

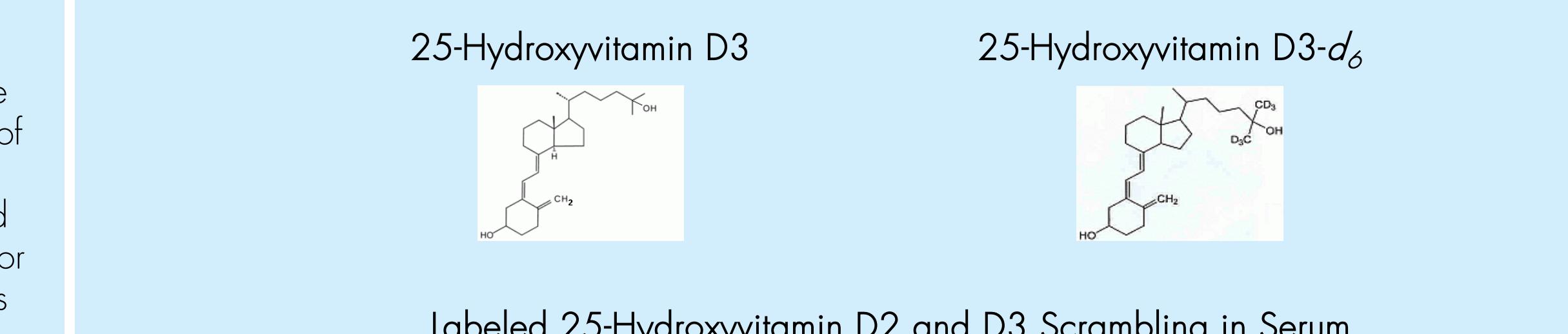
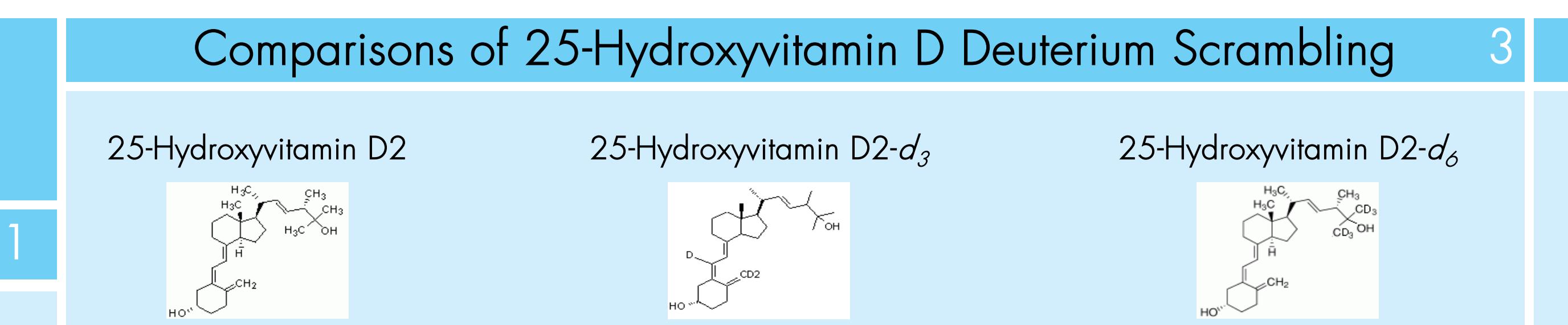
Evaluation of LCMSMS Deuterium Scrambling in Clinically Significant Small Molecules

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Introduction



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

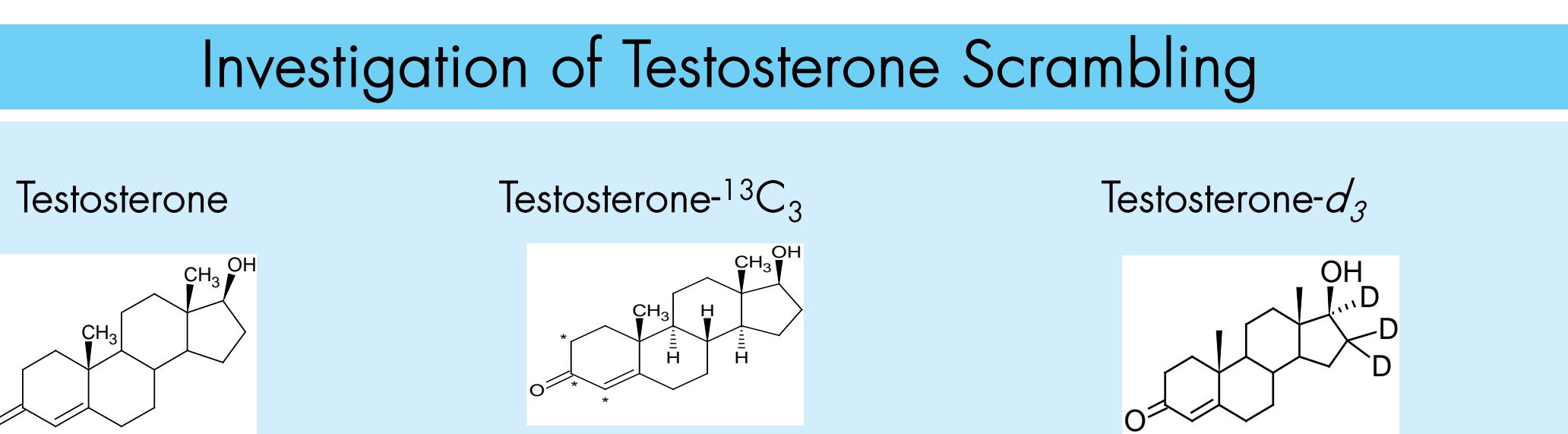
Parent → Water loss						
Compound	Label	Concentration $\mu\text{g/mL}$	Transition d_{n-1}	Transition d_n	Scrambling % d_{n-1}/d_n	
25-Hydroxyvitamin D2	d_3	100	416→397	416→398	2.9	
	d_6	100	419→400	419→401	2	
	native	50	413→394	413→395	0.5	
25-Hydroxyvitamin D3	d_6	50	407→388	407→389	4	
	native	100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

Parent → 2 Water losses						
Compound	Label	Concentration $\mu\text{g/mL}$	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 $\mu\text{g/mL}$	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_3	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.

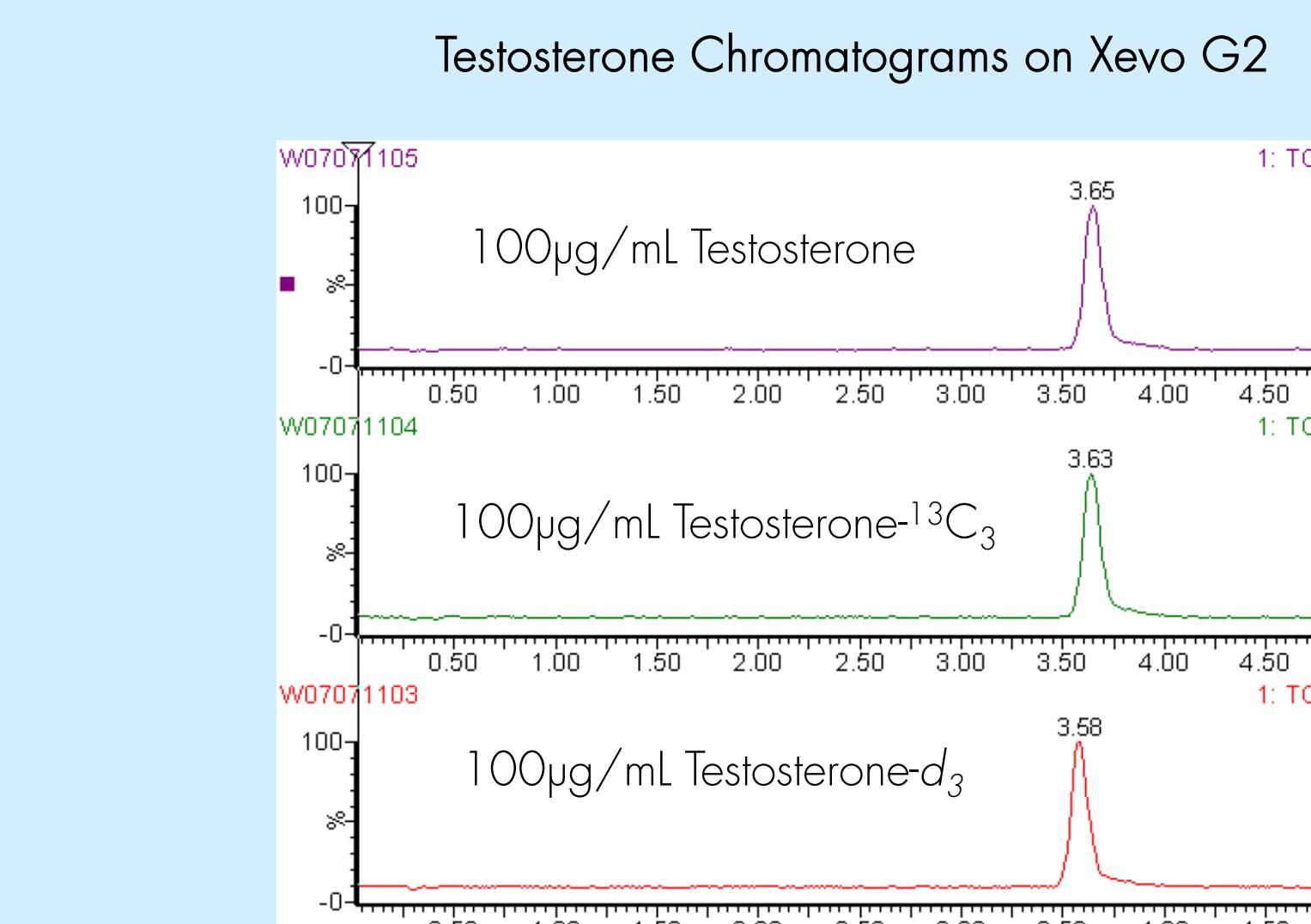
Selection of Transitions Greatly Impacts Observed Scrambling



Major transitions are:
Native: 289→97 & 289→109
Testosterone- d_3 : 292→97 & 292→109
Testosterone- $^{13}\text{C}_3$: 292→100 & 292→112
No scrambling at major transitions

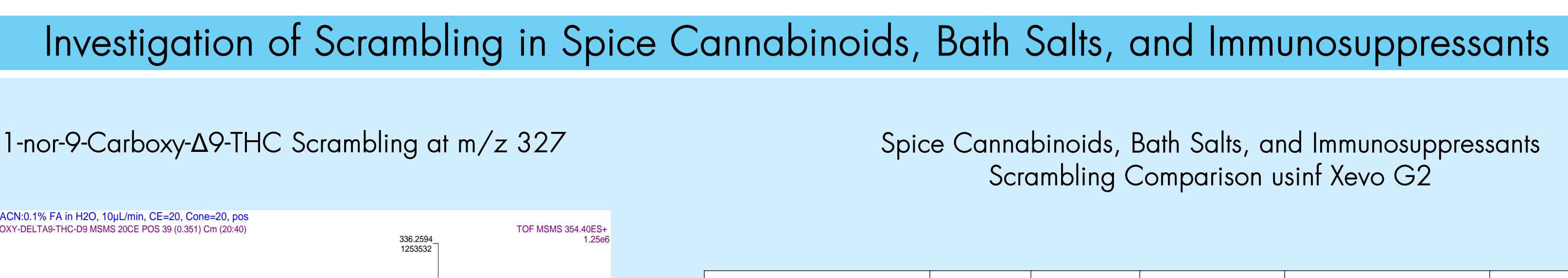
Testosterone Scrambling at Minor Transitions

label	Method	Instrument	Concentration $\mu\text{g/mL}$	Transitions D_{n-1} or $^{13}\text{C}_{n-1}$	Transitions D_n or $^{13}\text{C}_n$	*Scrambling % D_{n-1}/D_n
d_3	Q-ToF	Infusion	10			31.9
		100			36.5	
		10	292→255	292→256	35.7	
		100	292→255	292→256	37.7	
$^{13}\text{C}_3$	LC	10			36.3	
		100	289→252	289→253	0.1	
native		100	289→252	289→253	0.0	



CONCLUSIONS

- 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.



Spice Cannabinoids, Bath Salts, and Immunosuppressants Scrambling Comparison using 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
	native	neg	30	346→248	0
(±)-11-nor-9-Carboxy-Δ9-THC	d_3	neg	30	343→245	0
	native	neg	30	343→325	0
(±)-11-nor-9-Carboxy-Δ9-THC	d_9	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
	native	pos	20	348→302	0
Cannabinol	d_3	pos	20	345→299	0
	native	neg	30	316→248	0
Cannabinol	d_3	pos	20	318→262, 196	0
	native	pos	20	315→259, 193	0
JWH018 4-Hydroxypentyl metabolite	d_5	pos	20	363→345	22.83
	native	pos	20	358→340	0
JWH018 4-Hydroxypentyl metabolite	d_5	pos	20	363→155	0
	native	pos	20	358→155	0
JWH073 3-Hydroxybutyl metabolite	d_5	pos	20	344→216	0
	native	pos	20	344→155	0
3,4-MDPV HCl	d_8	pos	15	284→134	0
	native	pos	15	284→126	0
Ethylene 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
Methyline HCl	d_3	pos	10	211→163	0
	native	pos	10	208→160	0
Methyline 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

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