Waters

Application of ESCi™-UPLC™/MS/MS in Drug Discovery and Development by ACQUITY UPLC™-Quattro Premier™

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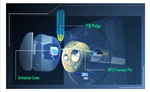
MICROMASS® MS TECHNOLOGIES

Introduction

Introducing ESCi™ Technology



- "ESCi™ multi-mode ionization source" refers to a combination ESI & APCI capability available on Waters mass spectrometers.
- In MS, identifiable differences do exist in spectra generated by different ionization methods. ESI vs APCI do occur in the source simultaneously dependent on mobile phase and other conditions.

The enabling of the ESCi source is driven by specifically designed electronics capitalizing on unique attributes of the source geometry.

- In ESCi, both ESI and the APCI modes can function alternating at MS switching speeds in both positive & negative polarities[1].

Physicochemical Profiling for Drug Discovery

- Physical chemical information plays an important role in drug discovery. Physicochemical properties can be used as predictors of ADME characteristics. These properties can be profiled at reduced expense, time, and animal usage to many ADME procedures.
- The need for speed as well as diversity and numbers are critical factors to consider when developing a pharmaceutical profiling program. Typical properties included in high throughput (HT) physicochemicl profiling are solubility, permeability, pKa, lipophilicity, stability, and integrity[3].
- The stability test screens compound stability in microsomes, plasma and buffers. It provides important information about potential liabilities of drug candidates. HT stability assays usual contain two components: robotic sample preparation, and rapid automated LC/MS analysis[4].

Overview

- The progress of modern drug discovery and development relies heavily on the application of LC/MS/MS technology. Typically, LC/MS utilizes atmospheric pressure ionization (API) as the ionization source with electrospray (ESI) or atmospheric pressure chemical ionization (APCI) as options. Polar compounds are more suitable for ESI and non-polar compounds are more appropriate for APCI.
- To determine an optimum ionization mode for a particular compound, one would need to optimize in both ESI and APCI. In general, about 80% compounds can be analyzed by ESI. APCI is the common option to analyze additional compounds. This requires the analyst to physically change the ionization source and re-optimize the ion source parameters, which results in lost analysis time, hence significantly reduces analytical throughput[2].

Project Goal

- To develop an ESCi-LC/MS/MS high throughput microsome stability test in order to demonstrate the advantages of ESCi™ multimode ionization and the Waters ACQUITY UPLC™ for higher throughput and broader compound coverage in drug discovery.
- To evaluate a protocol with the ESCi multimode ionization available on Waters mass spectrometers.
- To evaluate Waters ACQUITY UPLC™ impact on high throughput (HT) processes.

Experimental Conditions

- UPLC™ by Waters ACQUITY UPLC™ System
- Mobile Phase:
  A: 10 mM NH_4OAc in ACN/H_2O 10/90
  B: 10 mM NH_4OAc in ACN/H_2O 95/5
- Column: ACQUITY UPLC™ Column 1.7 mm, 2.1 x 50 mm, 40°C
- Sample Temperature: 5°C
- Flow Rate: 1.0 ml/min. (400 µl/min into the MS)
- Gradient:

Time (min.)	A%	Curve
0.0	90%	6
0.4	5%	6
0.5	5%	1

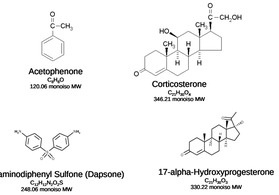
- MS by Waters Quattro Premier™
- Tune Page Parameters:

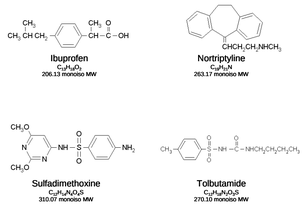
ESCi Multi-Mode Ionization enabled	
ESI capillary voltage [kV]:	3.00
APCI corona current [mA]:	4.0
Source Temp. [°C]:	130
Desolvation Temp. [°C]:	420
Desolvation Flow [L/Hr]:	980
Cone Gas Flow [L/Hr]:	50

- Data collection by MRM
- Inter-scan delay: 20 ms
- Inter-channel delay:

For Mixture:	ESI+:	5 ms
	ESI-:	20 ms
	APCI+:	10 ms
	APCI-:	10 ms
For single compound:		50 ms

Chemical Structures of the Standards





ESCi Enabled Tune Page

- ESCi is a standard option for the Waters Premier line of mass spectrometers. It requires MassLynx™ 4.0 Software and employs a single standard ESI probe.
- Once installed, analysts have an option to enable or disable the ESCi by going into the multimode ionization under option from tune page.
- Once ESCi is enabled, two sets of parameters will appear in tune page. The most significant parameters controlling the behavior of the source is the temperature and flow rate of the gas[4].

Method Optimization

Figure 1. ESCi MS Scan and MS/MS Scan

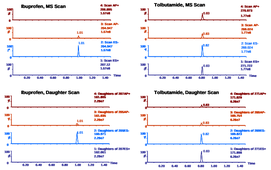
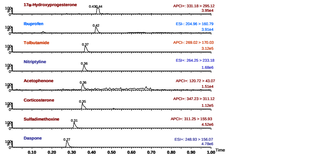


Figure 2. MRM of the 8 Compound Mixture

Simultaneous Four Mode in a Single Injection, 100 ng/



About 6-8 data points obtained across each peak. Inter-scan delay was 20 ms. Inter-channel delay was 5 ms, dwell time was as low as 5 ms for some compounds.

Figure 3. LC and MRM MS Methods for 4 Compound Mixture

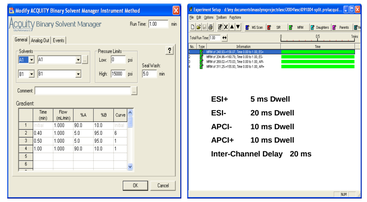


Table 2. ESCi Quantification Results of the 4 Compound Mixture

	Ionization Mode	Linear Range (ng/mL)	r^2	Limit of Detection (ng/mL, 5 µL Inj)
Daspone	ESI+	0.01 - 200	0.991	0.01
Ibuprofen	ESI-	0.1 - 100	0.984	0.1
Sulfadimethoxine	APCI+	0.1 - 1000	0.992	0.01
Tolbutamide	APCI-	0.1 - 1000	0.992	0.1

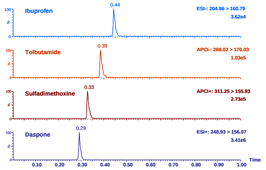
For optimization session, 3 levels of experimental results were displayed to demonstrate the feasibility of the ESCi multimode ionization. This allows the analyst to be confident enough to design experiments based on the application's requirements rather than based on the instrument capability. The fast scanning/switching speed enabled by the T-wave™ technology implemented in the Quattro Premier mass spectrometer allows ESCi to be performed in various types of data collection modes ranging from MS and MSMS scan to MRM.

Table 1. Optimization Results

	ESI+	ESI-	APCI+	APCI-	Ionization Mode	MRM Transition
Acetophenone	+	-	++	-	APCI+	120.72>43.07
Corticosterone	++	-	++	-	APCI+	347.11>310.87
Daspone	+++	-	+++	-	ESI+	248.97>156.07
17a-Hydroxy progesterone	++	-	++	-	APCI+	331.18>295.12
Ibuprofen	-	++	-	+	ESI-	205.29>161.07
Nortriptyline	++	-	++	-	ESI+	264.25>233.18
Sulfadimethoxine	+++	+	+++	+	APCI+	311.25>155.93
Tolbutamide	+++	+++	++	++	APCI-	269.02>170.03

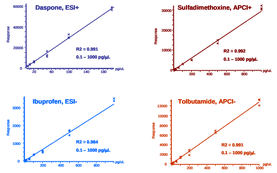
- Figure 1 shows example results of the MS and MS/MS scans. The two compounds displayed are Ibuprofen and the Tolbutamide.
- Once the MS parameters were optimized from the tune page, two injections were made with the optimized parameters for confirmation. The first injection was the MS scan of the standard, the second injection was the daughter scan of the standard.
- For each injection, the mass spectrometer switched among four ionization modes simultaneously: ESI+/ESI-/APCI-/APCI+. The inter-scan delay was 20 ms. About 7 to 10 data points were obtained for each chromatographic peak.
- The y-axis on figure 2 is normalized to the highest peak intensity. This offers a clear visualization of the optimum ionization mode for each compound. The choice we made based on the optimization is listed on table 1.

Figure 4. MRM Chromatograms of the 4 Compound Mixture



Simultaneous Four Modes in a single Injection, 100 ng/mL
About 15 data points obtained for each peak. Sufficient for quantification. Inter-scan delay was 20 ms, Inter-channel delay was 5 ms, dwell time was as low as 5 ms for some compounds.

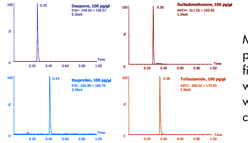
Figure 5. Calibration Curves of the 4 Compound Mixture



Response Type External Standard Area, Linear Curve, Exclude Origin, 1/x Weighting

Results and Discussion

Figure 6. MRM Chromatograms of the Single Compound Injection



More than 20 data points per peak. Ideal for quantification. Inter-scan delay was 20 ms, dwell time was 50 ms per compound.

Figure 7. MRM MS Method for Single Compound Injection

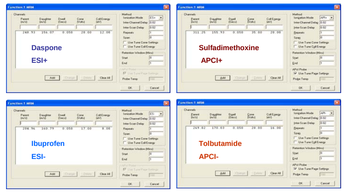
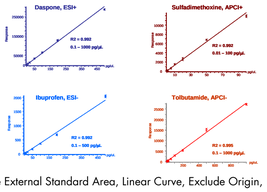


Figure 8. Calibration Curves for Single Compound Injection



Response Type External Standard Area, Linear Curve, Exclude Origin, 1/x Weighting

Fitness of Purpose

- The application of ESCi can become rather demanding for data acquisition of the mass spectrometer. This particularly true for MS or MS/MS scan experiments vs. the MRM experiments.
- However, the exact requirements for an experiment is dependent on the fitness of purpose. "All factors bearing on any analysis must be consistent with the overall purpose of the task"[5].
- For the MS and MS/MS scan experiments, the purpose was to optimize the best ionization mode and to choose the optimum parameters. This type of experiment is typically qualitative. Even though it is always better to obtain more data point across the peak, for qualitative work, 7-10 data points across the peak should be sufficient for the purpose.
- For ESCi MRM experiments, the number of data points obtained varied depending on the number of channels to be monitored. Less data points were obtained for higher number of compounds in a mixture. More analytes per mixture will result less the data points across the peak. In our example, 6-8 data points were obtained for an 8 compound mixture, while more than 15 data points were obtained for a 4 compound mixture.
- Mixture analysis can be useful for cassette dosing experiments. In drug discovery, the analytical requirement can be a semi-quantitative estimation of the ADME properties. As we shown in optimization session, the analyst can choose the number of analytes in a mixture for simultaneous quantification depending on the quantification requirement. Less demanding in figure of merit may allow more compounds to be analyzed simultaneously.
- For the microsomal stability test, there is no need for a mixture analysis. A typical experiment is carried out for a single unknown compound per well. Therefore, we only need to analyze a single compound per injection.

Table 3. Quantification Results for Single Compound

	Ionization Mode	Linear Range (ng/mL)	r^2	Limit of Detection (ng/mL, 5 µL Inj)
Acetophenone	APCI+	5.0 - 1000	0.990	10.0
Corticosterone	APCI+	2.0 - 1000	0.991	2.0
Daspone	ESI+	0.1 - 1000	0.996	<< 0.1
Hydroxyprogesterone	APCI+	0.2 - 200	0.990	0.2
Ibuprofen	ESI-	0.1 – 500	0.990	0.1
Nortriptyline	ESI+	1 - 500	0.980	1.0
Sulfadimethoxine	APCI+	0.01 - 100	0.995	0.01
Tolbutamide	APCI-	0.1 - 1000	0.991	0.1

Conclusion

- We have developed a high throughput ESCi-LC/MS/MS protocol:
  - HT separation enabled by the Waters ACQUITY UPLC™ System.
  - LC run time of 1.0 minute was obtained.
  - High speed capability of the Waters Quattro Premier allowing simultaneous four mode ESCi switching during a single injection.
- This method can be applied in screening a large number of compounds with high speed and covering diverse structures for drug discovery and development.

Acknowledgement

The authors wish to thank Dr. Rob Plumb and Mr. Michael Balogh for their help and useful discussions during this project.

Future Work

- We will apply a microsome incubation method on the 8 test compounds and compare the results with the HPLC-Quattro micro™ method that is currently applied at Wyeth Research for sample analysis
- The ESCi-ACQUITY UPLC™/MS/MS protocol will then be applied to discovery compounds in 96 well plate format.

References

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