drug applications to ensure that pharmaceutical industry productivity can be more realistically appreciated.

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Multidimensional separation and hyphenated techniques in pharmaceutical research: practical considerations

With the widespread use of combinatorial chemistry, HTS, genomics, proteomics and metabonomics, pharmaceutical research is entering a new era. The increasing complexity of pharmaceutical research has led to the development and application of multidimensional separation methods and hyphenated techniques. Analytical scientists are now faced with the challenge of choosing the most appropriate technologies for solving practical problems. In a recent edition of *Drug Discovery Today*, Guttman *et al*. [1] summarized the latest developments in multidimensional separation techniques. Multidimensional separation can be comprehensive or simple. In the case of a comprehensive analysis, every part of the sample is subject to separation in each dimension. In the case of a simple analysis, only a portion of the sample components might pass to the next separation dimension. In addition, there are different combinations of separation techniques coupled with various detection or hyphenation techniques. The coupling of separation and detection or hyphenation can be simple, as in the case of liquid chromatography–mass spectrometry (LC–MS), or more extensive, for example, LC–UV–evaporative light-scattering detector (ELSD)–chemiluminescent nitrogen detector (CLND)–MS, which is used for characterizing combinatorial library compounds. Different separation and detection techniques can be used together, either on-line or off-line, to solve a practical problem, with different advantages and limitations.

Throughput versus information content

Often there is a need to obtain comprehensive information from complex samples. In the case of proteomics and metabonomics, multidimensional separation might be necessary even if the overall throughput is low [2]. However, using MS or nuclear magnetic resonance (NMR) as the detection method potentially affords a higher throughput because these techniques are capable of selective and simultaneous detection of multiple components. For example, the use of high resolution Fourier transform–ion cyclotron resonance (FT–ICR)–MS coupled with one-dimensional separation for proteomics has been demonstrated with excellent coverage for the bacterial proteome [3]. Another example is the analysis of combinatorial library compounds. Good separation is always beneficial, but the need for high throughput necessitates the use of generic one-dimensional reverse-phase HPLC (RPLC) or supercritical fluid chromatography (SFC) methods to analyze large numbers of library compounds coupled with suitable spectroscopic detections (e.g. UV–MS) [4].

Degree of hyphenation

MS is generally used as one of the detectors in a hyphenated system because of its sensitivity and ability to generate information that can be used to identify target compounds. It is the detection technique of choice in proteomics involving LC separation. In metabonomics, NMR and MS are used as complementary detection techniques. For combinatorial library compound purification and analysis, the combination of RPLC or SFC with MS is commonly used and is complemented by one or two additional detection techniques including, UV, ELSD, CLND and NMR. These different detection methods often provide complementary capability in detection selectivity, range of sensitivity and linearity of response. A recent example by Yurek *et al*. [5] describes the simultaneous determination of identity, purity and concentration of library components that were produced by parallel synthesis. The system uses an HPLC with diode array detector (DAD), ELSD, CLND and time-of-flight (TOF)–MS detectors. The use of the exact mass capability of TOF–MS with CLND yields a synergistic combination that enables target and side-product structures to be elucidated and the concentration and purity of the compounds to be determined in a single analysis. We have used LC–UV–MS to determine the stability of 644 diverse compounds from the Abbott Laboratory repository. MS was used to identify the target compounds and decomposition products while UV was used for quantitation of these diverse compounds at a relatively high concentration [6].

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