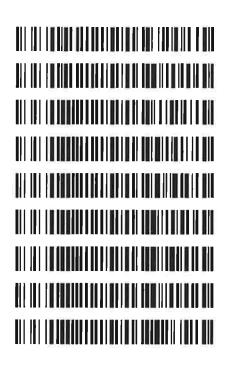
REVIEWED
By Celena Shrum at 2:32 pm, Feb 28, 2019

Worklist: 2906

LAB CASE	ITEM	TASK ID	DESCRIPTION
M2018-6344	1	138135	AM 27 Blood THC Quant by LC-QQQ
M2019-0057	2	138136	AM 27 Blood THC Quant by LC-QQQ
P2018-3370	1	138137	AM 27 Blood THC Quant by LC-QQQ
P2019-0111	1	138138	AM 27 Blood THC Quant by LC-QQQ
P2019-0127	1	138139	AM 27 Blood THC Quant by LC-QQQ
P2019-0133	1	138140	AM 27 Blood THC Quant by LC-QQQ
P2019-0153	1	138141	AM 27 Blood THC Quant by LC-QQQ
P2019-0154	1	138142	AM 27 Blood THC Quant by LC-QQQ
P2019-0195	1	138143	AM 27 Blood THC Quant by LC-QQQ

REVIEWED

By Sarah Pickle at 4:09 pm, Jan 30, 2019



1/28/2019

AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 01/29/19 Plate lot#: 0539904 Analyst: <u>Tamara Salazar</u> Plate Expiration: 09/10/19

Mobile phase A:0.1% Formic Acid in LCMS Water
LCMS MethanolMTBELCMS MethanolBlank Blood Lot:Hemostat 445283-1LCMS-QQQ ID:59740

Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane Column: UCT Selectra DA 100 x 2.1mm 3um

Pre-Analytic:

- ☑ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- \boxtimes 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.
- 3. Create worklist: Data Path: <u>C:\MassHunter\Worklists\012918 THCQ TS.wkl</u>

Analytic:

- ☑ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette 1000μL blood/urine (calibrated pipette) Pipette ID: 27 in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. Shaker ID: 067105
- 4. Pipette 500μL 0.1% formic acid in water in wells of analytical plate for blood samples.
- \boxtimes 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- ⊠ 6. Transfer 800µL of blood+acid mixture to corresponding wells of SLE+ plate.
- Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
 (Load at 85-100 PSI- Selector to the right) Manifold ID: 067104
- \boxtimes 8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- \boxtimes 10. Wait 5 minutes.
- ☑ 11. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ☑ 12. Add 2.25mL Hexane. (Add in 3 increments of 750uL)
- \boxtimes 13. Wait 5 minutes.
- ☑ 14. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ☑ 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID: 067103
- ⊠ 16. Reconstitute in 100µL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

 \boxtimes 1. Create batch and process data.

Worklist path: <u>C:\MassHunter\Data\2019\AM 27\012919 THCQ TS</u> Batch Name: THCQ 012919 TS

- \boxtimes 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r² values ≥ 0.98 for each analyte
- ☑ 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less). Ion ratios must be within +/- 20% of the averaged calibrators
- ☑ 4. Case sample response for THC and OH-THC 3ng/mL (quantitative), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- \boxtimes 5. Did all QCs pass for each analyte? Y / N
- \boxtimes 6 Enter QCs into control charting.
- 2 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Curves limited: THC-COOH 10-100



Idaho State Police Forensic Services

AM #27 Quantitative Analysis of THC and Metabolites in Blood by LCMS-QQQ

Component	Source	Source Lot Number	Expiration Date		
Methanol (LCMS)	Fisher	177145			
THC	Cerilliant	FE04231406	04/30/2019		
C-THC	Cayman	0497429	02/08/2019		
THC-OH	Cerilliant	FE01121503	01/31/2020		
Prepared:	10/24/18	10/24/18			
Prepared By:	Tamara Salaz	Tamara Salazar			
Expires:	02/08/19				

Methanol External Control Solution (Lot: WS102418) 10 ul of 1mg/mL THC, 100 ul of 100 ug/mL THC-OH, C-THC in 9790 ul MeOH

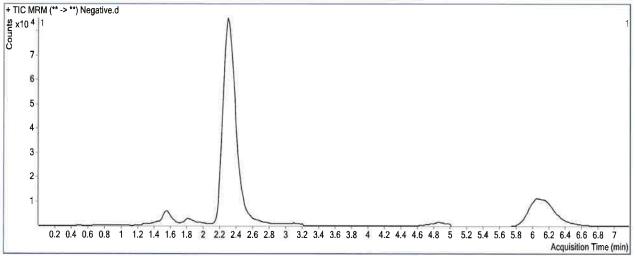
Blood External Control Solution (Lot: 102418)

100 ul of methanol external control solution was added to 9900 ul of blood. Approximately 10ng/mL of each compound.

Component	Source	Source Lot Number
Negative Blood	Lampire	18G207D7
Methanol External Control Solution		WS102418
Prepared:	10/24/18	
Prepared by:	Sarah Pickle	
Expires:	02/08/19	



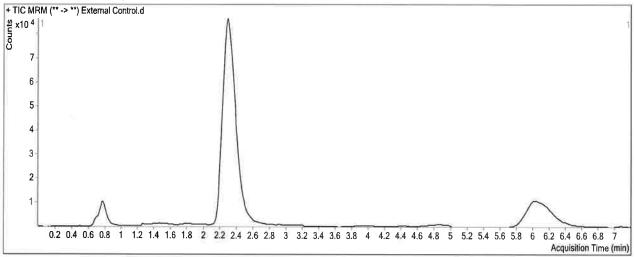
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Last Calib Update	1/30/2019 1:46 PM	Batch State	Processed
Analysis Info Acq Time	2019-01-29 15:17	Data File	Negative.d
Sample Type	Sample	Sample Name	Negative
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-A2	Sample Info	-
Inj Vol	-1	Comment	Hemostat 445283-1



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.519	3873	676219	0.0057	1.1371 🟒 3 🏾 🧖
THC-COOH	THC-COOH-D9	2.259	13810	238449	0.0579	1.9266 <10 TS

Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\20 1/30/2019 1:46 PM 1/30/2019 1:48 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 15:40	Data File	External Control.d
Sample Type	Sample	Sample Name	External Control
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-B2	Sample Info	
Inj Vol	-1	Comment	Lampire 18G207D7 + WS 102418

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.292	52674	599766	0.0878	8.4557
THC-COOH	THC-COOH-D9	2.392	35771	233040	0.1535	7.1813
THC	THC-D3	6.052	16699	224612	0.0743	9.0789



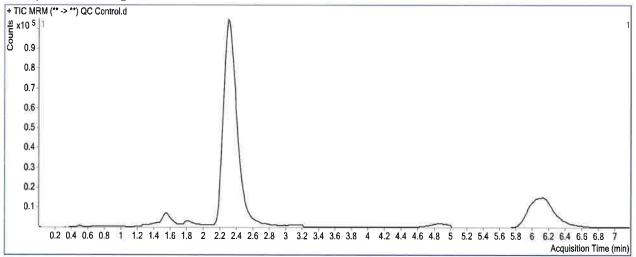
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Analysis Time	1/30/2019 1:46 PM	Analyst Name	datastor
Report Time	1/30/2019 1:47 PM	Reporter Name	datastor
Last Calib Update	1/30/2019 1:46 PM	Batch State	Processed
Analysis Info			
Acq Time	2019-01-29 14:53	Data File	QC Control.d
Sample Type	Sample	Sample Name	QC Control
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-H1	Sample Info	

Comment

Sample Chromatogram

-1

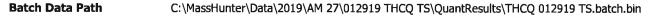
Inj Vol



Resul	ts
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Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.305	36332	784356	0.0463	4.7559
THC-COOH	THC-COOH-D9	2.406	55937	273091	0.2048	10.0031
THC	THC-D3	6.119	12259	326930	0.0375	4.8407

ISP Forensics **Calibration Curve Report**

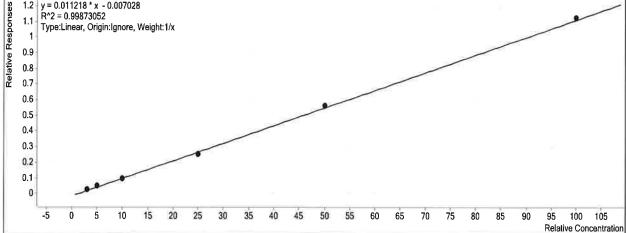


Last Calib Update

Analyst Name ISP TOX

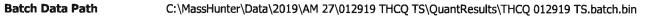
Target Compound THC-OH Internal Standard THC-OH-D3 THC-OH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 0 QCs 1.2 y = 0.011218 * x - 0.007028 1 1 R^2 = 0.99873052 1.1 Type:Linear, Origin:Ignore, Weight:1/x 1

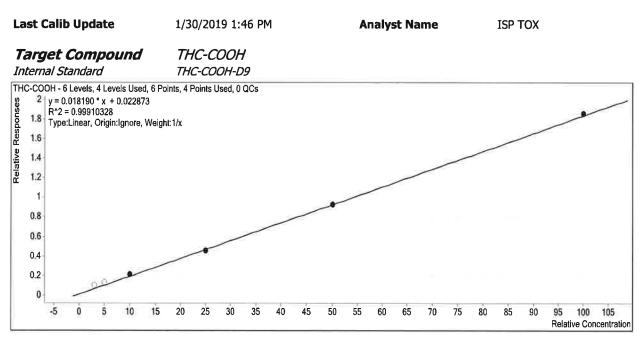
1/30/2019 1:46 PM



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1-3ng	1	A	3	3.1	105.0
Cal 2-5ng	2	$\mathbf{\nabla}$	5	5.2	103.8
Cal 3-10ng	3	\square	10	9.5	95.5
Cal 4-25ng	4	\square	25	23.3	93.0
Cal 5-50ng	5	V	50	50.8	101.7
Cal 6-100ng	6	Y	100	101.0	101.0

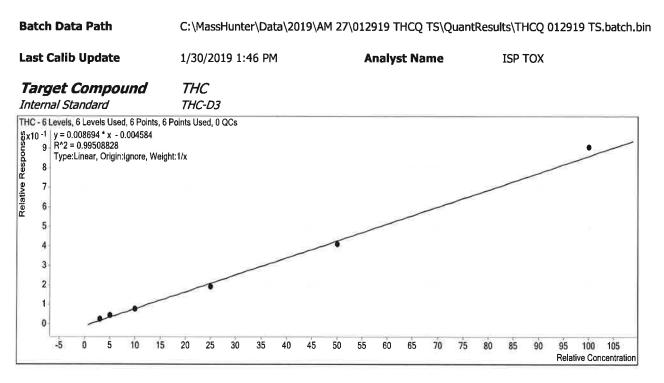
ISP Forensics Calibration Curve Report





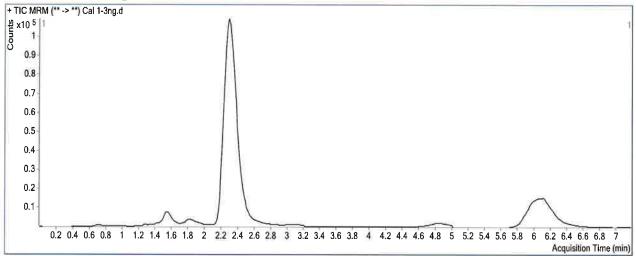
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1-3ng	1		3	4.4	145.0
Cal 2-5ng	2		5	6.0	120.0
Cal 3-10ng	3	Ø	10	10.4	104.1
Cal 4-25ng	4	\checkmark	25	23.8	95.2
Cal 5-50ng	5	Ŋ	50	49.9	99.8
Cal 6-100ng	6	Ø	100	100.9	100.9

ISP Forensics Calibration Curve Report



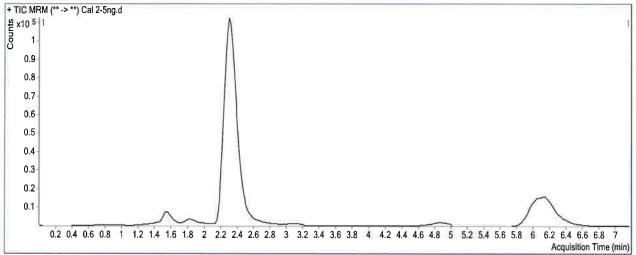
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1-3ng	1	\mathbf{N}	3	3.3	109.7
Cal 2-5ng	2	$\mathbf{\overline{A}}$	5	5.4	107.3
Cal 3-10ng	3	Ø	10	9.3	92.7
Cal 4-25ng	4	Ø	25	22.5	90.1
Cal 5-50ng	5	M	50	47.7	95.4
Cal 6-100ng	6	$\mathbf{\nabla}$	100	104.9	104.9

Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\2 1/30/2019 1:46 PM 1/30/2019 1:47 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 13:30	Data File	Cal 1-3ng.d
Sample Type	Calibration	Sample Name	Cal 1-3ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-B1	Sample Info	
Inj Vol	-1	Comment	



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.292	23899	844537	0.0283	3.1493
THC-COOH	THC-COOH-D9	2.392	28708	281411	0.1020	4.3509
THC	THC-D3	6.119	8054	335285	0.0240	3.2905

Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\20 1/30/2019 1:46 PM 1/30/2019 1:47 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 13:42	Data File	Cal 2-5ng.d
Sample Type	Calibration	Sample Name	Cal 2-5ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-C1	Sample Info	
Inj Vol	-1	Comment	



Results

Compound 🖻	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.305	43297	845572	0.0512	5.1912
THC-COOH	THC-COOH-D9	2.406	38775	293724	0.1320	6.0000
THC	THC-D3	6.132	13538	321891	0.0421	5.3650

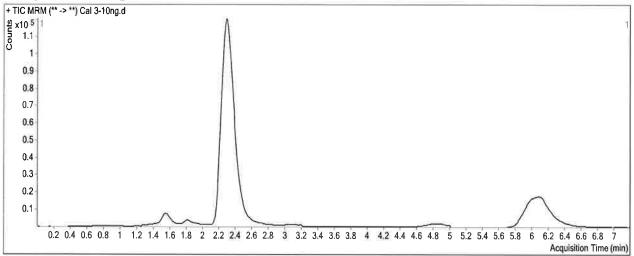
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Analysis Time	1/30/2019 1:46 PM	Analyst Name	datastor
Report Time	1/30/2019 1:47 PM	Reporter Name	datastor
Last Calib Update	1/30/2019 1:46 PM	Batch State	Processed
Analysis Info			
Acq Time	2019-01-29 13:54	Data File	Cal 3-10ng.d
Sample Type	Calibration	Sample Name	Cal 3-10ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-D1	Sample Info	

Comment

Sample Chromatogram

-1

Inj Vol

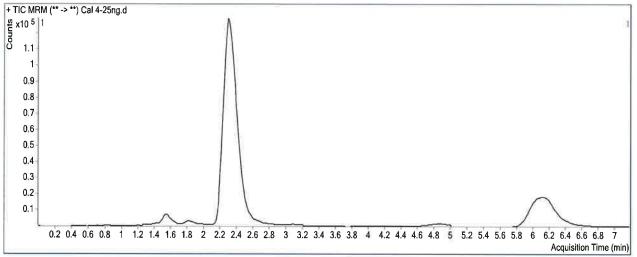


Resu	lts
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Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.292	86409	863425	0.1001	9.5480
THC-COOH	THC-COOH-D9	2.392	62718	295565	0.2122	10.4082
THC	THC-D3	6.106	26841	353125	0.0760	9.2706

Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\20 1/30/2019 1:46 PM 1/30/2019 1:47 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 14:06	Data File	Cal 4-25ng.d
Sample Type	Calibration	Sample Name	Cal 4-25ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-E1	Sample Info	
Inj Vol	-1	Comment	

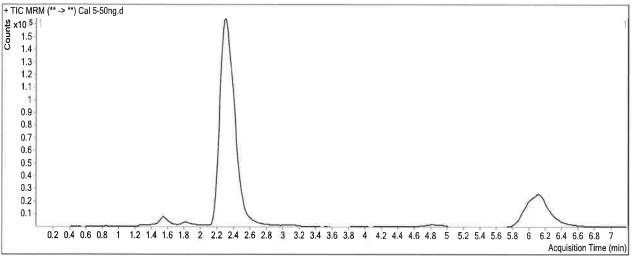
Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.305	204772	806487	0.2539	23.2612
THC-COOH	THC-COOH-D9	2.406	124769	273678	0.4559	23.8060
THC	THC-D3	6.146	62885	328904	0.1912	22.5200

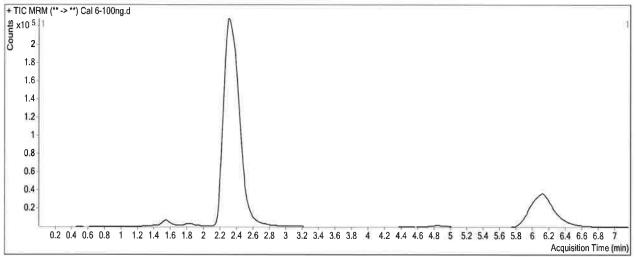
Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\2 1/30/2019 1:46 PM 1/30/2019 1:47 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 14:18	Data File	Cal 5-50ng.d
Sample Type	Calibration	Sample Name	Cal 5-50ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-F1	Sample Info	
Inj Vol	-1	Comment	



Result	S
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THC-OH THC-OH-D3 2,292 45	58621 814417 0.5631 50.8269
THC-COOH THC-COOH-D9 2.392 25	58503 277755 0.9307 49.9080
THC THC-D3 6.119 13	30733 318947 0.4099 47.6758

Batch Data Path Analysis Time Report Time Last Calib Update	C:\MassHunter\Data\2 1/30/2019 1:46 PM 1/30/2019 1:47 PM 1/30/2019 1:46 PM	019\AM 27\012919 T Analyst Name Reporter Name Batch State	HCQ TS\QuantResults\THCQ 012919 TS.batch.bin datastor datastor Processed
Analysis Info			
Acq Time	2019-01-29 14:29	Data File	Cal 6-100ng.d
Sample Type	Calibration	Sample Name	Cal 6-100ng
Dilution	1	Acq Method	THC Quant 051517 workingmm.m
Position	P1-G1	Sample Info	
Inj Vol	-1	Comment	



Results	5
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Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
ТНС-ОН	THC-OH-D3	2.305	947200	841049	1.1262	101.0235
THC-COOH	THC-COOH-D9	2.406	518828	279268	1.8578	100.8778
ТНС	THC-D3	6.119	292729	322678	0.9072	104.8781