Developing a pharmaceutical product has become increasingly difficult and expensive. With an emphasis on developing project knowledge at an earlier stage in development, the use of information-rich technologies (particularly MS) has continued to expand throughout product development. Continued improvements in LC/MS technology have widened the scope of utilizing MS methods for performing both qualitative and quantitative applications within product development. This review describes a multi-tiered MS strategy designed to enhance and accelerate the identification and profiling of both process- and degradation-related impurities in either the active pharmaceutical ingredient (API) or formulated product. Such impurities can be formed either during chemical synthesis, formulation, or during storage. This review provides an overview of a variety of orthogonal-mass spectrometric methodologies, namely GC/MS, LC/MS, and ICP-MS, in support of product development. This review is not meant to be all inclusive; however, it has been written to highlight the increasing use of hyphenated MS techniques within the pharmaceutical development area. © 2010 Wiley Periodicals, Inc., Mass Spec Rev

**Keywords:** mass spectrometry; product development; impurities; degradation products; drug substance; dosage form

## I. INTRODUCTION

The complex process of developing a pharmaceutical product that addresses unmet patient needs has become increasingly more difficult and expensive. At last count, the cost of taking a new molecular entity through the entire process, that is, from the identification of a validated target and generating a viable lead candidate to commercialization and global launch of the formulated dosage form, can exceed 10 years and at a cost in excess of $1 billion dollars (Dimasi, Hansen, & Grabowski, 2003; Suresh & Basu, 2008). Of the four major stages of drug development, which includes drug discovery, preclinical development, clinical development, and manufacturing, the longest stage is typically clinical development, which itself is divided into three major phases (I–III). During phase I, the compound’s safety and pharmacokinetic profile are defined. Phase II development focuses on compound efficacy, establishing the pharmacologically relevant dosage range, and obtaining tolerability and safety data. Finally, phase III of clinical development is focused on completing the human safety and efficacy programs and to ultimately secure regulatory approval for commercial marketing. This review focuses on the use of orthogonal-mass spectrometric methodologies, namely GC/MS, LC/MS, and ICP-MS, in support of clinical development. A few excellent reviews pertaining to the use of LC/MS across the entire drug development process have been written (Lee & Kerns, 1999; Ermer & Vogel, 2000).

Relative to drug discovery and metabolism areas, the use of mass spectrometry (MS) for early phase pharmaceutical product development is not well documented in the literature. This can be attributed to the proprietary nature of the work that is performed. Since much of the method development information at this stage will potentially be utilized within a quality control environment, there has been reluctance to implement MS as a robust, routine tool to control the synthetic process of the active pharmaceutical ingredient (API) as it moves towards manufacturing or as part of the control strategy for the release of the dosage form. However, as MS has matured and has become the methodology of choice for many other applications within drug development (particularly in drug discovery and ADME), the overall perception of incorporating MS into methods and control within product development has changed.

With the continuing improvements in LC/MS technology, the many advantages of MS for performing qualitative and quantitative analysis have widened the scope of applications within product development. Numerous strategies exist for using MS instrumentation to aid in the acceleration of drug development. Lee has documented many of the ideas, trends, and applications for using LC/MS across the drug development cycle in the book titled, “LC/MS Applications in Drug Development” (Lee, 2002). More complete overviews of the analytical techniques utilized for impurity analysis can be found in the book “Identification and Determination of Impurities in Drugs” (Görög, 2000) and in a review article by Qiu and Norwood (2007).

More recently, our laboratory has designed an MS strategy to enhance and accelerate the profiling and identification of process-related impurities and degradation compounds encountered during the chemical synthesis of the API/drug substance as well as the formulation of the dosage form. This strategy involves identifying the mass spectrometric instrumentation needed to acquire the desired information during various stages of the process chemical and formulation development. A flow scheme showing the tiered approach is exhibited in Figure 1. For example, at the early stages of a synthetic process, rapid analysis methods that provide nominal molecular weight data are essential. Nominal mass information, along with the process chemist’s knowledge of the synthetic scheme and associated chemistry, is often adequate to identify process-related impurities; thus, the first tier involves the use of simple and robust LC/MS and GC/MS quadrupole instruments that can routinely provide the required information and can be readily accessed.
by all scientists involved with product development. As the synthetic route moves closer to commercialization phase, more detailed structural data may be required to identify or characterize lower, trace level impurities. Thus, tier two in our strategy involves the use of accurate mass instrumentation such as time-of-flight (TOF) MS as a way to obtain molecular formula information for impurities and other process-related substances. The ability to obtain molecular formulas for identification of impurities early in process chemistry is paramount and can have substantial impact on the selection of the appropriate synthetic route. The final tier of the strategy involves the use of tandem MS techniques, including both nominal mass and exact mass instrumentation, along with advanced NMR techniques to fully characterize the structure of any impurities. This information is valuable for developing a more defined impurities and control strategy for commercialization. The key to the tiered approach is that the simplest, most robust instrumentation (nominal mass spectrometers) is provided to the largest number of scientists involved with product development for solving the most routine problems, while the most sophisticated instrumentation (tandem and higher resolution mass spectrometers) is utilized for resolution of the most difficult problems by the most experienced personnel.

This review has been written to reflect this multi-tiered approach established in our laboratory for analysis and characterization within pharmaceutical product development. It provides an overview of a variety of MS techniques used to analyze impurities and degradation products encountered in both drug substance and dosage form development. In addition, a brief review of quantitative assays commonly used within product development will be discussed, followed by some potential future directions and applications that utilize the many advantages of MS.

II. IDENTIFICATION OF PROCESS IMPURITIES AND DEGRADATION PRODUCTS IN API/DRUG SUBSTANCE

Mass spectrometry (MS) is an essential tool in the determination of chromatographic purity of the API during HPLC method development (Antonovich & Keller, 2002). Standard LC/MS methods provide a direct way of comparing impurities and other related substances from various lots of API, including the first toxicology lot, subsequent current Good Manufacturing Practices (cGMP) campaigns, process validation lots, etc., based on molecular mass of constituents rather than simply a retention time and UV spectrum. The limitations observed with UV or diode array detection (DAD), due primarily to similar absorption characteristics of closely related impurities and in some cases lack of a sufficient chromophore for low-level sensitivity, can often be overcome with the use of LC/MS methodology. LC/MS has the distinct advantage over other techniques in providing both selectivity and sensitivity and can also provide easy-to-interpret data. These attributes of LC/MS are valuable when developing methods for analyzing stressed samples and samples for stability testing. Various examples of using LC/MS for peak purity assessment of API can be found in the literature. In one such example LC/MS, reported by Bryant, Kingswood, and Belenguer (1996), demonstrated the effectiveness of this methodology for detection of low level impurities by using ESI (electrospray ionization) with an HPLC system to intentionally co-elute a number of impurity standards with the drug substance (API). Known co-eluting impurities down to 0.02% of the drug substance were detected. In most cases with LC/MS, peak purity can be verified down to impurity levels of at least 0.1%. This article clearly demonstrates the versatile, selective, sensitive, and rapid nature of LC/MS in the determination of LC peak purity.

The presence and quantity of impurities can have a significant impact on the quality and safety of the API during the drug development process. It has been pointed out that LC/MS can be an essential part of the comprehensive characterization of impurity profiles. This has been shown using a multidimensional evaluation with peptide drugs as an example (Ermer, 1998). This investigation focused on the tracking of impurities throughout a process (toxicological batches through clinical batches). Utilizing MS, the molecular mass can be used as a starting point for identification and further characterization. A standardized approach has also been promoted by Xue et al. (2004), using LC/MS for automated peak tracking during the impurity profiling process. This fully automated system employed orthogonal LC separations and hyphenated UV-MS detection. The system tracked individual impurity peaks across orthogonal HPLC methods automatically by using molecular weights of the separated components. Initial testing of over 500 drug impurities utilizing this automated peak tracking has yielded an 80% success rate.

The advantages of LC/MS can be further enhanced through the coupling of this methodology with NMR analysis. Many of the impurities detected in the API require more than just simple molecular weight information for characterization particularly when impurities have identical molecular weights. This can be
accomplished by isolation and collection of the impurities using preparative HPLC. MS can be used for confirmation of the isolated peaks before further characterization by NMR (Babjak et al., 2002). With the advances in NMR technologies, for example, introduction of flow probes to allow for LC-NMR analysis, higher sensitivity probes based on the use of microcoils (Olsen et al., 1995; Lacey et al., 2001; Henry et al., 2008) and cryocooling of the active probe (Keun et al., 2002; Rashid et al., 2002) the power of both MS and NMR can be coupled for more complete impurity profiling. Mistry et al. (1997), have shown how this methodology can reduce time-consuming isolation of individual impurities and eliminate the potential for degradation during the work-up process. This work evaluated the application of LC/MS and LC/NMR for efficient identification of novel impurities without the use of time-consuming and labor-intensive isolation and purification procedures.

Another more specialized methodology has been evaluated for impurity profiling of drug substances. This approach was also based on using standardized separation methods coupled with MS detection; however, instead of traditional LC/MS, the use of capillary zone electrophoresis (CE)/MS was utilized for impurity profiling (Vassort et al., 2005). This evaluation showed the complementary nature of using both established LC/MS and these CE/MS methods that together can produce a more comprehensive approach for identification of process-related impurities.

In addition to using advanced instrumentation, utilizing software tools to aid in data interpretation can prove valuable for analysis of process impurities or degradation products produced during the API development process. An example using 3D contour plots for evaluation of LC/MS data (retention time, m/z, and intensity) obtained from complex mixtures was described by Moser, Groeppel and Linder (2002). This work demonstrated the advantages of using LC/MS coupled with appropriate software for performing reaction monitoring to observe formation of the three tautomeric forms of pimecrolimus and for peak purity assessment from decomposition samples or mother liquors.

An essential part of the characterization and specifications of APIs has been the determination of potential metal contamination. Historically, these types of tests have been used to ensure that little to no inorganic-based material is incorporated into the final API material. The application of ICP-MS for the determination of heavy metals has been demonstrated (Lewen et al., 2004). ICP-MS provides the advantages of smaller sample size (therefore minimizing sample matrix effects), element specific information, quantitation, rapid sample throughput, and higher sensitivity for catalyst metals such as Pd as compared to ICP with atomic emission spectroscopy (AES). Additionally, higher recovery of most elements is observed with ICP-MS compared to ICP-AES due to the sample preparation methods utilized which result in the loss of the more volatile elements. Initially, a general screen can be set-up for analysis of a variety of elements. Once specific elements have been identified, a selective quantitative analysis can be performed on the elements of interest. Low-level detection limits in the ppb and sub-ppb levels are commonly achieved for most elements.

There are many instances during process development where nominal mass data is not sufficient for the determination of the structure of unknown impurities, particularly at trace levels. The ability to obtain accurate mass data on trace components in complex mixtures is an essential tool in determination of unknown impurities in the drug substance. Methods for analysis of materials and potential impurities formed throughout synthetic processes need to be suitably sensitive as well as selective for characterization of the structures.

Time-of-flight (TOF) MS was utilized to monitor the cyclization, metatization, and bromination reaction processes during the synthesis of phthalocyanine compounds (Chen et al., 2004). Lower resolution mass spectral data provided molecular weight information that could be used for comparison with theoretical molecular weights for simple identification. However, accurate mass data obtained with higher resolution was utilized to identify fragment ions formed during the ionization process allowing for detailed structural characterization of the phthalocyanine dyes. This example illustrates the practical utility of using accurate mass measurements for process monitoring.

The coupling of HPLC with accurate mass analysis can provide the advantage of separating the minor components from the main drug substance. The use of HPLC coupled to a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer for analysis of minor components in a mixture was first reported by Haskins et al. (1995). This work described the separation and identification of minor impurities in cimetidine utilizing the capabilities of accurate mass measurements on an FT-ICR instrument. Several minor components were detected and accurate mass measurements used to confirm their identity with better than 5 ppm accuracy. In addition, due to the large abundance of the cimetidine peak within the chromatogram, many impurities lower in concentration may be “masked” or co-elute with this large peak. However, utilizing the technique known as stored waveform inverse Fourier transform (SWIFT), protonated cimetidine was ejected from the trap in real-time prior to detection, therefore allowing for more accurate determination of the minor components. This technique provides a different method for selectively obtaining accurate mass measurements on trace level components of interest in complex mixtures.

Additional structural elucidation data for identification of impurities present within the drug substance can be obtained by utilizing the selectivity of tandem mass spectrometry (MS/MS). In general, MS/MS instrumentation allows for the selective fragmentation of a particular precursor (compound) ion of interest. The subsequent fragment ions that are produced are characteristic of the selected precursor ion and can be utilized for structural identification and/or confirmation of impurities or degradation products. A variety of MS/MS instrumentation is available for providing this type of structural information. One of these, triple quadrupole mass analyzer, has been shown to provide consistent and sensitive MS/MS data for structural elucidation. The advantages of LC/MS/MS on a triple quadrupole have been used for “fingerprinting” of active drug substance impurity profiling (Nicolas & Scholz, 1998). This fingerprinting process consists of a protonated molecular ion and characteristic fragment ions (3 or 4), combined with retention time for each drug substance impurity, allowing for tracking of impurities from lot-to-lot of material in the synthesis process. Utilization of consistent tandem MS conditions for collision energy and collision gas thickness results in MS/MS fingerprints with characteristic ions that can distinguish isomeric impurities at concentration levels between 0.04% and 0.012% based on UV area. Another more recent example of using MS/MS fingerprinting as a tool for structural identification/verification of a low level drug substance impurity was discussed by Li, Lin, and
Rustum (2008). In combination with appropriate stress studies, an impurity at slightly higher than 0.1% was identified without the need for comprehensive impurity purification and NMR. This example provides an effective general strategy for the rapid identification of impurities at low levels (~0.1%) in the drug substance.

MS/MS and LC/MS/MS have been utilized to characterize impurities and degradation products formed in butorphanol API (Volk et al., 1996) during long-term stability studies. This work illustrates the complementary nature of using HPLC, UV, full scan, and tandem MS to rapidly and accurately elucidate structures of unknowns and confirm the structures of known impurities and degradation products. This methodology allows for rapid and systematic characterization of trace level components and provides a potential foundation for drug substance and product monitoring, as well as a diagnostic tool for identification of new impurities and degradation products. In addition, the resultant data from this methodology can be utilized for creation of an impurity and degradant database (Rourick et al., 1996). Creation of such a database can become the predictive foundation for future development work involving the monitoring of the synthetic process as well as drug substance stability and can be utilized at any stage of the drug development process.

Another type of MS instrumentation commonly employed for structural elucidation and identification of impurities is the ion trap mass spectrometer. This instrument is capable of producing MS/MS data that can further elucidate the characteristic fragment ions encountered from MS/MS data. Many examples using ion trap methodology can be found in the literature for analysis of impurities and degradation products in the drug substance (Leader et al., 2002; Zhao, Qin, & Reed, 2002). An example of utilizing LC/ESI-MS and MS/MS data, along with complementary GC/MS electron impact data and NMR to characterize synthetic impurities in multi-step synthetic reactions was shown by Zhou et al. (2005). On-line LC/MS/MS was used for initial examination of the impurities of tri-clabendazole, followed by isolation using semi-preparative chromatography. Structural characterization of the impurities was performed utilizing various types of MS instrumentation, along with NMR. These identified and characterized impurities in the bulk drug substance are of importance for reaction monitoring during process development as well as for quality control.

Determining the potential pathways for formation of process impurities during chemical synthesis as well as the degradation products observed in the drug substance upon exposure to specific chemical and physical conditions can be beneficial. The ability to obtain an empirical formula for a pseudomolecular ion as well as obtaining the characteristic fragments of the molecular ion can allow the accurate confirmation of these potential pathways. Combining the advantages of tandem MS with accurate mass measurements, unknown compounds can be identified with a high degree of confidence. This has been demonstrated by Nagele and Moritz (2005) in the structure elucidation of the degradation products of amoxicillin. The fragments obtained through MS/MS experiments on an ion trap mass spectrometer were used along with ppm level mass accuracy obtained from a TOF mass spectrometer to establish elemental compositions for the degradation products along with the proposed pathways of their formation.

Additionally, one can obtain similar data from mass spectrometers that provide both accurate mass for both molecular ions and their subsequent fragments. One of these instruments utilized to obtain this type of data is a quadrupole time-of-flight (QTOF) mass spectrometer. In the case of a synthetic chemical process, the structural characteristics of potential impurities can be extremely diverse. Having the ability to obtain elemental compositions on the product (fragment) ions of low-level impurities can increase the degree of confidence of the identification of unknown impurities. The enhanced sensitivity of the TOF analyzer allows acquisition of product ions from MS/MS on trace level impurities that would be difficult for triple quadrupole tandem mass analyzers. Eckers, Haskins, and Langridge (1997) utilized LC-QTOF methodology for identification of trace impurities in the drug substance, cimetidine. Their work demonstrates the clear advantages of obtaining accurate mass data on fragment ions produced from product ion mass spectra. The key to obtaining low ppm accuracy with TOF analyzers is the use of the appropriate calibrants (internal/external). “Good” data typically obtained from a TOF analyzer will show mass accuracies of <5 ppm, with current TOF instrumentation routinely capable of producing mass accuracies between 1 and 2 ppm.

An FTICR-MS can readily provide high-resolution, exact mass analysis. Additionally, since FTICR is an ion trapping technique, MS^n data can be obtained for further structural elucidation information by a variety of means. LC coupled with FTICR-MS has been utilized to characterize pharmaceutical compounds (drug substance) and related impurities (Winger & Kemp, 2001). Both positive and negative ion ESI-MS^n data were obtained on potential degradation products observed in two different drug substances. Positive ion exact mass measurements (Table 1) were obtained for the protonated or nitrated molecular ions observed from the peaks corresponding to degradation products of a secretory phospholipase A2 inhibitor (Fig. 2). Additionally, one of the advantages of the FTICR-MS is the ability to generate exact mass data with multiple stages of mass analysis, MS^n. Elemental compositions for all fragments of a drug candidate for a cancer indication were obtained that yielded information pertaining to the atom-to-atom connectivity of the molecule (Table 2). Since, the FTICR-MS usually produces mass accuracies lower than 3 ppm for both molecular and fragment ions, these data can be used to unambiguously identify fragmentation pathways for drug substances and their corresponding impurities and degradation products. More recently, Qui et al. (2009) utilized the high resolution and mass accuracy capabilities of an LTQ-FT instrument to identify an unknown oxidative impurity observed a new drug substance. The data obtained was utilized to assist in the determination of the origin of the impurity from a side-reaction of the last intermediate with an oxidant used in the synthesis of the drug substance. The ability to obtain this type of information with an on-line experiment has drastically reduced the need for isolating and purifying substantial quantities of impurities for mass spectral characterization.

Another more recent hybrid MS available for performing high-resolution accurate LC/MS and MS^n measurements is the LTQ-Orbitrap. The LTQ-Orbitrap is capable of providing structural information with low ppm mass accuracies on complex mixtures. Several impurities were characterized within a drug substance during the course of a large-scale production with the
LTQ-Orbitrap (Chen et al., 2007). In spite of the large differences in ion abundance for the impurities and drug substance (API) found in the sample, the mass accuracy data for the measured elemental compositions were within 1 ppm. This level of mass accuracy allows for the differentiation of isomeric impurities and shows the utility of this instrumentation in performing rapid and accurate structural characterization.

### III. IDENTIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS IN DOSAGE FORM FORMULATIONS

Development of single methods that allow for the detection, identification, and quantitation of low-level impurities in pharmaceutical formulations is essential. The standard method involves the use of HPLC with UV detection due to its ease of use, cost, sensitivity, and low level of chemical background. However, with its lack of selectivity and ability to provide limited qualitative information, UV has limitations as applied to the identification of unknown impurities and degradation products.

An example utilizing GC/MS for the analysis of volatile impurities in both API and dosage form formulations in process chemistry has been described (Marinković et al., 1997). This method was applicable for both routine qualitative and quantitative determination of all impurities of a frequently used coronary vasodilator in drug substance and various dosage formulations. The method demonstrates the utility of GC/MS for stability studies providing a simple, straightforward, and sensitive assay.

### TABLE 1. Exact mass LC-FT/MS data for LY315920 bulk material stressed at pH 11 and 70°C for 3 days

<table>
<thead>
<tr>
<th>Eluted Peak (min)</th>
<th>Measured m/z</th>
<th>Predicted Elem. Comp.</th>
<th>Calculated m/z</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7</td>
<td>359.1597</td>
<td>C_{19}H_{23}N_{2}O_{5}</td>
<td>359.1602</td>
<td>-1.2</td>
</tr>
<tr>
<td>7.2</td>
<td>342.1333</td>
<td>C_{19}H_{20}NO_{5}</td>
<td>342.1336</td>
<td>-1.0</td>
</tr>
<tr>
<td>7.7 (Parent)</td>
<td>381.1446</td>
<td>C_{21}H_{21}N_{2}O_{5}</td>
<td>381.1445</td>
<td>0.2</td>
</tr>
<tr>
<td>9.0</td>
<td>382.1279</td>
<td>C_{21}H_{20}NO_{6}</td>
<td>382.1285</td>
<td>-1.5</td>
</tr>
<tr>
<td>11.6</td>
<td>354.1332</td>
<td>C_{20}H_{20}NO_{5}</td>
<td>354.1336</td>
<td>-1.3</td>
</tr>
<tr>
<td>12.2</td>
<td>397.1388</td>
<td>C_{21}H_{21}N_{2}O_{6}</td>
<td>397.1394</td>
<td>-1.5</td>
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<tr>
<td>14.3</td>
<td>310.1054</td>
<td>C_{16}H_{16}NO_{2}Na</td>
<td>310.1050</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The predicted elemental compositions were determined by performing a search of the measured m/z for each eluting peak over the following range of elements: \(^{12}\text{C}: 0–30; \(^{1}\text{H}: 0–30; \(^{14}\text{N}: 0–5; \(^{16}\text{O}: 0–8; \) and \(^{23}\text{Na}: 0–1.\)


![Figure 2](https://wileyonlinelibrary.com)
TABLE 2. Exact mass MS/MS data for LY231514

<table>
<thead>
<tr>
<th>Measured m/z</th>
<th>Predicted Elem. Comp.</th>
<th>Calculated m/z</th>
<th>Error (ppm)</th>
</tr>
</thead>
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<tr>
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<tr>
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<tr>
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<td>84.0455</td>
<td>C_{6}H_{2}O</td>
<td>84.0455</td>
<td>2.8</td>
</tr>
</tbody>
</table>

The exact mass value of the deprotonated molecule (m/z 426.1419) was used to adjust the electric field term in the calibration equation. The predicted elemental compositions were determined by performing a search of the measured m/z for each eluting peak over the following range of elements: 1C: 0–20; 1H: 0–20; 14N: 0–5; 16O: 0–6, which is the composition of deprotonated LY231514. Reproduced with permission from Winger et al. (2001). Copyright 2001 American Pharmaceutical Reviews.

A comparison between LC/UV and LC/MS for characterization of impurities and degradation products in trimethoprim tablets has been shown (Barbarin, Henion, & Wu, 2002). This work showed that MS is well suited for detection and characterization of impurities and degradation products present in drug formulations. A program called Component Detection Algorithm (CODA) (Windig, Phalp, & Payne, 1996) can be utilized for automating the identification of relevant mass chromatograms from complex dosage form mixtures. This algorithm was developed to reduce random noise and high background encountered in a typical LC/MS analysis. The use of CODA can enhance the detection limits of LC/MS when using full scan data often allowing identification of material well below required levels. It has been shown that by coupling UV (PDA) detection with MS a comprehensive set of data can be provided that can be utilized to establish impurity and degradation profiles. Hong et al. (2003) used this methodology during forced degradation studies to improve the accuracy of quantitation and mass balance determinations within a dosage form matrix. An optimized gradient HPLC method coupled with MS and PDA detection accurately determined API concentrations, mass balance, and total impurities and was ultimately utilized for release and stability testing of the dosage form. This methodology can be utilized for tracking process impurities and eventually be transferred to manufacturing sites for quality control methodology.

As aforementioned, molecular weight data obtained from LC/MS methods provides essential information in the drug development process. However, additional information is sometimes required for further structural characterization. And in the case of dosage forms, a variety of excipients and/or polymers can be used in the manufacturing of API for optimum drug performance. In many cases, the additional excipients or ingredients used can catalyze the degradation of the API and formation of impurities. An interesting example of this is found with duloxetine hydrochloride (Jansen et al., 1998). An enteric polymer coated formulation (dosage form) was developed to prevent degradation in the acidic environment of the stomach. In this study, it was determined that polymer degradation products from the enteric coating were reacting with the API (duloxetine hydrochloride) to produce two impurities. Both nominal mass (LC/MS) and accurate mass high-resolution (FAB/MS and EI/MS) data were obtained to facilitate the identification of these impurities.

The identification of dosage form impurities can, at times, prove to be more complicated than with drug substance (API). Therefore, the ability to perform accurate mass analysis can be even more valuable. Obtaining both nominal mass and accurate mass data can assist in the structural elucidation of impurities, but at the same time create large volumes of chromatographic and spectroscopic data. Researchers as well as vendors have begun to develop software algorithms to aid in the interrogation, interpretation, and management of this data. Many of these programs (MetaboLynx™, LightSight™, and Metlab Profiler to name a few) have focused on the area of metabolite identification. The feasibility of using this software designed for metabolite identification to assist with the identification of impurities and degradation products has been studied (Freed et al., 2004). Ten pharmaceutical compounds representing a wide diversity of chemical properties were tested to examine the power of combining LC/UV/MS with a program from Waters called MetaboLynx™ to detect and identify analytes at low concentrations. Data were obtained on both an LC-TOF and LC-quadrupole (Q), and the resulting spectra were searched by MetaboLynx™ to determine its processing and data manipulation capabilities for this task. It was determined that this program has the ability to identify unknown degradation products at low concentrations buried in the chemical noise of a mass chromatogram. In addition, with the advantages of accurate mass, elemental compositions of the identified degradation products could be obtained and used for further characterization of the proposed impurity. However, the method is time consuming to optimize and requires numerous points of visual inspection to check for peaks of interest. Further refinement of these types of algorithms is needed for this methodology to become a reliable tool for analysis of impurities and degradation products.

The importance of obtaining elemental compositions for both molecular ions and fragment ions was shown by Vuletić, Cindrić, and Korunžnjak (2005) using Q-TOF instrumentation for identification of unknown impurities in simvastatin tablets. Levels of impurities (or products that are not the chemical entity or an excipient) need to be identified and qualified higher than 0.05% or 0.1% in the drug substance and 0.1%, 0.2%, or 0.5% in drug product depending on the maximum daily dose (ICHQ3A, 2008; ICHQ3B, 2006). The capabilities of the Q-TOF (coupled with LC) were utilized to determine the structures of the unknown impurities. By comparing the accurate MS/MS data obtained from simvastatin with that obtained from the unknown impurities, areas of the molecule that had undergone structural modification could be readily determined. The use of the elemental compositions obtained for the characteristic fragment (product) ions allowed for the elucidation of the structures of the unknown impurities observed in the simvastatin tablets. Utilizing the lock spray real-time mass calibration feature on the Q-TOF,
mass accuracies around 3 ppm were obtained for the precursor ions and between 4 and 13 ppm for the subsequent observed fragment ions.

When performing definitive stability or accelerated stress degradation studies, new and often quite unique impurities may need further characterization utilizing detailed structural elucidation methods. In addition, since these impurities can occur at low levels and, in the case of dosage form, in the presence of large amounts of excipients, it is critical to use instrumentation that is both sensitive and selective. Because, nominal mass LC/MS provides only molecular weight data and limited structural information, LC/MS/MS is an ideal choice for this work. The use of MS/MS methods for performing rapid screening and identification of unknowns has been well documented. In the case of drug development, comparison of the fragmentation patterns observed with standards compared to the unknown impurities or degradation products can provide a valuable tool for performing stress degradation studies. Zhao et al. (1999) give an example of this in the identification of losartan degradation products in stress tablets. Three low-level degradation products, shown in Figure 3, were identified by comparison of the fragmentation of the unknown degradation products to the fragmentation of the standard API (losartan). An example of this comparison is shown in Figures 4 and 5 for degradant I.

As pointed out previously, more detailed and thorough characterization may require other techniques. The combination of MS/MS fragmentation and NMR data provides a powerful set of tools for structural characterization. The use of the combination of techniques was demonstrated in the identification of oxidative degradation products in a dosage form shown by Wu et al. (2003). This study examined the interactions between the API and its counterion, in this case the TRIS salt. There work demonstrates that the combination of LC/MS/MS and along with NMR techniques is essential in solving complicated problems encountered in the drug development process.

The use of LC/MS/MS as a high-throughput (HT) tool in drug substance and dosage form development was demonstrated by van de Kamp et al. (2004). Studies involving salt form selection and forced degradation studies for drug substance were shown. This work utilized the capabilities of ion trap MS for acquisition of both full scan and MS/MS data for elucidation of degradation products. In addition, dosage form optimization was performed utilizing quantitative LC/MS methods to determine the concentration of impurities in final formulations. This work further demonstrates the advantages of MS techniques for performing a variety of studies involving identification of impurities and degradation products encountered in dosage form development.

The investigation of leachables (chemical species that can “migrate” from containers, closure systems, and other packaging components) and extractables (chemical species that can be “released” from containers, closure systems, and other packaging components) has been determined to be important during the course of the drug development process. Since these materials have the potential for contaminating the dosage form, characterization of potential leachables and extractables is required within the pharmaceutical industry for the approval of new dosage forms. Möller, Olsen, and Vo (2003) have utilized the power of tandem MS to characterize potential leachables from a common stabilizer know as Irganox 1010. Both ion trap MS data as well as QTOF analysis was performed to obtain characteristic fragments from the leachables for structural elucidation. The combined capabilities of MS and accurate mass data to provide elemental composition of fragment ions provided a fast and reliable method for elucidation of the leachables.

IV. QUANTITATIVE ASSAYS

Liquid chromatography/mass spectrometry (LC/MS) and LC/MS/MS have become “gold standard” for performing quantitative bioanalysis (Brewer & Henion, 1998; Lee & Kerns, 1999; Ackermann, Berna, & Murphy, 2002). For more than 10 years, the utility of using MS detection for quantifying the drug substance in the presence of complex biological matrices have been reported in the literature. These assays utilize the unmatched sensitivity and selectivity that can be obtained with MS detection. In the process of development, manufacture and release of a dosage form, very few examples of using MS detection for quantitation have been reported. One example though, by Kolodick, Rossi, and Kingsmill (2003), compared quantitative results obtained with LC/MS/MS to previously validated LC/UV methods for precision, linearity, selectivity, accuracy, and sensitivity and found them to be equivalent. However, even with this type of information available, LC/UV remains the workhorse for quantitative analysis within drug development.

There has been an overall lack of acceptance for using LC/MS methods for quantitation when performing such analytical tests as content uniformity analysis of the drug substance, assay of the drug substance within the formulated dosage form, and cleaning validation of manufacturing equipment. Some of the objections are: (1) that MS methods lack the precision typically required for analysis during the drug development process, (2) that MS instrumentation is too expensive and too difficult to operate, (3) UV only responds to things that are UV active, therefore, the UV detector can effectively ignore background components, such as excipients, that are not of interest to the analysis and, (4) UV methods can utilize phosphate buffers,
which are UV-transparent as well as improving peak shape, reducing baseline noise, and providing good buffering capacity for robust method reproducibility. The cost issue has been addressed by many instrument manufacturers, producing low cost (approx. $100K) and simple to use (“black box”) LC/MS instruments. Additionally, the precision issue as well as accuracy has been improved by incorporating labeled and non-labeled internal standards to the typical LC/MS methods. In fact, it is common practice in bioanalytical assays to utilize an isotopically labeled internal standard to account for differences in chromatography, ionization, or in the case of MS/MS detection, ion fragmentation.


In product development, it is sometimes necessary to continue to explore different synthetic routes for preparation of the drug substance. During the course of this work it can be advantageous to examine the quantitative aspects of the initial starting materials for optimization and selection of the appropriate synthetic route. Many of these starting materials and their potential by-products can be analyzed by GC/MS methods due to their volatility. A capillary GC/MS method was utilized by Robbins et al. (2003) to analyze 4-benzotrifluoride tert-butyl ether as a reaction by-product in the synthesis of fluoxetine. Selected-ion monitoring (SIM) was used to compare fluoxetine samples generated from both sodium hydride (NaH) and potassium tert-butoxide. Trace levels of 4-benzotrifluoride tert-butyl ether were detectable at low ppm levels in fluoxetine free base samples.

Other uses of quantitative MS analysis in product development have been reported for determination of the API in a pharmaceutical preparation. As stated previously, the use of isotopically labeled internal standards can aid in the precision and accuracy of the MS method. This methodology was utilized by Mick and Winger (2004) to perform rapid HT MS assays for quantitation using both flow injection analysis and isocratic HPLC for analysis of API in drug substance (drug purity) and for related substances analysis, respectively. Using SIM, two pharmaceutical compounds in development were analyzed with both methodologies and produced similar accuracy and precision data to that of traditional LC/UV detection.

Due to the expense and effort required to synthesize labeled reference standards for this type of analysis, Wade and Miller (2005) employed similar flow injection methods with non-labeled reference standards. This study examined the analysis of caffeine within a typical pharmaceutical preparation and within commercially available tablets, as well as the analysis of creatine within a typical pharmaceutical preparation. Creatine was specifically selected for this study due to its lack of a strong chromophore. Recoveries were accurate to within ±3% with precision of 3% relative standard deviation (RSD) or less obtained by this methodology. These studies demonstrate the capabilities and advantages of MS-based methods for performing quantitative assays within the drug development process.

V. FUTURE DIRECTIONS AND APPLICATION FOR PRODUCT DEVELOPMENT

Numerous advances in new instrumentation and software have been developed in recent years that will aid in the application of MS methods within product development. Recently there has been the need to both identify and quantitate trace level impurities (0.1% or less) found in both drug substance and dosage form. Having the ability to perform both quantitation and confirmatory identification within a single analysis would potentially provide an increase in productivity and reduction in cost. With this in mind, a study using the Q Trap™ that has the capability for performing rapid scanning single run identification, characterization, and confirmation of impurities and degradation products has been reported (Biesenthal, Pace, & Impey, 2003). Both quantitative and qualitative information (using selective software) can be obtained on a single instrument without compromising performance.

Recently, a new method for performing desorption ionization on a variety of materials in an ambient environment has been reported by Takać et al. (2004). This technique, known as...
desorption electrospray ionization (DESI), has the potential for performing on-line, HT monitoring of pharmaceutical samples without prior sample manipulation. R. Graham Cook’s group at Purdue (Chen et al., 2005) has shown that this methodology can analyze up to three samples/sec in an ambient environment. Figure 6 demonstrates the rapid analysis capabilities of this technique involving the analysis of Claritin tablets on a moving belt. Additionally, this methodology is capable of detecting compounds or impurities as low as ~0.1% without significant carry-over effects. This technique has the advantage of being able to sample a variety of objects with a minimum of sample manipulation. DESI is relatively simple to implement and allows for both in situ and in vivo analysis and retains the advantages of high sensitivity and selectivity that characterize MS detection methods.

A variant of this technique known as “direct analysis in real-time” or DART has recently been reported and commercialized (Cody, Larameé, & Durst, 2005). The basic difference between the two techniques (DESI and DART) is that DART exposes a sample to a stream of excited gas where DESI utilizes an electrosprayed liquid to produce ions. The basic design and various applications have been reported in the literature (Williams et al., 2006; Morlock & Ueda, 2007). The DART ion source is versatile and has demonstrated the ability to rapidly analyze samples on surfaces, liquids, and gases. Another version of this has been reported by McEwen, McKay, and Larsen (2005) and overlaps significantly with both DESI and DART technology. In principle, it utilizes slightly modified electrospray (ESI) or atmospheric pressure chemical ionization (APCI) ion sources. All of these techniques show the ability to perform rapid HT qualitative analysis of volatile and semi-volatile compounds desorbed from various surfaces. These technologies could prove increasingly valuable in the area of process analytical chemistry and quality control.

Another potential application of MS within product development is in the area of characterization of pharmaceutical materials, particularly in regard to counterfeit drugs. Recently, there has been an increase in the number of counterfeit medicines which have raised concerns with regulatory agencies and pharmaceutical manufacturers. With counterfeit drugs in mind, a variety of MS and MS/MS techniques have been applied to the authentication or “fingerprinting” of API as well as excipients and polymer layers or coatings found within pharmaceutical dosage formulations. A recent review by Olsen and Kiehl (2006) summarizes a variety of methodologies used to address this issue. An example of the identification of the “wrong” API in a counterfeit sample of an antimalarial drug, Halfan™ was discussed by Wolff, Thomson, and Eckers (2003). A range of mass spectrometric techniques, including accurate mass LC/MS and MS/MS, were employed to successfully identify the unknown API in the counterfeit drug product as the antibacterial agent, sulfamethazine, instead of antimalarial drug Halfan™.

These more “classical” hyphenated MS techniques though suffer from low throughput due to sometimes lengthy sample preparation and analysis steps. The two ambient ionization techniques mentioned earlier, DESI and DART, offer higher sample throughput with no sample preparation. The use of both of these techniques for analysis of counterfeit pharmaceuticals have been employed and discussed in the literature (Fernández et al., 2006; Nyadong et al., 2009). In some cases, an even more highly specific means of differentiating pharmaceutical products is necessary in the current regulatory environment. Recently, stable isotopic characterization of API by isotope ratio mass spectrometry (IRMS) has been shown to provide highly specific
methodology capable of identifying the source of pharmaceutical starting materials. Jasper et al. (2004) have reported on the analysis of four APIs using IRMS to distinguish between those produced by different manufacturers and different batches by the same manufacturer. Their work concluded that the compounding effect of individual dynamic ranges of multiple stable isotope values generates a highly specific “fingerprint” of any given API.

VI. SUMMARY

This review is not meant to be all inclusive; however, it has been written to highlight the overall use and significant increase of hyphenated MS techniques within the pharmaceutical development area over the last 10 years. With an emphasis on developing project knowledge at an earlier stage in development, the use of information-rich technologies (particularly MS) will continue to expand throughout product development (ICHQ8, 2006). One can imagine that in the very near future LC/MS will become “gold-standard” for implementation of the control strategy for the drug substance, especially for early phase development, as well as for route method development.

REFERENCES


