HPLC-ESI-MS/MS in bioanalytical chromatography: Can we forget chromatography?

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AIMS: LC-MS/MS has become the prominent analytical technique for quantitative determination of pharmaceuticals and drugs of abuse in biological matrices in bioanalytical and forensic laboratories in the recent past. But a closer look into scientific publications in this field shows a strong trend to simplify liquid chromatographic separations in favour to the very high power in specificity of tandem mass spectrometry.
This resulting diminished chromatographic performance is generally compensated in higher (more costly) needs in mass spectrometric performance. A short rehearsal of basic chromatographic knowledge shows that this has not necessarily to be the only solution. On several examples in trace analysis of drugs of abuse in hair samples, the most prominent analytical problems in LC-MS are discussed, which can also solved more economically by applying more consequently basic chromatographic know-how.

METHODS AND RESULTS: *Ion suppression*, still overlooked in tandem MS especially when short run times with short separation columns are targeted, is most prominent when analytes co-elute with sample matrix components in the zone of the chromatographic dead volume. By choosing, in contrary, maximal column separation power with low retention factors by increasing the elution power of the mobile phase, also polar analytes are easily moved out of this ion suppression zone. ESI-ionisation efficiency is at the same time enhanced due to a higher organic fraction of the mobile phase!
Because ion suppression effects are concentration dependant processes, they are consequently attenuated by ('simple') dilution of the sample. Choosing the appropriate diluent, allows at the same time to compensate for the loss in sensitivity, by applying proper peak compression mechanisms during the injection process.
While *complex gradients* are often applied for the same reason, they have the consequences of more variable and longer analysis time and the need in additional HPLC instrumentation. Simple reproducible step gradient profiles are easily generated in contrary by exploiting the possibilities of multiple loops on the injection switching valve.
Ion Efficiency in many ESI sources is strongly dependant on volume flow, thus decreasing the diameter of the separation column always leads to a much enhanced overall detection sensitivity even for less sensitive MS instruments, when injecting identical sample amounts. Observing the basic chromatographic rules for injection and separation, most applications can be easily modified to a reduced scale on a micro-bore columns (≤ 1mm diameter), also when 'standard' HPLC equipment is utilized.
On-line sample extraction has also become a common sample preparation tool in LC-MS, but generally at the cost of additional and bulky HPLC pumping equipment. An example will be shown, how this is integrated into the separation system, by just utilizing the complex control features available with modern automated sample injection systems.