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Achieve confident synthesis control with the Thermo Scientific ISQ EC single quadrupole mass spectrometer

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Keywords

Single quadrupole mass spectrometer, Medicinal chemistry, Synthesis control, Quantitative LC-MS, High throughput

Goal

Demonstrate quantitative synthesis control with the Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer and show its benefit for medicinal chemistry.

Introduction

Medicinal chemistry has a central place in drug discovery, lead optimization, and drug development. It enables pharmaceutical companies to research and develop new active pharmaceutical compounds or even compound classes. The success of organic synthesis of new molecules or of the isolation of naturally sourced compounds needs to be established. An important tool for this is LC-MS, which allows verification of the analyte by mass confirmation to determine the yield of the reaction and to check for impurities related to the proposed drug candidate in the same analysis. Another requirement is fast analysis to achieve high throughput. Often large numbers of candidates need to be screened, putting a strain on LC-MS resources.

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Modern single quadrupole mass spectrometers, such as the Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer (ISQ EC MS), are reliable workhorses designed for routine applications. The ISQ EC MS can operate in Full Scan and Single Ion Monitoring (SIM) mode, to either scan a mass range for all detectable analytes or focus on a specific compound. It can run at scan rates suitable for fast UHPLC applications while delivering picogram detection limits. The new orthogonal source design provides high levels of instrument robustness, even with difficult matrices. Full integration into the Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) and the Thermo Scientific™ AutoSpray™ smart method setup make LC-MS operation straightforward and intuitive.

The synthesis of acetaminophen, also known as paracetamol, was selected as an example to prove that the ISQ EC MS can be used for fast, quantitative synthesis control. Acetaminophen is produced in a one-step synthesis by acetylation of 4-aminophenol (Figure 1). The ratios of pre-mixed reactant to product sample mixes were determined and compared with the theoretical values to determine the accuracy and precision of the presented workflow.

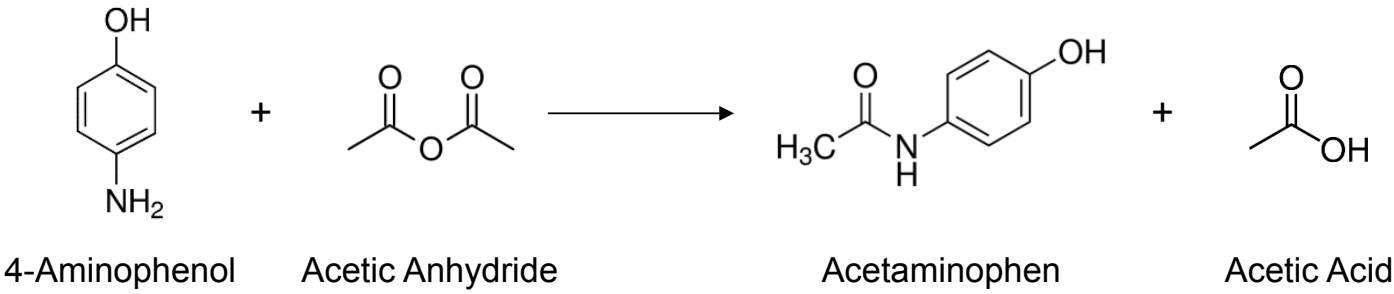

Figure 1. Reaction equation of acetaminophen (paracetamol).

Experimental

Sample reagents were purchased from Sigma-Aldrich. Table 1 lists the analytes used and Table 2 lists the solvents and additives.

Table 1. Analytes.

Analyte	Purity	CAS	Chemical Formula	Monoisotopic Mass [M]	$[M+H]^+$
4-Aminophenol	≥98%	123-30-8	C_6H_7NO	109.05	110.06
Acetaminophen	98.0–102.0%	103-90-2	$C_8H_9NO_2$	151.06	152.07

Table 2. Solvents and additives.

Reagent	Grade	Supplier	Part Number
Acetonitrile	Optima™ LC-MS	Fisher Chemical	A955-212
Formic acid	Optima™ LC-MS	Fisher Chemical	A117-50
Methanol	Optima™ LC-MS	Fisher Chemical	A456-212
Water	Ultra-Pure, 18.2 MΩ at 25 °C	Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System	

Chromatographic separation was performed on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system consisting of a binary pump, an autosampler, a column compartment, and a variable wavelength detector (Table 3). A 75 cm long MP35N capillary with 100 µm inner diameter (P/N 6042.2390) was used for connecting to the ISQ EC MS. The HPLC conditions are listed in Table 4, and the MS conditions are listed in Table 5.

Table 3. Vanquish Flex Binary UHPLC system modules.

Module	Part number
Vanquish System Base F	VF-S01-A
Vanquish Binary Pump F (with 35 µL mixer)	VF-P10-A (6044.3870)
Vanquish Split Sampler FT	VF-A10-A
Vanquish Column Compartment H	VH-C10-A
Vanquish Variable Wavelength Detector F (2.5 µL SST flow cell)	VF-D40-A (6074.0360)

Table 4. HPLC conditions.

Parameter	Value
Column	Thermo Scientific™ Hypersil GOLD™, 1.9 µm, 2.1 x 50 mm (P/N 25002-052130)
Mobile phase	A: Water with 0.1% formic acid B: Acetonitrile with 0.1% formic acid
Gradient	0–2 min: 5–50% B 2–3 min: 50% B 3–5 min: 5% B
Flow rate	0.6 mL/min
Column temperature	Forced air, 40 °C Active preheater, 40 °C
Injection volume	1 µL
UV detection	280 nm, 50 Hz, easy mode

Table 5. MS conditions.

Parameter	Value
Vaporizer temperature	550 °C
Ion transfer tube temperature	350 °C
Source voltage	+1000 V
SIM scan	
Compound Time Mass Source CID voltage	4-Aminophenol 0–0.5 min 110.1 *m/z* 20 V
Compound Time Mass Source CID voltage	Acetaminophen 0.5–1.18 min 152.1 *m/z* 20 V
Full Scan	
Time Mass range Source CID voltage	0–5 min 105–250 *m/z* 20 V

The ISQ EC MS is fully integrated into the Chromeleon 7.2 CDS, which was used for system operation and subsequent data analysis.

Calibration standards (10 ppb–10 ppm) were prepared by serially diluting stock solutions (1 µg/µL 4-aminophenol and acetaminophen in 50:50 methanol:water) in 5% methanol in water. Samples for measuring the reactant to product ratios were prepared diluting the stock solutions in 5% methanol in water. Prepared samples are listed in Table 6.

Table 6. Sample analyte ratios and used sample concentrations (1 ppm = 1 ng/µL).

Theoretical Ratio (Reactant:Product)	4-Aminophenol (ppm)	Acetaminophen (ppm)
100:1	10	0.1
50:1	5	0.1
20:1	2	0.1
10:1	1	0.1
5:1	0.5	0.1
2:1	0.2	0.1
1:1	0.1	0.1
1:2	0.1	0.2
1:5	0.1	0.5
1:10	0.1	1
1:20	0.1	2
1:50	0.1	5
1:100	0.1	10
1:200	0.05	10
1:500	0.02	10
1:1000	0.01	10

Results and discussion

Synthesis control requires fast, quantitative analysis of the reactants and products while they are present in different ratios. In this work, ratios between 100:1 and 1:1000 (reactant to product) were premixed and quantified in order to mimic the time course of acetaminophen synthesis from the very start to near completion. To assure the best quantitation results, a high-throughput method was developed, which provided baseline separation of 4-aminophenol and acetaminophen (Figure 2).

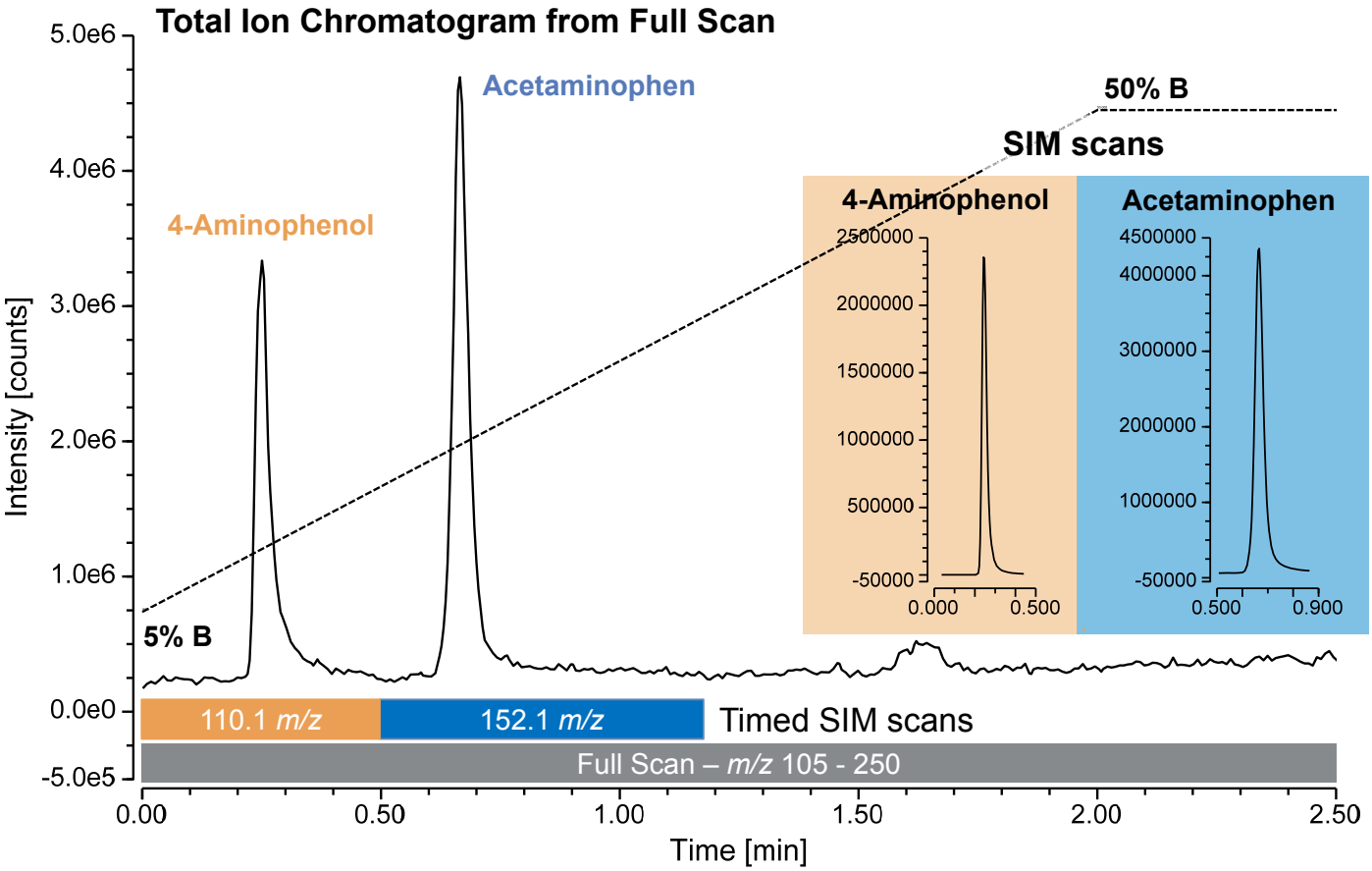

Figure 2. Total ion chromatogram of 4-aminophenol and acetaminophen (50 pg) in full scan acquisition. The gradient part of the high throughput method is depicted. The gradient starts with 5% B and increases to 50% B. The acquisition windows for the SIM scans are depicted in orange and blue.

The analytes were detected with SIM mode to improve detection limits. The SIM window (0.4 amu) and dwell time (0.2 s) parameters were selected to increase signal intensity and to assure at least 15 scans over the peak for good quantitation results. The SIM scans for 4-aminophenol (110.1 *m/z*) and acetaminophen (152.1 *m/z*) were timed using retention time windows fully encompassing the retention time of the expected chromatographic peaks (Figure 2). For initial method verification, full scan acquisition together with SIM mode acquisition was performed. For quantification, only SIM scans were performed to assure optimal analyte detection.

Mass spectrometric detectors usually outperform UV detectors in terms of detection limits. Thus, the detection limits of the ISQ EC MS and the Vanquish Flex variable wavelength UV detector (VWD) were compared.

Looking at the signal responses of an ISQ EC MS and a VWD revealed differences in detetction limits of up to three orders of magnitude (Figure 3). With the VWD, 5 ng acetaminophen on column were detectable with a signal-to-noise ratio (peak to peak) of 18. Therefore, a detection limit of ~1 ng can be expected (S/N 3). Using the ISQ EC MS extracted ion chromatograms from the Full Scan afforded detection down to 50 pg of analyte; in SIM mode signal-to-noise was 55 at 50 pg and 4 at 5 pg. Thus, 5 pg is the lower limit of detection.

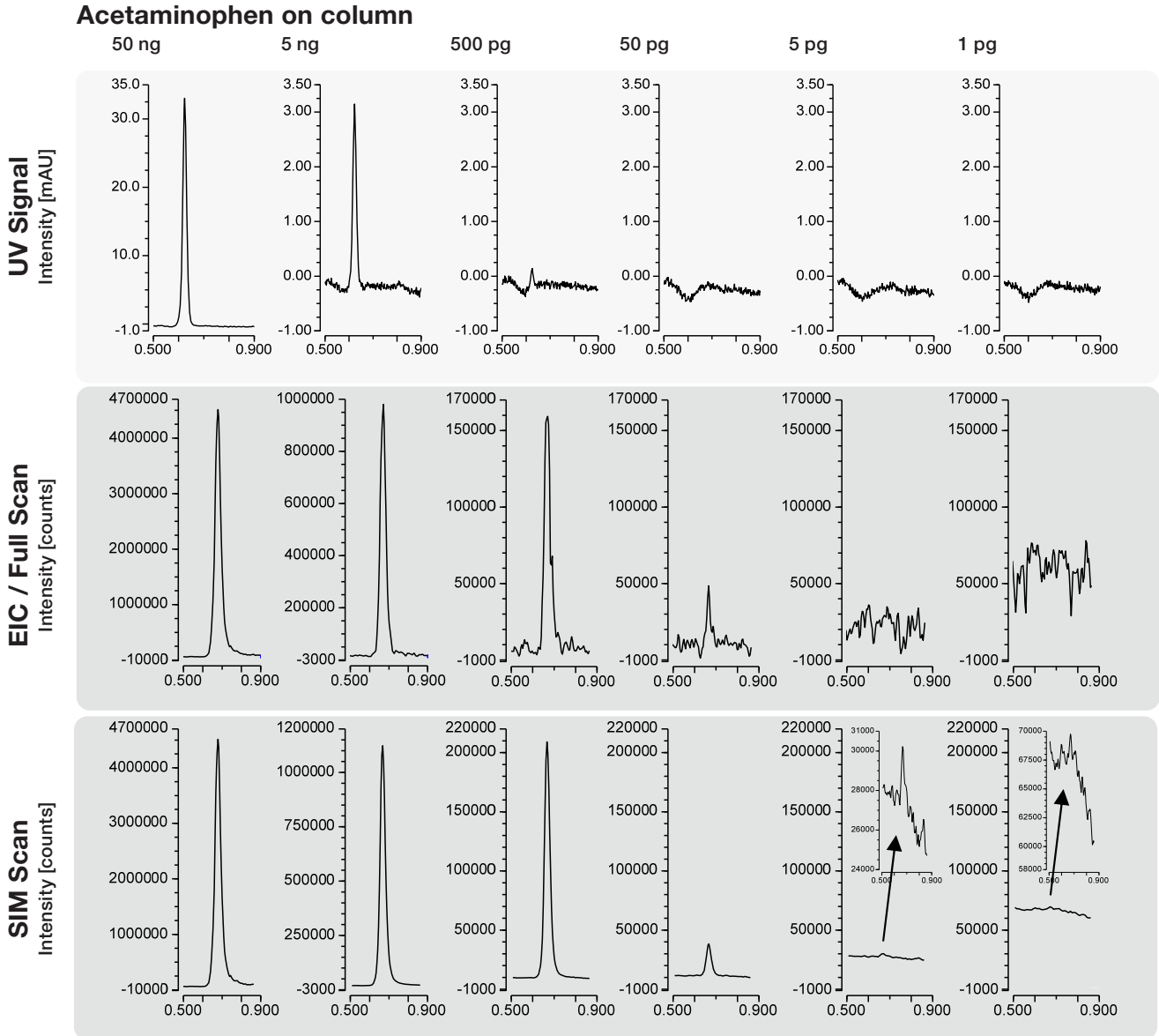

Figure 3. Comparison of signal response between UV and ISQ EC detector for acetaminophen (EIC: Extracted Ion Chromatogram).

Calibration curves for 4-aminophenol and acetaminophen spanning the relevant sample concentrations were generated (Figure 4). All injections were done in quintuplicate. Afterwards reinjections of calibrants were done in triplicate to verify the accuracy of the calibration.

4-Aminophenol and acetaminophen showed good recovery rates. The standard deviation between the reinjection replicates was below 5% indicating high precision (data not shown).

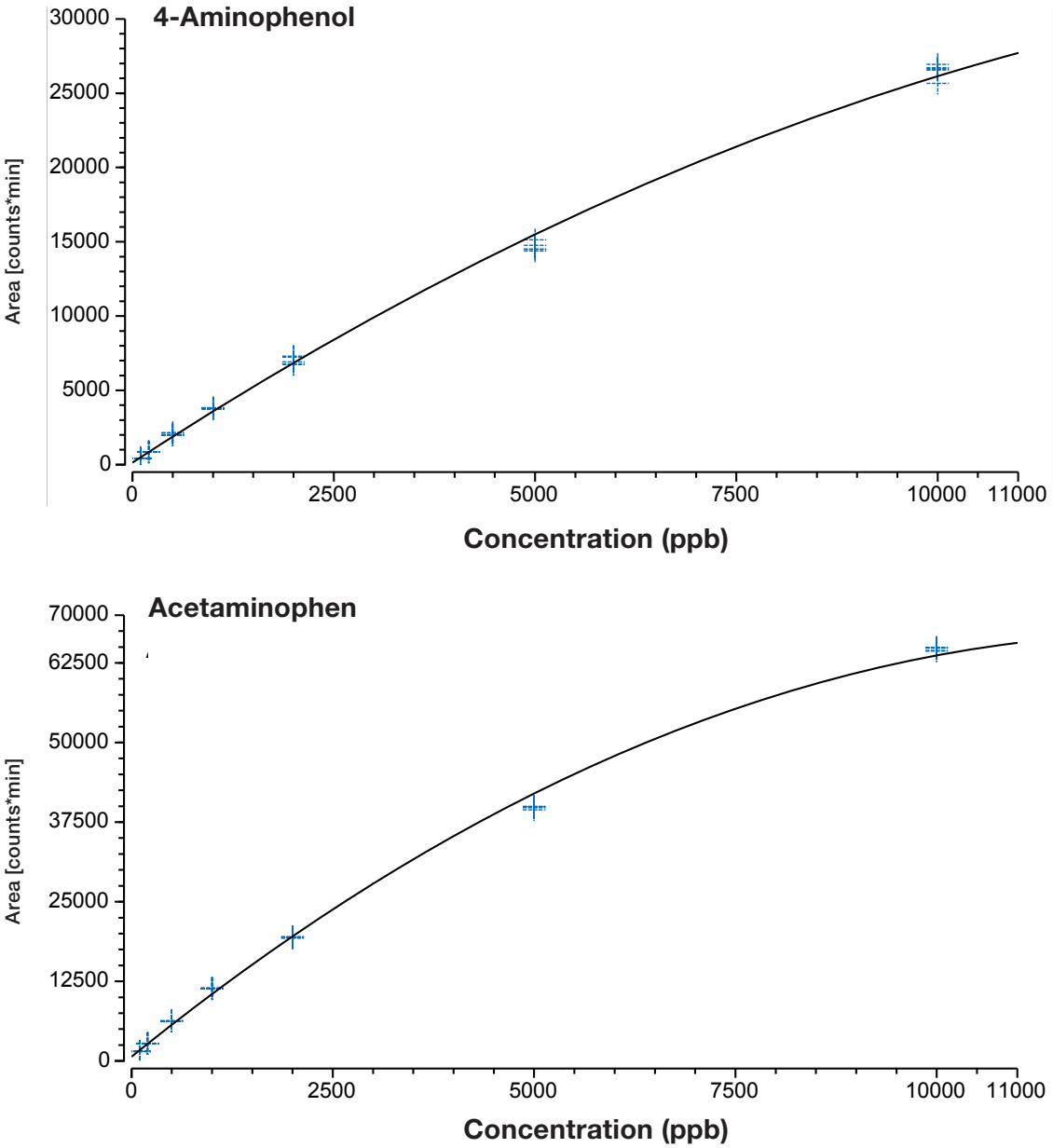

Figure 4. Calibration curves for 4-aminophenol and acetaminophen. 1 ppb equals 1 pg on column since 1 µL was injected. Quadratic fit with 1/x weighting was applied. 4-Aminophenol: $3.7220x - 0.0001x^2$, $R^2 = 0.9962$; Acetaminophen: $663.3313 + 10.2319x – 0.0004x^2$, $R^2 = 0.9966$.

For synthesis control, 4-aminophenol / acetaminophen mixtures at different ratios were analyzed starting with 100:1 and finishing with 1:1000 (Table 6 and Figure 5). All samples were analyzed in quintuplicate. The change in the composition of the samples could be accurately detected. With changing ratios, the relative amount of acetaminophen increased in the expected manner. Even relative abundances of 4-aminophenol below 1 percent could be accurately determined (zoom-in, Figure 5). These results show that the ISQ EC MS can accurately quantify analytes. This highlights that the presented single quadrupole mass spectrometer is a suitable tool for high-throughput monitoring of chemical syntheses in medicinal chemistry.

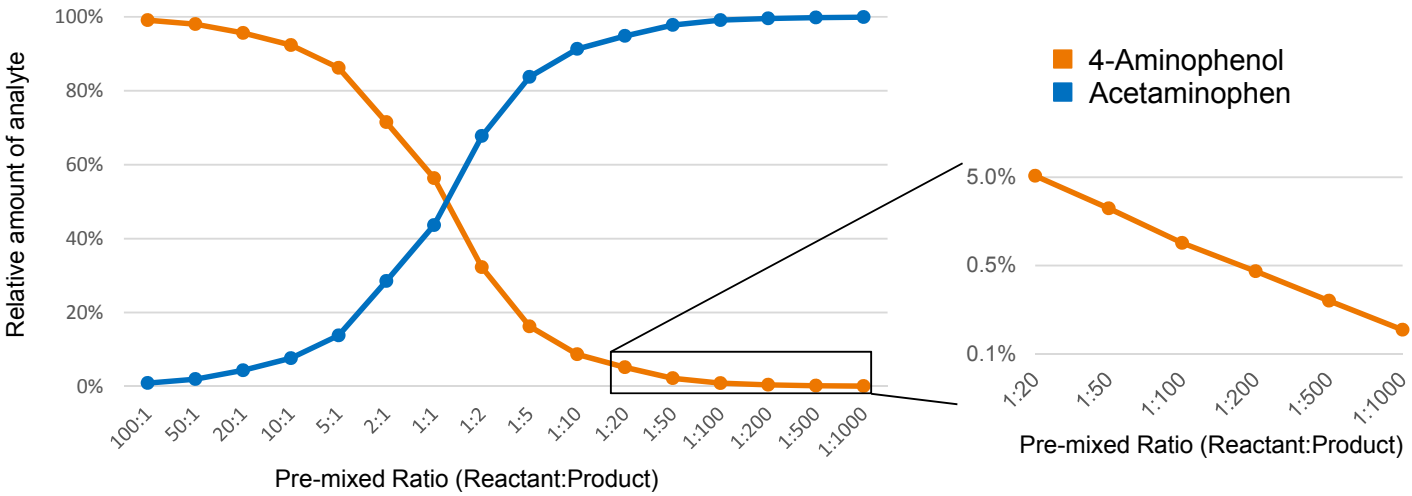

Figure 5. Synthesis control. Observed 4-aminophenol:acetaminophen ratios are plotted. The pre-mixed ratios are stated on the x-axis. Zoom-in shows 1:20 to 1:1000 ratios for 4-aminophenol on a logarithmic scale.

Conclusion

- High-throughput, quantitative synthesis control can be done with the ISQ EC single quadrupole mass spectrometer.
- SIM mode greatly increases sensitivity over UV detection and can be used for targeted quantitative analyses.
- Full Scan mode results in general detection of present analytes, e.g. monitoring other potential products, and can be used for generic methods.

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