Mass spectrometry_Layout 1 27/09/2010 13:57 Page 73

Mass Spectrometry in Drug Discovery and Development

Mass Spectrometry is a mature technology predicated on a premise demonstrated almost a century ago. It is widely used in most scientific disciplines involving basic research or industrial endeavours, that require accurate and precise measurement of elemental and molecular components. More recently it has become one of the foundation technologies in high throughput omic analyses. Approximately 27 companies manufacture and supply a broad range of Mass Spectrometry equipment. Annual sales, service and ancillary efforts involving such instrumentation constitute a $3.3 billion global market. The use of Mass Spectrometry in the pharmaceutical sector associated with the Drug Discovery and Development process is rich and varied. Many of the initial efforts were associated with online high performance liquid chromatography-mass spectrometry in drug metabolism, pharmacokinetic and pharmacodynamic studies. There have been numerous innovative efforts to apply various mass spectrometric techniques in early drug discovery, preclinical and clinical development, as well as in Phase 0 studies using Accelerator Mass Spectrometry. Today there is a re-evaluation and refocusing on how to efficiently adopt, adapt and use modern Mass Spectrometry instrumentation in the Drug Discovery and Development process.

By Dr Stephen Naylor and Paul T. Babcock

Productivity in the pharmaceutical sector continues to undergo withering scrutiny[1-8]. In a recent supplement of ***Drug Discovery World (DDW Insights***, Summer 2010*)*, a panoply of pharmaceutical, scientific, technology and business experts opined on the necessity of improving drug safety and efficacy as well as significantly reducing costs associated with the drug discovery and development (DDD) process[9]. A number of these authors suggested specifically that an increasing role of technologies would enhance productivity and directly address critical DDD issues. However, Naylor and others have argued that the haphazard adoption and untrammeled use of technology has had limited impact on productivity and costs[3,10-12], and "…has been overhyped as a possible cure for the industry's productivity woes"[3]. This is due primarily to the poor understanding of new technology introduction into the DDD process. It is important to recognise that a complex array of factors must be considered in any technology adoption and includes the Technology

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 74

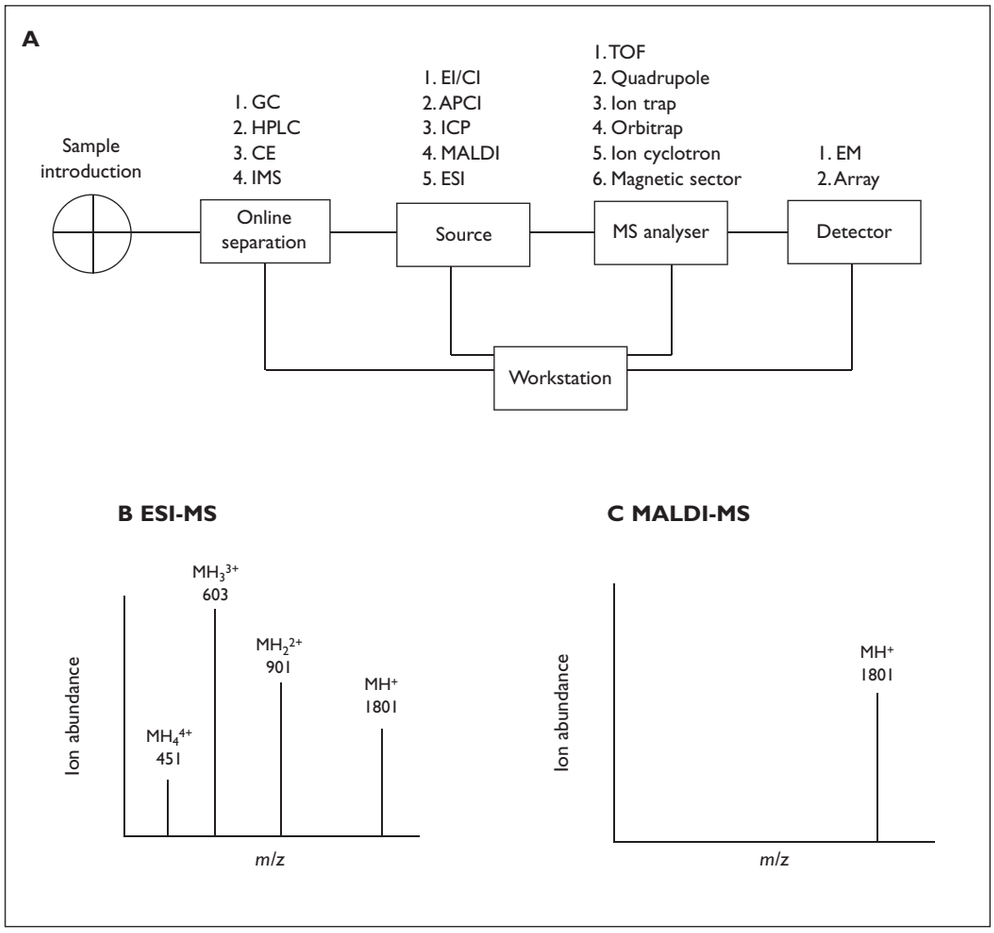

Figure 1
(A) Generic schematic of the component parts required in modern mass spectrometric analysis. (B) Representative example of an ESI mass spectrum of a single compound with MH^+=1801, highlighting the multiple charging that occurs in this ionisation process. (C) Representative example of a MALDI mass spectrum of the same compound with MH^+=1801

Development Cycle (TDC), Technology Hype Cycle (THC), and the Technology Assimilation and Innovation Adoption Curves (TAC and TIAC respectively). These processes have been described in detail elsewhere, and inadequate consideration of their consequences leads to poor integration and misuse of new technologies in the DDD process[3].

Cursory consideration of Mass Spectrometry (MS) suggests it should not be subject to the same vagaries as other 'new technologies' when utilised in the DDD process. MS is a mature analytical technology predicated on the ability to separate charged analytes (ions) based on their mass to charge (*m/z*) ratio, and was first demonstrated by J.J. Thompson in 1913[13]. The first commercially viable MS instrument was developed by A.J. Dempster in 1918[13]. In addition five Nobel Prizes have been awarded to J.J. Thompson (1906), F. Aston (1922), E.O. Lawrence (1939), jointly to H.G. Dehmelt and W. Paul (1989), and jointly to J.B. Fenn and K. Tanaka (2002) in areas pertaining to the development of MS over the past century[13]. Today it is widely used in basic research across a diverse array of disciplines as well as industries and specialty areas including food, environmental, forensic, toxicological and geochemical sciences as well as all aspects of the health and life sciences[14]. In particular MS is now the foundation technology of high throughput omic analyses for proteomics, metabolomics and to a lesser extent some aspects of genomics such as SNP determination[14].

There are numerous examples of MS facilitating the DDD process and historically they include pharmacokinetic and pharmacodynamic analyses as well as Phase I and II drug metabolism studies[15-18]. However the overall impact of MS in the pharmaceutical sector over the past 15 years has sometimes been uneven and unpredictable. An immediate question that arises is how can a mature, 100 year-old technology not be utilised in an efficient manner? In this paper we will attempt to address the issue as well as describe in some detail the role of MS in the DDD process.

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 75

Mass Spectrometry

Mass Spectrometry has emerged as a powerful analytical tool applied to the health life sciences and the pharmaceutical sector[14,15-18]. The essence of a modern MS platform consists of a sample introduction port and/or online separation device, a source region coupled to the mass spectrometer and a detection system, all under workstation control complete with software packages to assist in data acquisition and interpretation as shown in **Figure 1A**. More extensive reviews and details are available elsewhere[14,19] but for convenience, a glossary of common terms used in MS and discussed in this article are provided in the Glossary Box.

Online separation chromatography-MS

The use of direct sample introduction or online chromatographic-MS analysis is determined by the sample complexity. The analysis of complex biological mixtures encountered in the DDD process is greatly aided by additional stages of separation prior to analysis by MS (**Figure 1A**). There are a myriad of such online chromatography technologies but three are commonly used in the pharmaceutical sector and include Gas Chromatography (GC), Reversed Phase High Performance Liquid Chromatography (HPLC) or Capillary Electrophoresis (CE). Currently, a powerful new gas phase separation technology, Ion Mobility Spectrometry (IMS), is being used in the separation of complex biological-derived peptide mixtures. The development of IMS-MS has been pioneered by David Clemmer at Indiana University and Waters Corporation recently introduced the SYNAPT™ commercial MS instrument.

Gas Chromatography: GC-MS is used in conjunction with either Electron Impact (EI) or Chemical Ionisation (CI) as the primary ionisation source. Complex mixtures of organic molecules are separated in a capillary tube coated with a suitable hydrophobic material using a carrier gas as the mobile phase to transport the individual molecules through the capillary and into the mass spectrometer. The dynamic and individual interactions of molecules with the capillary surface induce separation as they traverse the capillary. The separated molecules are eluted directly into the source region where ionisation occurs followed by subsequent separation in the actual mass spectrometer.

HPLC: Reversed-phase high performance liquid chromatography-MS is routinely used in the pharmaceutical sector since it is well suited to separation of biological mixtures and can be readily interfaced to an Electrospray Ionisation (ESI) ion source. HPLC separates analytes based upon their hydrophobicity. The complex biological mixture is initially adsorbed, from an aqueous solution, in a narrow band at the beginning of a column packed with a stationary support. A liquid mobile phase is then pumped through the column, while the hydrophobic nature is increased by adding an organic solvent (typically acetonitrile). This causes peptides to differentially migrate through the column as a function of their hydrophobicity. When the HPLC is connected to MS, the MS continually analyses the HPLC effluent and detects the peptides as they elute from the HPLC column. Great strides in HPLC-MS have occurred over the past decade through miniaturisation of both the HPLC technique and ESI interfaces, since for every 50% reduction in column diameter, a four-fold increase in sensitivity occurs.

Capillary Electrophoresis: CE-MS actually consists of a family of CE techniques that include capillary zone electrophoresis (CZE), isotachophoresis (ITP), isoelectricfocusing (IEF), micellar electrokinetic chromatography (MEKC), affinity electrophoresis (ACE) and capillary electrochromatography (CEC). All have been used coupled to a mass spectrometer[20]. Analyte mixtures are separated in a capillary tube containing a conductive liquid medium subjected to a high voltage. Individual analytes are separated, to a first approximation based on their charge (or partial charge) to mass ratio and directly sprayed into the ESI source.

IMS: Ion Mobility Spectrometry is a separation technique where ions are subject to an applied electrical potential gradient in the presence of a neutral carrier gas such as argon. Analyte ions separate as a function of 'drift time' based on their ionic radius to charge of the ion. Since separations in the IMS drift tube take on the order of milliseconds and MS analysis occurs at a much faster rate, then it is possible to sample analyte ions from the IMS analysis in real time to obtain an IMS-MS spectrum rich in information content.

Basic principles of MS

A mass spectrometer is an analytical instrument that separates ions based on their *m/z* ratio and determines the molecular weight of elemental, chemical and biological compounds to a high degree of precision and accuracy (~10^{-3}-10^{-6}%) as well as sensitivity (detection of 10^{-9}-10^{-21} moles of sample required). A simple schematic is shown in

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 76

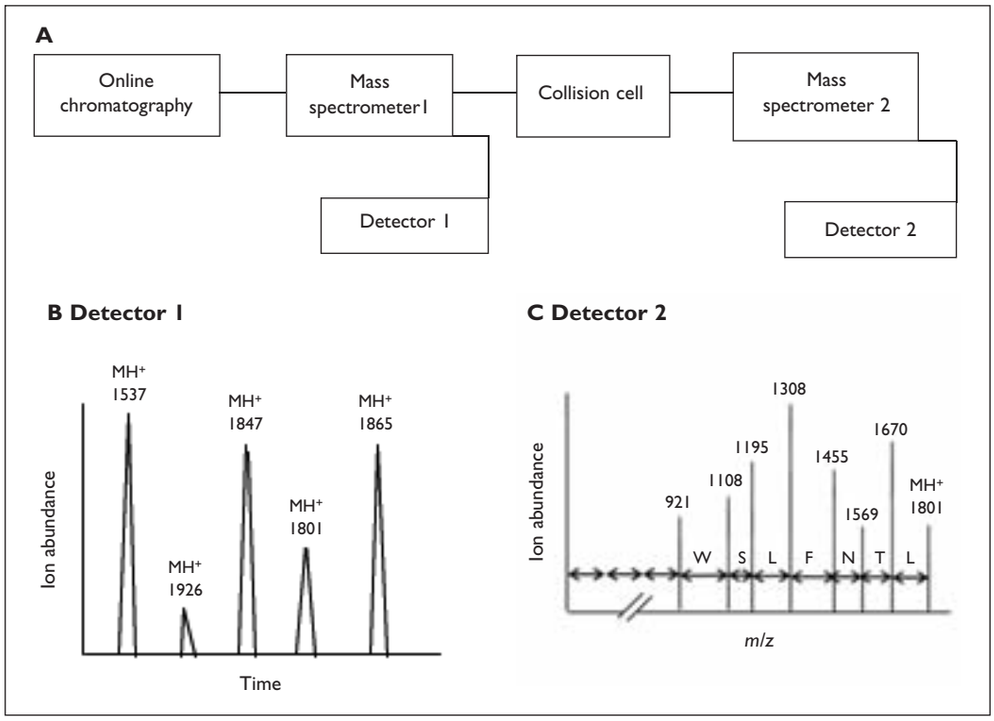

Figure 2
(A) Schematic of a typical set-up to conduct MS/MS analysis of peptides derived from a biological complex mixture. (B) Ion chromatogram of all individual peptides separated by both HPLC and then mass spectrometer 1, with detection at Detector 1. (C) Representative MS/MS spectrum of MH^+=1801, denoting sequence information, obtained at Detector 2

Figure 1A. A limitation of mass spectrometry is that compounds can only be analysed in the gas phase, either as negatively- or positively-charged ions. Hence the source region serves as both the sample inlet and ionisation chamber (**Figure 1A**). In the pharmaceutical sector commonly used ionisation techniques include EI, CI, Atmospheric Pressure Chemical ionisation (APCI) and Inductively Coupled Plasma (ICP) ionisation. These ionisation approaches are used to analyse small organic chemicals and metabolites (EI, CI and APCI) or elements and electrolytes (ICP).

More recently workers in pharmaceutical companies have focused primarily on using two common 'soft' ionisation techniques known as Electrospray Ionisation (ESI) and Matrix-Assisted Laser Desorption Ionisation (MALDI) for the analyses of biologically derived molecules. Soft ionisation refers to the ability to ionise and volatilise thermally labile compounds, such as peptides proteins, oligosaccharides, drug metabolites and other chemically fragile moieties, without inducing fragmentation or decomposition. The ESI process generates charged, micro-droplets containing analyte ions. Gentle evaporation of the droplets in the source region ultimately results in a charge-transfer from the water droplet surface to the analyte, leading to the creation of gas phase ions. Such ions are detected as a series of multiply charged ions (**Figure 1B**). In order to determine the molecular weight (M_r) of the compound, a simple algorithm 'transforms' this ion series into a single value M_r. In the case of MALDI, the analyte is mixed and co-crystallised with a photoactive organic acid matrix, which readily absorbs energy from laser irradiation. Hence, when the target containing analyte and matrix is placed in the source region and subjected to laser bombardment, analytes are projected into the gas phase, typically as singly charged ions (**Figure 1C**). Different ionisation methods can be used with MS analysers. However MALDI is most commonly associated with a time-of-flight (TOF) analyser (MALDI-TOF-MS). ESI is frequently coupled to a quadrupole, ion-trap, magnetic sector, quadrupole-TOF analyser, Fourier transform ion cyclotron resonance mass spectrometer (FTMS) or Orbitrap. In all cases, the MS analyser functions to separate the ions produced in the source region, based on their *m/z* ratio, which are then counted at a detection device, typically an electron multiplier or ion counting detector, as delineated in **Figure 1A**.

Structural information-tandem Mass Spectrometry or MS^n

Soft ionisation techniques such as ESI, or its various analogues such as nanospray-ESI or picospray-ESI, and MALDI do not induce any significant

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 78

fragmentation of compounds in the source region. Therefore, in order to acquire structural information on a biological compound of interest, such as a peptide sequence, it is necessary to induce fragmentation of the molecules/ions in the mass spectrometer. This can be achieved using tandem mass spectrometry (MS-MS), or multiples of MS (MS^n), and this is shown schematically in **Figure 2A**. For example, a complex mixture of peptides is individually ionised in the source region using ESI or MALDI. These peptides are then further separated, based on their *m/z* ratio, by Mass Spectrometer 1 and detected at Detector 1 (**Figure 2**). Subsequently, one peptide, eg MH^+=1801 is selected and subjected to fragmentation, and commonly referred to as collision-induced dissociation (CID) (**Figure 2B**). All the ions having an *m/z* 1801 are allowed into the collision cell, which is filled with an inert gas such as xenon or argon. The resulting collisions between ions and gas cause fragmentation of the peptide. These fragment ions (also known as product ions) are separated on their *m/z* values in Mass Spectrometer 2 and detected at Detector 2. For peptides the fragment ions represent loss of individual amino acids, and hence can provide valuable sequence/structural information as shown schematically in **Figure 2C**. It should be noted that there a number of methods now available to induce fragmentation and they include Electron Capture Dissociation (ECD), Electron Transfer Dissociation (ETD), Surface Induced Dissociation (SID), and Infrared Multiphoton Dissociation (IRMPD) and they are discussed in detail elsewhere[14,19].

Data

As shown in **Figure 1A**, data acquisition, representation and many aspects of data interpretation are under workstation control complete with a suite of software tools and some of the offerings provided by the MS instrument companies as detailed in **Table 3** discussed below.

Data output: The most common form of data output from a modern MS instrument is in the form of a mass spectrum schematically shown in **Figure 1B** or **1C**. The x-axis designates the *m/z* value of the ions detected and the y-axis indicates the relative ion abundance compared to the largest (base) peak. If an on-line chromatography-MS analysis is undertaken then the data representation can also be in the form of a mass chromatogram. In this case the x-axis is time (typically in minutes for GC, HPLC or CE and milliseconds for IMS) and the y-axis is still relative ion abundance. In addition there is now software commonly available to represent the data in three-dimensional plots where the x-axis is *m/z*, the y-axis is relative ion abundance and the z-axis is typically time.

Data analysis and interpretation: This broad and complex topic has received much attention and undergone massive development over the past decade. It is beyond the scope of this article, but the interested reader is referred to detailed reviews on the subject published elsewhere[14]. Suffice it to say that the over-interpretation of data and a poor understanding of the data that a MS instrument can produce has led to an over-extension of MS capability and this is discussed in more detail later.

Mass Spectrometry companies

The global market for MS instrumentation has grown rapidly over the past decade into a multibillion annual sales market. In 2009 global sales, instrument service contracts and ancillary services were approximately $3.3 billion[21]. The sector has continued strong growth prospects and is projected to experience annual growth of 8-10% through 2012. At least 27 different companies manufacture and sell a wide range of MS instrumentation. The global distribution breaks down in terms of numbers into 14 USA, nine European, three Asian (all Japan) and one Australian-based companies They range in size and market cap from the giant conglomerate Thermo-Fisher Scientific Inc (Waltham, MA, USA) with annual corporate revenues of $10.11 billion (2009) to Vitalea Sciences (Davis, CA, USA) which manufactures custom-built Accelerator-MS instruments. All these companies are listed in **Table 1** along with contact website information and the date the company was either founded or created.

Bullish sales and growth have led to a flurry of mergers and acquisitions (M&A) over the past decade in this vibrant MS market. This was preceded by much more modest M&A activity. For example, in 1989 Shimadzu (Kyoto, Japan) acquired one of the pioneers of modern mass spectrometry, Kratos Analytical, based in Manchester UK. Then in 1997 Waters Corporation (Milford, MA, USA) bought Micromass Ltd (Manchester, UK), another early MS leader, for $178 million in cash. In the 00s (2000 and beyond) several strategies have emerged as companies seek to either enter into or leverage existing MS capability. In the former case, Biorad (Hercules, CA, USA), a major reagent and instrument supplier for the life sciences, purchased Ciphergen's SELDI proteomics platform in 2006 for $20 million cash and

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 79

Table 1: Companies that manufacture and sell mass spectrometry instruments

COMPANY NAME	HEADQUARTERS	DATE FORMED	PRODUCT WEBSITE	ANNUAL REVENUE (2009)
Agilent Technologies Inc	Santa Clara, California, USA	1999	www.agilent.com	4,481 million
Austrailian Scientific Instruments Ltd	Fyshwick, Australia	1998	www.asi-pl.com	
Bergmann Messgeräte Entwicklung KG	Murnau, Germany	1991	www.bme-bergmann.de	
Biorad	Hercules, CA	1952	www.bio-rad.com	1,784.24 million
Bruker Corporation	Billerica, Massachusetts, USA	1980	www.bruker.com	1,114 million
CAMECA	Gennevilliers, France	1929	www.cameca.fr	
Comstock Inc.	Oak Ridge, Tennessee, USA	1979	www.comstockinc.com	
Danaher Corporation/ AB Sciex	Washington, DC, USA	1980s	www.danaher.com	1,150 million
GSG Analytical Instruments	Bruchsal, Germany	1982	www.gsg-analytical.com	
Hiden Analytical	Warrington, UK	N/A	www.hiden.co.uk	
Hitachi High-Tech	Tokyo, Japan	1910	www.hitachi.com	8,968,546 million (yen)
JEOL	Tokyo, Japan	1948	www.jeol.com	83,872 million (yen)
LECO	St Joesph, Michigan, USA	1936	www.leco.com	
KORE Technology	Cambridgeshire, UK	1992	www.kore.co.uk	
Monitor Instruments	Cheswick, PA, USA	1992	www.monitorinstruments.com	
MKS Instruments Inc	Andover, Massachusetts, USA	1961	www.mksinst.com	411.4 million
NU Instruments	North Wales, UK	1995	www.nu-ins.com	
PerkinElmer Inc	Waltham, Massachusetts, USA	1937	www.perkinelmer.com	1,812 million
Physical Electronics	Chanhassen, MN, USA	1969	www.phi.com	
SerCon	Cheshire,UK	N/A	www.sercongroup.com	
Shimadzu	Kyoto, Japan	1909	www.shimadzu.com	238.3 billion (yen)
Spectromat Massenspektrometer GmbH	Bremen, Germany	N/A	www.spectromat.de	
Stanford Research Systems	Sunnyvale, CA, USA	1980	www.thinksrs.com	
Thermo Fischer Scientific Inc	Waltham, Massachusetts, USA	2006	www.thermofisher.com	10,109.7 million
Thermolinear	Bremen, Germany	1998	www.thermolinear.de	
Vitalea Sciences	Davis, CA, USA	2003	www.vitaleascience.com	
Waters Corporation	Milford, Massachusetts, USA	1958	www.waters.com	1,575.1 million

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 80

$3 million equity. A good example of the latter situation was the acquisition of Ionspec by then Varian Corporation in 2006 for $17.3 million in cash and assumed debt. This was subsequently followed by the acquisition of Varian into Agilent Technologies (Santa Clara, CA, USA) in 2009 for $1.5 billion. As part of the deal and stipulated by the monopoly concerns, Varian also sold its ICP-MS and GC-MS line of instruments to Bruker Daltonics (Billerica, MA, USA) for an undisclosed sum. In the same year Applied Biosystems merged with the life sciences reagent behemoth Invitrogen to create a new entity, Life Technologies Inc (Carlsbad, CA, USA), in a deal worth $6.7 billion in cash and stock. As part of the deal structure, Life Technologies Inc sold the MDS Sciex MS instrument division to Danaher (Washington DC, USA) for $1.1 billion to create AB Sciex. All the M&A activity of the past decade in the MS instrumentation sector is summarised in **Table 2**.

The MS manufacturing companies listed in **Table 1** produce a diverse array of MS instruments designed to fulfill a variety of analytical demands. In addition they provide a plethora of software tools that enable facile data acquisition, processing, analysis and interpretation. All of this is captured and summarised in **Table 3**. Many of these companies produce instruments required for specific types of analysis including surface analysis and material sciences using Secondary Ion Mass

Table 2: Mergers & Acquisitions that have occurred in the past decade involving Mass Spectrometry companies

COMPANY ACQUIRED	COMPANY ACQUIRING	ACQUISITION YEAR	TYPE OF DEAL	RESULT
Dexter and Life Technologies	Invitrogen	2000	Cash and Stock 1.9 billion	Divisions of Invitrogen
Ionalytics Corporation	Thermo Electron Corporation	2005	N/A	N/A
Thermo Electron Corporation	Fisher Scientific International Inc	2006	Stock-for-Stock	Thermo Fisher Scientific Inc
IonSpec Corporation	Varian Inc	2006	Cash and Assumed Debt: 17.2 million	Integration into Varian's Product Lines
Ciphergen's Proteomics Section	Bio-Rad	2006	Cash: 20 million	
Equity: 3 million	Division of Bio-Rad			
Bruker Optics	Bruker BioSciences	2006	Cash and Stock: 135 million	Integration into Bruker BioSciences
SwissAnalytic Group	Thermo Fisher Scientific	2007	N/A	N/A
CAMECA SAS	AMETEK	2007	Cash: 112 million	Joined Electronic Instruments Group
Applied Biosystems	Invitrogen	2008	Cash and Stock: 6.7 billion	Companies merged forming Life Technologies
Applied Biosystems/MDS Sciex	Danaher	2009	Cash: 1.1 Billion (650 million to MDS/450 million to Life Technologies)	Creation of ABSciex
Varian	Agilent	2009	Cash: 1.5 Billion total	Division of Agilent
Varian ICP-MS; LabGC; GC-QQQ	Bruker Corporation	2010	N/A	N/A

Mass spectrometry_Layout 1 27/09/2010 13:57 Page 81

Table 3: Mass Spectrometry products and areas of specialisation offered by MS companies.
[a] The instrument applications are not meant to imply exclusive use in these areas only, but to indicate some of the primary areas of market focus

COMPANY	TYPES OF INSTRUMENTATION	SOFTWARE	INSTRUMENTATION APPLICATIONS
Agilent Technologies Inc	GC/MSD; LC/MS; ICP-MS	Deconvolution Reporting Software; Fiehn GC/MS Metabolomics RTL Library; Mass Profiler Professional Software; MassHunter BioConfirm; MassHunter Workstation; MSD Productivity ChemStation; MSD Security ChemStation	Drug Discovery; Drug Development; Security; Foods; Environmental
Australian Scientific Instruments Ltd./ANU Enterprise Pty Ltd	SIMS; HIP; ICP-MS	Datalogging using LabView; Neo Vista System Integrators Pty Ltd Software Package	Environmental; Surface Chemistry
Bergmann Messgeräte Entwicklung KG	EI-TOF; LILBID-TOF; Cluster-TOF; Linear-TOF	N/A	Surface Chemistry
Biorad	SIMS-TOF	Protein Chip Software	Biomarker Discovery
Bruker Corporation	Qq-FTMS; MALDI-TOF/TOF; LC-MALDI; LC-MS/MS; ITMS; ESI-TOF; ESI-Qq-TOF; UHR-TOF; ICP-MS; GC-MS	Compass; Compass Open Access; ProteinScape; BioTools; WARP-LC; ClinProTools; flexImaging; GenoTools; MetaboliteTools; Profile Analysis; Target Analysis; PolyTools; MALDI Biotyper	Pharma; Biomarker Discovery; Environmental; Foods; Forensics
CAMECA	SIMS	WinCurve; WinImage	Materials; Life Sciences
Comstock Inc.	TOF; MALDI; EI	LabView	Surface Analysis
Danaher Corporation/AB Sciex	Qqq-LC/MS/MS; Qq-TOF; MALDI TOF-TOF; QQQ	Analyst; BioAnalyst; ChemoView; Cliquid; DiscoveryQuant; LightSight; LipidView; MarkerView; Voyager; TissueView; MetabloitePilot; Metabloite ID; MRM Pilot; MIDAS; MRMPilot; MultiQuant; ProtienPilot; SimGlycan; 4000 Series Explorer; Data Explorer	Pharma; Biomarker Discovery; Environmental; Foods; Forensics
GSG Analytical Instruments	LC/MS/MS; IMS-TOF; PTR-MS; Q-MS	Analyst	Forensics; Environmental;
Hiden Analytical	SIMS; Q-MS	N/A	Surface Analysis; Chemical
Hitachi High-Tech	LC/MS; q-TOF; MS(n)-TOF	Analog to Digital Converter; BA	Pharma; Biomarker Discovery
JEOL	MALDI-TOF; ESI; GC-TOF; EI; Q-GC/MS	Polymerix	Foods; Drug Analysis; Forensics; Environmental
LECO	GC-TOFMS/MS; GCxGC-TOF/MS; GCxGC	ChromaTOF	Chemical; Environmental; Foods
KORE Technology	TOF-SIMS; TOF-MS; PTR/APCI TOF-MS; TOF-MS EI; PTR TOF-MS Q	NIST; GRAMS/AI; CASA XPS	Surface Analysis; Chemical; Environmental
Monitor Instruments	MS	Supervisory Control and Data Acquisition	Isotope Ratio
MKS Instruments Inc	GC/MS; Q-MS	TOOLweb; Umetrics; SenseLink	Chemical; Environmental
NU Instruments	ICP-MS; Glow Discharge-MS	N/A	Environmental; Surface Chemistry
PerkinElmer Inc	ICP-MS; HPLC and UHPLC; Q-LC/MS; Q-GC/MS	TurboMass; Ion Signature Quantitative Deconvolution; Chromera; ELAN Enhanced Security	Chemical; Pharma; Foods; Environmental
Physical Electronics	TOF-SIMS; Q-SIMS	MultiPak; Quantum 2000 XPS Microprobe	Surface Analysis; Pharma; Chemical
SerCon	EI-MS	N/A	Isotope Ratio

Mass spectrometry_Layout 1 27/09/2010 13:58 Page 82

Table 3 (continued)

COMPANY	TYPES OF INSTRUMENTATION	SOFTWARE	INSTRUMENTATION APPLICATIONS
Shimadzu	QQQ-LC/MS/MS; GXxGC-MS; TOF-q; LC-MS;	GC Image; Compound Composer; NIST; FFNSC	Foods; Environmental; Pharma
Spectromat Massenspektrometer GmbH	N/A	RunI26xt; DoIt26x; Eval26x	Environmental; Surface Chemistry;
Stanford Research Systems	Q	N/A	Gas Analysis
Thermo Fischer Scientific Inc	LC-MS/MS; q-MALDI; FTMS; QQQ-LC/MS	PEAKS Studio; ProMass Deconvolution; SIEVE Automated Label-Free Differential Expression; BioWorks; Mass Frontier Spectral Interpretation Software; MetWorks Automated Metabolite ID;	Foods; Environmental; Pharma; Chemical
Thermolinear	q-MS;	N/A	Environmental; Surface Chemistry;
Vitalea Sciences	AMS	N/A	Pharma; Chemical; Physics; Pharma; Isotope Ratio
Waters Corporation	UPLC; HPLC; LC/MS TOF EI; Q-LC/MS/MS; GC/MS TOF; MALDI;	MassLynx; Quanpedia; QCMonitor; TrendPlot; TargetLynx; NeoLynx; OpenLynx; FractionLynx; ChromaLynx; MassFragment; Metabolynx; QuanOptimize; MarkerLynx; ProfileLynx; POSI IVE; BiopharmaLynx; ProteinLynx	Pharma; Environmental; Surface Analysis

spectrometry (SIMS) (including ASI, BME, CAMECA, Comstock Inc and Hiden Analytical); environmental analysis (including GSG, Kore Technologies, MKS Instruments, NU Instruments and Spectromat Massenspektrometer GmbH) or isotope ratio analysis (Monitor Instruments and Sercon). The pharmaceutical sector is primarily served by only 33% of the MS companies which produce MS instrumentation. The list includes Agilent Technologies, Bruker Daltonics Corporation, Danaher/AB Sciex, Hitachi High Tech, JEOL, Perkin Elmer Inc, Shimadzu, Thermo Fisher Sciences Inc and Waters Corporation. Companies such as Biorad and Leco Corporation (St Joseph, MI, USA), which offer an interesting range of MS instrument capabilities that would be of value to pharmaceutical companies, have not yet penetrated this lucrative sector. Finally, Vitalea Sciences custom-manufactures AMS instruments used in the burgeoning field of Phase 0 clinical studies and mass balance analyses in ADME Toxicology.

The MS companies which offer products to the pharmaceutical sector produce a diverse and powerful array of instrumentation that potentially meets all DDD needs, as summarised in **Table 3**. Single stage HPLC-MS instruments are routinely used in PK/PD and drug metabolism studies and are used primarily as a detector for the HPLC instrument. HPLC-MS/MS tandem instruments continue to garner significant technological development and are considered the workhorses of MS in drug discovery and pre-clinical studies. MALDI-TOF-MS instrumentation continues to play a significant role in drug discovery but the significant growth patterns of the early 2000 period have long subsided. FT-MS continues to attract considerable interest from pharmaceutical companies as they struggle to employ the superior capabilities of such instrumentation, whereas there is minimal interest and growth in the use of magnetic sector instruments in DDD. More recently there has been increasing demand for the new IMS-MS instrument offered by Waters Corporation.

Mass Spectrometry in DDD

Mass Spectrometry has evolved into a widely used technology across a number of diverse industrial sectors[14]. In a parallel process there continues to be rapid new developments in sample introduction, online chromatography-MS, creation of multifaceted separation approaches such as HPLC-nanoESI-Q-IMS-TOF-MS/MS (eg Waters SYNAPT™ instrument), enhanced performance mass analysers and detectors and ever more sophisticated software for data analysis and interpretation. Pharmaceutical

Mass spectrometry_Layout 1 27/09/2010 13:58 Page 83

Table 4: Summary of Mass Spectrometry-based approaches in the analysis of pharmaceutical and biological drugs in the DDD process (adapted in part from reference 15)

APPLICATION IN DDD	MS METHOD
1. Library generation and use	
Identify and Determine Purity	Flow Injection-MS Multi-column LC-MS Ultrafast HPLC-ESI-TOF-MS
Purify NCE	Dual column *m/z*-triggered fraction collection
2. High-Throughput Screening	
Screen combinatorial libraries for biological activity	Affinity-CE-MS Affinity-MALDI-TOF
3. ADME	
Determine PK in animal models	HPLC-MS/MS 96 well solid phase extractions CE-MS
In vitro screens for permeability and metabolism	On-line 96 well SPE coupled with HPLC-MS
Metabolite Identification	ESI-QQQ-MS, ITMS, ESI-Q-TOF-MS/MS, FTMS
Mass Balance	Continuous Flow Isotope Ratio-MS Accelerator-MS
4. Biomolecules/omics	
Proteomics	Various assorted on-line Chromatography-MS/MS
Variant and Degradation Products	MALDI-TOF-TOF-MS HPLC-ESI-MS/MS
QC Testing-Batch to Batch	MALDI-TOF-MS
In Process Monitoring	HPLC-ESI-Q-TOF-MS
Metabolomics	Various assorted on-line Chromatography-MS/MS

companies have attempted to utilise these improved technologies in their complex, multifaceted DDD process. Indeed, Papac and Shahrokh have argued that "Mass Spectrometry has significantly altered how the pharmaceutical and the biotechnology industries discover new therapeutics and develop them into safe and marketable drugs"[15]. There is no doubt that MS has been successfully utilised in a variety of different roles primarily in the Discovery and Preclinical phases of the DDD process. For example, in the late 1990s and early 2000 period combinatorial libraries and high throughput screening were in vogue. MS was used in a variety of different ways to characterise compounds from libraries, determine purity and in conjunction with bioassays perform high throughput screening. New generation HPLC-MS/MS and CE-MS/MS instruments have significantly enhanced throughput capability in the PK, PD and metabolism studies of NCEs. In addition the use of Accelerator-MS is providing the opportunity to carry out Phase 0 clinical trials[22]. Since Accelerator-MS has such exquisite sensitivity the *in vivo* metabolic distribution of a drug can be readily determined with minimal exposure levels of the radioactive compound. MS has been heavily used in the arena of omic analysis and biological therapeutics, but with more mixed indicators of success. In the case of simple QC and in-process monitoring, as well as the structural determination of recombinant protein variants and

References

1 PriceWaterhouseCoopers-Pharma 2020. I) The vision; ii) Which Path will you Take?; iii) Marketing the Future; iv) Challenging Business Models; v) Taxing Times Ahead. http://www.pwc.com/pharma-life-sciences/pharma-2020/index-html.

2 Naylor, S. Systems Biology: Information, Disease and Drug Discovery. Drug Discov. World. Winter Edition: 23-33 (2004/2005).

3 Naylor, S, Culbertson, AW and Valentine, SJ. Technology-Bane or Bonanza for the Pharmaceutical Industry? Drug Discov. World. Fall Edition: 51-58 (2007).

4 Goodman, M. Pharmaceutical Industry Financial Performance. Nature Rev. Drug Discovery. 8: 927-928 (2009).

5 Pharmaceutical Research and Manufacturers of America (PhRMA). http://www.phrma.org/.

6 Liebman, MN. Personalized Medicine-End of the Blockbuster? Pharma Focus Asia. 9: 4-8 (2008).

7 Naylor, S and Cole, T. Overview of Companion Diagnostics in the Pharmaceutical Industry. Drug Discov. World. Spring Edition: 67-79 (2010).

8 Naylor, S and Cole, T. Companion Diagnostics in the Pharmaceutical Industry: Part II-Business Models. Drug Discov. World. Summer Edition: 61-67 (2010).

9 Jordon, R. (Editor). Insight: Back to the Future. Drug Discov. World. Summer Edition-Supplement: 1-44 (2010).

10 Accenture Report. The Pursuit of High Performance Through Research and Development. Understanding Pharmaceutical Research and Development. http://www.pharma.org/files/Accenture%20R&D%20Report-2007.pdf June 17 (2007).

11 Bains, W. Failure Rates in Drug Discovery and Development: Will We Ever Get Any Better? Drug Discov. World. Fall Edition: 9-18 (2004).

Continued on page 85

Mass spectrometry_Layout 1 27/09/2010 13:58 Page 84

Glossary of common terms used in Mass Spectrometry

Accelerator Mass Spectrometry (AMS): Ions are separated into carbon-12, carbon-13 and carbon-14 in order to undertake Phase 0 clinical studies of drug distribution.
Atmospheric Pressure Chemical Ionisation (APCI): Ionisation process generated by spraying vapour droplets at atmospheric pressure into reagent gas ions produced by a corona discharge method.
Base peak: Normally the most intense ion peak in a mass spectrum used for determining relative ion abundance.
Capillary Electrophoresis Mass Spectrometry (CE-MS): Online analysis undertaken using capillary electrophoresis coupled directly to a mass spectrometer.
Chemical Ionisation (CI): Process of ionisation performed between reagent gas ions and the analytes sprayed into the source region.
Collision-Induced Dissociation (CID): Fragmentation process typically caused by collision of ions produced in source with an inert gas.
Electron ionisation (EI): Ionisation process in which high energy electrons impact analytes introduced into the source region.
Electrospray Ionisation (ESI): Ionisation process in which charged droplets in the source region containing analytes are evaporated and concomitantly transferring charge.
Fourier-Transform ion cyclotron resonance Mass Spectrometry (FT-MS): Ions trapped in a magnetic field and separated. The resulting signals of ions moving in such a field are subject to a Fourier transform to produce a mass spectrum.
Gas Chromatography Mass Spectrometry (GC-MS): Online analysis undertaken using gas chromatography coupled directly to a mass spectrometer.
High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS): Online analysis undertaken using reverse phase high performance liquid chromatography coupled directly to a mass spectrometer.
Inductively Coupled Plasma (ICP): Analytes such as elements and electrolytes are sprayed into plasma region at 5-7000 K and ionised.
Ion: Particle such as an atom or molecule that contains a net charge either positive or negative.
Ion mobility spectrometry: Ions are separated in a tube containing an inert gas based on their drift time down the tube when high voltage applied. Ions separated as a function of ion radius to charge ratio.
Ion Trap Mass Spectrometer (ITMS): Ions are trapped in a cell and ejected using an alternating voltage as a function of their *m*/*z* value.
Ionisation: In MS terminology conversion of a solid, liquid or gas into ions in the gas phase.
Mass Spectrometer (MS): Mass analyser that separates, detects and records ions.
Mass Spectrometry/Mass Spectrometry (MS/MS): Also known as tandem mass spectrometry. Employs two MS instruments in series to fragment ions in order to produce structural information.
Mass spectrum: Is a representation of a mass spectrometric analysis as a function of *m*/*z* and relative ion abundance.
Mass-to-charge ratio (*m*/*z*): Value determined after dividing the ion mass by the charge of that ion.
Matrix-Assisted Laser Desorption Ionisation (MALDI): Ionisation method using an organic matrix mixed with the analytes and subsequently subjected to a laser beam to introduce a specific energy frequency.
Multiple Reaction Monitoring (MRM): Method using tandem mass spectrometry to determine presence of specific analytes as well as quantitate them.
Product ion: Ions generated from a fragmentation (CID) process. The original ion from which the fragments are created is known as the precursor ion.
Protonated molecule: An ion generated upon proton transfer to generate MH^+.
Quadrupole mass spectrometer (Q): A specific type of mass spectrometer employing quadrupole rods to separate ions based on their *m*/*z* values.
Time-Of-Flight Mass Spectrometer (TOF-MS): Ions separated based on their flight velocities.
Sector instrument: Mass spectrometer employing a combination of a magnetic and electrostatic components to separate ions.

degradation products, MS has been a useful tool. All of this is summarised in **Table 4** and has been discussed in more detail elsewhere[14,15-18].

The hype surrounding the advent of proteomic and metabolomic profiling using MS-based platforms has infiltrated the pharmaceutical sector over the past decade[2]. This led to an outlay of considerable capital by all the major pharmaceutical companies to purchase the latest MS technologies as well as build in-house technical expertise. Unfortunately, this expenditure was not translated into significant breakthroughs in early discovery efforts predicated on MS analyses. Armed with the clarity of hindsight it is interesting to observe that an interesting phenomenon occurred that explains the poor return on MS investment for proteomic and metabolomic analyses. The MS technologies, were evolving and constantly improving predicated on the efforts of the MS manufacturers during the past 10 years. Nevertheless, proteomic and metabolomic analyses were performed on relatively mature MS platform technologies. It was not the performance characteristics of the MS platform(s) that were poorly understood. Unfortunately, it was the application of MS to proteomic and metabolomic analyses that was not adequately understood and hence subject to the Hype Cycle. This led to poorly thought through experimental protocols and mismatched expectations of MS capability versus the quality and usefulness of data output. For example, in the differential analysis of blood samples (plasma or serum) from a control group versus a specific disease state cohort, little or no consideration was given to the well-known dynamic range limitations of MS. It should not have been surprising to learn that irrespective of the disease state being investigated, the same high abundant proteins typically associated with the inflammatory response were always found to be the differentiators between control and diseased populations.

MS companies will continue to innovate and develop better instruments as well as firmware and software packages that control MS hardware and aid in data output and interpretation. However, the pharmaceutical sector is wisely re-evaluating and refocusing the role of MS in certain areas of the DDD process. In the case of early discovery using proteomic and metabolomic approaches, there needs to be a specific biological question being asked that requires a defined answer. Understanding the limitations and capabilities of the instrumentation, firmware and software in concert with the biological question is imperative for expectations to be met and satisfied. The days

Mass spectrometry_Layout 1 27/09/2010 13:58 Page 85

of fishing expeditions in the DDD process employing MS platform technologies are hopefully over.

Conclusions

MS predicated platform technologies will continue to play a significant role in specific areas of discovery and preclinical processes. Well-defined processes that require specific information content will continue to be served well by employing MS. The role of MS in clinical development will continue to be severely limited. However, by determining a specific need, for example in the newly emerging area of Companion Diagnostics[7] and avoiding the Hype Cycle associated with application of MS to the problem, one can foresee future roles for MS in the clinical development process.

Finally, it is important for pharmaceutical companies to understand the limitations of current technologies and software. Much of the MS platform technologies are designed to serve two customer bases with very different expectations. The basic research community apply different standards to the interpretation of data compared to pharmaceutical companies which ultimately must satisfy stringent regulatory requirements imposed on them by FDA or EMA. This can sometimes lead to a mismatch of expectations in terms of MS capability. For example, many of the commercially available MS/MS data analysis programs used for peptide sequencing determination and protein identification, such as SEQUEST, Mascot, X! Tandem and others are much less than 100% accurate. Often times this level of inaccuracy may be tolerable for the basic researcher, but for the pharmaceutical company, such inaccuracies waste time, money and may jeopardise the progress of a NCE. Pharmaceutical companies need to understand such problems and limitations at the outset and seek ways to alleviate such shortcomings. In summary, the role of MS in the DDD process is assured provided realistic expectations are set and practical heads determine the decision making process.

Acknowledgements

Dr Naylor acknowledges the support of the National Institutes of Health through an SBIR Grant (5R44-HL082382-03) in partial support of this work. DDW

Dr Stephen Naylor *is a Founder, Chairman of the Board and CEO of D2D Inc, a start-up Companion Diagnostics Company. He is also Founder, President and Chairman of MaiHealth Inc, a molecular bioprofile diagnostics company in the health and wellness sector, as well as Founder, Chairman and former CEO of Predictive Physiology and Medicine Inc, a personalised medicine company offering a series of information content tools to consumers.*

Paul T. Babcock *is a public health professional working for the Marion County Health Department based in Indianapolis (Indiana, USA). He has a Master's in Public Affairs with a focus on drug control and delivery policy and is involved in a series of early stage start-up ventures.*

Continued from page 83

12 Naylor, S. NostraPharmus Revisited: A Future of Splendid Isolation or Multilevel Participation for Pharmaceutical Companies. Drug Discov. World. Summer Edition-Supplement: 24-26 (2010).
13 Griffiths, J. A Brief History of Mass Spectrometry. Analy. Chem. 80: 5678-5683 (2008).
14 Gross, ML and Caprioli, R. (Eds). The Encyclopedia of Mass Spectrometry 10 Volume Set. Elsevier Press. 2003-2010.
15 Papac, DI and Shahrokh, Z. Mass Spectrometry Innovations in Drug Discovery and Development. Pharm. Research 18: 131-145 (2001).
16 Janiszewski, JS, Liston, TE and Cole, MJ. Perspectives on Bioanalytical Mass Spectrometry and Automation in Drug Discovery. Curr. Drug Metabol. 9: 986-994 (2008).
17 Rossi, DT and Sinz, MW (Eds). Mass Spectrometry in Drug Discovery. Marcel Dekker Inc. 2002.
18 Korfmacher, WA (Ed). Using Mass Spectrometry for Drug Metabolism Studies (2nd Edition). CRC Press/Taylor Francis Group. 2010.
19 De Hoffmann, E and Stroobant, V. Mass Spectrometry: Principles and Applications (3rd Edition) Wiley & Sons 2007.
20 Landers, JP (Ed). Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques (3rd Edition) CRC Press/Taylor Francis Group 2008.
21 Strategic Directions International Inc. Market Research Report on Mass Spectrometry 2009. http://www.strategic-directions.com/apps/.
22 Marusina, K. Accelerator MS is a Powerful New Tool. Genetic Eng. News 27: 1-3 (2007).