Gold standard

Mass spectrometry and chromatography

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Mass spectrometry (MS) interfaced with liquid chromatography (LC) was once considered a technology that was reserved for rather specific applications that appeared to work in sequence with the phases of the moon. Early adventures with thermospray and particle beam interfaces proved to be of limited use, and it was not until atmospheric pressure ionization established itself that we could regard LC–MS as the analytical tool of choice for a considerable range of challenges. Indeed, advances in source design and increased ion transmission have presented a new generation of instruments that use hybrid technology such as quadrupole-time of flight (QToF), ToF–ToF and quadrupole ion-trap ToF, which provide even higher levels of confidence as a consequence of higher mass accuracy and enhanced structural information.

Although MS technology has accelerated at a bewildering pace, it is somewhat of a paradox to observe working practices in defining the optimal conditions for ion introduction into the MS following LC. The 'pot of gold' in LC–MS is often considered to embrace a single 'generic' column with a single 'generic' mobile phase. This view has been cited on a number of occasions and a number of papers have been published in an attempt to increase sample throughput and reduce method development costs without compromising the validity of the data. While other techniques such as capillary electrophoresis or super-critical fluid chromatography may provide an acceptable solution to this quest, the preferred pathway is to redefine the conventional bonded phase approach. Seeking a bonded phase material that may be used as a 'generic' column undoubtedly has some merit given the widespread use of the technology. However, a number of factors must be taken into account before embarking on this pathway, particularly when considering quantitative analysis. These factors include resolution and tuning of the mass analyser, linearity of the signal, matrix suppression, sample preparation, validity of the internal standard(s), effects of carry-over from the autosampler, and effects from metabolites. Additionally, the factors required to enhance ion signal intensity as a result of the mobile phase composition used in chromatography must be analysed.

Experience normally identifies the critical factors for analysis, which include choice of ionization mode. Electrospray is a condensed phase ionization process that preferentially ionizes polar compounds, whereas atmospheric pressure ionization is a gas phase ionization process that is considered to ionize rather non-polar compounds. In practice, the choice of ionization mode is also highly dependent on instrument design and not simply on the ionization process itself.

With such a large number of variables to consider in optimizing signal intensity, one approach is currently being redefined: column-switching. The advantages of column switching have been well documented, but in environments where high throughput is required it is a technology that has now been integrated into LC–MS data management software that provides a simple solution to complex separation problems.

Use of column switching in product characterization

Integrated column-switching technology was initially applied to routine LC–NMR (analysis within a pharmaceutical research context). The natural evolution was then to consider applications in LC–NMR–MS and high throughput LC/MS for impurity characterization. In the case of the impurity characterization, ICH guidelines require structural characterization of actual impurities present in the new drug substance at or above an apparent level of 0.1%. Often, methods have been developed to separate out impurities or by-products that cannot simply be transferred to LC–MS detection, as the mobile phases include non-volatile buffers or ion-pairing reagents. Although desalting the mobile phase is an approach that has been applied off-line, it is both inefficient and labour intensive. Column switching techniques can

solve such a problem, providing on-line analysis for LC–MS by replacing solvents with a pre-concentration function. This enabling technology allows mobile phase compositions that provide the optimum response and performance in both LC and MS to be selected.

Components were separated in a mobile phase of 10 mM phosphate buffer containing 200 mM $NaClO_4$. Such a mobile phase would be inappropriate for LC–MS as both salts would not only affect ion evaporation, but also contribute to space charge effects at the ion entrance resulting in a limited ion signal and a high-maintenance cycle. To maintain chromatographic integrity and provide an ion signal three HPLC segments are connected by switching valves.

The first LC separates the compounds of interest under optimal conditions using conventional non-volatile buffer solutions. Target components are subsequently fractionated using a six-way valve into a sample loop and transferred onto a trapping column after diluting with water, as part of the second LC system. Buffer constituents that are inappropriate for MS are washed out using the trapping column. The retained compounds are then eluted from the trapping column to the third LC, and are re-separated using an appropriate mobile phase for LC–MS. For the concentration effect, a semi-micro column may be applied for the third LC. MS experiments can now be undertaken using a 'generic' column and a 'generic' mobile phase.

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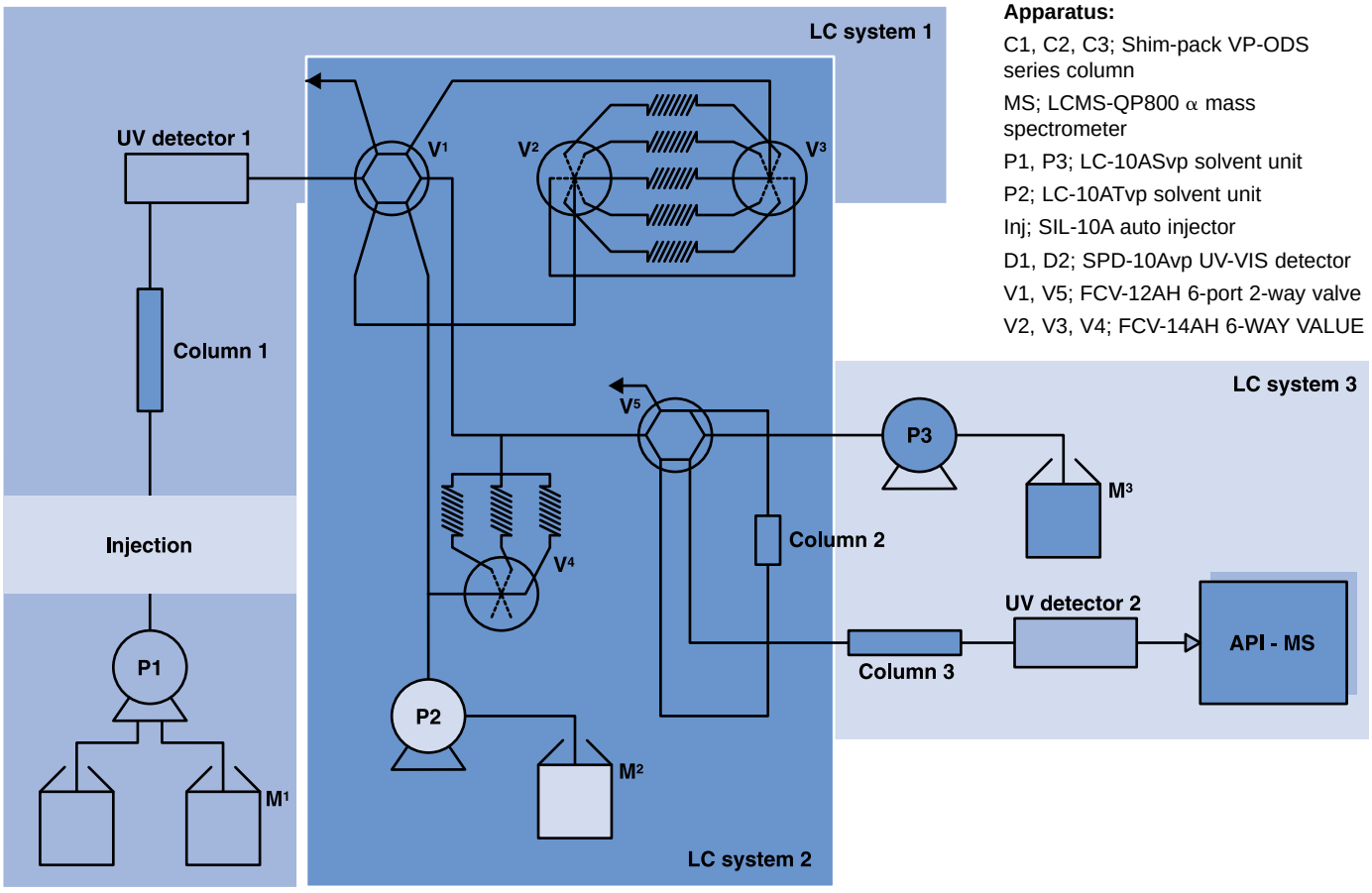
Using test compounds in a mobile phase of 10 mM phosphate buffer the initial trapping efficiency is typically higher than 98%. Following the desalting step at the trapping column, the concentration of phosphoric acid in the eluted fractions is minimal (the initial concentration was equivalent to 5000 p.p.m., after washing the column the residual concentration was 1.2 p.p.m. — equivalent to 1/4180 of the initial value).

In practice, the MS analysis of an impurity present in the model drug substance above a level of 0.1% (calculated using the UV peak area of the drug) shows that methods that require non-volatile buffers



Apparatus:

C1, C2, C3; Shim-pack VP-ODS series column
MS; LCMS-QP800 α mass spectrometer
P1, P3; LC-10ASvp solvent unit
P2; LC-10ATvp solvent unit
Inj; SIL-10A auto injector
D1, D2; SPD-10Avp UV-VIS detector
V1, V5; FCV-12AH 6-port 2-way valve
V2, V3, V4; FCV-14AH 6-WAY VALUE

present in the mobile phase can be successively transformed for routine LC–MS analysis. A further advantage in the system design is that components can be concentrated rapidly. Clearly, such an approach also has a significant impact on the quality of spectra compared with conventional modes of analysis and once again results in higher levels of confidence in data reporting.

Conclusions

This system was applied to trace drug analysis and the following advantages were observed:

1. The optimal mobile phase for LC separation can be used routinely without restricting the mobile phase composition.
2. Mobile phases appropriate for LC–MS analysis can be used routinely.
3. Target components can be pre-concentrated.
4. All procedures are automated providing an on-line solution which is rapid and simple.
5. Sample pre-concentration ensures all trace components are identified.
6. Further purification and concentration of the separated compounds are achieved by a third LC column.
7. Unstable compounds that may decompose during an off-line procedure are effectively analysed by this system.

This system can be a powerful generic tool in automating on-line desalting and pre-concentration, it provides LC–MS with a simple solution to working with conventional approaches in product characterization and in operating with conventional mobile phase compositions. It may even show the way to the end of the rainbow.

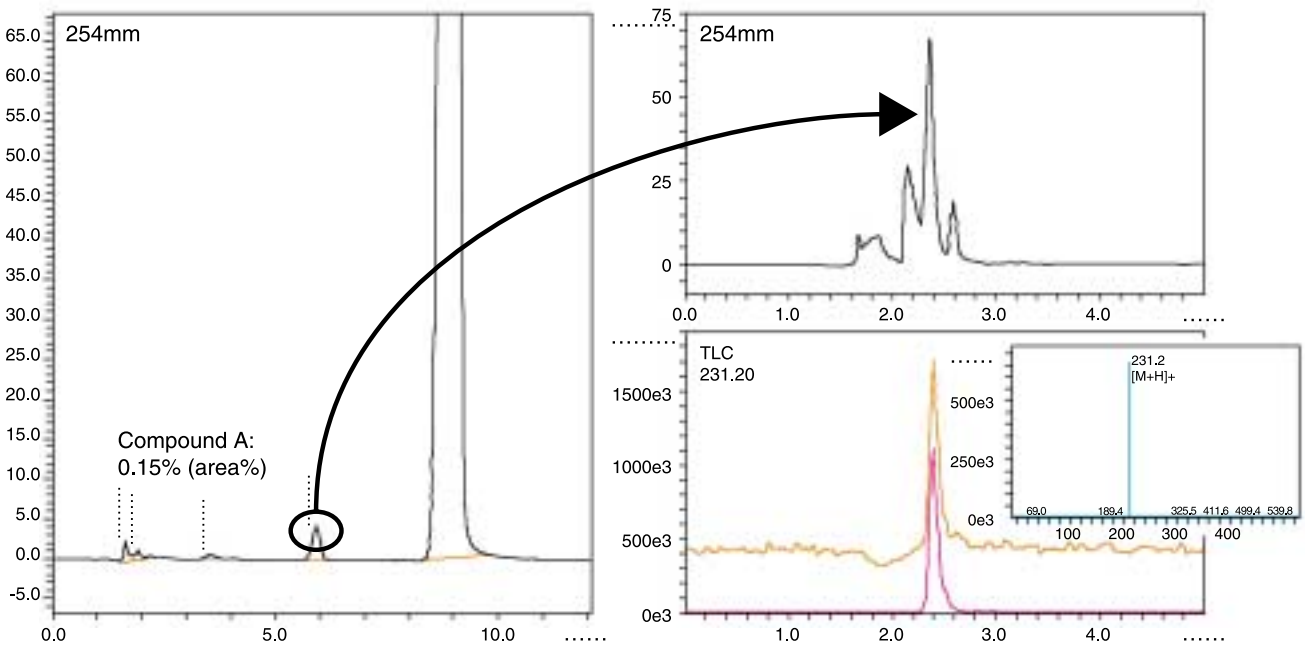
References

1. N. Asakawa et al., (1991) J. Chromatogr. **541**, 231
2. Y. Yokoyama et al., (2000) Anal. Sci. **16**, 1183

Neil Loftus is a Business development Manager for Shimadzu Biotech who works principally on MS product strategy for life science and drug discovery markets. With a background in biochemistry he has worked for a number of years on hyphenated techniques applied to the pharmaceutical sector.

For more information visit Shimadzu's Biotech's website: www.shimadzu-biotech.net

Photo courtesy of Photodisc



Initial chromatography

Column: Shim-pack VP-ODS 4.6mmI.D.x150mmI.
Mobile phase: 20mM (Na)phosphate : methanol (45 : 55)
Flow rate: 1.0 ml/min
Trapping colum: VP-ODS 2.0mmI.D.x5.mmI.

Rechromatography and MS analysis of compound A.

Column: Shim-pack VP-ODS 2.0mmI.D.x150mmI.
Mobile phase: methanol
Flow rate: 0.2 ml/min
Ionization: ESI (+), Scan m/z 10-550