dreds of lead compounds per month instead of just one or two in the same period of time.

Accompanying this new drug discovery paradigm, new scientific journals have been established such as ***Combinatorial Chemistry & High Throughput Screening, Journal of Combinatorial Chemistry, Journal of Biomolecular Screening,*** and ***Molecular Diversity*** (see list of journal websites in Section 4). The variety of topics published in these journals reflects the multidisciplinary nature of the current drug discovery process and ranges from organic chemistry, medicinal chemistry, molecular modeling, molecular biology, and pharmacology, to analytical chemistry. As described below, the most significant analytical component of drug discovery has become mass spectrometry. Only mass spectrometry has become an essential element at all stages of the drug discovery and development process.

Although a variety of spectroscopic and chromatographic techniques, including infrared spectroscopy, nuclear magnetic resonance spectroscopy, fluorescence spectroscopy, gas chromatography, HPLC, and mass spectrometry, are being used to support drug discovery in various capacities, some of them, such as gas chromatography and fluorescence spectroscopy, are not applicable to most new chemical entities, some are not specific enough for chemical identification (e.g., infrared spectroscopy), and other techniques suffer from low throughput (e.g., nuclear magnetic resonance spectroscopy). Unlike gas chromatography, HPLC is compatible with virtually all drug-like molecules without the need for chemical derivatization to increase thermal stability or volatility. In addition, mass spectrometry provides a universal means to characterize and distinguish drugs based on both molecular weight and structural features while at the same time providing high throughput. With the development of routine LC-MS interfaces and ionization techniques such as electrospray and APCI, mass spectrometry has also become an ideal HPLC detector for the analysis of combinatorial libraries (11), and LC-MS, MS-MS, and LC-MS-MS have become fundamental tools in the analysis of combinatorial libraries and subsequent drug development studies (12–14).

The application of combinatorial chemistry and high-throughput screening to drug discovery has altered the traditional serial process of lead identification and optimization that previously required years of human effort. Consequently, neither the synthesis of new chemical entities nor their screening is limiting the pace of drug discovery. Instead, a new bottleneck is the verification of the structure and purity of each compound in a combinatorial library or of each lead compound obtained from an uncharacterized library using high-throughput screening. Because the number of lead compounds entering the drug development process has increased, in part because compounds are entering development at earlier stages than in the past, the traditional drug development investigations concerning absorption, distribution, metabolism, and excretion (ADME) and even toxicology evaluations of new drug entities have become additional bottlenecks. As a solution to the drug development bottlenecks, high-throughput assays to assess the metabolism, bioavailability, and toxicity of lead compounds are being developed and applied earlier than ever during the drug discovery process, so that only those compounds most likely to become successful drugs enter the more expensive and slower preclinical pharmacology and toxicology studies. In support of these new combinatorial chemistry synthetic programs and new high-throughput assays, mass spectrometry has emerged as the only analytical technique with sufficient throughput, sensitivity, selectivity, and robustness to address all of these bottlenecks.

2.1 LC-MS Purification of Combinatorial Libraries

Although combinatorial libraries were originally synthesized as mixtures, today most libraries are prepared in parallel as discrete compounds and then screened individually in microtiter plates of 96-well, 384-well, or 1536-well formats. To facilitate subsequent structure-activity analyses and to assure the validity of the screening results, many laboratories verify the structure and purity of each compound before high-throughput screening. Semi-preparative HPLC has become the most