Maximizing efficiency in UHPLC-LC-MS/MS method development for multi component analysis

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1. Introduction

With the development of high sensitive and ultra-fast LC-MS/MS instruments, the triple quadrupole technology has found its way into a huge area of applications. Nowadays, it is the method of choice for trace level analysis and identification of various compounds. The steadily increasing number of applications in different fields like pharmaceutical, environmental, food, forensic and clinical analysis demands fast and efficient development of new LC-MS/MS methods. The basis for stable generation of high quality data is a well optimized chromatographic separation. Fully automated optimization of the UHPLC/HPLC method using Shimadzu's method scouting software (Figure 1) in combination with automated MS optimization for MRM and interface parameters are the perfect platform for the generation of new triple quad MS methods. Here, we report a fast procedure for LC-MS/MS method optimization for multi component analysis of a drug standard.

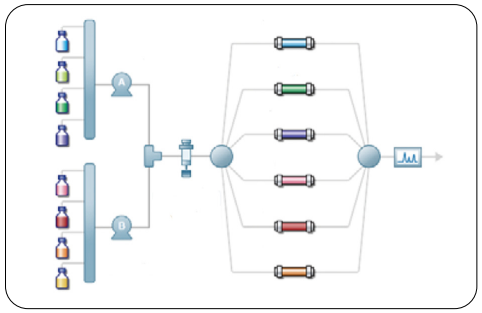

Figure 1 Combination of columns and solvents during the method scouting process.

LCMS-8050 triple quadrupole mass spectrometer

2. Materials and Methods

2-1. LC-MS/MS parameters

One of the first steps during this automated process is the precursor ion selection, followed by the m/z adjustment of the precursor. The collision energy is optimized for the most abundant fragments and finally the fragment m/z adjustment. Six optimization steps were performed via flow injection analysis, each taking 30 seconds (Figure 2). The result of these automated steps was the automatic generation of a final MRM method

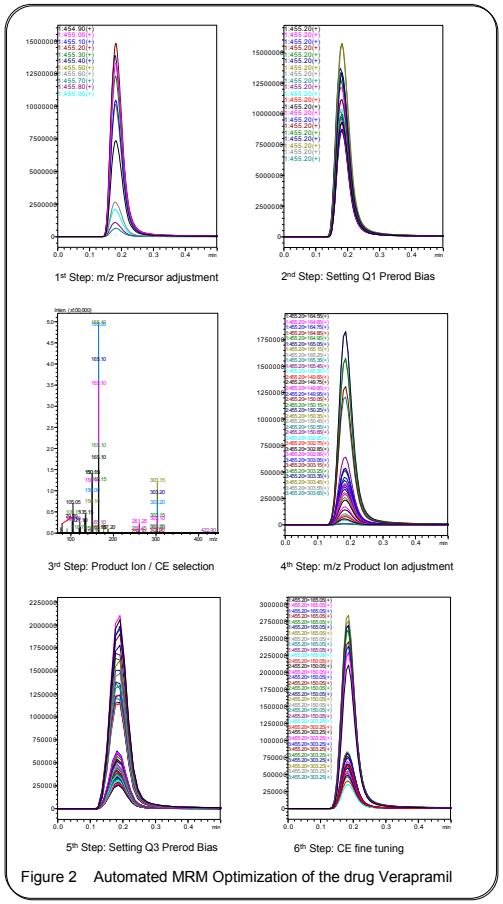

Figure 2 Automated MRM Optimization of the drug Verapramil

2-2. UHPLC parameters

Choosing the best UHPLC/HPLC column and composition of eluents are often the most important but time-consuming steps during method development. This can influence sensitivity and separation from potentially interfering matrix effects. The Shimadzu Method Scouting System in combination with the ACE Excel Method Scouting Column Kit was used in order to elucidate the best UHPLC/HPLC parameters for the analysis of different compounds. The Method Scouting System allows the combination of up to 6 UHPLC/HPLC columns with up to 16 different eluent combinations, resulting in the investigation of a maximum of 96 different combinations, which requires only a fraction of the time required by traditional approaches. The ACE Excel Method Scouting Column Kit includes a variety of unique phases that exploit multiple mechanisms of separation and have been specially developed to provide alternative selectivity to C18 phases.

2-3. Ion Source parameters

Analysis results with higher sensitivity are obtained by optimizing the interface parameters. The Interface Setting Support Software from Shimadzu automatically creates batch files to perform analysis and acquire data while changing the heater temperature and gas flow rates in the LC-MS/MS interface. By comparing the data obtained from the batch analysis, it is possible to determine the heater temperature and gas flow rate that are optimal for the target compounds, resulting in higher sensitivity for analysis.

Table 1. The ACE Excel HPLC/UHPLC Method Scouting Column Kit

ACE Excel 2 C18	Ultra-high purity, base deactivated ACE columns provide guaranteed reproducibility and excellent column lifetime. Traditionally, C18 bonded phases with mainly hydrophobic character have been preferentially used in the majority of RP applications due to their versatility and superior stability.
ACE Excel 2 C18-AR	Provides alternative selectivity to "standard" C18 columns – it is recommended for compounds with aromatic functionality and compatible with highly aqueous mobile phases to enable the retention and separation of polar compounds.
ACE Excel 2 C18-PFP	Combines a C18 chain with a PFP functionality to provide the hydrophobicity, stability and low bleed of a C18 phase as well as the advantages in selectivity of a pentafluorophenyl phase.
ACE Excel 2 C18-Amide	The polar amide group offers complementary selectivity for improved separations with polar, acidic, basic and phenolic compounds, while the extended spacer technology provides extended column lifetime.
ACE Excel 2 CN-ES	Combines a polar CN group with an extended spacer that provides retention similar to a C18 column. The extended spacer technology provides increased column lifetime compared to traditional CN bonded phases with short alkyl spacer (typically C_3/propyl)

3. Results

An automated method scouting procedure in combination with the fully automated MRM-optimization by flow injection and the use of the Interface Setting Support software was used for fast development of a method. Figure 3 exemplarily shows the influence on the separation of a mixture of drugs on 5 different columns.

The first step is the evaluation of the optimal column / solvent combination using a generic gradient starting with 5% of organic solvent increasing to 95% within a specified time. This initial stage generates a viable method requiring some further optimization.

The second step optimizes the slope of the gradient as well as the solvent conditions. As a last step the optimal interface parameters were determined with the help of the Interface Setting Support Software. Figure 4 shows the influence of different temperatures of the ion source (ESI) with a single compound (Simvastatin) as an example.

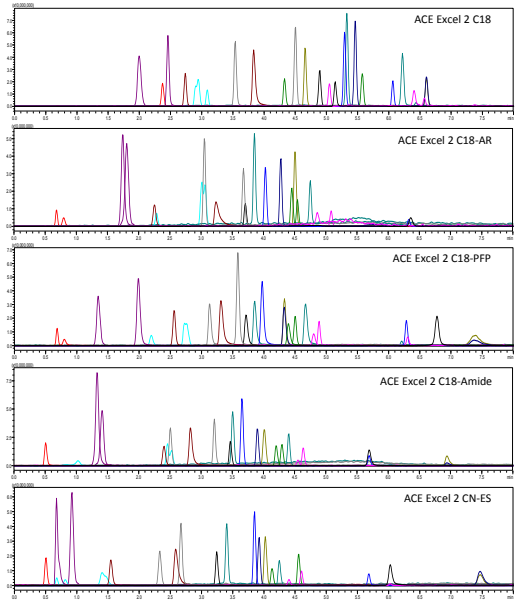

Figure 3 Examples on the separation of a mixture of drugs on 5 different columns (Conditions: Mobile phase A: 0.0025% NH4OH, B: MeOH, 5 to 95 % B in 5 min, 0.4 ml/min)

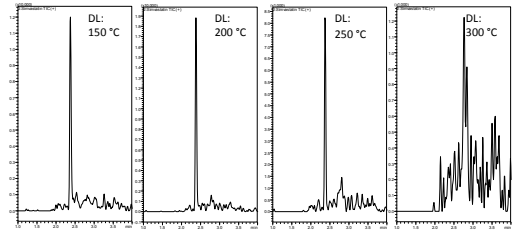

Figure 4 Influence of different temperatures of the ion source (ESI) shown for Simvastin

4. Conclusion

Shimadzu's MS control software LabSolutions offers the possibility to select MS parameters like precursor ion selection, collision energy and fragment optimization for MRM via flow injection analysis in an automated way while the Interface Setting Support Software is a useful tool to find the most suitable Interface settings. All steps combined lead to a fully automatically generated optimized method for the analysis of multi components.