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Combining UHPLC and high-resolution MS: A viable approach for the analysis of complex samples?

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ABSTRACT

The coupling of ultra-high-performance liquid chromatography (UHPLC) with high-resolution mass spectrometry (HRMS) has been well received within the analytical community. Both technologies have experienced significant advances in recent years. Not only have the resolution power and sensitivity improved, but the increased robustness, which includes prolonged column lifetime, extended dynamic range, easier mass calibration, and enhanced software handling capabilities, is making this coupling more attractive to a larger user base. In this article, we discuss possibilities and current limitations of the UHPLC-HRMS coupling. We also review the application of UHPLC-HRMS in a variety of fields, where it has been widely accepted or where we anticipate more extensive use in the near future.

Contents

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1. Introduction

There is probably no common consensus when a sample should be considered complex. For many analysts, the combined presence of compounds belonging to entirely different substance groups (e.g., proteins, carbohydrates, plant secondary compounds, and exogenous compounds) may present one dimension of complexity. The other dimension may be that some analytes are only present at trace levels, while the concentration of others may be in the high g/kg range. Things become even more complex when one analyte represents a trace compound in one sample, but features as a major ingredient in the next sample.

At the moment, no single, one-dimensional analytical technology is capable of handling such samples, so the utilization of coupled, orthogonal technologies has been extensively explored. The most successful coupling was probably the combination of a chromatographic separation and mass spectrometry (MS)-based detection. Such nearly orthogonal techniques {e.g., gas chromatography coupled with MS [GC-MS] or liquid chromatography coupled with MS [LC-MS]} were successfully commercialized. However, coupled techniques consisting of more than two dimensions have hardly expanded beyond research laboratories, with the possible exceptions of comprehensive GC or LC coupled with ion-mobility-MS. This can be explained by incompatibility factors or conflicting requirements for a successful coupling, such as mobile phase, and flow rate. It also has to be stressed that there are time constraints within a comprehensive three-dimensional system. This results in unacceptably long run times or the utilization of a fast, yet poorly resolving, dimension. Often, the addition of a third dimension increases the selectivity far less than expected. This can mostly be traced to true orthogonality among the employed dimensions being seldom achieved [1]. Last, but not least, monitoring and processing three-dimensional data are extremely complex.

Clearly, it is easier to improve the resolution of existing two-dimensional technologies (e.g., chromatography and MS) than to put additional dimensions into an analytical instrument. Thus, GC-MS was faster and easier to implement than LC-MS in this respect. The step to replace packed columns with capillary columns provided significantly higher resolution and quickly made GC-MS the premiere technique for monitoring residues and environmental contaminations. The much higher viscosity of LC eluents made it much more difficult to replace separation columns with capillaries. Hence, capillary chromatography or capillary electrophoresis never really became a mainstream technology.

It was only the commercial introduction of the Waters UPLC system that made significant inroads [2]. This instrument finally raised the long-held 400-bar pressure limit of LC pumps to 1000 bar. Furthermore, dead volumes within the instrument (pump, injector, and detector) were significantly reduced. The technology was well received by a wide community of analytical chemists, since the hardware was shipped together with pressure-stable, sub-2-μm-particle columns. All major LC companies have now launched similar UHPLC instruments. As a consequence, classic LC instruments have lost much of their market share. They are sold for applications where separation power or separation speed is not critical, or to laboratories with a tight financial budget. This does not mean that the majority of analysts buying a UHPLC system have installed sub-2-μm-particle columns in their UHPLC instruments. Some still use their unchanged methods, relying on old, lower-resolving columns.

The need for higher MS selectivity was soon realized by the research community as well. However, this was initially attempted by using tandem quadrupole instruments. Certainly, LC-MS/MS provides a much higher selectivity, but it does not provide a third dimension, when compared to single-stage MS. LC-MS/MS is not even a truly comprehensive two-dimensional technique, since MS/MS in the selected reaction monitoring (SRM) mode is capable of monitoring only a few mountain summits (mass peaks), but fails completely in describing the wider landscape in which they are located.

MS/MS was so well received because it provided the analyst with a previously unknown degree of selectivity and sensitivity. Hence, complex, labor-intensive, sample-processing techniques could be significantly simplified. Analysts soon considered it normal that their instrument only saw the things that it was told to see. However, they also realized that monitoring a few known compounds does not provide a comprehensive description of the sample as a whole. For example, there was the need to monitor toxic metabolites of a particular drug. The abuse of certain veterinary drugs could not be proved by monitoring the short-living active drugs, but rather by detecting metabolites that showed much longer clearance times. In addition, the number of pesticides used had increased significantly, so classic targeted analysis became more and more challenging.

Such challenges motivated researchers and, eventually, commercial analytical instrument companies to develop technologies, which provide MS/MS-like selectivity and sensitivity, while maintaining comprehensive full-scan information. Classic high-resolution MS (HRMS) instrumentation (sector or Fourier-transform ion-cyclotron resonance [FT-ICR]) were too slow, too complex to handle, and probably too expensive to buy and to maintain. It was only the introduction of modern time-of-flight (TOF), followed by Orbitrap instrumentation, which provided high mass resolution in combination with a sufficient dynamic range and speed. TOF and Orbitrap have undergone tremendous technological advances. The rate of HRMS innovation has certainly been much faster than that of conventional tandem quadrupole mass spectrometers. Tandem MS (QqQ) is now a mature technology, where the rate of innovation has slowed down considerably. The high sensitivity provided by modern QqQ has actually become a liability. Quadrupole-based MS/MS is approximately two orders of magnitude more sensitive than it was 10 or 20 years ago. However, the selectivity provided by these unit-resolving instruments has remained virtually unchanged. Hence, users are increasingly confronted with a number of peaks that require confirmation with a second or even third selected SRM trace. However, the performance of HRMS has improved in a number of aspects. In parallel, sensitivity and selectivity (mass-resolving power and mass accuracy) have been improved in recent years. Hence, HRMS is a promising technology with a number of previous limitations that have been resolved so as to permit its use in routine environments. However, there are still a number of issues to be addressed, so that analysts can fully benefit from the powerful possibilities provided by HRMS.

2. Technical constraints related to coupling UHPLC with HRMS

2.1. Generation of fast, well-resolved chromatographic peaks

UHPLC-HRMS techniques were initially the domain of university research laboratories. Separations were performed with home-built UHPLC systems [3]. Such systems consisted of 15 cm x 30–100-μm i.d. capillaries packed with 1.5-μm non-porous silica particles, which were coupled with a TOF system [3]. The instrumental hardware (required pumping pressure) and the sample capacity of the utilized non-porous silica particles were not designed for routine use. Hence, such early reports were basically intended to serve as a proof of principle.

Average chromatographers had no access to such applications, since, at that time, commercial HPLC pumps had an upper pressure limit of 400 bar. That limit was not only defined because of pump-seal leaking issues, but also because of the pressure stability of the silica particles used for packing the analytical columns.