The Incredible Impact that Electrospray had on the Pharmaceutical Industry. 'A Personal Perspective'

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Nowadays within the Pharmaceutical Industry, we consider LC-MS as a routine analytical tool. When referring to LC-MS, terms like Atmospheric Pressure Ionisation, APCI and Electrospray are banded around as if they have been with us since the birth of mass spectrometry. LC-MS is now probably one of the most important analytical techniques used throughout the Drug Discovery and Development process, and it is hard to imagine a time when this was not the case. However, when I started out as a young mass spectrometrist in that industry in the early 1970s, the concept of interfacing liquid chromatography to mass spectrometry was just a 'wild dream' and considered by many at that time to be impossible to achieve. Back then, there were a small number of academic groups within the world attempting to marry these two apparently incompatible techniques, but they were seen by many of the mass spectrometry community to be out on the 'lunatic fringe'. In fact it was one of the early pioneers of direct liquid introduction, Patrick Arpino, who was responsible for the now iconic cartoon shown in *Figure 1* which implied that the chances of interfacing liquid chromatography to mass spectrometry was about as likely as the bird marrying the fish and living happily ever after [1].

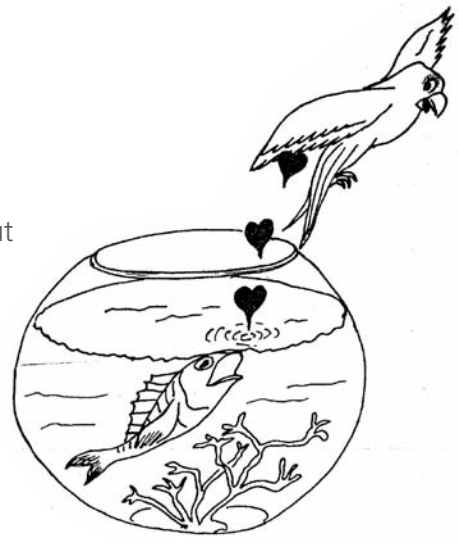

Figure 1. LC and MS, will the two ever come together.

Highlighting the incompatibility between liquid and gas phase (vacuum based) systems. Patrick Arpino, 1974.

These pioneering academic groups did, however, produce a wide variety of interesting interfaces, all of which were sensational for their time, but were not seen by the mass spectrometry community within the pharmaceutical industry, as reliable enough to be considered as routine tools within the Drug R&D process [2-8]. However, as the exploration for new drug entities evolved in the late 70s and into the 80s, the type of molecules that we were investigating became more polar in nature, and the only separative technique capable of resolving reaction mixtures and detecting metabolites in biological fluids was reverse phase liquid chromatography (RP HPLC), therefore the drive to link LC to MS became the 'Holy Grail' of that time.

Many approaches to overcome the incompatability of the techniques were attempted by the mass spectrometrists withiin the pharmaceutical industry. One of the most common was 'off line' peak collection, followed by laborious 'dry down/solvent evaporation. This was cumbersome, slow and very labour intensive, and so many of us resorted to applying some of the afore mentioned 'interesting interfaces' in a desperate attempt to speed up the analysis of drug candidates. Some exciting work was accomplished and published by the pharmaceutical industry sector using these wacky techniques. Thermospray was the first technique that really showed there was a future for LC-MS within the Pharmaceutical sector [9]. This technique was embraced extensively across Drug Discovery and Development, and even with its limitations on solvent composition and reliability, a considerable amount of work was carried out, and the start of the 'open access' MS revolution was developed using thermospray as the ionising medium [10]. Thermospray became the 'benchmark' for LC-MS and remained so for a number of years until atmospheric pressure ionisation appeared on the scene and then the whole world changed.

The API revolution, as it is now referred, was not a tidal wave or even the exclusive domain of one research group, it was, however, the culmination of a number of minor events and developments that happened in tandem across the world which, when brought together, changed the LC-MS landscape completely. It is not often in science, that events coincide in this way, but this is an example one of those wonderful periods. Here is a brief catalogue of these events. Firstly, Jack Henion (of DLI fame circa 1978) was working in the veterinary medicine arena at Cornell University had linked up with Bruce Thomson (of ion evaporation fame circa 1978) who was working for a small mass spectrometry company called Sciex in Toronto who build air monitoring mass spectrometers. They published a seminal paper in Anal Chem titled Determination of sulfa drugs in biological fluids by LC-MSMS [11], which used atmospheric pressure chemical ionisation in front of a Sciex mass spectrometer. This approach to LC-MS demonstrated incredible sensitivity at trace levels. This was quickly followed by improvements in the design of the APCI source and further papers by Henion et al [12,13] followed in quick succession. Around the same time John Fenn at Yale University, had developed the first atmospheric pressure electrospray source based on the original work of Dole back in the 1960s [14]; and he reported on the multicharging of large bioorganic molecules such as proteins (see *Figure 2*) [15].

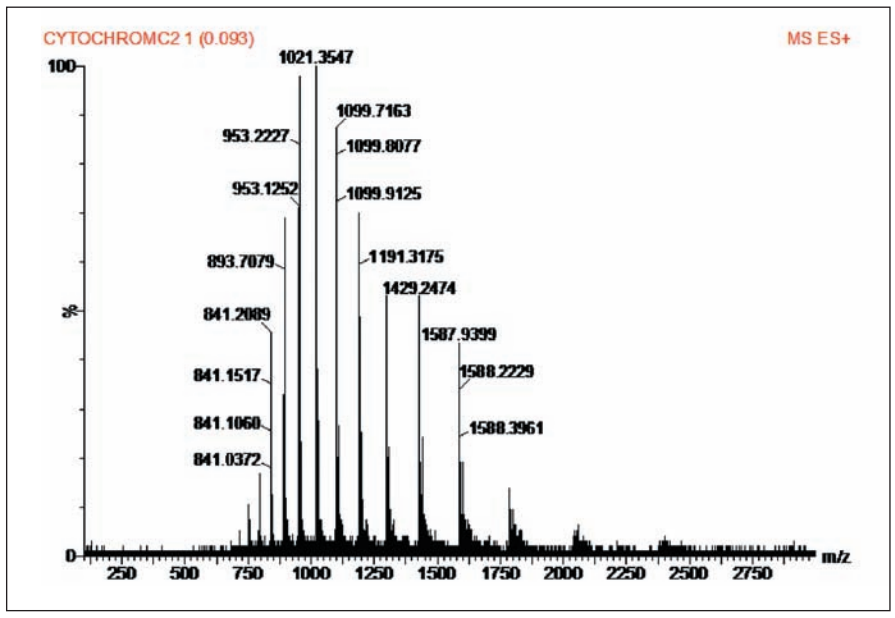

Figure 2. API Electrospray MS spectrum of the protein Cytochrom C.

So suddenly and by two independent research groups the world had been exposed to two techniques both using ionisation at atmospheric pressure; both showing incredible sensitivity, and both capable of being linked to reverse phase LC. It was also fortunate that through Thomson and his access to an instrument company with a mass spectrometer that sampled at atmospheric pressure, meant that all the planets were in alignment and a whole new era of LC-MS was born. The first publications started appearing in the literature under the titles of 'ion spray and APCI [16] and all were demonstrating sensitivities at least one to two orders of magnitude greater than other techniques which was particularly interesting to the drug metabolism industry, and by the time Sciex launched their first API MSMS instrument in 1989, and the whole landscape of LC-MS changed irreversibly. This new breed of instruments were simple to use, had no real constraints on mobile phase composition or flow rate; the spectra were simple to interprete, as the protonated molecular ion predominated the spectrum *(Figure 3)*, with virtually no fragmentation being detected. All of this meant that LC-MS was no longer wholly the domain of the mass spectrometrist, and biochemists, drug metabolism specialists, chromatographers, all now access this technology, and in a very small space of time all the other mass spectrometer manufacturers follow suit and produced mass spectrometers exclusively dedicated to these API techniques.

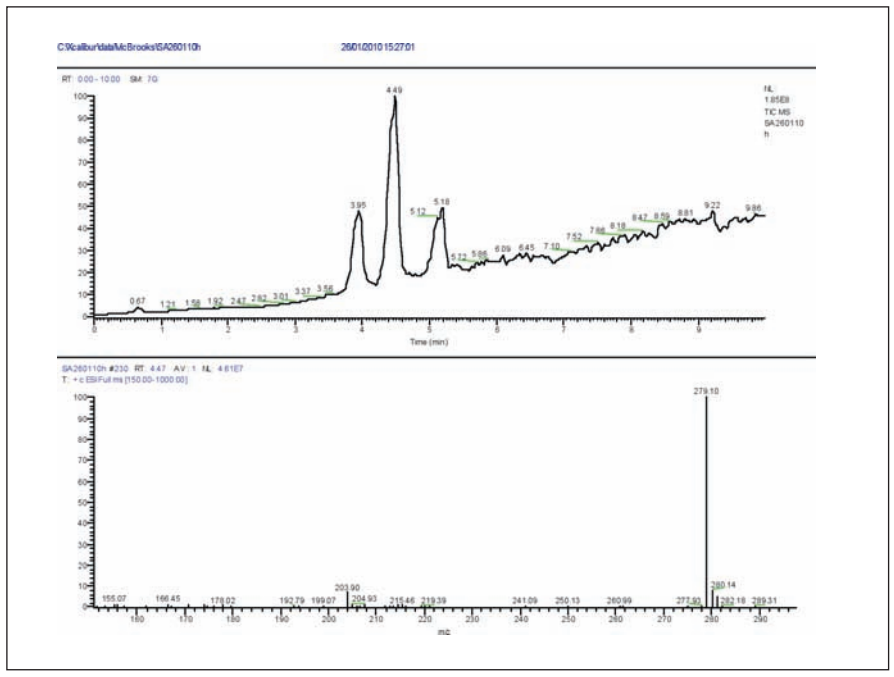

Figure 3. LC-MS analysis of three sulphonamides; MS spectrum shows the predominant MH+ from peak 2.

It was the Drug Metabolism community within the Pharmaceutical Industry that were the first to catch on to the potential of Electrospray and APCI for the detection of key drug metabolites in biological fluids. I was fortunate to be working for the pharmaceutical company who purchased the first two SCIEX API 3 instruments to be used for drug metabolism, one based in Groton USA and one in Sandwich UK [17]. The simplicity of use, combined with the incredible sensitivity achieved enabled us to detect metabolites at levels previously not considered possible. Nowadays, when you tour around a modern PDM or Drug Metabolism department within a big pharmaceutical company and see rows of triple quadrupole mass spectrometers carrying out met id and quantitative analysis; it is impossible to believe that without the development of electrospray less than thirty years ago, this type of analysis would still be a slow and laborious process.

It was not just the Drug Metabolism groups that embraced this new era of LC-MS. Within the Drug Discovery environment the 'open access' revolution post the development of electrospray, really took off [18]. Nowadays, synthetic chemists think nothing of simply walking up to an 'open access' MS and use it as a quick way to confirm reaction intermediates and final products. We all think it quite normal to see mass spectrometers adjacent to synthetic chemistry labs carrying out routine analysis, but less than thirty years ago this was just a wild dream. Electrospray LC-MS is also used as the key component in prep HPLC, and mass directed fraction collection is a technique in common use within Drug Discovery [19]. Drug Development also use LC-MS as a routine tool when carrying out reaction optimisation, or for detecting trace level components in the final drug. LC-MS using electrospray has become an essential component in drug stability profiling and due to is incredible sensitivity, it can detect impurities and degradants at below the 0.5% level of the main drug; no other analytical technique can match the power of LC-MS. In fact it was the sensitivity of electrospray ionisation and its ability to detect low-level components in mixtures that led to the development of on-line LC-NMR-MS, a tool which was developed within the pharmaceutical industry to increase the structural information that was not possible to obtain by LC-MS alone [20].

It is not just in the small molecule arena that electrospray has made an impact. The whole area of protein and peptide analysis was revolutionised by the development of this technique. Electrospray MS could ionise and detect proteins way beyond the mass range of the mass spectrometer. Multiple charging has allowed us to extend the mass range capability and the analysis of proteins and olignucleotides is becoming more important as the pharmaceutical industry embrace biomolecules for therapeutic purposes [21,22]. It is a complementary technique to protein X ray crystallography and these two techniques are used hand in hand, along with 'in silico' structure prediction to help understand the shape of protein 'pockets' when exploring future drug targets. I predict that ion mobility linked to mass spectrometry will become increasingly important within this area of the pharmaceutical industry as it also provides important information to help understand molecular interactions.

I often wonder if the late John Fenn and co-workers ever realised what impact the discovery of electrospray was going to have on mass spectrometry community? Within the Pharmaceutical Industry it has single handedly changed the whole drug analysis process. It took mass spectrometry from a 'lunatic fringe' technique, into one of the main analytical techniques used across that industry.

That is an awesome claim to fame, and those pioneers at Yale have deservedly carved out their names in the Mass Spec 'Hall of Fame'. I feel proud to have been working in the pharmaceutical industry during this exciting period of LC-MS development. We all owe John Fenn a debt of gratitude. I can remember visiting John at his lab just after the ASMS meeting in Miami Beach in 1987. His students showed me his old quadrupole mass spectrometer, which had one of the first electrospray interfaces attached to it. We ran some protein samples that I had brought with me, and we printed out the spectra an old dot matrix printer. Little did I know that I was looking at the future of LC-MS, and that this humble instrument with its 'Heath Robinson' ionisation source would revolutionise my and most other mass spectrometrists lives. Oh how I wish that I had kept those dot matrix spectra.

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