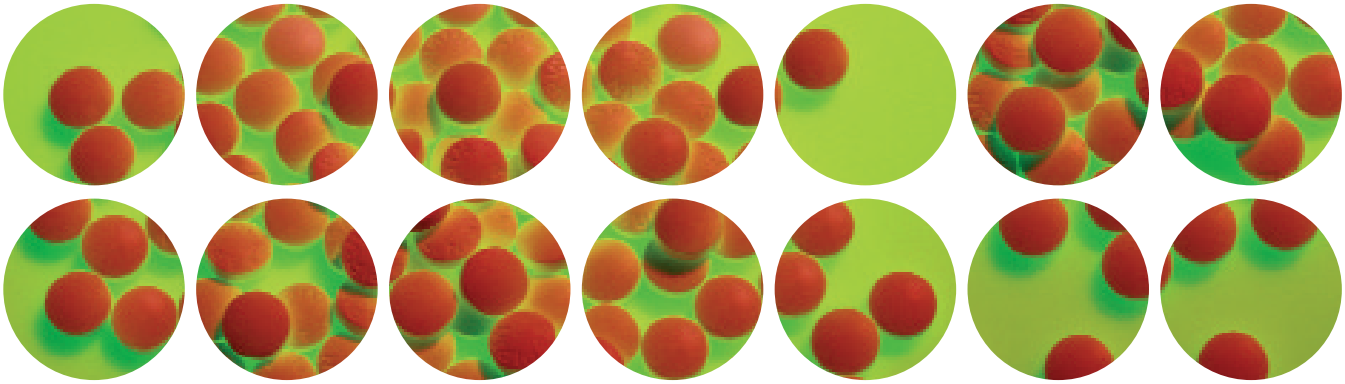

The Role of LC–MS in Drug Discovery

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Introduction

The implementation of new tools within the discovery process, including combinatorial chemistry, proteomics and ADME/tox profiling, demand the creation of new strategies for pharmaceutical analysis. Sample generation has increased very rapidly and, as a consequence, traditional analytical approaches are unable to fulfil the requirements set by lead generation. Therefore, a great challenge has developed to create new, rapid, high-throughput analytical methods to speed-up the whole discovery process.

CE–MS

Capillary electrophoresis (CE) is a relatively new tool for chiral analysis. Chiralty is very important in biochemistry because different enantiomers can produce diverse pharmacological and toxicological actions. Fujiware and Honda described the use of CE in the pharmaceutical industry.[1] Once a screening method has been developed, CE can be used as an additional technique for the detection of impurities. In the discovery process CE can help with the quantification of enantiomers. The first coupling of CE with mass spectrometry (MS) was demonstrated by Lane et al.[2] CE–MS combines the advantages of CE (highly efficient separation) and MS (structural information). However, optimizing the interfacing of CE with MS is a real challenge because of the low flow-rates (10–100 nL/min) required in CE, which is achieved by using a make-up liquid. CE–ultraviolet detection (UV) has been widely used but CE–MS has led to applications in the field of qualitative chiral analysis in order to clearly identify unknown compounds and impurities.

GC–MS

In GC–MS electron impact (EI) is commonly used in addition to chemical ionization (CI). Mass spectra obtained by this hyphenated technique offer more structural information based on the interpretation of fragmentations. Fragmentation spectra with different relative abundances can be compared with library spectra. The information obtained by GC–MS is totally different than from LC–MS. Although it is not so applicable to a wide range of compounds (Figure 1), GC–MS can help in following reactions and is especially suited for analysing small and/or volatile molecules not easily detected by electrospray LC–MS. Orthogonal time of flight (TOF) mass spectrometry coupled with GC is used for confirmation of purity and identity by measuring exact mass and calculating elemental composition. Mostly, a lock mass, such as chloropentafluorobenzene, is used to calculate the exact mass. Nowadays, developments in GC–MS relate to decreasing analysis time.[3,4]

SFC–MS

In supercritical fluid chromatography (SFC) a gas, often carbon dioxide, is used above its critical pressure and temperature. Coupling of SFC with MS is, in most instances, compatible with standard probes on LC–MS instruments. In fact, only recently this technique has been used for combinatorial library screening.[5]

LC–MS

The recent improvements in LC–MS technology have been staggering. Ten years ago there was no competitor in the field of analytical chemistry that could satisfy the requirements of high-throughput, sensitivity and selectivity. Additionally, LC–MS instruments have become easy to use, cost effective and robust, and helped by the readiness of the instrument manufacturers to meet the requirements of their customers, the different techniques and instruments have been optimized. Consequently, MS is becoming step by step a kind of universal detector for HPLC. In parallel to this evolution in LC–MS instrumentation there has been tremendous growth in LC–MS methodologies for pharmaceutical analysis; so much so that LC–MS is now accepted as a routine tool within the

pharmaceutical industry. The following sections give a short overview of the wide field of LC–MS applications in drug discovery and medicinal chemistry.

Obtaining structural information in the early stages of drug development has been enabled by LC–MS.

LC–MS in ADME/tox: The process of bringing a drug candidate to market is both expensive and time consuming, and speeding up drug discovery has become a major goal for all pharmaceutical companies. To save time and improve the selection of candidates, physicochemical properties such as absorption, distribution, metabolism, elimination and toxicity (ADME/tox), and pharmacokinetic screening are evaluated early in drug discovery. The availability of this information leads to faster decision making. For this purpose, high-throughput LC–MS enables quick monitoring of these parameters for the large set of compounds generated. Triple quadrupole technology coupled with fast gradients on narrow-bore columns is commonly used in the bioanalytical laboratory for quantification. Alternatively, ion traps provide repetitive fragmentations of product ions that is fundamental for metabolite idenfication.[10]

LC–MS in proteomics: The screening of proteins as potential targets for drug interaction is a rapidly developing field. Recently, HPLC methodologies have been investigated to analyse tryptic digests of large proteins. Coupled with ESI-MS complex mixtures can be analysed. Fully automated data interpretation using bioinformatic systems can help in the identification and quantification of proteins. Instruments such as matrix-assisted laser-desorption ionization (MALDI)-TOF and tandem mass spectrometry (Q-TOF) are commonly used in the field of proteomics.[11]

The challenge to increase innovation and productivity in drug discovery starts in medicinal chemistry.

LC–MS in medicinal chemistry: MS has a significant role to play in medicinal chemistry. Only a decade ago, chemists would identify their compounds based on thin-layer chromatography and purify their compounds in order to perform NMR. At that time large amounts of compounds were synthesized. From 1970 to 1990 NMR and IR were the analytical tools used to monitor chemical reactions, with other analytical techniques such as elemental analysis and titrations being used for a complete confirmation of the structure and purity of the compounds. MS was mostly used as an additional technique. Before 1990 the most used ionization methods were electron ionization (EI), chemical ionization (CI) and fast atom bombardment (FAB).[6,7] At that time HPLC was the method of choice for the analysis of complex mixtures. As packing techniques made it possible to prepare reproducible columns from 10 µm packing material in the early 1970s, HPLC was used for different applications in the pharmaceutical industry. Nowadays, HPLC is competitive with GC and is able to analyse a broader range of compounds.

The development of an interface for the introduction of the liquid flow from an HPLC system into a mass spectrometer was unique.[8,9] Today, most chromatographic analysts have become mass specialists because of the growing trend of coupling LC with MS. Obtaining structural information in the early stages of drug development has been enabled by LC–MS for the medicinal chemist.

Open-access LC–MS: One of the first articles concerning the application of open-access MS was written by Taylor et al.[12] Recently Greaves described the introduction of an open-access MS facility in a university environment.[13]

Previously, the chemists in our medicinal chemistry department submitted their samples to the analytical laboratory, which was a full service department, and following analysis received an interpretation of their spectra or analytical data. However, such a central analytical service unit with many analysts specialized in a certain technique is not flexible enough and not customer focused. After an internal reorganization an open-access concept has been implemented. This was realized after an in-depth analysis of its feasibility.

It is important to know

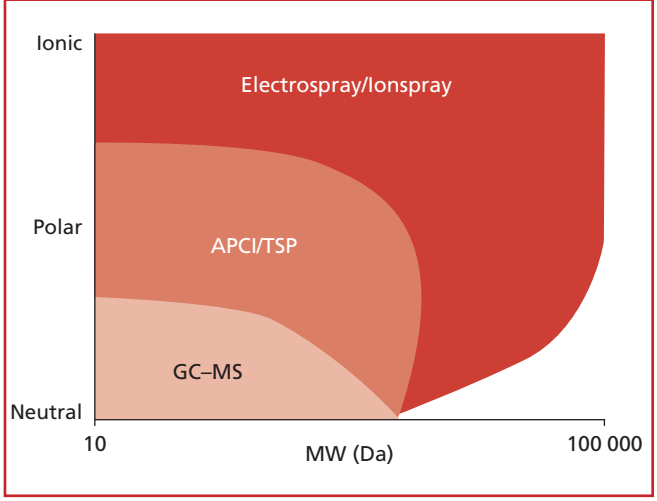
- which instruments could be transferred in open access?
- what kind of transformation is needed to obtain a user-friendly analysis laboratory?
- which training needs to be organized ?
- which analytical data can be interpreted by a synthetic chemist with no or little experience?

The robustness of current LC–MS instruments, the availability of user-friendly, open-access software and the advantages of electrospray ionization giving molecular weight information made LC–MS suitable for use in an open-access laboratory. Four LC–MS instruments are currenly operating in an open-access environment in our laboratories and are used by approximately 50 chemists.

The open-access operating procedure for LC–MS was presented in a lecture to all medicinal chemists followed by hands-on laboratory training in small groups and a theoretical training or refresher course for customers. Additionally, we have reorganized our laboratory to facilitate this new working practice. A wide range of applications from reaction monitoring and reaction optimization to characterization of intermediates is covered by LC–MS. In most instances molecular weight confirmation can be sufficient.

Nowadays software is a significant tool for analysts and chemists who require an easy way to control their LC–MS systems. All our LC–MS systems are from Waters-Micromass

Figure 1: Application of different ionization methods.



(Milford, Massachusetts, USA) and run with Masslynx software. Creating an environment where chemists can have an access to all LC–MS systems, we need to have a simplified software allowing only those input fields that are essential for the measurement. Normally most of the features of the operating software for controlling the mass spectrometer and the HPLC pump require the input of different parameters needed for the acquisition and the tuning of the instrument. Those parameters are essential for the measurement but are too complicated for the open-access user. If the non mass specialist uses this operating software we run the risk of chemists who will unconsciously change parameters that can damage the instruments. For this reason we use Openlynx software (Waters-Micromass), a dedicated package for open-access integrated within the Masslynx operating software. It is not necessary for chemists to have access to all LC and MS parameters. By using Openlynx we can use predefined methods incorporating all acquisition, tune and process parameters.

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Sample preparation is performed by the chemist and samples can be introduced into the system whenever is convenient and results are available immediately after the experiment. This process enables chemists to rapidly and effectively perform LC–MS analysis without the intervention of an MS specialist. Open access creates fast turn around and frees the analytical chemist to concentrate on more challenging tasks.

Figure 2: Openlynx login — inputting necessary information such as chemist, sample ID, method and vial position is enabled by different screens.

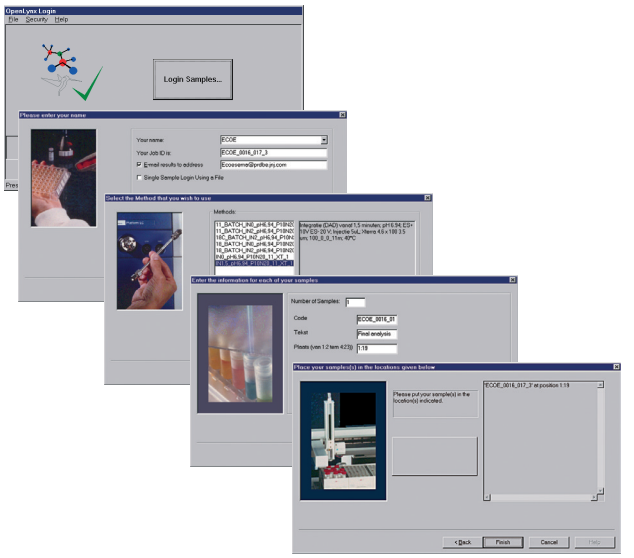


Figure 2 shows the different steps in the sample login procedure. The first step is identification of the chemist by the combined use of an individual user name coupled with a definable e-mail address. The next step involves specification of the LC–MS method. The following screen allows the chemist to add a sample ID, some text and the vial position. In its simplest form a "button push" is enough to start the experiment.

As we support a great community of users with compounds from different projects, it is not easy to cover every type of compound by a single generic LC–MS method. First, achieving the best separation is the most critical step in this way of working. Delivering quality in the obtained chromatograms is essential because the chemists do not have time to look in detail at the mass spectra. It is therefore better to concentrate on a longer analysis time instead of creating a fast analysis with the possibility of coeluting peaks that are difficult to interpret quickly. Initially, a standard generic gradient method was used for all LC–MS systems. However, to create flexibility because of the different classes of compounds we have equipped different instruments with other types of columns and gradients to obtain different selectivities on the LC–MS instruments. For MS parameters we also use a generic set-up with alternate positive/negative switching.

Triggering on a specific mass to automate the reporting, and highlighting the mass found with 'green' is possible but not advisable because the chemist will trust the depicted mass. In this instance it is important to look at the mass spectrum to check the molecular weight.

After measurement the raw data are processed using an Openlynx process file (olp) to create a simple report with a customizable layout including all necessary chromatograms and mass spectra of the most important peaks available. The report as depicted in Figure 3 is electronically sent by e-mail to the chemist. Every chemist has the ability to open the MS report via the Openlynx browser software and perform interpretation. It could be said that this way of electronic reporting creates a non-communicative environment. However, as far as we have experienced, chemists are willing to address an analytical expert when a problem arises in mass spectral interpretation. Analysts can assist the chemist in the interpretation and/or perform

Figure 3: Openlynx browser — MS and UV data are displayed. The blue light indicates that no trigger mass was specified during sample login.

additional experiments on dedicated instruments. Those experiments are performed to solve problems with separations, ionization efficiency or with the technique itself.

Every chemist is trained in basic mass-spectral interpretation. They need to know the information generated by API-LC–MS. Although it looks very simple (positive ions are detected as protonated species MH^+ while negative ions are deprotonated $M\text{-}H^-$) it is important to know that some compounds may cause adduct formation and ions including $MNH4^+$, MNa^+ and MK^+ can occur in the spectrum. In addition, chemists more familiar with average molecular masses must calculate the formula with monoisotopic masses.

From Parallel Synthesis to Parallel Analysis

As a result of the acceleration of the drug discovery process and to identify potential lead compounds new strategies have been introduced such as combinatorial chemistry. In our high-throughput chemistry laboratory series of compounds ranging from fifty to five-hundred compounds are generated in parallel by a solution- or solid-phase approach.

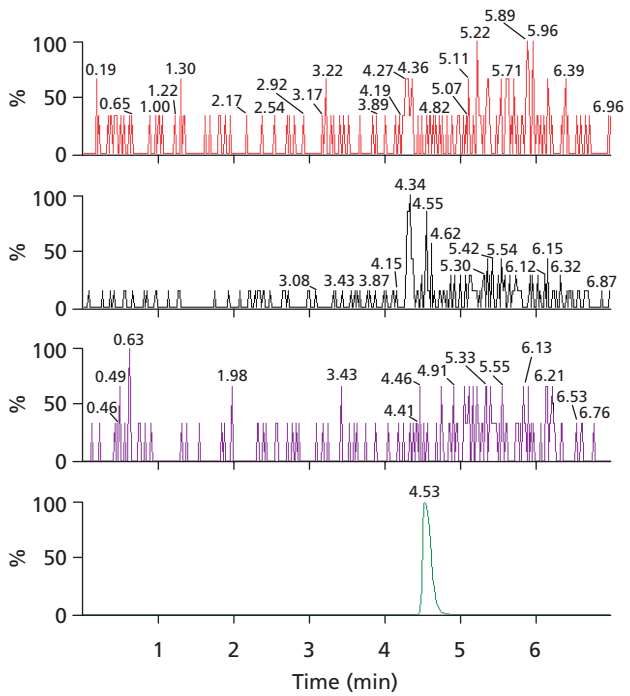
The recent developments in automatic synthesis instrumentation has led to a huge interest in the combinatorial chemistry field. There, the real problem starts for the analyst, who is confronted with a huge amount of samples requiring analysis in a short period of time. Definitely, as we want to deliver high-quality compounds, a good separation is required. One obvious solution would be to invest in new LC–MS instruments. But there are new innovative improvements that enable high-throughput LC–MS in a more flexible, cost-effective and time-saving manner. For example, high-throughput LC–MS can be realized by using a multiplexed electrospray interface (MUX). We have decided to implement this parallel interface on an LCT spectrometer (Waters-Micromass). The aim is to combine speed of analysis, through parallel LC–MS, with the quality of data generated by a TOF spectrometer. By using erythromycin as a lock mass we can realize exact mass measurements in a routine manner on the LCT spectrometer. A set of emperical formulae can be derived from the exact mass spectra.

In principle, with the 4+1 MUX interface each individual electrospray inlet enters the analyser virtually simultaneously. In practice, a sampling rotor is used (propelled by a variable-speed stepping motor). If the dwell time for spectral acquisition is set to 100 ms and the stepping is at 50 ms then the cycle time for a four-way MUX is 600 ms. To be as flexible as possible in sample throughput we have evaluated a four-way MUX interface.

Figure 4 shows four MS spectra from four consecutive sprays. In the first spray a sample was injected. In spray two and three the effect was checked using a blank injection. Inter-channel crosstalk was found to be negligible.

An additional advantage of this set-up is the minimal bench-space required to install such parallel LC–MS systems. Figure 5 shows the different components in the whole set-up. This system is equipped with four LC pumps instead of using one gradient pump and splitting to four columns.

Figure 4: Inter-channel cross contamination.

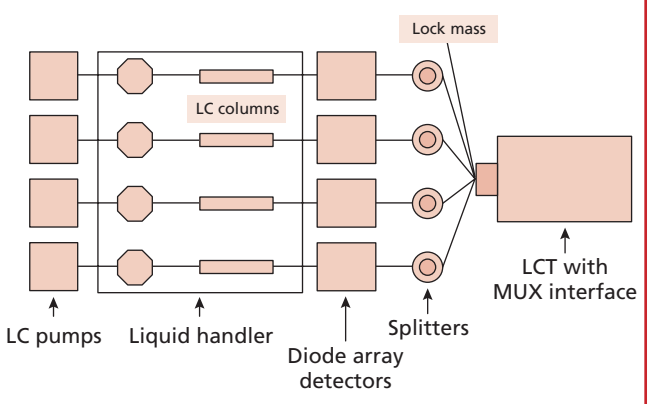


MS-Triggered HPLC

Because the mass spectrometer was known as a selective and sensitive detector in analytical LC–MS, its use for mass-triggered fraction collection became popular. Previously, UV detection was used to trigger the collection of fractions during preparative HPLC analysis. In this way purification would provide many fractions that need to be identified by a separate LC–MS or flow injection analysis–MS experiment. Collecting every UV-sensitive peak was time-consuming work. Recently, the introduction of mass-directed fraction collection has evolved into an intelligent alternative for the autopurification used in reversed- and normal-phase separations. This results in a small set of fractions that are collected based on a predefined trigger mass, adduct mass or multiple masses. The MS-directed preparative platform is outlined in Figure 6.

The implementation of such UV/MS-based LC–MS systems has improved efficiency and reduced cycle time compared with classic preparative UV-directed HPLC analysis. A few to hundreds of milligrammes product can be loaded using a gradient pump (Waters 4000) and injected using a Gilson 215 autosampler. Sample tracking is achieved by Fractionlynx

Figure 5: Schematic of a parallel LCT system.



software (Waters-Micromass), specially designed for autopurification systems. A dedicated splitter with high split ratio (1:10 000) is an essential part of the whole set-up. A make-up pump is used to increase flow to the detectors. The collection of fractions is monitored by Fractionlynx, which controls the switching valve (load-inject position) on the collector.

The Future

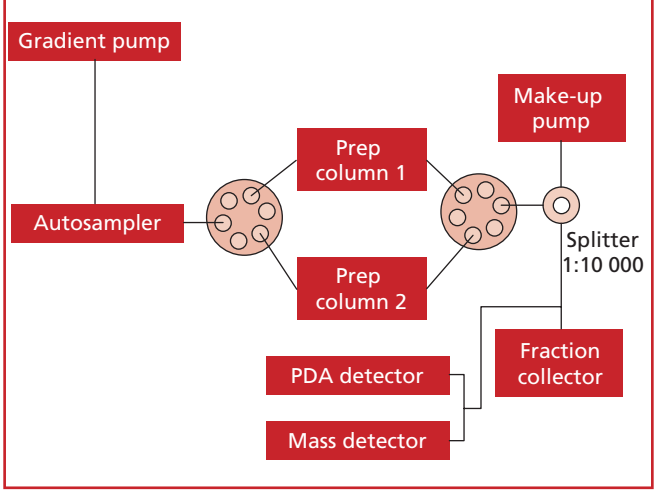
The field of LC–MS and other hyphenated techniques is continuously developing. There is still a trend to move towards faster LC separations because high-thoughput lead generation must go hand in hand with analytical identification. Many manufacturers are focusing on automation but it must be emphasized that data quality and measurement accuracy is sometimes the real paradigm in a high-throughput oriented industry.

At present there is a lack of quantitative data determination using UV and/or MS detection. Both detectors have there own disadavantages. If you are using UV for quantitative work you need to make reference standards. So, here the question arises: can we find a universal detector? Obviously, a universal detector is not available but evaporative light scattering detection (ELSD) and chemiluminescent nitrogen detection (CLND) allow purity determination of combinatorial libraries more accurately than a UV-based system. Nevertheless, these detectors also have disadvantages. For ELSD, volatile compounds can be evaporated. For CLND, working in a nitrogen-free medium is essential, and nitrogen impurities from the system or solvents can drastically influence results. A combination of UV, ELSD and CLND in one set-up could be an alternative and workable solution for determination of the purity and identity of the combinatorial library.[14,15] Alternatively, new ionization sources such as atmospheric pressure photoionization (APPI) enable an improved ionization of compounds that are not feasible by ESI or APCI.

Using LC–MS as the only tool for high-thoughput analysis has its pitfalls. Coelution of peaks has already been mentioned and determination of stereochemistry cannot be solved. What if a library of compounds contains positional isomers? Recent developments in the field of coupling LC–MS with NMR have allowed the elucidation of structures of individual compounds.[16] Hyphenated NMR can also be helpful and time-saving in the idenfication of metabolites.

To conclude we may never forget that such high-throughput analysis is generating large amounts of data, which has evolved the introduction of information management systems. Obviously sharing knowledge by using data mining tools enables analysts and chemists to interpret analytical data as spectra, chromatograms and reports obtained from different techniques. Data capturing, cataloguing, reporting and retrieving tools combined with spectral interpretation, processing and visualization tools merged in a common software platform delivers quality in decision making about the identity and purity of compounds.

Figure 6: Mass-directed purification scheme using two preparative columns.



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Dr David Corens gained a PhD degree in organic synthesis at the Catholic University Leuven (KUL), Belgium, under the supervision of the late Professor G. L. Abbé and Professor G. Hoornaert in 1997. In this year he started at Janssen Pharmaceutica and became responsible for the mass spectrometry subgroup of the analysis medicinal chemistry group. In 2001 he was promoted to Teamleader Analysis in the Department of Medicinal Chemistry and became responsible for building a state-of-the-art analytical laboratory in an open-access environment. His team has an expertise in a diverse range of analytical techniques such as NMR, MS, IR, VCD, CE, elemental analysis, ion chromatography and polarimetry.