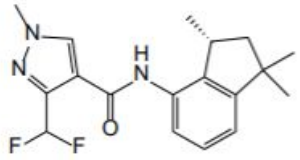
IR-4 Project | NATIONAL EDUCATION CONFERENCE February 3-5, 2026

Inpyrfluxam Coffee Method Development

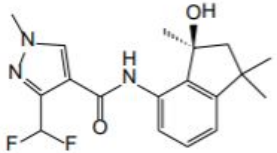
Joel McCray

Inpyrfluxam Background

Mix A

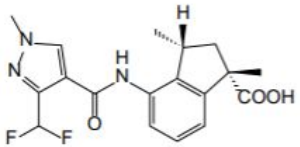


S-2399 (MW = 333.4)

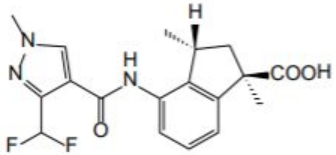


3'-OH-S-2840 (MW = 349.4)

Mix B

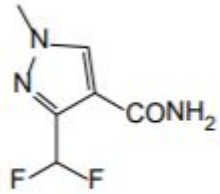


1'-COOH-S-2840-A (MW = 363.4)

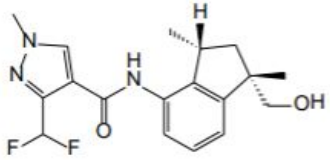


1'-COOH-S-2840-B (MW = 363.4)

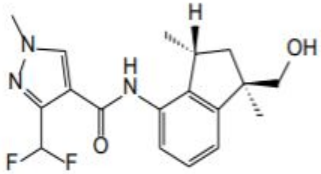
DFPA



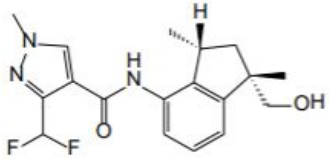
DFPA-CONH$_2$ (MW = 175.1)



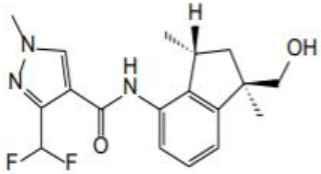
1'-CH$_2$OH-S-2840-A (MW = 349.4)



1'-CH$_2$OH-S-2840-B (MW = 349.4)

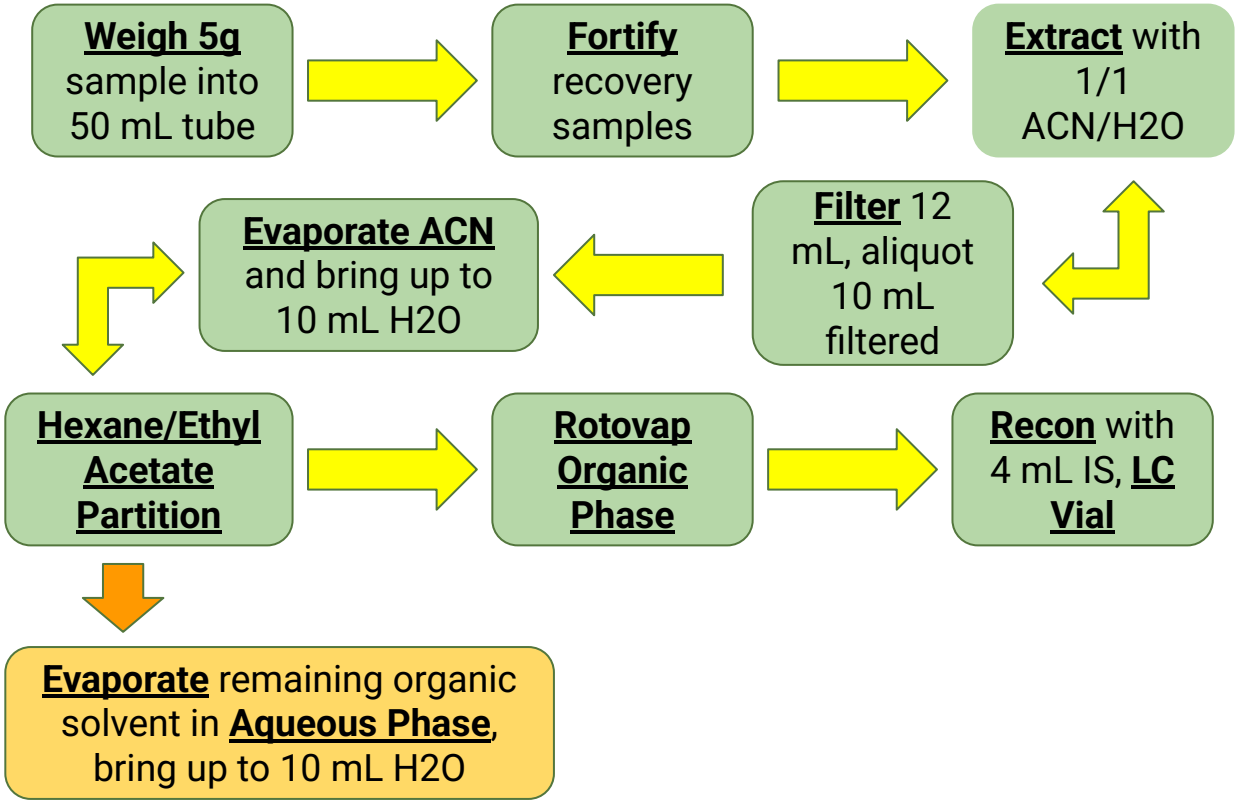


1'-CH$_2$OH-S-2840-A (MW = 349.4)

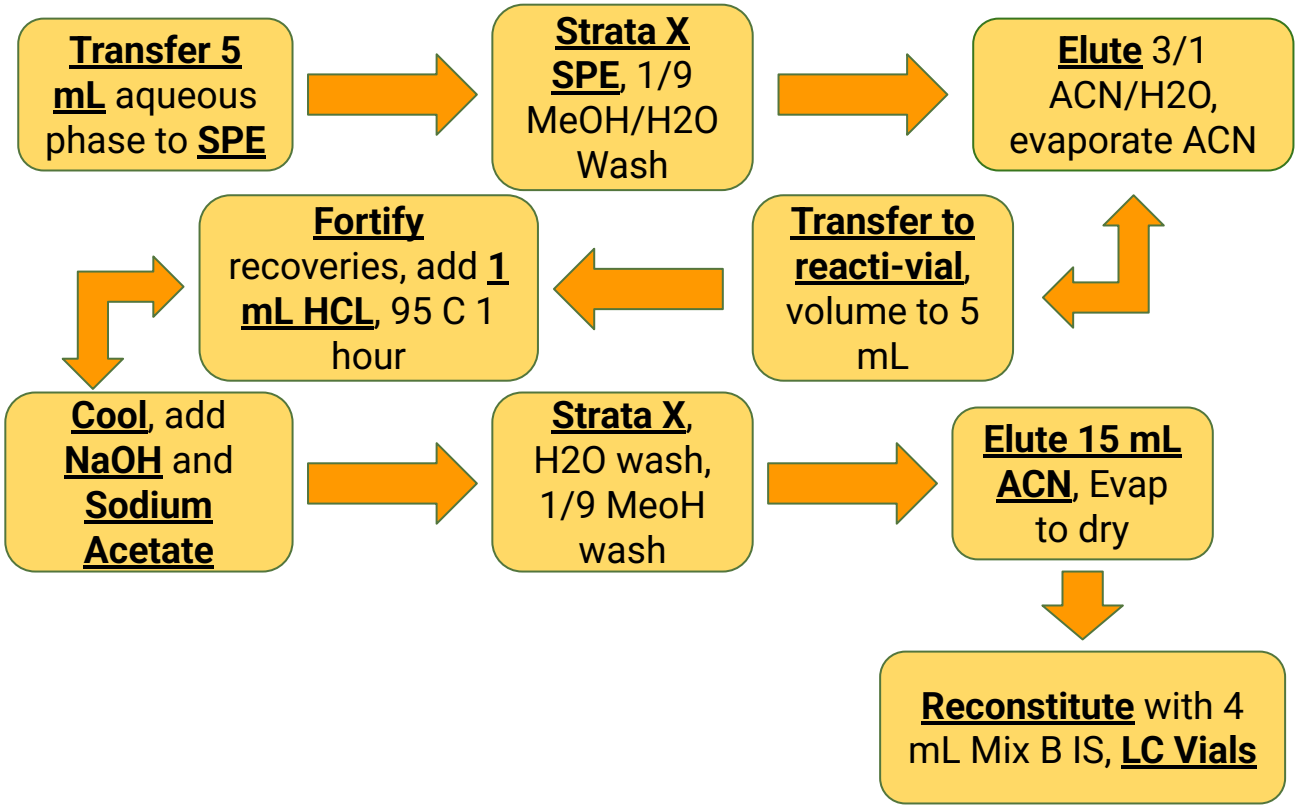


1'-CH$_2$OH-S-2840-B (MW = 349.4)

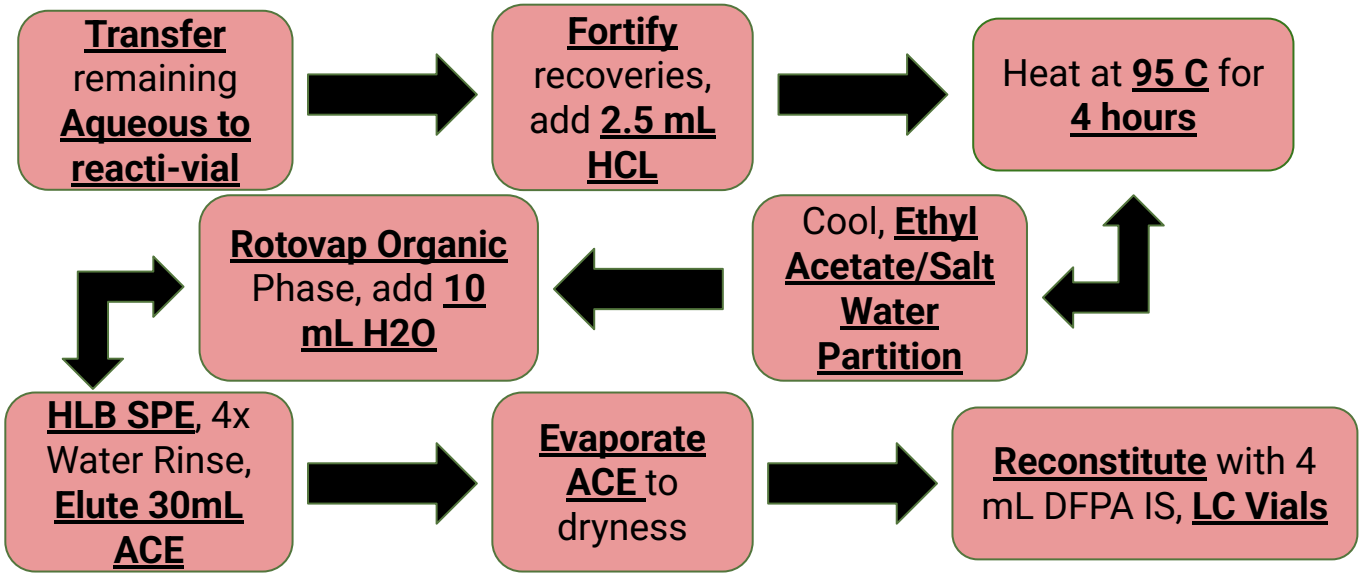
Mix A Extraction



Mix B Extraction



DFPA Extraction



Green Bean Passed Mix A Method Validation

Calculations **S-2399 (Inpyrfluxam)**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024035 M	----	----	<0.01	----
Recovery				
2024035 N	2.20	0.0440	0.00880	88
2024035 O	2.21	0.0443	0.00886	89
2024035 P	2.22	0.0443	0.00887	89
2024035 Q	21.5	0.430	0.0860	86
2024035 R	21.4	0.429	0.0858	86
2024035 S	21.7	0.433	0.0867	87
2024035 T	215	4.30	0.861	86
2024035 U	226	4.51	0.902	90
2024035 V	218	4.36	0.872	87

Sample ID Lab # - Rep.	Peak Area % Difference
2024035 N	-0.17
2024035 O	-0.47
2024035 P	-0.36
2024035 Q	-0.50
2024035 R	0.21
2024035 S	-1.07
2024035 T	-0.23
2024035 U	-0.35
2024035 V	0.05

IR-4 Project

Green Bean Passed Mix B Method Validation

Calculations **COOH-A**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024035 M	----	----	<0.01	----
Recovery				
2024035 N	1.21	0.00483	0.00966	97
2024035 O	1.21	0.00485	0.00970	97
2024035 P	1.23	0.00493	0.00985	99
2024035 Q	12.1	0.0483	0.0966	97
2024035 R	12.3	0.0491	0.0983	98
2024035 S	12.2	0.0488	0.0977	98
2024035 T	122	0.487	0.974	97
2024035 U	126	0.505	1.01	101
2024035 V	125	0.501	1.00	100

Sample ID Lab # - Rep.	Peak Area % Difference
2024035 N	0.89
2024035 O	3.17
2024035 P	-1.81
2024035 Q	-0.61
2024035 R	-1.16
2024035 S	-0.44
2024035 T	0.09
2024035 U	0.02
2024035 V	-0.78

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Green Bean Passed DFPA Method Validation

DFPA

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024035 M	----	----	<0.01	----
Recovery				
2024035 N	2.46	0.00985	0.0197	98
2024035 O	2.17	0.00868	0.0174	87
2024035 P	2.20	0.00881	0.0176	88
2024035 Q	11.4	0.0455	0.0909	91
2024035 R	11.2	0.0447	0.0895	89
2024035 S	11.5	0.0460	0.0920	92
2024035 T	106	0.423	0.845	85
2024035 U	126	0.506	1.01	101
2024035 V	138	0.552	1.10	110

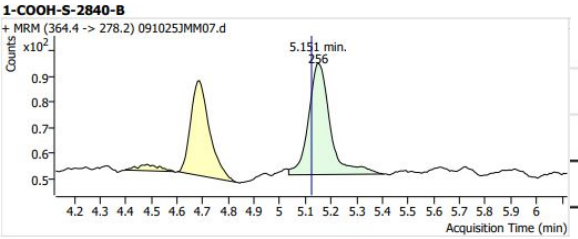
Sample ID Lab # - Rep.	Peak Area % Difference
2024035 N	-10.29
2024035 O	-0.41
2024035 P	1.43
2024035 Q	-2.87
2024035 R	1.27
2024035 S	-0.75
2024035 T	4.28
2024035 U	-4.19
2024035 V	0.06

IR-4 Project

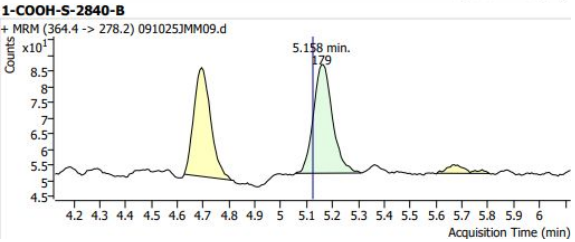
Problems with Roasted Bean/Freeze Dried

COOH compounds inconsistent in both matrices

Roasted Bean

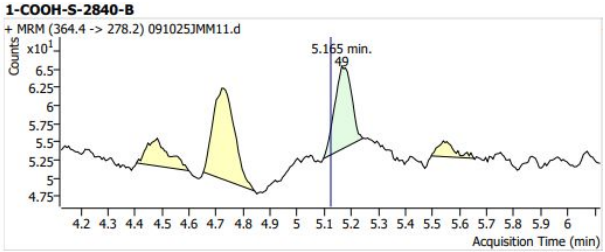


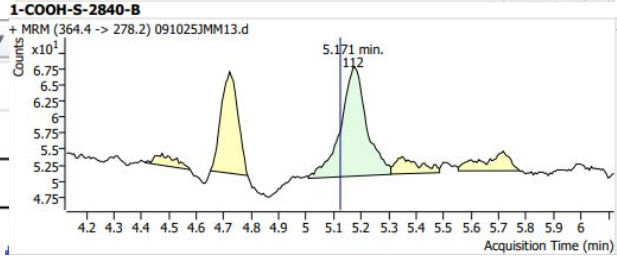
Sample	Peak
ID	Area
Lab # - Rep.	% Difference
2024042G	29.50
2024042H	-0.79



Sample	Peak
ID	Area
Lab # - Rep.	% Difference
2024044G	-76.08
2024044H	-4.79

Freeze Dried





Problems with Roasted Bean/Freeze Dried

DFPA Recoveries inconsistent or below minimum threshold

Roasted Bean

Calculations:

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024042F	----	----	<0.01	----
Recovery				
2024042G	1.85	0.0074	0.0148	74
2024042H	12.05	0.0482	0.0964	96

Freeze Dried

Calculations:

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044F	----	----	<0.01	----
Recovery				
2024044G	1.75	0.00699	0.0140	70
2024044H	7.27	0.0291	0.0582	58

Mix B NH2 SPE Testing

> Mix B Extraction performed as normal until 2nd round of SPE, **Strata X replaced with NH2 500 mg 6 mL cartridge**, rinse with H2O and elute with MeOH

Roasted Bean COOH-A

Calculations Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024042F	----	----	<0.01	----
Recovery				
2024042G	0.0531	0.000212	0.000424	4
2024042H	0.400	0.00160	0.00320	3

Freeze Dried COOH-A

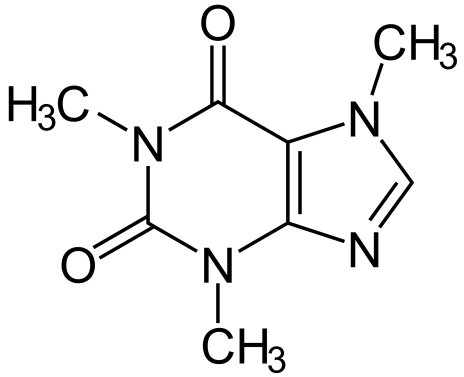
Calculations Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044F	----	----	<0.01	----
Recovery				
2024044G	0.05	0.00021	0.00042	4
2024044H	0.7	0.0028	0.0056	6

> The analysis revealed that **NH2 would significantly worsen the recovery** of Mix B analytes

Problematic compound in Coffee?

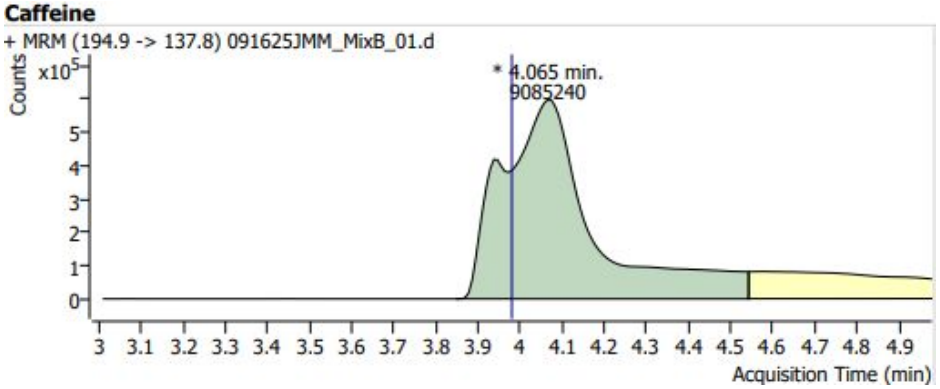
Caffeine

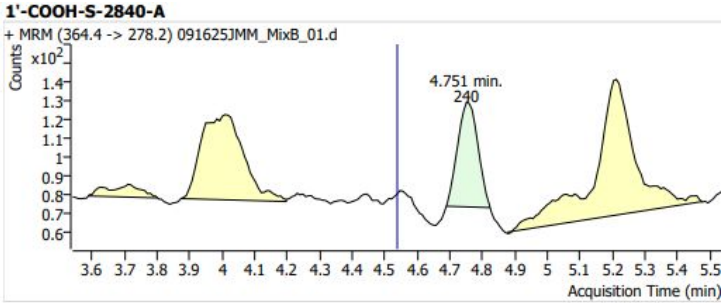
Precursor Ion: 194.9 m/z
Product Ion: 137.8 m/z

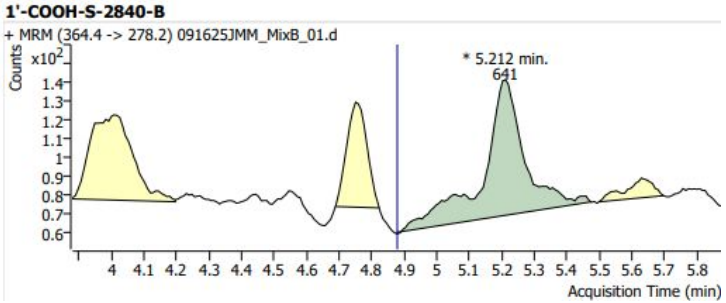


Caffeine Test Mix B

- Caffeine was found to elute with a large peak, which trailed off and eluted with COOH-A and COOH-B
- This same phenomenon occurred in DFPA samples as well



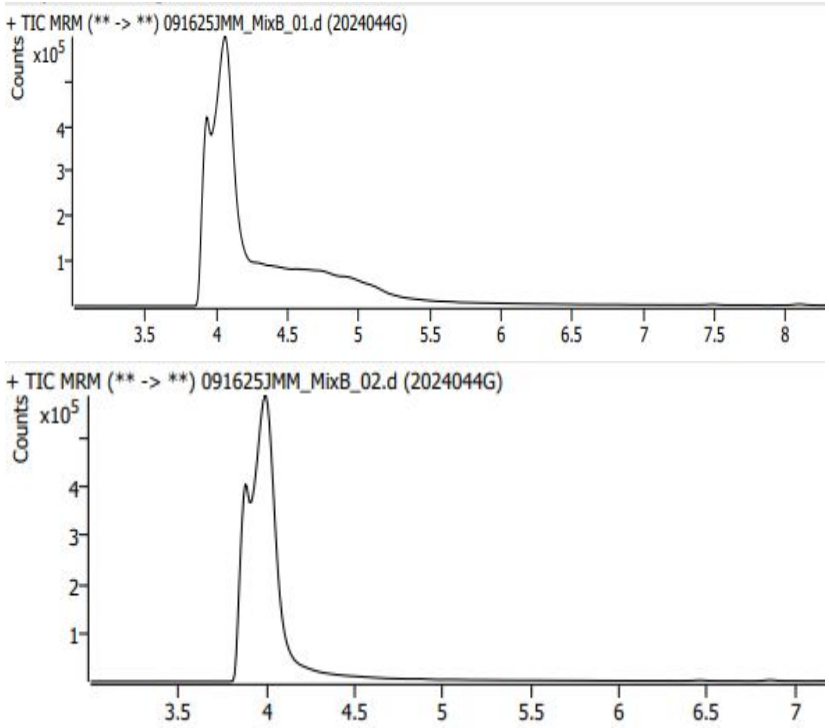




Chromatography Testing Mix B Roasted

Time	A%	B%
0	55	45
0.1	55	45
8	20	80
10	20	80
10.1	5	95
15.5	5	95
15.6	55	45
18.6	55	45

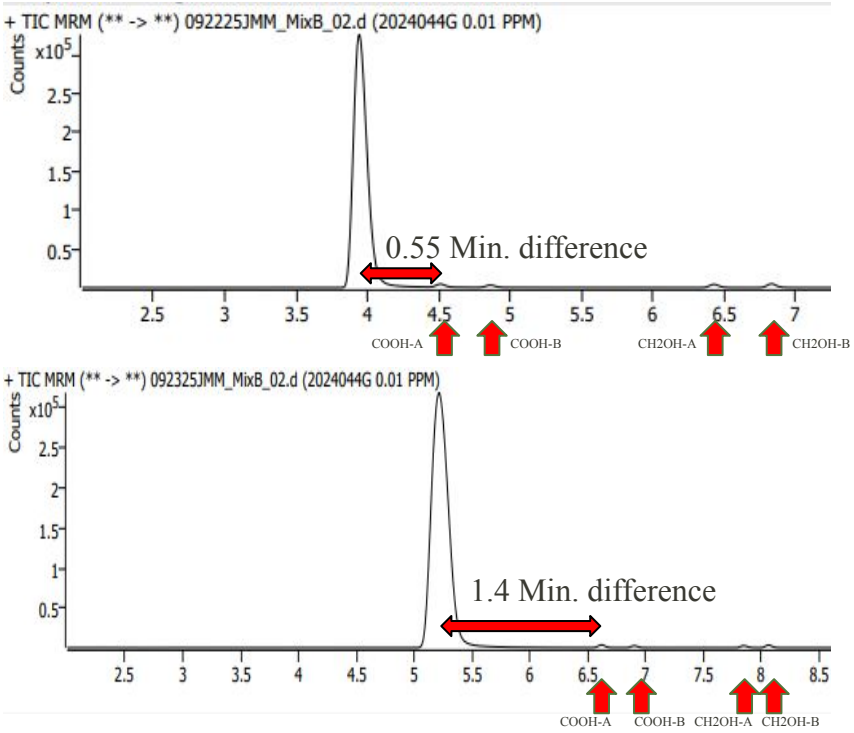
Time	A%	B%
0	55	45
0.1	55	45
5	20	80
10	20	80
10.1	5	95
15.5	5	95
15.6	55	45
18.6	55	45



Chromatography Testing Mix B Roasted

Time	A%	B%
0	55	45
0.1	55	45
5	20	80
10	20	80
10.1	5	95
15.5	5	95
15.6	55	45
18.6	55	45

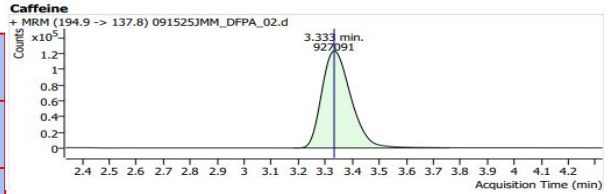
Time	A%	B%
0	65	35
2	65	35
5	20	80
10	20	80
10.1	5	95
15.5	5	95
15.6	65	35
18.6	65	35

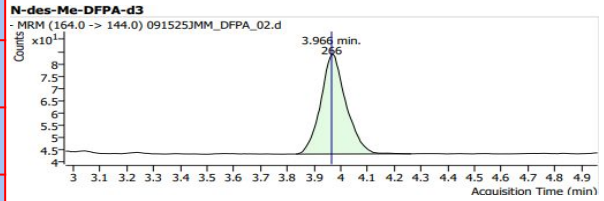


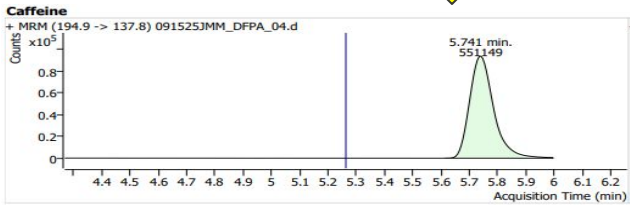
Chromatography Testing DFPA Roasted

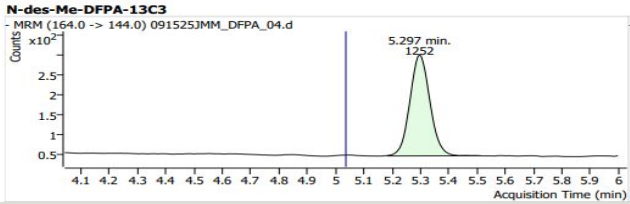
Time	A%	B%
0	80	20
3	80	20
4	5	95
15	5	95
15.1	80	20
80	80	20

Time	A%	B%
0	90	10
1	90	10
3	80	20
4	80	20
4.1	65	35
6	65	35
6.1	5	95
15.5	5	95
15.1	90	10
21	90	10









Modifications to Extraction Method

- **Samples were centrifuged at 5000 RPM for a minimum of 2 minutes to separate layers** during hexane ethyl acetate partition **instead of allowing the samples to separate themselves**
- **Samples were centrifuged at 5000 RPM for 3 minutes before** transferring 5 mL for the **first round of Mix B SPE cleanup** instead of transferring directly after adjusting volume to 10 mL with water.

Roasted Bean Passed Mix B Method Validation

COOH-A

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024042 I	----	----	<0.01	----
Recovery				
2024042J	0.994	0.00397	0.00795	79
2024042K	1.31	0.00523	0.0105	105
2024042L	1.10	0.00439	0.00879	88
2024042M	11.2	0.0446	0.0893	89
2024042N	11.2	0.0447	0.0894	89
2024042O	11.5	0.0461	0.0923	92
2024042P	118	0.471	0.943	94
2024042Q	116	0.463	0.926	93
2024042R	115	0.460	0.920	92

Sample ID Lab # - Rep.	Peak Area % Difference
2024042J	10.47
2024042K	18.76
2024042L	-21.23
2024042M	5.38
2024042N	-2.54
2024042O	9.14
2024042P	1.28
2024042Q	1.21
2024042R	0.29

Roasted Bean Passed DFPA Method Validation

DFPA

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024042I	----	----	<0.01	----
Recovery				
2024042J	2.16	0.00865	0.0173	86
2024042K	2.10	0.00840	0.0168	84
2024042L	2.01	0.00805	0.0161	80
2024042M	9.84	0.0394	0.0787	79
2024042N	10.0	0.0400	0.0799	80
2024042O	10.4	0.0418	0.0836	84
2024042P	101	0.406	0.812	81
2024042Q	107	0.429	0.857	86
2024042R	112	0.449	0.898	90

Sample ID Lab # - Rep.	Peak Area % Difference
2024042J	-6.66
2024042K	-4.09
2024042L	-4.29
2024042M	-8.89
2024042N	-7.16
2024042O	1.53
2024042P	4.16
2024042Q	0.92
2024042R	-4.72

Freeze Dried Failed Mix B Method Validation

COOH-A

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044J	0.694	0.00278	0.00555	56
2024044K	1.087	0.00435	0.00869	87
2024044L	0.557	0.00223	0.00446	45
2024044M	9.17	0.0367	0.0734	73
2024044N	10.0	0.0399	0.0798	80
2024044O	10.1	0.0404	0.0808	81
2024044P	103	0.413	0.826	83
2024044Q	101	0.403	0.807	81
2024044R	88.5	0.354	0.708	71

Sample ID Lab # - Rep.	Peak Area % Difference
2024044J	7.06
2024044K	0.00
2024044L	6.15
2024044M	-2.16
2024044N	1.82
2024044O	-0.54
2024044P	-3.01
2024044Q	-0.47
2024044R	-0.78

Freeze Dried Failed DFPA Method Validation

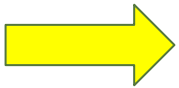
Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044J	1.71	0.00682	0.01365	68
2024044K	1.56	0.00622	0.01245	62
2024044L	2.10	0.00842	0.01683	84
2024044M	10.3	0.0411	0.0821	82
2024044N	8.26	0.0331	0.0661	66
2024044O	10.9	0.0435	0.0870	87
2024044P	101	0.405	0.811	81
2024044Q	97.8	0.391	0.782	78
2024044R	103	0.411	0.822	82

Sample ID Lab # - Rep.	Peak Area % Difference
2024044J	4.24
2024044K	-3.89
2024044L	-1.13
2024044M	4.55
2024044N	-3.46
2024044O	-1.52
2024044P	-1.32
2024044Q	1.60
2024044R	-1.94

Chromatography Testing Mix B Frz Dry

Time	A%	B%
0	65	35
2	65	35
5	20	80
10	20	80
10.1	5	95
15.5	5	95
15.6	65	35
18.6	65	35



Time	A%	B%
0	65	35
3.5	65	35
5	25	75
10	25	75
10.1	5	95
18.5	5	95
18.6	65	35
21.6	65	35

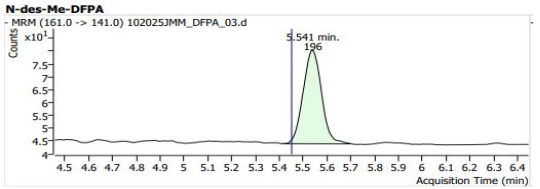
Name	RT	Resp.	ISTD	ISTD RT	ISTD Resp.	RR
Caffeine	~5.25					
1'-COOH-S-2840-A	6.624	120	1'-COOH-S-2840-A-d3	6.616	1067	0.1126
1'-COOH-S-2840-B	6.911	123	1'-COOH-S-2840-B-d3	6.902	762	0.1617
1'-CH2OH-S-2840-A	7.869	250	1'-CH2OH-S-2840-A-d3	7.860	1119	0.2232
1'-CH2OH-S-2840-B	8.080	290	1'-CH2OH-S-2840-B-d3	8.072	1410	0.2055

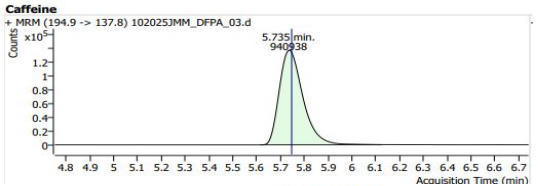
Name	RT	Resp.	ISTD	ISTD RT	ISTD Resp.	RR
Caffeine	5.001	15542185				
1'-COOH-S-2840-A	7.478	246	1'-COOH-S-2840-A-d3	7.476	1768	0.1390
1'-COOH-S-2840-B	7.776	214	1'-COOH-S-2840-B-d3	7.766	1156	0.1855
1'-CH2OH-S-2840-A	8.513	661	1'-CH2OH-S-2840-A-d3	8.511	2682	0.2466
1'-CH2OH-S-2840-B	8.760	721	1'-CH2OH-S-2840-B-d3	8.750	3668	0.1966

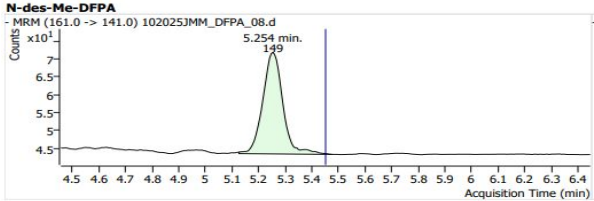
Chromatography Testing DFPA Frz Dry

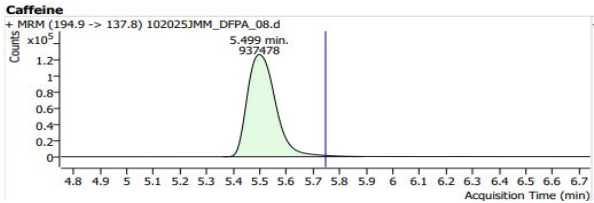
Time	A%	B%
0	90	10
1	90	10
3	80	20
4	80	20
4.1	65	35
6	65	35
6.1	5	95
15.5	5	95
15.1	90	10
21	90	10

Time	A%	B%
0	90	10
1	90	10
3.5	80	20
5	80	20
5.1	45	55
7	45	55
7.1	5	95
16.5	5	95
16.1	90	10
21	90	10









Mix B SPE Rinse Testing

- Original: Rinse with 1/9 MeOH/Water **(044J)**
- Testing: 2/9 **(044K)**
- Testing:1/3 MeOH/Water **(044L)**

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044J	1.272	0.00509	0.01017	102
2024044K	1.185	0.00474	0.00948	95
2024044L	1.175	0.00470	0.00940	94

Sample ID Lab # - Rep.	Peak Area % Difference
2024044J	-80.58
2024044K	51.90
2024044L	27.79

- **Peak area percent differences decrease** with **increasing MeOH concentration**, little to **no recovery loss**, so can attempt higher concentration levels

Mix B SPE Wash Testing

Mix B fortification standard spiked into water, put directly onto SPE

- Original: Rinse with 1/9 MeOH/Water
- Testing: 4/9 MeOH/Water
- Testing: 5/9 MeOH/Water
- Testing: ⅔ (6/9) MeOH/Water
- Testing: 10% Acetonitrile
- Testing: 10% Acetone

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
1/9 Control	----	----	<0.01	----
Recovery				
1/9 MeOH	1.15	0.00459	0.00918	92
4/9 MeOH	1.14	0.00457	0.00915	91
5/9 MeOH	1.21	0.00485	0.00971	97
6/9 MeOH	1.17	0.00470	0.00939	94
10% ACN	1.19	0.00476	0.00952	95
10% ACE	1.19	0.00476	0.00952	95

Sample ID Lab # - Rep.	Peak Area % Difference
1/9 MeOH	0.55
4/9 MeOH	0.35
5/9 MeOH	-1.04
6/9 MeOH	0.34
10% ACN	0.66
10% ACE	-0.80

Mix B SPE Wash Testing

Mix B regular extraction performed

- Original: Rinse with 1/9 MeOH/Water 0.01 PPM **(044J1)**
- Testing: 6/9 MeOH/H2O 0.01 PPM **(044J2)**
- Testing: 10% Acetonitrile 0.01 PPM **(044K)**
- Testing: 10% Acetone 0.01 PPM **(044L)**

COOH-A

Calculations

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044I	----	----	<0.01	----
Recovery				
2024044J1	0.93	0.00372	0.00745	74
2024044J2	1.17	0.00468	0.00937	94
2024044K	1.26	0.00505	0.01010	101
2024044L	2.51	0.01005	0.02009	201

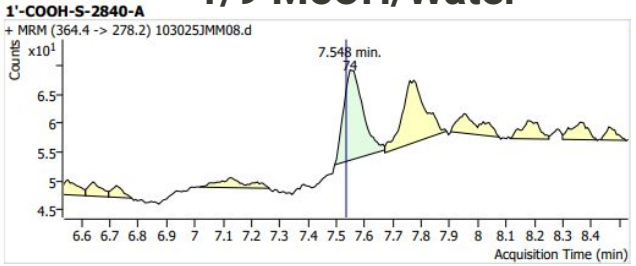
Sample ID Lab # - Rep.	Peak Area % Difference
2024044J1	-35.01
2024044J2	-19.19
2024044K	-78.54
2024044L	128.33

Mix B SPE Wash Testing

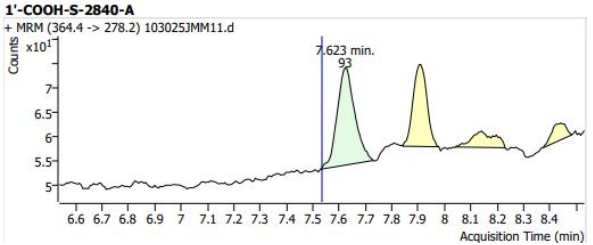
Mix B regular extraction performed

- Original: Rinse with 1/9 MeOH/Water 0.01 PPM **(044J1)**
- Testing: 6/9 MeOH/H2O 0.01 PPM **(044J2)**
- Testing: 10% Acetonitrile 0.01 PPM **(044K)**
- Testing: 10% Acetone 0.01 PPM **(044L)**

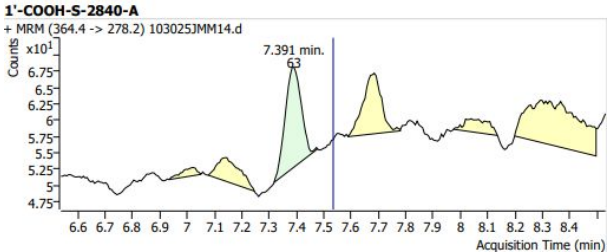
1/9 MeOH/Water



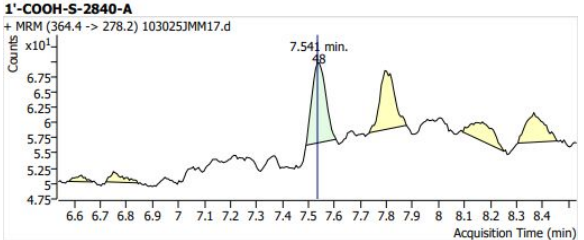
6/9 MeOH/Water



10% Acetonitrile



10% Acetone



DFPA SPE Rinse Testing

- Original: Rinse with 5 mL Water 4x **(044J)**
- Testing: Rinse with 5 mL 1/9 MeOH/H2O 4x **(044K)**
- Testing: Rinse with 5 mL 2/9 MeOH/H2O 4x **(044L)**

Calculations **DFPA**

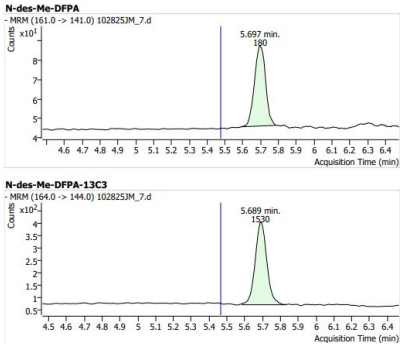
Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044J	1.39	0.00556	0.0111	56
2024044K	1.68	0.00673	0.0135	67
2024044L	0.32	0.00130	0.00260	13

Sample ID Lab # - Rep.	Peak Area % Difference
2024044J	-0.83
2024044K	-0.75
2024044L	0.36

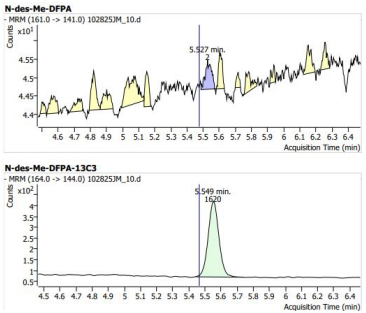
DFPA SPE Rinse Testing

- Original: Rinse with 5 mL Water 4x
- Testing: Rinse with 5 mL 10% Acetone 4x
- Testing: Rinse with 5 mL 10% Acetonitrile 4x
- Testing: Rinse with 5 mL Hexane 4x
- Testing: Rinse with 5 mL Ethyl Acetate 4x

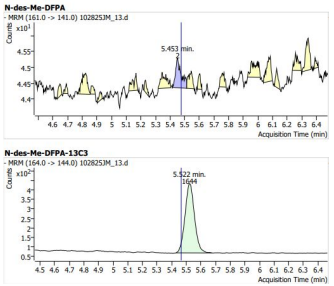
Water



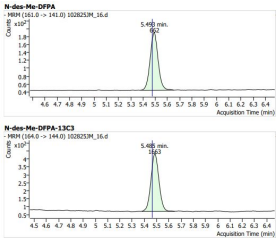
10% Acetone



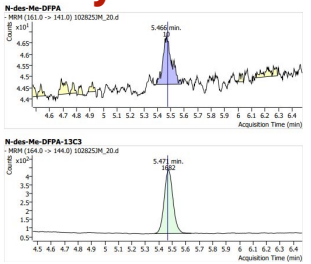
10% Acetonitrile



Hexane

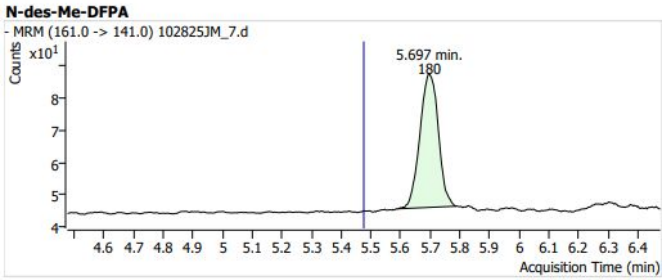


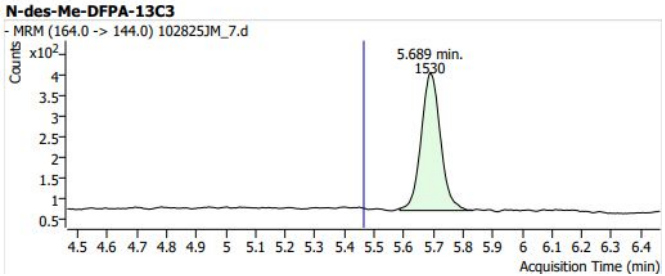
Ethyl Acetate



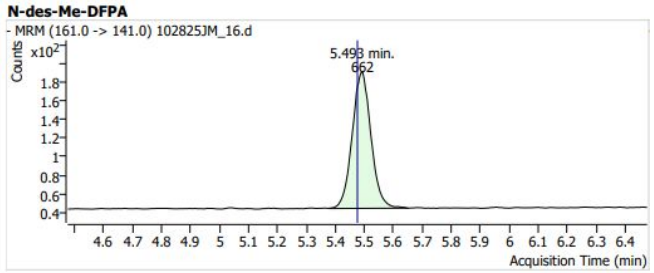
DFPA SPE Rinse Testing

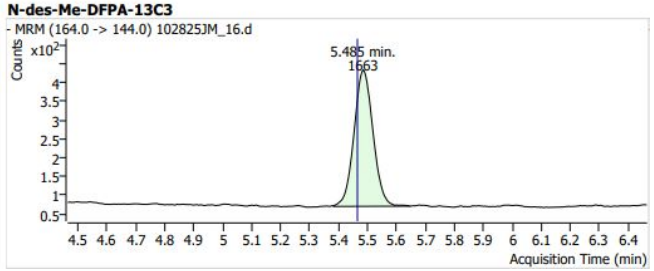
- **Hexane** was the only solvent to not elute DFPA, and even appeared to give a higher area count than water





Water





Hexane

DFPA SPE Rinse Testing

- Original: Rinse with 5 mL Water 4x **(044N & 044O)**
- Testing: Rinse with 5 mL **1/19 MeOH/H2O** 4x **(044P & 044Q)**
- Testing: **Dilute samples** before LC Vials **1:1(044N2 & 044O2**
- A **new calibration level of 0.25 ng/mL** was made to accommodate the 1:1 dilution

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 M	----	----	<0.01	----
Recovery				
2024044N	1.60	0.00640	0.0128	64
2024044O	1.39	0.00556	0.0111	56
2024044P	1.60	0.00639	0.0128	64
2024044Q	1.20	0.00479	0.00958	48
2024044N2	1.57	0.00629	0.0126	63
2024044O2	1.40	0.00560	0.0112	56

Sample ID Lab # - Rep.	Peak Area % Difference
2024044N	5.38
2024044O	-3.00
2024044P	-1.92
2024044Q	9.95
2024044N2	10.70
2024044O2	-3.42

DFPA SPE Testing

- Original: Rinse with 5 mL **Water** 4x **(044N)**
- Testing: Elution with **Acetonitrile (044O)**
- Testing: SAX Ion Exchange Cartridge **(044P)**
- Testing: Rinse with 5 mL **Hexane** 4x **(044Q)**

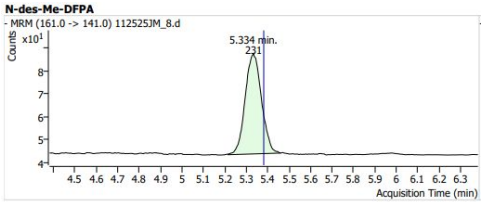
Calculations **DFPA**

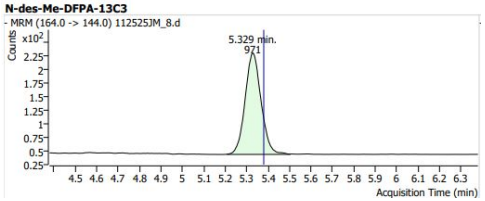
Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 M	----	----	<0.01	----
Recovery				
2024044N	1.29	0.00515	0.0103	51
2024044O	1.28	0.00510	0.0102	51
2024044P	0.63	0.00250	0.0050	25
2024044Q	1.70	0.00680	0.0136	68

Sample ID Lab # - Rep.	Peak Area % Difference
2024044N	2.66
2024044O	2.52
2024044P	13.02
2024044Q	-1.90

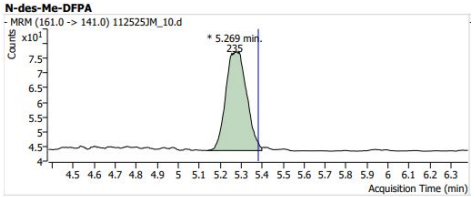
DFPA SPE Testing

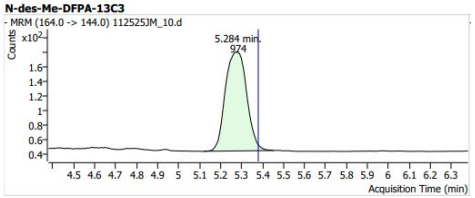
Water Rinse



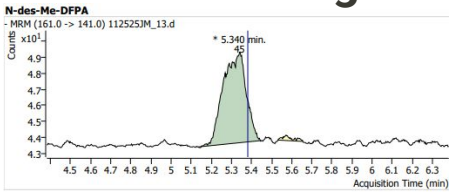


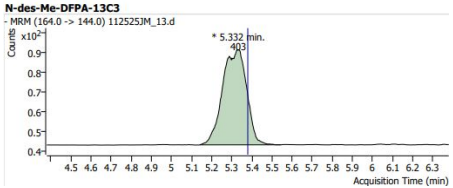
Acetonitrile Elution



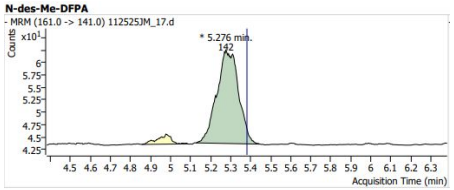


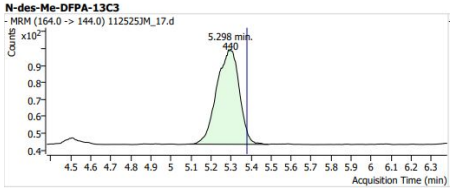
SAX Cartridge





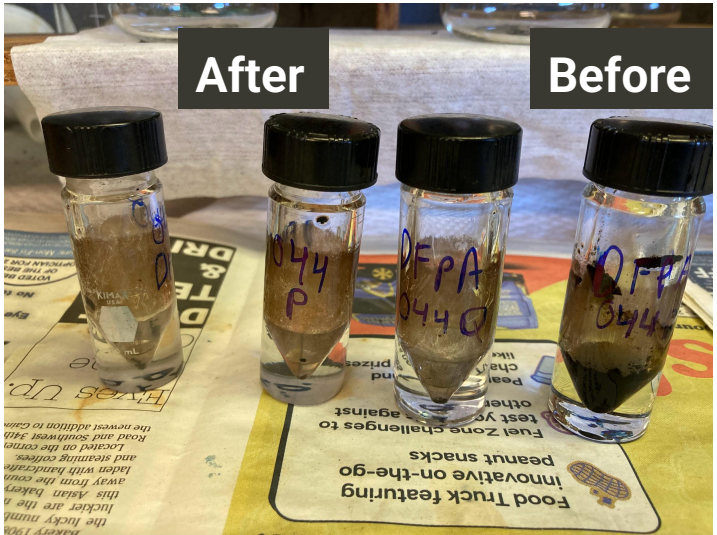
Hexane Rinse





DFPA Saltwater Reactivial Rinse Test

- After **observing that a large amount of "gunk"** gets **left behind** during the ethyl acetate partition step, I wanted to test **rinsing the reacti vials with the 5% saltwater** which was already a part of the partition to **dissolve the "gunk" better and transfer it** to the set funnel to **reduce loss.**



DFPA Saltwater Reactivial Rinse Test

- Original: Water SPE **No Mix (044N)**
- Testing: Water SPE **(Saltwater Rinse) (044O)**
- Testing: Hexane SPE **No Mix (044P)**
- Testing: Hexane SPE **(Saltwater Rinse) (044Q)**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 M	----	----	<0.01	----
Recovery				
2024044N	1.65	0.00659	0.0132	66
2024044O	1.76	0.00704	0.0141	70
2024044P	1.61	0.00646	0.0129	65
2024044Q	1.85	0.00738	0.0148	74

Sample ID Lab # - Rep.	Peak Area % Difference
2024044N	0.45
2024044O	-5.41
2024044P	-3.23
2024044Q	3.45

DFPA SPE Cartridge Test

- Original: HLB Cartridge 5 mL Water 4x **(044 J)**
- Testing: Strata X 5 mL 1:9 MeOH/H2O 4x **(044 K)**
- Testing: NH2 5 mL Water 4x **(044 L)**
- Testing: NH2 Hexane 4x **(044 M)**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044 J	1.70	0.00680	0.0136	68
2024044 K	0.60	0.00241	0.0048	24
2024044 L	0.11	0.00045	0.0009	5
2024044 M	0.12	0.00047	0.0009	5

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 J	-2.10
2024044 K	-4.08
2024044 L	115.79
2024044 M	-127.27

DFPA SPE Cartridge Test

- Original: HLB Cartridge 5 mL Water 4x **(044 J)**
- Testing: Strata X Water, 1% Formic Acid in Acetone **(044 K)**
- Testing: NH2 Water, 1% Formic Acid in Acetone **(044 L)**
- Testing: SAX Water, 1% Formic Acid in Acetone **(044 M)**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 I	----	----	<0.01	----
Recovery				
2024044 J	2.10	0.00841	0.0168	84
2024044 K	0.51	0.00203	0.0041	20
2024044 L	-0.07	-0.00029	-0.0006	-3
2024044 M	-0.09	-0.000351	-0.0007	-4

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 J	7.56
2024044 K	-3.64
2024044 L	66.7
2024044 M	80.0

DFPA SPE Cartridge Test

- **Original:** HLB Cartridge 5 mL Water 4x **(044 O & 044 P)**
- **Testing:** HLB Cartridge 5 mL Hexane 2x and 5 mL H2O 2x **(044 Q & 044 R)**

DFPA

Calculations Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 N	----	----	<0.01	----
Recovery				
2024044 O	1.33	0.00531	0.0106	53
2024044 P	1.72	0.00687	0.0137	69
2024044 Q	1.41	0.00564	0.0113	56
2024044 R	1.50	0.006018	0.0120	60

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 O	0.90
2024044 P	-2.97
2024044 Q	2.6
2024044 R	8.9

- Observed more “gunk” in O and Q after ethyl acetate partition, possible correlation to lower recovery?

DFPA Filtering Gunk Out

More Transferred, lower recovery

Less Transferred, higher recovery

DFPA Filtering Gunk Out

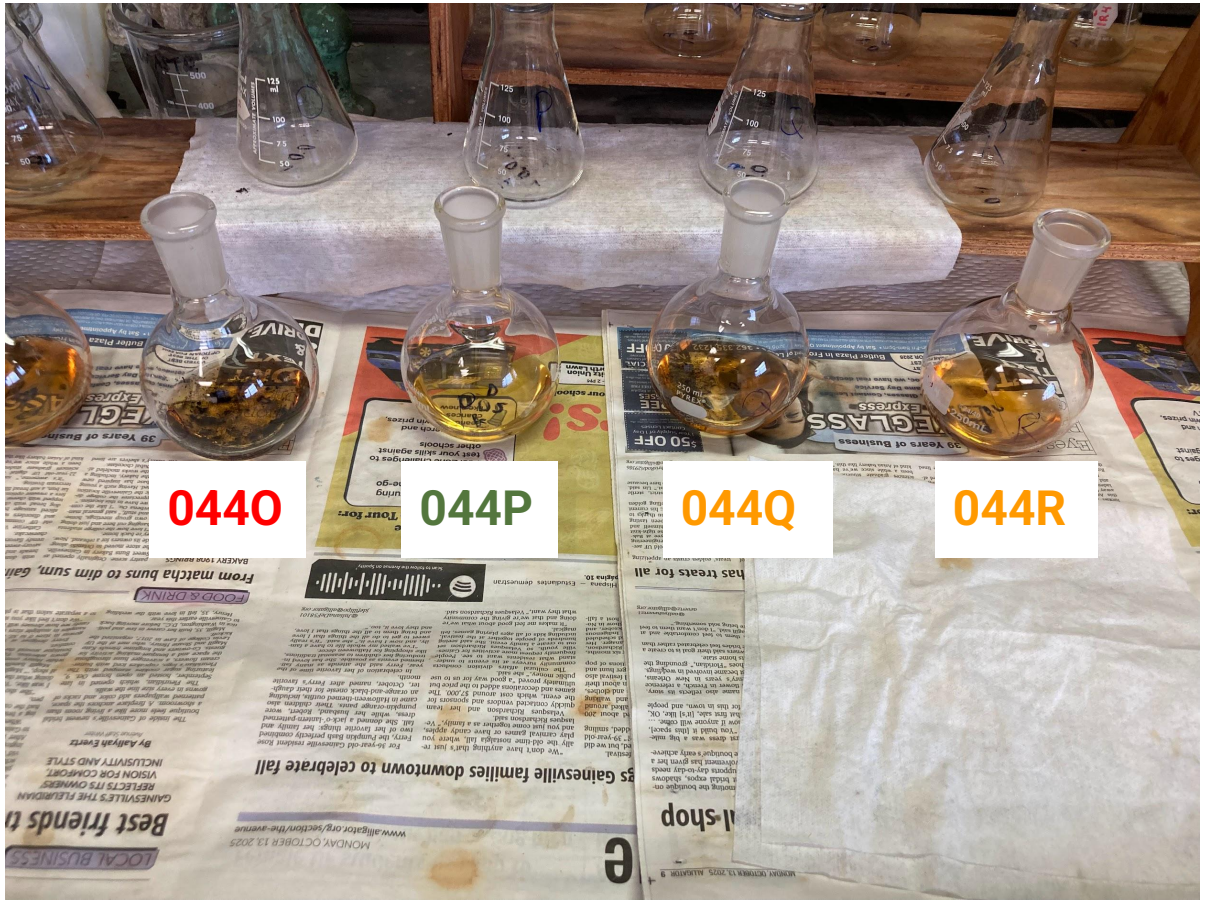
- Original: Quickly drain set funnel to maximize gunk transferred **(044 O)**
- Testing: Filter sample after 4H cook into set funnel **(044 P)**
- Testing: Filter before spiking **(044 Q)**
- Testing: Slowly drain set funnel to prevent gunk from proceeding **(044 R)**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044 N	----	----	<0.01	----
Recovery				
2024044 O	2.04	0.00816	0.0163	82
2024044 P	1.02	0.00408	0.0082	41
2024044 Q	1.59	0.00637	0.0127	64
2024044 R	1.53	0.006127	0.0123	61

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 O	-1.53
2024044 P	-2.02
2024044 Q	-2.2
2024044 R	-2.4

DFPA Filtering Gunk Out



DFPA Maximize Gunk

- For all samples, **rinse set funnel with 20 mL ethyl acetate** after partition to pull gunk down from the walls
- **044 R2: 0.02 PPM**
- **044 R3: 0.1 PPM 1:5**
- **044 R4 1 PPM 1:20**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044R1	----	----	<0.01	----
Recovery				
2024044R2	1.47	0.00589	0.0118	59
2024044R3	5.68	0.02272	0.0454	45
2024044R4	107.47	0.42989	0.8598	86

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 O	-2.62
2024044 P	-4.93
2024044 Q	0.0

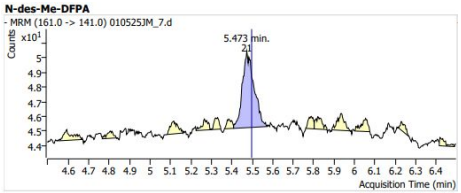
DFPA Maximize Gunk

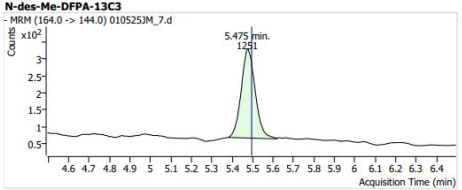
DFPA Maximize Gunk Part 2

- SPE Cartridges from previous experiment were rinsed with Methanol, elution collected **(R2, R3, R4)**
- Round bottom flasks were rinsed with Methanol and put onto SPE cartridges, elution collected separately in order to find missing DFPA **(R2-2, R3-2, R4-2)**
- **This was done after winter break, so I wasn't expecting much after sitting for roughly two weeks**

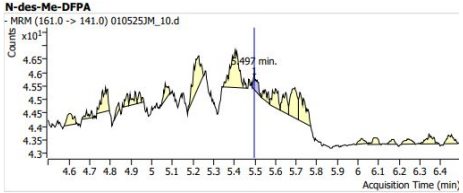
DFPA Maximize Gunk Part 2

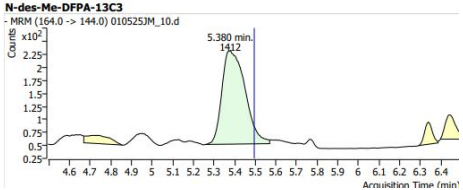
R2 0.02 PPM



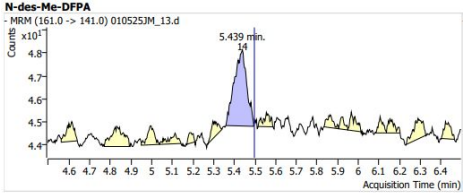


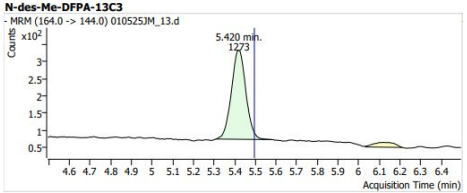
R3 0.1 PPM 1:5



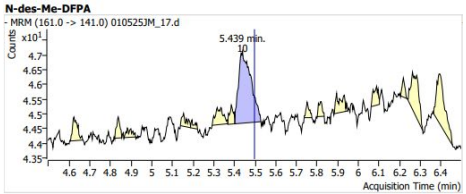


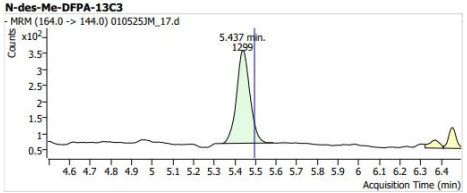
R4 1 PPM 1:20



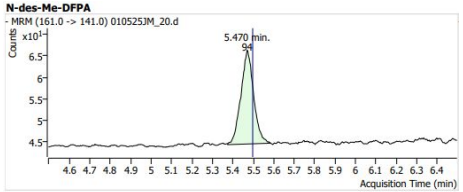


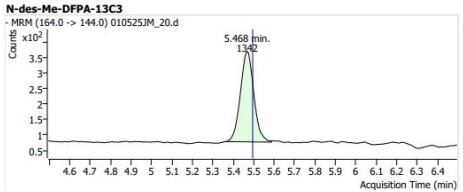
R2-2 0.02 PPM



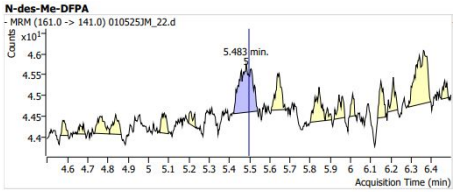


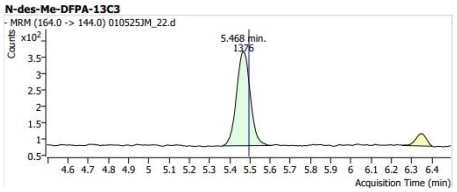
R3-2 0.1 PPM 1:5





R4-2 1 PPM 1:20





Latest Mix B 6/9 MeOH Test

- For all samples, perform extraction like normal, second round of SPE use two rinses of 6/9 MeOH/H2O
- **044T, 044U and 044V all at 0.01 PPM**

Calculations **COOH-A**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044S	----	----	<0.01	----
Recovery				
2024044T	1.08	0.00432	0.00865	86
2024044U	1.03	0.00413	0.00825	83
2024044V	0.87	0.00346	0.00693	69

Sample ID Lab # - Rep.	Peak Area % Difference
2024044T	-1.24
2024044U	7.14
2024044V	-4.44

- **Sample 044V dried down much faster than others, potential loss?**

DFPA Acetone Rinse Test

- For all samples, use the saltwater rinse before ethyl acetate partition, rinse set funnel with ethyl acetate, and rinse round bottom flask with acetone during elution SPE step

- **044T, 044U and 044V all at 0.02 PPM**

Calculations **DFPA**

Sample ID Lab# - Rep.	Final Ext. Conc. (ng/mL)	Amount Found (µg)	Amount Found (ppm)	Fort. Recovery (%)
Control				
2024044S	----	----	<0.01	----
Recovery				
2024044T	1.80	0.00718	0.0144	72
2024044U	1.93	0.00772	0.0154	77
2024044V	1.50	0.00600	0.0120	60

Sample ID Lab # - Rep.	Peak Area % Difference
2024044 J	-9.01
2024044 K	-2.60
2024044 L	0.58

- Again, sample 044V dried down much faster, look into using a different position on air dryer?

Future Testing

- Test drying samples on a different position on the air dryer where they will not dry down so quickly
- Test higher concentrations of Methanol in the MeOH/Water SPE wash step for Mix B to test if it can be further improved
- Retest rinsing round bottom flasks in DFPA SPE step with 1/9 MeOH/Water to see if recovery improves when more “gunk” is present

Inpyrfluxam Coffee Key Takeaways

- By **adjusting the chromatography gradient**, the **caffeine peak** was able to be moved **further away from COOH-A, COOH-B and DFPA reducing suppression**, enough for Roasted Bean to pass.
- **Freeze dried required more cleanup in Mix B** by **increasing the Methanol concentration** in the SPE wash from **1/9 to 6/9 MeOH/Water.**
- **DFPA in freeze dried samples is easily removed** by any solvent that isn't water or hexane from the column, **HLB works best for DFPA.**
- **Moving "gunk" forward** appears to **somewhat reduce loss of DFPA** from step to step
- If **samples are reduced to dryness** and left alone **for too long, recovery is significantly reduced.**

Method Development Key Takeaways

- Ask colleagues for input
- Take ideas from other similar projects and build on what has been done before
- Don’t be afraid to throw things at the wall and see what sticks
- Make note of any observations, what might be different sample to sample that could affect recovery and build off that for the next test
- If something isn’t working, take a step back and look for something else that can be improved

Thank you!

Questions? Comments? Suggestions?

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IR-4 Project