

The optimizing quantitative analysis in high pressure chromatography coupled with mass spectrometry

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Introduction

The high pressure liquid chromatography coupled with mass spectrometry method became more and more used to realize quantitative analysis. To optimize this analysis method for a better quantification, it is necessary to test several parameters; such as column characteristics, mobile phase nature and spectrometer lens potentials. Those tests were realized in order to create concrete examples to introduce this method of optimization to the university and industries that work with the laboratory. This article focuses on the solvent nature changes.

Experimental methods

Material used for manipulations was an Api300 (Applied Biosystem) with an electrospray source and an Agilent HPLC with a Waters X-Bridge column (100mm*2.1mm, 3 μ m).

First, mass lens parameters (declustering and focusing potentials, collision energy) were optimized and MRM transitions are selected for each product. So as to optimize the mobile phase, the organic solvent nature was modified and different flow rates of this solvent were experimented.

Results

Figure 1 represents the influence of organic solvent natures on mobile phase. This chromatogram proves that the methanol solvent gives a fifteen times more sensitive signal than acetonitrile. In this experiment, organic solvent rates are chosen so that compounds had the same retention time so as to realize a better sensibility comparison (without retention influence).

Figure 2 illustrates that the sensibility response of diuron increases with the percentage of methanol. So it is demonstrated that with a higher and higher organic solvent percentage, in mobile phase, as in this experiment with diuron (hydrophobic component), the peak intensity was better when there is more organic solvent.

Conclusion

This study authorizes to conclude that the elute phase nature affects compound peak intensities. Indeed, with methanol as eluent, the intensity is higher than with acetonitrile. Furthermore, the signal sensibility gets better thanks to the increase in the quantity of organic solvents in HPLC. As a result, it permitted a better quantification of the analytes. Nevertheless each parameter (column nature, sources, detection system) has to be adapted and tested to optimize completely the analysis.

Figure 1: chromatogram of Propanolol intensity (cps)
Blue: 72%water28% acetonitril
Red: 50%water 50% methanol

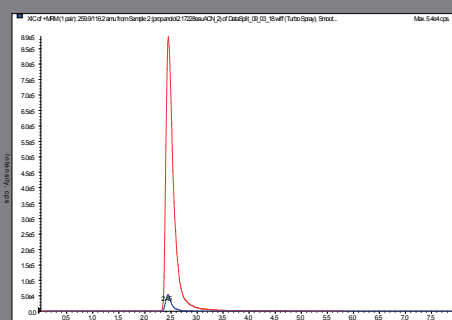


Figure 2 : chromatogram of Diuron intensity (cps)

Blue : 20% water 80% methanol
Red : 30% water 70% methanol
Green : 40% water 60% methanol

