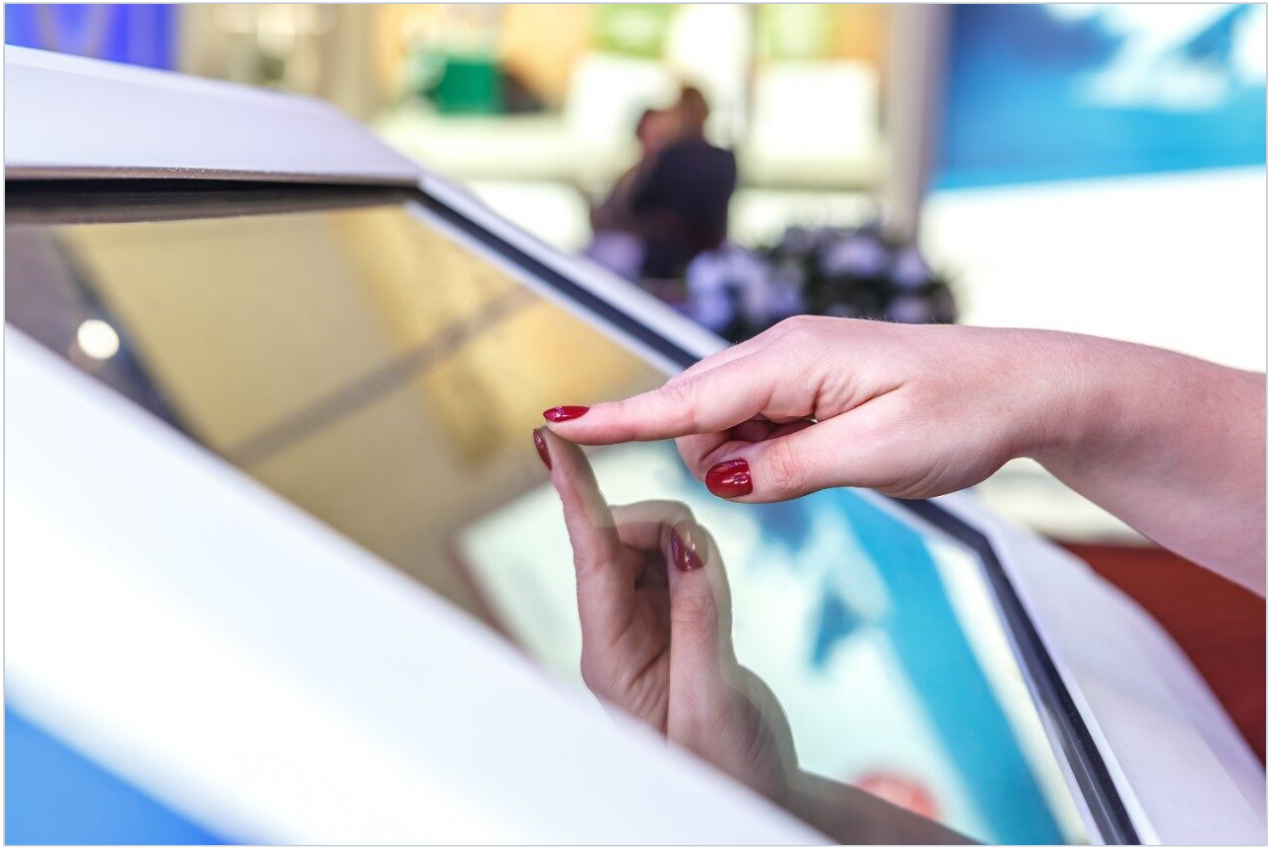
Waters™

□□□□

UltraPerformance Convergence Chromatography (UPC^2) with MS Detection Using Three Different Atmospheric Pressure Ionization Techniques Using Liquid Crystal Intermediates as a Model

Jane Cooper

Waters Corporation



Abstract

This study demonstrates the options available to collect MS data with UPC^2, using the analysis of liquid crystal intermediate compounds as an example.

Considering three ionization modes ESI, APCI, and APPI, optimizing the choice of modifier and tune parameters to illustrate how the high speed and unique selectivity of UPC^2 can be combined with the greater selectivity and specificity that can be achieved using MS detection.

Benefits

- This document demonstrates ACQUITY UPC^2 System interfaced with MS detection, considering various atmospheric pressure ionization modes, for the analysis of liquid crystal intermediate compounds.
- To illustrate the capability of combining the high speed and unique selectivity of UPC^2, with the greater selectivity and specificity of MS.

Introduction

Convergence Chromatography (CC) is a separation technique that uses carbon dioxide as the primary mobile phase, with a co-solvent such as acetonitrile to give similar selectivity as normal phase LC. Waters UltraPerformance Convergence Chromatography (UPC^2) builds upon the potential of CC, utilizing the benefits of sub-2 µm particle size stationary phases, while using proven and robust Waters UPLC technology.

Various detection methods can be used with UPC^2 including UV and Evaporative Light Scattering Detection (ELSD). But there is also the option of interfacing UPC^2 with Mass Spectrometry (MS) detection as illustrated in Figure 1. UPC^2 can easily and quickly be connected to a wide-range of MS systems, with the addition of a MS splitter to the system, which introduces a controlled leak to the system and enables the maintenance of the CO_2 pressure, while maintaining peak shape and width.

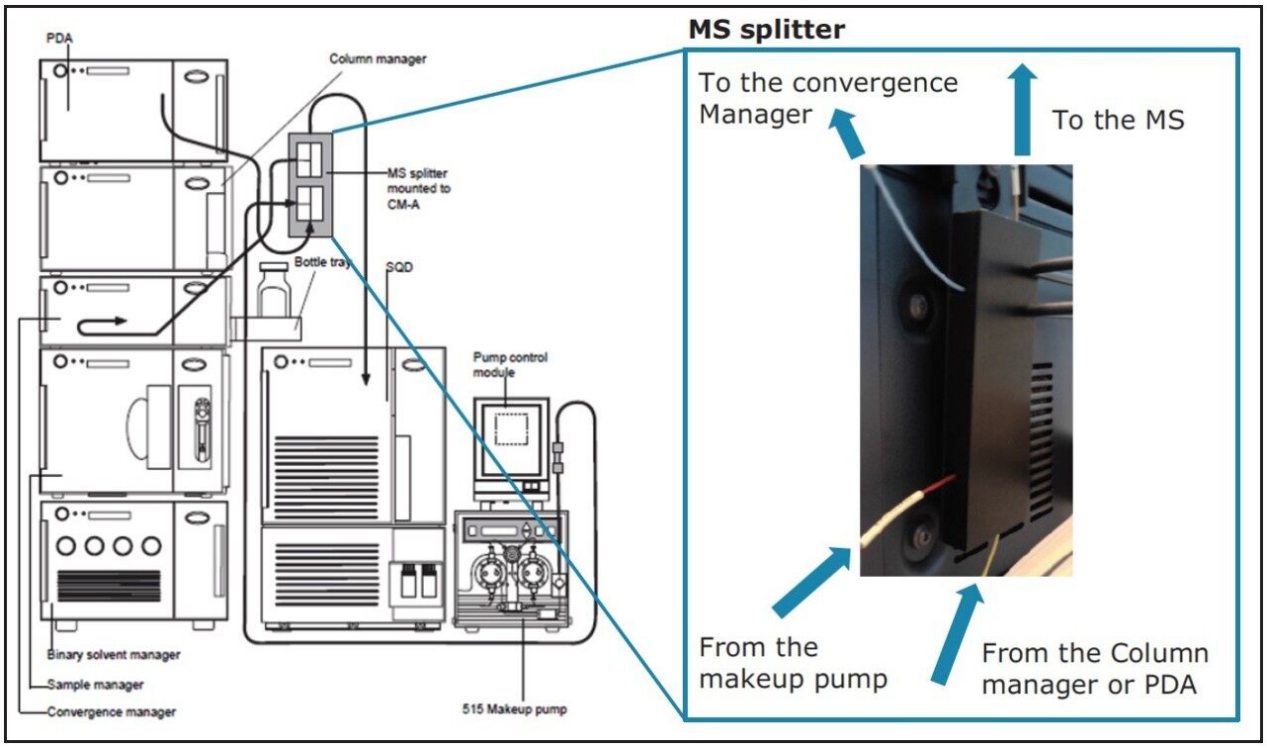


Figure 1. ACQUITY UPC^2 configured with PDA and MS detection (here illustrated with an SQD), including MS splitter and a makeup pump.

The MS splitter provides sufficient back pressure to maintain the CO_2 in the liquid state and maintain its supercritical properties such as density and solvating power which could alter factors such as compound solubility and the selectivity of the stationary phase. The additional option to add a makeup solvent via a makeup pump to the flow prior to MS detection can be used to provide greater solvating powers, to enhance the selectivity and sensitivity of MS detection, and also to influence ionization.

When using electrospray ionization (ESI) where an electrically charged field is used to generate charged droplets, then analyte ions are formed by evaporation prior to MS analysis. The addition of a protonation source such as formic acid to the makeup solvent can be used to enhance ionization and increase sensitivity. In atmospheric pressure photo ionization (APPI), ultraviolet light produced from a krypton lamp ionizes gas phase analytes and dopants leading to gas-phase reactions. Therefore, the addition of a dopant such as toluene to the makeup solvent can enable and enhance ionization. Whereas when using atmospheric pressure chemical ionization (APCI), the solvent present, from both the co-solvent and the makeup solvents, acts as chemical ionization reagent gas in order to ionize the sample.

Waters' Xevo family of MS systems share a universal source platform which enables easy, quick, and tool-

free source exchange, without venting the system.[1] This facilitates the quick optimization and screening of the compounds of interest using different ionization modes when using UPC^2.

This document demonstrates interfacing UPC^2 with Xevo TQD, considering three atmospheric pressure ionization modes ESI, APCI, and APPI for the analysis of liquid crystal intermediate compounds as a model. This illustrates how the high speed and unique selectivity of UPC^2 can be combined with the greater selectivity and specificity that can be achieved using MS detection.

Experimental

UPC2 conditions

System:	ACQUITY UPC^2
Run time:	5.00 min
Column:	ACQUITY UPC^2 BEH 2-EP, 3.0 mm x 100 mm, 1.7 µm
Column temp.:	65 °C
CCM back pressure:	2000 psi
Sample temp.:	20 °C
Mobile phase A:	CO_2
Mobile phase B:	Methanol
Flow rate:	2.0 mL/min
Injection volume:	1 µL

UPC2 conditions

Vials:	Waters Amber Glass 12 x 32 mm Screw Neck Vial, 2 mL

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	2.00	98.0 (96.0*)	2.0 (4.0*)	-
2	1.00	2.00	86.6	13.4	6
3	3.00	2.00	60.0	40.0	6
4	4.00	2.00	60.0	40.0	6
5	4.01	2.00	98.0 (96.0*)	2.0 (4.0*)	6
6	5.00	2.00	98.0 (96.0*)	2.0 (4.0*)	6

Table 1. ACQUITY UPC^2 mobile phase gradient.

**Conditions used for APCI analysis, when no makeup solvent is used.*

PDA conditions

UV system:	ACQUITY UPC^2 PDA Detector
Range:	210 to 450 nm
Resolution:	1.2 nm
Sampling rate:	20 pts/sec

PDA conditions

Filter time constant:	Slow (0.2 sec)

Sample description

The liquid crystal intermediate compounds were purchased from Sigma-Aldrich. Individual stock solutions were prepared to a concentration of 5 mg/mL, dissolved in either heptane/ethanol (9:1) or methanol. Serial dilutions of the stock solutions were preformed in heptane/isopropanol (9:1) in order to prepare mixed standards, or in methanol to prepare infusion MS tuning standards.

Instrument control, data acquisition, and results processing

MassLynx Software, v4.1, SCN 882, was used to control the ACQUITY UPC^2 and the Xevo TQD and also for data acquisition.

MS and MS splitter conditions

MS conditions				
Mass spectrometer	Xevo TQD			
Ionization mode	**APCI** (with makeup solvent)	**APPI** (with makeup solvent)	**ESI** (with makeup solvent)	**APCI** (without makeup solvent)
Corona	3.0 µA	N/A	N/A	3.0 µA
Capillary	N/A	N/A	5.00 kV	N/A
APCI probe temp.	300 °C	300 °C	N/A	300 °C
Source temp.	150 °C			
Repeller	N/A	0.50 kV	N/A	N/A
Desolvation gas	800 °C	600 °C	600 °C	800 °C
Cone gas	0 L/hr			
Acquisition	Multiple Reaction Monitoring (MRM)			
MS splitter conditions				
Makeup pump	Waters 515			Not used
Solvent	Methanol	Methanol + 10% toluene	Methanol + 1% formic acid	
Flow	0.4 mL/min			

Table 2. MS and MS splitter conditions using different ionization modes and MS splitter conditions.

Results and Discussion

The UPC^2 conditions were optimized for the analysis of five liquid crystal intermediate compounds along with one internal standard using the Waters ACQUITY UPC^2 System equipped with an ACQUITY UPC^2 PDA Detector. Different UPC^2 columns, co-solvents, column temperatures, mobile phase temperatures, and system CO_2 back pressures were considered. Retention times were established by analyzing single component standards, as described in Table 3.

Chemical substance	CAS number	Molecular weight	Retention time (minutes)	UV optimum absorbance (nm)
4,4'-Azoxyanisole-d_{14}	39750-11-3	272.36	1.03	346
4-Butylbenzoic acid	20651-71-2	178.23	2.23	235
4-Octylbenzoic acid	3575-31-3	234.33	2.40	235
4-Cyanobenzoic acid	3575-31-3	147.13	2.97	252
4-Butoxybenzoic acid	1498-96-0	194.23	2.35	252
4-(Octyloxy)benzoic acid	2493-84-7	250.33	2.50	235

Table 3. Liquid crystal intermediate compounds, associated CAS number, molecular weight, measured retention times, and the UV optimum absorbance.

Using the on-board fluidics system on the Xevo TQD, 2-ppm individual standards were infused into the source using APCI ionization in order to optimize the APCI MS conditions (Table 2) and the MRM conditions (Table 4). The established MRM conditions were also used in APPI and ESI ionization modes. The established MRM method is shown in Figure 2.

Compound	APCI /APCI /ESI (+/-)	Cone voltage (V)	MRM transition (*m/z*)	Collision energy
4,4'-Azoxyanisole-d_{14}	+	30	251.2 > 139.0	20
			273.2 > 114.1*	25
4-Butylbenzoic acid	+	30	195.0 > 121.0	20
			179.1 > 123.0	10
4-Octylbenzoic acid	+	35	235.0 > 123.0*	15
			235.0 > 217.0	20
4-Cyanobenzoic acid	-	25	179.1 > 161.0*	15
			146 > 102.0*	15
4-Butoxybenzoic acid	+	30	273.2 > 142.1	20
			195.0 > 95.0*	20
4-(Octyloxy)benzoic acid	+	40	251.2 > 121.0*	20
			251.2 > 139.0	20

Table 4. Five liquid crystal intermediate compounds and one internal standard, ionization mode, cone voltage, MRM transitions, and associated collision energy values.

**Refers to the quantification transitions.*

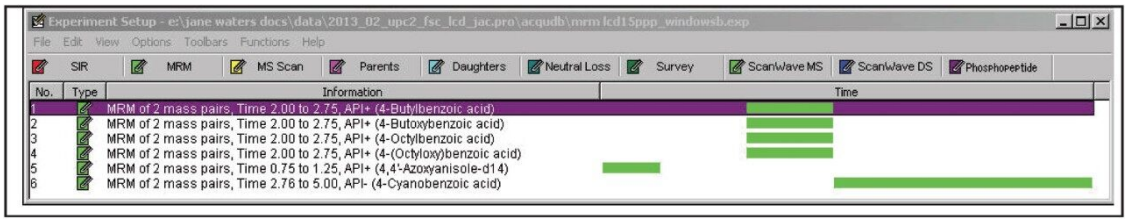


Figure 2. MRM method for five liquid crystal intermediate compounds and one internal standard.

The APPI, ESI MS conditions, and the MS splitter conditions were optimized (Table 2) by analyzing mixed 0.1 mg/mL calibration standards using the optimized UPC^2 conditions, and MRM method using either APCI, APPI, or ESI ionization modes as required. MS conditions considered included desolvation temperature and flow, cone gas flow, repeller voltage (APPI), and capillary voltage (ESI). When considering the MS splitter conditions, the makeup solvent and flow required for each ionization mode were optimized. Using ESI formic acid was added as a protonation source to the makeup solvent to enhance ionization and increase sensitivity. Whereas, when using APPI a dopant (toluene) was added to the makeup flow to enable and enhance ionization. When considering APCI the solvent present, from both the co-solvent and the makeup

solvents, were considered in order to optimize the ionization of the sample.

Results with makeup solvent (APCI, APPI, and ESI)

Mixed 0.1 mg/mL calibration standards were analyzed using the optimized UPC^2, MS and MS splitter conditions as detailed (Table 2) with the addition of a makeup solvent via the MS splitter, using APCI, APPI, and ESI ionization modes. The resulting MRM chromatograms are shown in Figure 3.

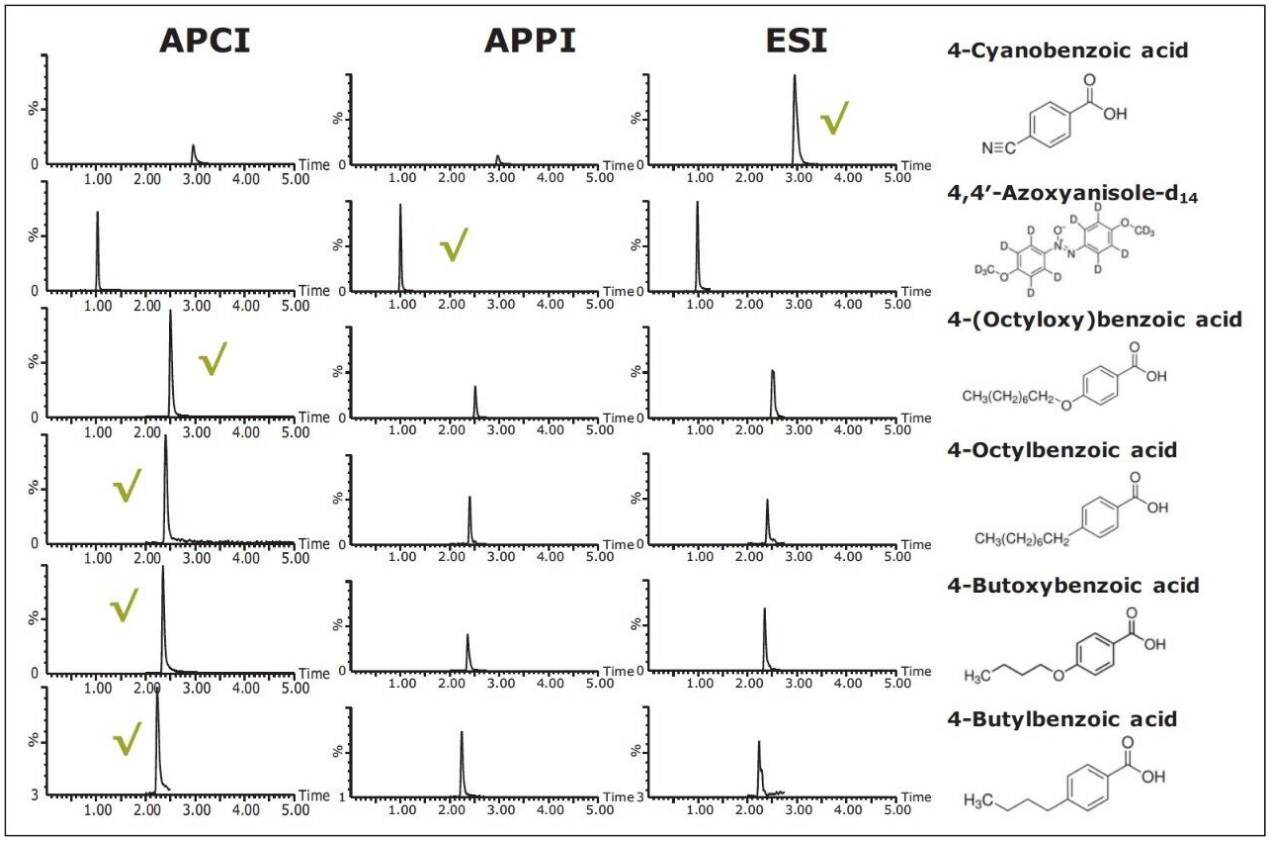


Figure 3. MRM chromatograms using APCI, APPI, and ESI ionization modes for the five liquid crystal intermediate compounds and one internal standard in a mixed 0.1 mg/mL calibration standard (√ refers to ionization mode which gave the highest peak area response for each compound).

Comparing results with and without makeup solvent (using APCI for illustration)

There is also the option when optimizing conditions of not adding an additional makeup solvent via the MS splitter, and purely using the co-solvent to aid ionization. This was considered using APCI ionization mode, where no additional buffers or dopants are required to aid ionization.

Using the UPC^2, MS and MS splitter conditions as detailed in Table 2, APCI (without makeup solvent) mixed

0.1 mg/mL calibration standard were analyzed. No additional solvent was added via the MS splitter, the same system configuration was used, with the exception that the tubing from the makeup pump to the MS splitter was isolated off (Figures 4a and 4b).

When using the initial 2% co-solvent the response of the earlier eluting peak was markedly reduced. In this example by increasing the initial co-solvent percentage from 2% to 4% and using no additional makeup solvent overall higher response values were observed for all the compounds considered. The MRM chromatograms using APCI with and without additional makeup solvent are shown in Figure 4c.

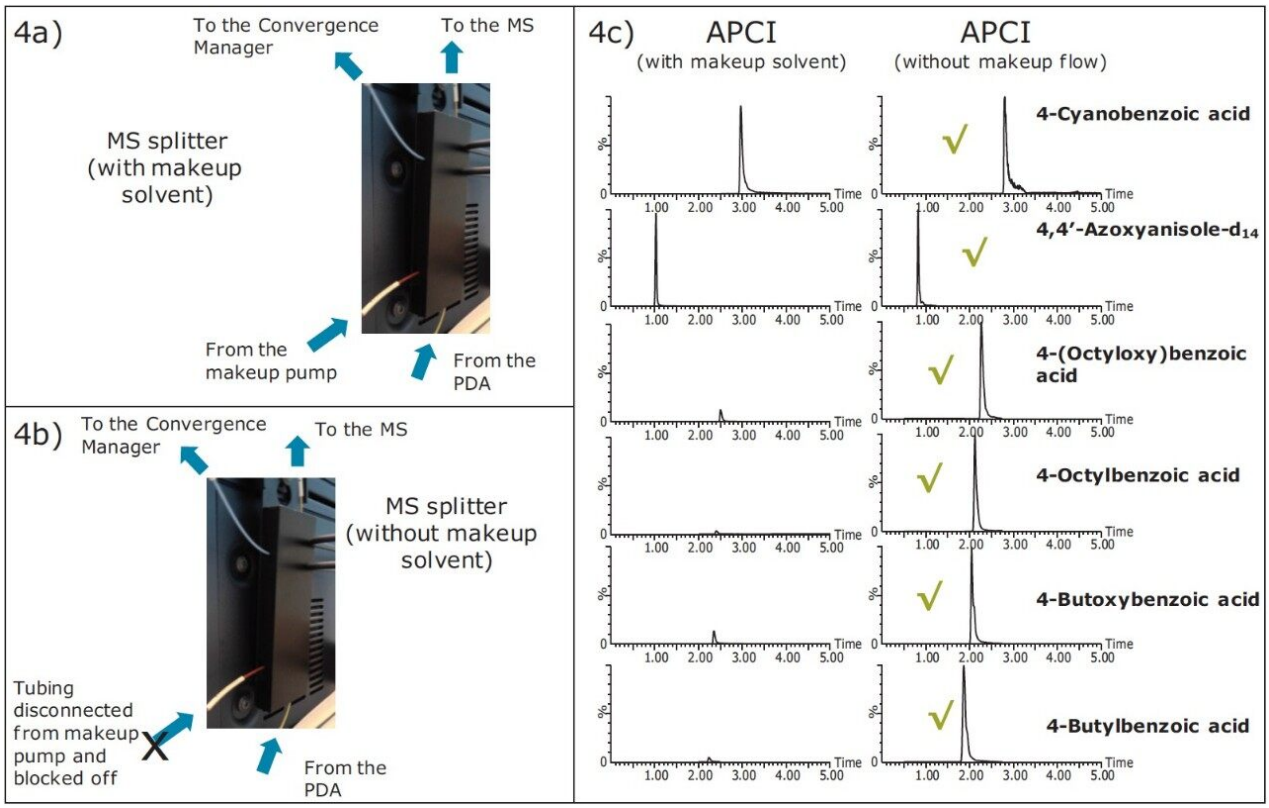


Figure 4. MS splitter, example configuration for the analysis using APCI with (4a) and without (4b) an additional makeup solvent. 4c) MRM chromatograms using APCI (with and with out additional makeup flow) for the five liquid crystal intermediate compounds and one internal standard in a mixed 0.1 mg/mL calibration standard (√ refers to the conditions which gave the largest response for each compound).

Conclusion

This document demonstrates the options available to collect MS data with UPC^2, using the analysis of liquid crystal intermediate compounds as an example.

UPC^2 can easily and quickly be connected to a Xevo TQD with the addition of an MS splitter, which introduces a controlled leak to the system and enables the maintenance of the CO_2 pressure. That makes available the option to add a modifier to the flow prior to MS detection, which can provide greater solvating powers, enhance the selectivity and sensitivity of MS detection, and also influence ionization.

Waters' Xevo family of MS systems share a universal source platform which enables easy, quick and tool free source exchange, allowing different ionization modes to be quickly optimized and screened for the compounds of interest when using UPC^2. Here we considered three ionization modes ESI, APCI, and APPI, optimizing the choice of modifier and tune parameters to illustrate how the high speed and unique selectivity of UPC^2 can be combined with the greater selectivity and specificity that can be achieved using MS detection.

References

1. E Riches. Xevo TQ MS with Atmospheric Pressure Photo Ionization (APPI) Source: The Ionization of Compounds with Diverse Structures Using Vitamins as a Model. Waters Technical Note 720003276en. 2010 Jan.

Featured Products

ACQUITY UPC2 System <https://www.waters.com/134658367>

Xevo TQD Triple Quadrupole Mass Spectrometry <https://www.waters.com/134608730>

MassLynx MS Software <https://www.waters.com/513662>

720004781, September 2013