. Graph. Model.* 17, 261–274
41 Wold, S. (2002) New and old trends in chemometrics. How to deal with the increasing data volumes in R&D&P (research, development and production) – with examples from pharmaceutical research and process modeling. *J. Chemometrics* 16, 377–386
42 van de Waterbeemd, H. and Gifford, E. (2003) ADMET *in silico* modelling: towards prediction paradise? *Nat. Rev. Drug Discov.* 2, 192–204
43 Lappin, G. and Garner, R.C. (2003) Big physics, small doses: the use of AMS and PET in human microdosing of development drugs. *Nat. Rev. Drug Discov.* 2, 233–240
44 Combes, R.D. *et al.* (2003) Early microdose drug studies in human volunteers can minimise animal testing: Proceedings of a workshop organised by Volunteers in Research and Testing. *Eur. J. Pharm. Sci.* 19, 1–11
45 Garner, R.C. *et al.* (2002) Evaluation of accelerator mass spectrometry in a human mass balance and pharmacokinetic study – experience with 14C-labeled (R)-6-[amino(4-chlorophenyl)(1-methyl-1H-imidazol-5-yl)methyl]-4-(3-chlorophenyl)-1-methyl-2(1H)-quinolinone (R115777), a farnesyl transferase inhibitor. *Drug Metab. Dispos.* 30, 823–830
46 Knight, J. (2002) Microfluidics: honey I shrunk the lab. *Nature* 418, 474–475
47 Kricka, L.J. (1998) Miniaturization of analytical systems. *Clin. Chem.* 44, 2008–2014
48 de Mello, A.J. (2002) Miniaturization. *Anal. Bioanal. Chem.* 372, 12–13
49 Kricka, L.J. (2001) Microchips, microarrays, biochips and nanochips: personal laboratories for the 21st century. *Clin. Chim. Acta* 307, 219–223
50 Wallenborg, S.R. *et al.* (2000) On-chip chiral and achiral separation of amphetamine and related compounds labelled with 4-fluoro-7-nitrobenzofurazane. *Electrophoresis* 21, 3257–3263
51 Yuan, C.H. and Shieal, J. (2001) Sequential electrospray analysis using sharp-tip channels fabricated on a plastic chip. *Anal. Chem.* 73, 1080–1083
52 Jiang, Y. *et al.* (2001) Integrated plastic microfluidic devices with ESI-MS for drug screening and residue analysis. *Anal. Chem.* 73, 2048–2053
53 Sorouraddin, H.M. *et al.* (2001) Use of a thermal lens microscope in integrated catecholamine determination on a microchip. *Fresenius J. Anal. Chem.* 371, 91–96
54 Verpoorte, E. (2002) Microfluidic chips for clinical and forensic analysis. *Electrophoresis* 23, 677–712
55 Woelke, J. and Ullmann, D. (2001) Miniaturized HTS technologies - µHTS. *Drug Discov. Today* 6, 637–646

56 Jakeway, S.C. *et al.* (2000) Miniaturized total analysis systems for biological analysis. *Fresenius J. Anal. Chem.* 366, 525–539
57 Bogunia-Kubik, K. and Sugisaka, M. (2002) From molecular biology to nanotechnology and nanomedicine. *Biosystems* 65, 123–138
58 Curtis, A. and Wilkinson, C. (2001) Nanotechniques and approaches in biotechnology. *Trends Biotechnol.* 19, 97–101
59 Guetens, G. *et al.* (2000) Nanotechnology in bio/clinical analysis. *J. Chromatogr. B Biomed. Appl.* 739, 139–150
60 Laval, J.M. *et al.* (2000) Nanobiotechnology and its role in the development of new analytical devices. *Analyst* 125, 29–33
61 Ansell, R.J. *et al.* (1996) Towards artificial antibodies prepared by molecular imprinting. *Clin. Chem.* 42, 1506–1512
62 Lee, S.B. *et al.* (2002) Antibody based bio-nanotube membranes for enantiomeric drug separations. *Science* 296, 2198–2200
63 Mosbach, K. and Ramstrom, O. (1996) The emerging technique of molecular imprinting and its future impact on biotechnology. *Biotechnology* 14, 163–170
64 Martin, C.R. and Kohli, P. (2003) The emerging field of nanotube biotechnology. *Nat. Rev. Drug Discov.* 2, 29–37
65 Bayley, H. and Martin, C.R. (2000) Resistive-pulse sensing from microbes to molecules. *Chem. Rev.* 100, 2575–2594
66 Lehman Brothers and McKinsey & Co. (2001) The fruits of genomics: Drug pipelines face indigestion until the new biology ripens. Lehman Brothers, New York
67 Schmid, E.F. (2003) Trust me – I'm a doctor! *Drug Discov. Today* 8, 439–440