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Applikationsbericht

Rapid Detection and Quantification of Selected Microsomal Substrates and Metabolites Using the Waters ACQUITY UPLC I-Class System and Xevo TQ-S micro

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Abstract

In the work presented here, we show the development and validation of a rapid and sensitive LC-MS method for the simultaneous detection and quantification of multiple, selected CYP450 substrates and metabolites.

Benefits

- The Xevo TQ-S micro offers excellent sensitivity and expansive linear range with fast MRM and polarity switching that can accommodate multiple transitions on UPLC-scale analyses
- Discrete assays were combined into a single method, reducing cycle time and enabling high throughput
- Limits of quantification (LOQs) as low as 0.200 nM were obtained for analytes

Introduction

High-throughput absorption, distribution, metabolism, and excretion (ADME) screening for the properties of drug candidates have become an essential part of modern drug development. Such screening provides a basis for better information and, consequently, better decision-making in the drug discovery and development process.[1] As part of these studies, incubating candidate compounds together with liver microsomes containing cytochrome CYP450 is relied upon extensively to determine compound metabolism, stability, and possible drug-drug interactions (DDI).

The use of CYP450 to determine drug-drug interactions is carried out through inhibition and induction studies and is of particular importance because as the number of prescribed drugs increases, the greater the probability that an adverse drug reaction could exist. Also, it has been stated that there could be up to a 40% probability of a DDI when a patient is administered ten drugs or more.[2] In a standard CYP450 inhibition and induction assay, multiple test compounds are evaluated to determine their ability to alter or influence the metabolism of known CYP450 specific substrates.

In the work presented here, we show the development and validation of a rapid and sensitive LC-MS method for the simultaneous detection and quantification of multiple, selected CYP450 substrates and metabolites.

Figure 1. Waters ACQUITY I-Class UPLC and Xevo TQ-S micro.

Experimental

Method conditions

LC conditions

LC system:	ACQUITY UPLC I-Class
Vials:	Waters Maximum Recovery
Column:	ACQUITY UPLC BEH C_{18}, 3.0 x 50 mm, 1.7 µm
Column temp.:	35 °C
Sample temp.:	Ambient
Injection volume:	5 uL
Flow rate:	0.600 mL/min
Mobile phase A:	2 mM NH_4CH_4COOH, 0.1% NH_4OH in H_2O
Mobile phase B:	2 mM NH_4CH_3COOH, 0.1% NH_4OH in CH_3OH
Gradient:	1% to 90% B over 2 minutes; curve 4

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI+
Capillary voltage:	1 kV

Acquisition mode: MRM

Data management

MassLynx Mass Spectrometry Software

Compound	Transition	Dwell time ms	Cone voltage	Collision energy
Acetaminophen	152>110	5	20	13
Acetaminophen-d4	156>114			
Hydroxymephenytoin	235>150	5	25	14
Hydroxymephenytoin-d3	238>150			
Hydroxybupropion	256>131	5	30	26
Hydroxybupropion-d6	262>131			
Dextorphan	258>199	5	20	29
Dextorphan-d3	261>199			
Hydroxytestosterone	305>269	5	20	11
Hydroxytestosterone-d3	308>272			
Hydroxydiclofenac	312>231	5	20	17
Hydroxydiclofenac-13C6	318>237			
Hydroxymidazolam	342>203	5	30	23
Hydroxymidazolam-d4	346>203			
6a-Hydroxypaclitaxel	870>286	5	30	4
6a-Hydroxypaclitaxel-d5	875>291			

Results and Discussion

The number and diversity of new drug candidates entering the pharmaceutical pipeline has increased, necessitating strategies such as rapid ADME assays for obtaining information that can be used to better qualify candidates. The rapid determination of potential DDIs is of particular importance. If they are discovered late in the development process or after the drug goes to market, DDIs can cost pharmaceutical companies millions of dollars in lost revenue. Figure 2 depicts the separation of two substrates and six metabolites routinely monitored in testing for potential DDIs through inhibition and induction CYP450 assays. Good separation is shown with base widths of chromatographic peaks on the order of three seconds.

These results were obtained under generic conditions, illustrating the ease and time for method development.

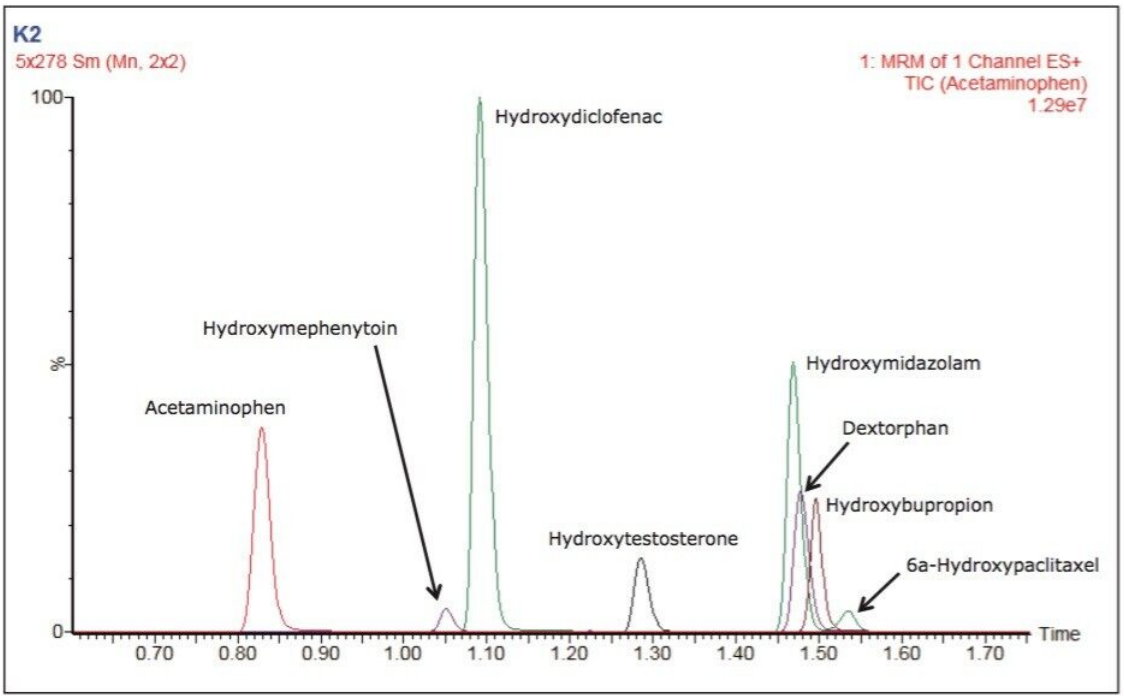

Figure 2. Rapid separation of CYP450 substrates and metabolites with the Waters ACQUITY UPLC I-Class and Waters Xevo TQ-S micro.

Figure 3 shows the calibration curve for the CYP450 isoform 1A2 substrate acetaminophen. It is linear well beyond three orders of magnitude, and it produces an R^2 value of 0.998935 (Table 1 shows the R^2 values for the other compounds used in this assay) Figure 3 also shows a lower limit of quantification (LLOQ) of 0.2 nM, with greater than 14 times the signal-to-noise ratio, as measured from the baseline of the separation.

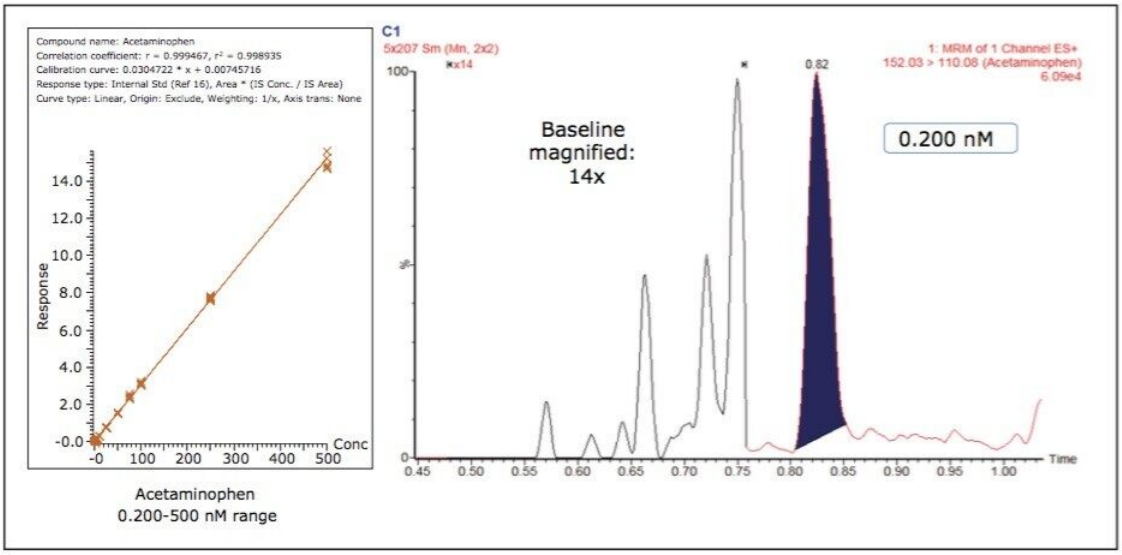

Figure 3. Calibration curve and LLOQ for acetaminophen.

Compound	Calibration Curve R^2 Value
Hydroxymephenytoin	0.996465
Hydroxybupropion	0.993304
Dextorphan	0.998533
Hydroxytestosterone	0.995952
Hydroxydiclofenac	0.996227
Hydroxymidazolam	0.997798
6a-Hydroxypaclitaxel	0.997247

Table 1. Calibration curve R^2 values of selected CYP450 substrate and metabolites utilized in this study.

The developed method was then tested for accuracy and precision by LC-MS, as prescribed in the USFDA guidance on bioanalytical method validation. Five determinations were made for 10 concentrations ranging from 0.2 to 5000 nM. The data in Table 2 show that the accuracy and precision for all of the analytes tested falls well within the 15% CV limit (3).

	Acetaminophen			Hydroxymephenytoin			Dextorphan			6α-Hydroxypaclitaxel		
[Nominal]nM	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV
0.5	0.456	-11	10				0.446	-11	9	0.534	7.6	8
1	1.05	5.1	5	1.05	4.6	8	0.944	-5.7	5	1.06	5.5	7
2	1.98	-1.1	4	1.93	-3.4	13	1.97	-1.3	5	1.96	-2.2	6
5	4.89	-2.2	2	4.68	-6.4	12	5.17	3.3	3	4.82	-3.7	12
10	10.2	1.6	4	10.3	2.7	11	10.5	5	5	9.61	-3.9	10
25	24.9	-0.55	2	24.9	-0.38	6	25.5	2	3	24.5	-1.9	9
50	50.4	0.73	2	51.3	2.5	4	50.8	1.6	4	48.8	-2.5	8
75	80	6.7	4	73.7	-1.8	3	76.5	2	5	75.8	1.1	7
100	101	1.5	2	102	1.7	4	103	2.6	4	103	3	10
250	252	0.94	1	253	1.3	4	254	1.6	4	251	0.23	4
500	491	-1.8	3	495	-0.93	7	490	-2	2	498	-0.43	4

	Hydroxymidazolam			Hydroxytestosterone			Hydroxydiclofenac			Hydroxybupropion		
[Nominal]nM	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV	[Mean]	Accuracy %	%CV
0.5	0.484	-0.1	12	4.85	-3	12	0.454	13.4	9	0.872	-13	6
1	0.93	-7	7	10.4	3.7	10	0.8	0.04	9	1.95	-2.4	11
2	2.17	8.3	5	20.1	0.45	7	1.93	-3.5	8	4.94	-1.2	4
5	5.12	2.3	11	50.1	0.13	6	4.01	0.2	11	9.96	-0.38	1
10	10.4	4.3	9	102	2.3	8	7.85	-1.9	10	27.7	11	4
25	24.7	-1.1	12	252	0.69	9	19.4	-2.8	9	51.5	3	2
50	48.9	-2.2	8	508	1.5	6	39.6	-1	8	79.2	5.6	3
75	73.4	-2.2	7	813	8.5	8	97.7	-2.3	9	96.8	-3.2	8
100	98.4	-1.6	5	1045	4.5	7	195	-2.4	10	258	3.1	2
250	240	-3.8	5	2528	1.1	4	302	0.57	8	487	-2.5	3

Table 2. Accuracy and precision for selected CYP450 substrates and metabolites.

The sensitivity of a bioanalytical LC-MS assay can depend on matrix suppression, chromatographic peak shape, and the duration over which the mass spectrometer samples the chromatographic peak (or peaks) of interest. The sensitivity in this work can also be attributed to the incubation conditions: longer incubation times or higher enzyme concentrations can lead to the development of greater amounts of detected, metabolized substrate. The narrow peak widths produced by the sub-2-µm LC separation require a sufficient number of points across the chromatographic peak for good quantification. Figure 4 shows that the Xevo TQ-S micro is fully capable of acquiring more than 20 points across these narrow peak widths while subsequently monitoring multiple MRM transitions.

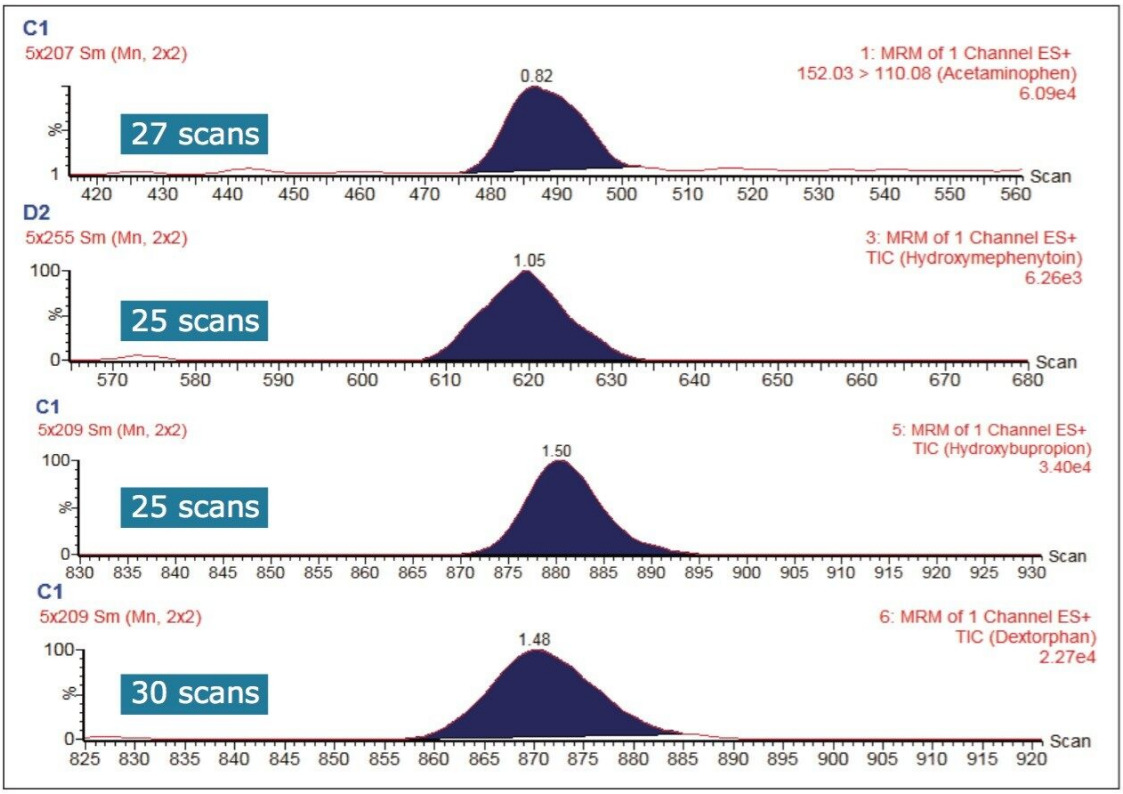

Figure 4. Replicate injections of acetaminophen illustrating the ability of the Xevo TQ-S micro to acquire high scan rates with narrow chromatographic peak widths as produced by UPLC.

The Xevo TQ-S micro can also be operated in RADAR acquisition mode. This allows the simultaneous acquisition of full-scan and MRM data in a single experiment. In Figure 5, we observe the data from the MRM channels monitored in this experiment and also the data from a full MS scan from *m/z* 50–600. We also observe co-elution of the target analyte with components in the matrix. Thus, the ability to monitor MRM channels and full-scan data can aid in the rapid development of robust methods that minimize the potential for co-elutions and possible matrix suppression. The capability of monitoring full-scan and MRM-channel data could be exploited for drug stability or metabolite identification studies. Though certain chemical entities would lay outside the specific MRM channels, they would nevertheless be subject to detection during full-scan acquisition. Data obtained in this way could then be further verified to determine positive identification.

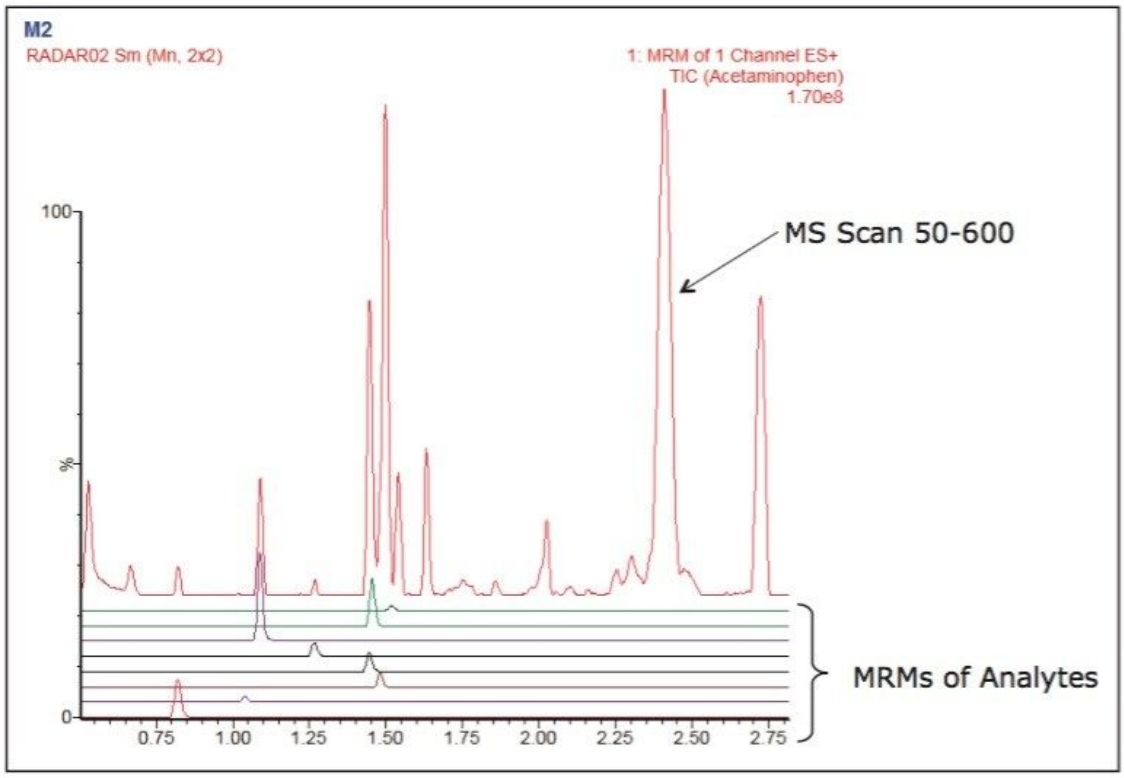

Figure 5. Chromatogram illustrating RADAR capability of the Xevo TQ-S micro. Here a full MS scan and multiple MRM channels can be simultaneously monitored.

Conclusion

The combination of the Xevo TQ-S micro and ACQUITY UPLC I-Class produced a rapid, sensitive, and robust method for the separation and detection of multiple, selected CYP450 substrates and metabolites often utilized in DDI studies. The accuracy and precision of the data produced from the method was well within the limits designated by the USFDA guidance for bioanalytical method validation. Linear responses for the calibration curves were obtained, revealing an LLOQ for the analytes of 0.2 nM. Further, the RADAR acquisition mode of the Xevo TQ-S micro enables the acquisition of full-scan and MRM data in a single run; a feature that can aid in method development and other applications.

References

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