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AUTOMATED DEVELOPMENT OF MRM METHODS FOR BIOANALYTICAL ASSAYS

Paul D. Rainville, Joanne Mather, and Robert S. Plumb
Waters Corporation, Milford, MA, U.S.

INTRODUCTION

Liquid Chromatography coupled with tandem quadruple mass spectrometry (LC/MS/MS) and operated in multiple reaction monitoring (MRM) mode is often the analytical method of choice for the determination and quantification of drugs and their metabolites in biofluids and tissues. This is due to the strong specificity, and therefore high levels of sensitivity, that LC/MS/MS is capable of achieving.

However, the use of this technique often requires companies to hire LC/MS specialists, or to train current personnel, both of which represent significant investments in time and resources. Developing a robust MRM method can be time consuming and requires a high level of expertise in order to make informed decisions.

The development of user-friendly and intuitive software tools that can assist or completely automate the operation of LC/MS/MS instrumentation can provide an essential service in several ways:

- Automates processes, saving time and allowing experienced personnel to direct their time towards tasks that require a higher level of expertise
- Facilitates the confident generation of robust assays and quality data by non-experts
- Provides consistency in the way instrumentation is utilized and information is transferred, thus reducing potential for error

In the development of an LC/MS/MS method, the analyst must first optimize the operational parameters for the mass spectrometer. Traditionally this has required a high level of expertise in mass spectrometry instrumentation. Parameters that must be determined for successful mass spectrometric detection and quantitation of a chosen compound are:

- Ionization mode; ESI or APCi, positive or negative
- MRM transition ions (precursor and product)
- Capillary voltage
- Cone voltage
- Desolvation gas flow
- Source temperature
- Collision energy

Determining the optimum operating conditions generally requires some trial and error, which, for non-experts, can be time-consuming and daunting. In particular, choosing the optimum ionization mode has involved physically changing the source.

In this application note, we describe the use of innovative software, Waters® IntelliStart™, and hardware tools, the ACQUITY® TQD tandem mass spectrometer, to rapidly develop an MRM method for the non-steroidal anti-inflammatory drug (NSAID) ibuprofen with subsequent incorporation into an LC/MS/MS method for separation, detection, and quantitation.

Figure 1. The ACQUITY TQD System.

EXPERIMENTAL

A generalized workflow for the development of an MRM method in bioanalysis is shown in Figure 2. These are the steps that are generally taken to determine the correct parameters for running an MS method subsequent to LC separation.

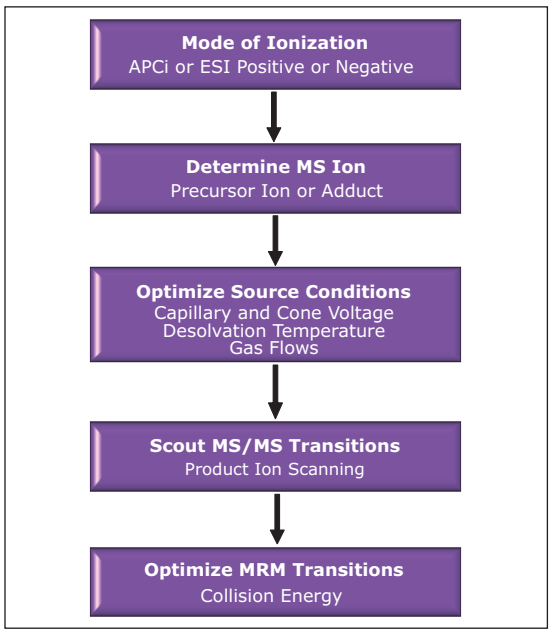

Figure 2. General workflow for development of MRM method for bioanalysis.

This process is both simplified and automated by using ESCi® Technology and IntelliStart Software. ESCi is an ionization technique that allows both positive and negative ion APCi and ESI to be carried out simultaneously – without physical changes to the ion source. When used in conjunction with IntelliStart, a software tool designed to facilitate fast and accurate optimization of parameters for MRM method development, ESCi greatly reduces the amount of time involved in optimizing the operating parameters for the mass spectrometer. Figure 3 illustrates the few steps required to quickly develop an MRM method.

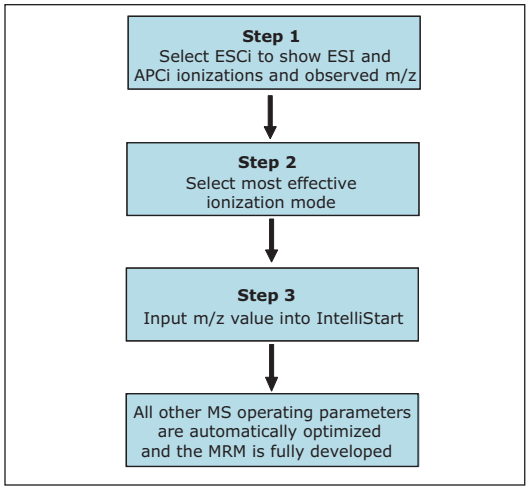

Figure 3. Steps required for a fully-developed MRM method.

RESULTS AND DISCUSSION

Using ESCi in Steps 1 and 2 of the optimization process allowed the user to determine the best ionization source for analysis of the compound in less than one minute and three keystrokes, as shown in Figure 4.

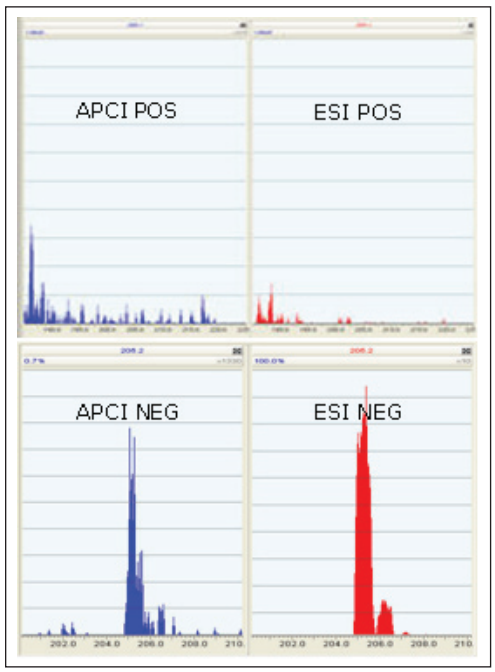

Figure 4. ESCi for ibuprofen.

Figure 4 clearly illustrates that ibuprofen ionized in both APCi and ESI negative modes; ESI negative yielded the best sensitivity and was therefore chosen. This step in the optimization process also told us the molecular ion observed was m/z 205.

With the use of IntelliStart software, the critical parameters for optimization of the MS method could now be obtained.

The molecular ion mass was entered into IntelliStart (Step 3) and the default ranges for cone voltage and collision energy were utilized to enable IntelliStart to automatically determine all other parameters, such as optimized voltages, desolvation temperatures, gas flows, and MRM transition, all in less than 5 minutes.

A report was automatically generated specifying the optimized settings for the MRM method. The report also detailed the daughter ion spectra along with cone voltage and collision energy values, as shown in Figure 5.

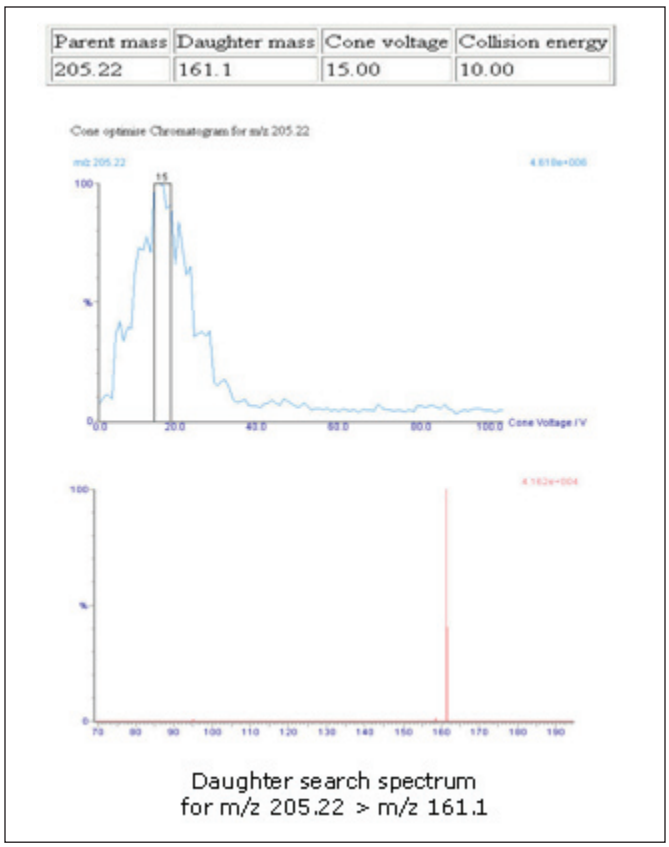

Figure 5. Abbreviated sample report showing parent mass.

Having successfully and quickly developed the MS method for the detection of ibuprofen, the MRM method parameters determined by IntelliStart are now inserted into the instrument method (Figure 6) for subsequent use in the method for optimizing the chromatographic conditions for ibuprofen. Because data are automatically transferred, transcription errors are avoided and time is saved. IntelliStart is available for both Empower™ and MassLynx™ software platforms.

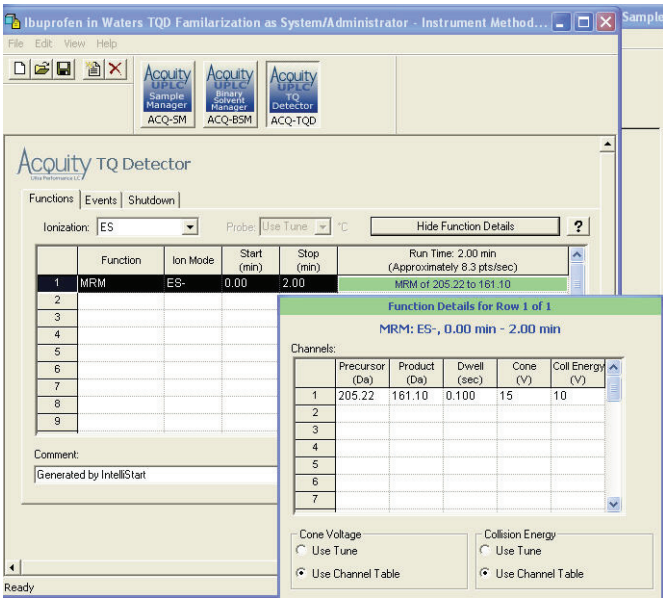

Figure 6. MRM method populated automatically into Empower Software.

A complete LC/MS/MS method for the determination of ibuprofen in biological fluids can now be developed, with all the optimized parameters for detection and quantitation in place, as shown in Figure 7.

LC conditions

LC system:	Waters ACQUITY UPLC® System
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Flow rate:	450 µL/min
Mobile phase A:	0.1 % Ammonium Hydroxide in H_2O
Mobile phase B:	MeOH

MS conditions

MS system:	Waters TQ Mass Spectrometer
Ionization mode:	ESI negative
Capillary voltage:	3.8 KV
Cone volyage:	15 V
Desolvation temp.:	450 °C
Desolvation gas:	900 L/Hr
Source temp:	150 °C
Acquisition mode:	MRM Transition 205 > 161
Collision energy:	10 eV

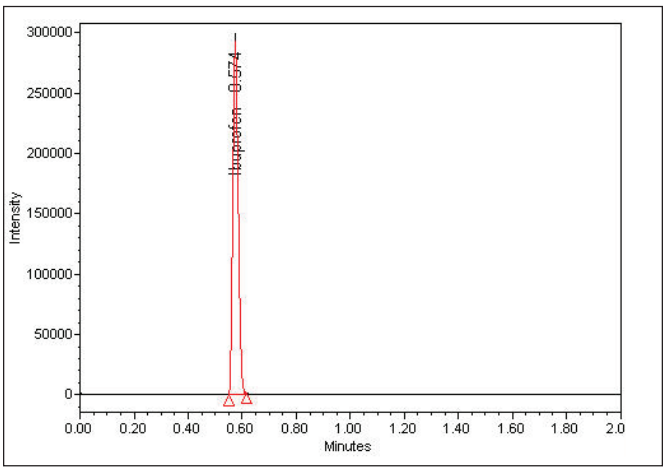

Figure 7. Resulting chromatogram for ibuprofen.

CONCLUSION

- The combination of ESCi Technology and IntelliStart Software provides an efficient, rapid, and effective approach to developing MRM methods for bioanalytical assays.
- IntelliStart saves time by allowing experienced personnel to direct their time towards tasks that require a higher level of expertise.
- The user-friendly, intuitive software allows an inexperienced user to generate quality data in a short time frame with confidence.
- Reports are created automatically for compliant regulated environments.
- IntelliStart is available on both Empower and MassLynx software platforms.

References

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
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