Evaluation of LCMSMS Deuterium Scrambling in Clinically Significant Small Molecules

Authors: Joshua Cooper, Isil Dilek, and Uma Sreenivasan

Cerilliant Corporation, 811 Paloma Drive, Suite A, Round Rock, TX 78665

Introduction 1

Introduction and Objective:

LC-MS/MS is a powerful tool that brings numerous benefits to the clinical sample analysis arena. However, due to the complexity of the instrumentation there are some unique challenges that also accompany these benefits. Even following sample extraction and cleanup, matrix effects from the samples can cause interferences or impact ionization efficiency. Deuterium-labeled internal standards are the most common and prevalent labeled internal standards used to compensate for matrix effects. Some deuterium labeled compounds may exhibit hydrogen-deuterium scrambling/exchange in the collision cell which can impact MS/MS transition selection.

In this study we investigated numerous variables that potentially contribute to scrambling in order to ascertain reproducibility and impact on scrambling ratios: influences of different LC-MS systems (tandem quadrupole vs. quadrupole time-of-flight), matrix selection, concentration, with and without HPLC, collision energies, and deuterium placement in the internal standard. Numerous small molecules of clinical importance were investigate including: hydroxyvitamin D, testosterone, immunosuppressants, bath salts, and spice cannabinoids.

Materials and Procedures 2

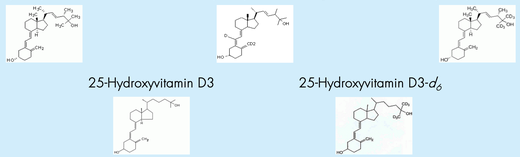
LCMS Systems:
Waters Alliance UPLC-Xevo G2 Q-Tof
Agilent 1100 HPLC-6410 triple quad

Cerilliant Solution Standards Used:
25-Hydroxyvitamin D3, Cat# H-083
25-Hydroxyvitamin D3-d_6, Cat# H-074
25-Hydroxyvitamin D2, Cat# H-073
Testosterone, Cat# T-037
Testosterone-d_3, Cat# T-046
Testosterone-$^{13}C_3$, Cat# T-037
(-)-Δ9-THC, Cat# T-005
(-)-Δ9-THC-d_3, Cat# T-003
(±)-11-Hydroxy-Δ9-THC, Cat# H-026
(±)-11-Hydroxy-Δ9-THC, Cat# H-041
(±)-11-nor-9-Carboxy-Δ9-THC, Cat# T-006
(±)-11-nor-9-Carboxy-Δ9-THC-d_3, Cat# T-004
(±)-11-nor-9-Carboxy-Δ9-THC-d_9, Cat# T-007
Cannabinol, Cat# C-045
Cannabinol-d_3, Cat# C-084
JWH-018 4-Hydroxypentyl metabolite, Cat# S-035
JWH-018 4-Hydroxypentyl metabolite-d_5, Cat# S-039
JWH-073 3-Hydroxybutyl metabolite, Cat# S-037
JWH-073 3-Hydroxybutyl metabolite-d_3, Cat# S-040
3,4-MDPV HCl, Cat# M-146
3,4-MDPV-d_8 HCl, Cat# M-146
Ethylone HCl, Cat# E-071
Ethylone-d_5 HCl, Cat# E-072
Butylone HCl, Cat# B-045
Butylone-d_3 HCl, Cat# B-046
Mephedrone HCl, Cat# M-138
Mephedrone-d_3 HCl, Cat# M-139
Methylone HCl, Cat# M-140
Methylone-d_3 HCl, Cat# M-140
Everolimus-d_4, Cat# E-070
Mycophenolic acid, Cat# M-106
Mycophenolic acid-d_3, Cat# M-137

Serum Extraction:
200µL of sample in serum + 200µL of methanol, vortexed to mix.
Added 1mL of heptane, vortexed for 30sec,
Centrifuged for 4min at 3000rpm
900µL of top layer dried under nitrogen
Reconstituted in 100µL of ethanol

Comparisons of 25-Hydroxyvitamin D Deuterium Scrambling 3

25-Hydroxyvitamin D2 | 25-Hydroxyvitamin D2-d_3 | 25-Hydroxyvitamin D2-d_6



25-Hydroxyvitamin D3 | 25-Hydroxyvitamin D3-d_6

Labeled 25-Hydroxyvitamin D2 and D3 Scrambling in Serum

Compound	Label	System	Concentration µg/mL	Transition d_{n-1}	Transition d_n	Scrambling % d_{n-1} / d_n
25-Hydroxyvitamin D2	d_3	Xevo G2	2	398→379	398→380	28.6
			0.2	398→379	398→380	35.4
		6410	5	416→397	416→398	2.8
				416→379	416→380	19.7
				398→379	398→380	30.4
			50	416→397	416→398	2.8
				416→379	416→380	20
				398→379	398→380	30.5
	d_6	6410	5	419→400	419→401	2
				419→382	419→383	8.8
				401→382	401→383	5.9
			50	419→400	419→401	2
				419→382	419→383	9
				401→382	401→383	5.4
25-Hydroxyvitamin D3	d_6	6410	2.5	407→388	407→389	4
				407→370	407→371	18.8
				389→370	389→371	9.2

Transitions Comparisons for Native and Labeled 25-Hydroxyvitamin D2 and D3 in EtOH on 6410

Parent → Water loss

Compound	Label	Concentration µg/mL	Transition d_{n-1}	Transition d_n	Scrambling % d_{n-1} / d_n
25-Hydroxyvitamin D2	d_3	100	416→397	416→398	2.9
	d_6	100	419→400	419→401	2
	native	50	413→394	413→395	0.5
25-Hydroxyvitamin D3	d_6	50	407→388	407→389	4
	native	100	401→382	401→383	0.5

Parent → 2 Water losses

Compound	Label	Concentration µg/mL	Transition d_{n-1}	Transition d_n	Scrambling % d_{n-1} / d_n
25-Hydroxyvitamin D2	d_3	100	416→379	416→380	19.5
	d_6	100	419→382	419→383	8.9
	native	50	413→376	413→377	0.5
25-Hydroxyvitamin D3	d_6	50	407→370	407→371	18.9
	native	100	401→364	401→365	0.3

Water Loss → 2 Water losses

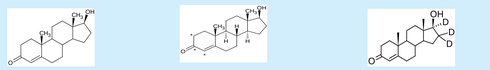
Compound	Label	Concentration µg/mL	Transition d_{n-1}	Transition d_n	Scrambling % d_{n-1} / d_n
25-Hydroxyvitamin D2	d_3	100	398→379	398→380	30.4
	d_6	100	401→382	401→383	5.4
	native	50	398→376	398→377	0.4
25-Hydroxyvitamin D3	d_6	50	389→370	389→371	11.2
	native	100	383→364	383→365	0.3

Notes: 25-Hydroxy D2-d_6 water loss→2 water loss has same transition as 25-Hydroxyvitamin D3 parent→water loss. Can be problem if compounds are not well resolved chromatographically.

Selection of Transitions Greatly Impacts Observed Scrambling

Investigation of Testosterone Scrambling 4

Testosterone | Testosterone-$^{13}C_3$ | Testosterone-d_3



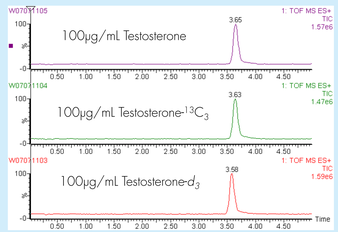
Major transitions are:
Native: 289→97 & 289→109
Testosterone-d_3: 292→97 & 292→109
Testosterone-$^{13}C_3$: 292→100 & 292→112
No scrambling at major transitions

Testosterone Scrambling at Minor Transitions

Label	Method	Instrument	Concentration µg/mL	Transitions D_{n-1} or $^{13}C_{n-1}$	Transitions D_n or $^{13}C_n$	*Scrambling % D_{n-1} / D_n
d_3	Infusion	Q-Tof	10	292→255	292→256	31.9
	LC		100			36.5
			10			35.7
		6410	100			37.7
			10			36.3
$^{13}C_3$			100			0.1
native			100	289→252	289→253	0.0

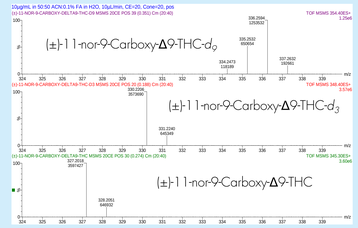
* or Scrambling % $^{13}C_{n-1}$ / $^{13}C_n$

Testosterone Chromatograms on Xevo G2

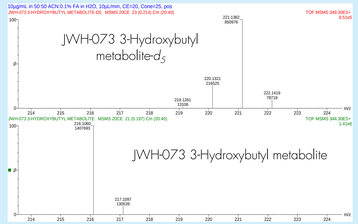


Investigation of Scrambling in Spice Cannabinoids, Bath Salts, and Immunosuppressants 5

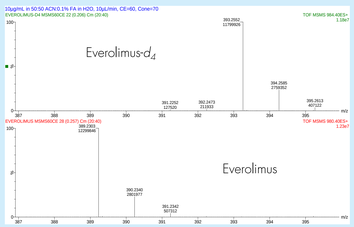
(±)-11-nor-9-Carboxy-Δ9-THC Scrambling at m/z 327



JWH-073 3-Hydroxybutyl Metabolite Scrambling at m/z 216



Everolimus Scrambling at m/z 389



Spice Cannabinoids, Bath Salts, and Immunosuppressants Scrambling Comparison usinf Xevo G2

Compound	Label	Polarity	Collision Energy	Transition(s) d_n	Scrambling % d_{n-1}/d_n
(-)-Δ9-THC	d_3	pos	25	318→262,196	0
	native	pos	25	315→259,193	0
(-)-Δ9-THC	d_3	neg	30	318→262,196	can't determine
	native	neg	30	315→259,193	can't determine
(±)-11-Hydroxy-Δ9-THC	d_3	pos	15	334→316	3.12
	native	pos	15	331→313	0
(±)-11-Hydroxy-Δ9-THC	d_3	pos	25	334→any	can't determine
	native	pos	25	331→any	can't determine
(±)-11-nor-9-Carboxy-Δ9-THC	d_9	neg	30	352→254	0
	d_3	neg	30	346→248	0
	native	neg	30	343→245	0
(±)-11-nor-9-Carboxy-Δ9-THC	d_9	neg	20	352→334	0
	d_3	neg	20	346→328	0
	native	neg	20	343→325	0
(±)-11-nor-9-Carboxy-Δ9-THC	d_9	pos	20	354→336	51.91
	d_3	pos	20	348→330	0
	native	pos	20	345→327	0
(±)-11-nor-9-Carboxy-Δ9-THC	d_9	pos	20	354→308	48.88
	d_3	pos	20	348→302	0
	native	pos	20	345→299	0
Cannabinol	d_3	neg	30	316→248	0
	native	neg	30	313→245	0
Cannabinol	d_3	pos	20	318→262,196	0
	native	pos	20	315→259,193	0
JWH-018 4-Hydroxypentyl metabolite	d_5	pos	20	363→345	22.83
	native	pos	20	358→340	0
JWH-018 4-Hydroxypentyl metabolite	d_5	pos	20	363→155	0
	native	pos	20	358→155	0
JWH-073 3-Hydroxybutyl metabolite	d_5	pos	20	349→221	25.44
	native	pos	20	344→216	0
JWH-073 3-Hydroxybutyl metabolite	d_5	pos	20	363→155	0
	native	pos	20	358→155	0
3,4-MDPV HCl	d_8	pos	15	284→134	0
	native	pos	15	284→126	0
Ethylone HCl	d_5	pos	15	227→209	0
	native	pos	15	222→204	0
Butylone HCl	d_3	pos	15	225→209, etc	0
	native	pos	15	222→204, etc	0
Mephedrone HCl	d_3	pos	10	181→163	0
	native	pos	10	178→160	0
Methylone HCl	d_3	pos	10	211→163	0
	native	pos	10	208→160	0
Methylone HCl	d_3	pos	10	211→135	0
	native	pos	10	208→132	0
Everolimus	d_4	pos	60	984→393	1.80
	native	pos	60	980→389	0
Mycophenolic acid	d_3	neg	15	322→278	0
	native	neg	15	319→275	0

CONCLUSIONS 6

- Scrambling was observed for several of the analytes at select transitions. In all cases, scrambling was mitigated or eliminated by optimizing instrument conditions and transition selection.
- Awareness of potential scrambling is important for proper internal standard selection.
- Scrambling was observed on both the Agilent 6410 triple quadrupole and the Waters Xevo G2 Q-Tof to approximately the same degree. For a specific transition, scrambling ratios were consistent between solvent and serum. No matrix effects on scrambling.
- Direct infusion can provide rapid and accurate determination of scrambling ratios. Infusion and chromatographic injection results were consistent.
- Scrambling may be mitigated or eliminated by altering instrument conditions and transition selection. However, there is a need to consider potential impact of scrambling on transitions chosen for optimal sensitivity.
- Deuterium-labeled internal standards are a viable option for LC-MS/MS analysis with selection of the appropriate transition. Deuterated standards can be more cost effective than ^{13}C labeled internal standards, more widely available and with lower cost per test. ^{13}C labeled internal standards are most effective when deuterium scrambling issues can not be resolved.

Cerilliant® Analytical Reference Standards
a SIGMA-ALDRICH company