

Removal of Purple Pigmentation from Cannabis using QuEChERS Extraction, ChloroFiltr® dSPE Clean-up and LC-MS/MS



UCT Part Numbers

ECMSSC-MP

Mylar pouch containing 4g
MgSO₄ and 1g NaCl

SLAQ100ID21-3UM

Selectra® Aqueous C18 HPLC
column, 100 × 2.1 mm, 3 µm

CUMPSGGC182CT

2mL dSPE tube containing
150 mg MgSO₄, 50 mg PSA, 50
mg C18 and 50 mg ChloroFiltr®

SLAQGDC20-3UM

Selectra® Aqueous C18 guard
cartridge, 10 × 2.1 mm, 3 µm

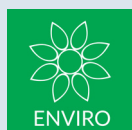
SLGRDHLDR-HPOPT

Guard cartridge holder

Summary:

Cannabis testing laboratories have the challenge of removing a variety of unwanted matrix components from plant material prior to running extracts on their LC-MS/MS or GC-MS. The complexity of the cannabis plant presents additional analytical challenges that do not need to be accounted for in other agricultural products. Up to a third of the overall mass of cannabis seed, half of usable flower, and nearly all of extracts can be contributed to essential oils such as terpenes, flavonoids and actual cannabinoid content (1). The biodiversity of this plant is exhibited in the over 2,000 unique strains that have been identified, each with their own pigmentation, cannabinoid profile and overall suggested medicinal use (2). While novel methods have been developed for the removal of chlorophyll, few, if any, sample preparation methods have been devoted to removal of other colored pigments from cannabis.

This application note outlines a QuEChERS extraction procedure for the analysis of 5 mycotoxins and 47 pesticides in cannabis. Using first an unbuffered QuEChERS salt blend, 7 dSPE configurations were evaluated for the removal of purple pigmentation, in addition to chlorophyll present in the plant material. Most of the LC-MS/MS amenable compounds on the Massachusetts and Nevada monitoring lists of pesticides and mycotoxins are included. Liquid chromatography, using a Selectra® Aqueous C18 column, coupled to tandem mass spectrometry (LC-MS/MS) is used for analysis of the pesticides and mycotoxins.



QuEChERS Procedure:

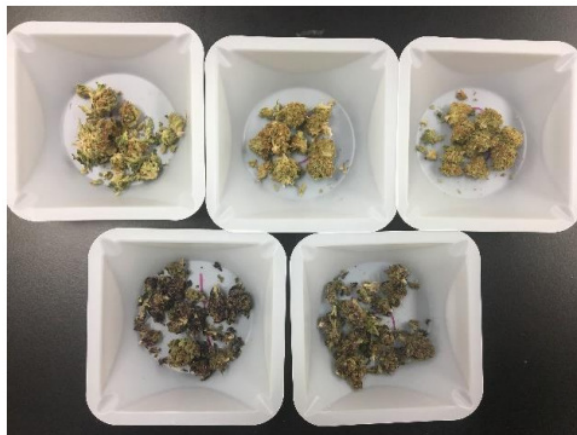


Figure 1. Cannabis strains used (clockwise from top left): Agent Orange, Tahoe OG, Blue Skunk, Grand Daddy and Grape Drink

Sample Extraction:

- Weigh 1 g of cannabis sample into a 50 mL polypropylene centrifuge tube.
- Add internal standard(s).
- Add 10 mL ultrapure water, vortex briefly, and allow sample to hydrate for 15 min (improves extraction efficiency).
- Add 10 mL acetonitrile containing 2% formic acid.
- Add the contents of the ECMSSC-MP Mylar pouch and shake for a minimum of 5 minutes (by hand or mechanically). For this work a Spex 2010 Geno/Grinder® was used (1500 RPM).
- Centrifuge the sample at ≥ 3000 rcf for 5 minutes.

dSPE Clean-up:

- Transfer 1 ml of supernatant to a dSPE cleanup tube (CUMPSGGC182CT).
- Vortex the sample for 30 seconds.
- Centrifuge the sample at ≥ 3000 rcf for 5 minutes.
- Transfer the purified and filtered sample extract into an autosampler vial for analysis.

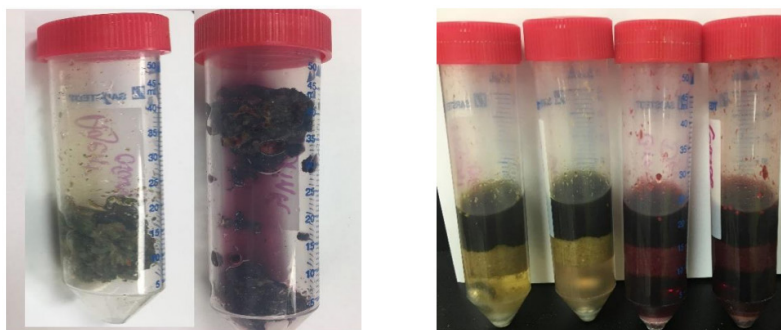


Figure 2. Cannabis samples following hydration (left) and QuEChERS extraction (right)

Table 1. dSPE Blends Evaluated

A	150 mg MgSO ₄ , 50 mg PSA, 50 mg C18, 50 mg ChloroFiltr®
B	150 mg MgSO ₄ , 50 mg PSA
C	150 mg MgSO ₄ , 25 mg C18
D	150 mg MgSO ₄ , 50 mg PSA, 50 mg C18
E	150 mg MgSO ₄ , 25 mg PSA, 7.5 mg GCB
F	150 mg MgSO ₄ , 50 mg PSA, 50 mg C18, 50 mg GCB
G	150 mg MgSO ₄ , 50 mg C18, 50 mg ChloroFiltr®

LC-MS/MS Parameters:**Table 2. Instrumentation**

HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® Aqueous C18, 100 × 2.1 mm, 3 µm (p/n: SLAQ100ID21-3UM)
Guard column	UCT Selectra® Aqueous C18, 10 × 2.1 mm, 3 µm (p/n: SLAQGDC20-3UM)
Guard column holder	p/n: SLGRDHLDLDR
Column temperature	40°C
Flow rate	300 µL/min
Injection volume	5 µL

Table 3. Mobile Phase Gradient

Time (min)	% Mobile Phase A (Water + 5mM NH ₄ HCO ₂ + 0.1% formic acid)	% Mobile Phase B (Methanol + 5mM NH ₄ HCO ₂ + 0.1% formic acid)
0.0	100	0
2.0	50	50
5.0	50	50
5.5	40	60
9.0	40	60
12.0	0	100
15.0	0	100
15.1	100	0
20.0	100	0

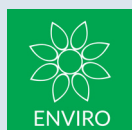


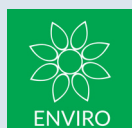
Table 4. MS Parameters and Retention Times

Analyte	RT	Parent Ion	Product 1	CE 1	Product 2	CE 2
Abamectin (M+NH ₄)	14.00	890.5	305.0	24	567.2	9
Acephate	3.75	184.0	95.1	23	143.0	6
Acequinocyl	13.75	384.4	119.1	31	177.1	13
Acetochlor	11.00	270.1	148.1	18	224.1	10
Aflatoxin B1	7.65	313.0	241.0	36	285.1	21
Aflatoxin B2	7.25	315.1	259.1	28	287.1	24
Aflatoxin G1	6.65	329.0	199.0	48	243.0	25
Aflatoxin G2	6.35	331.1	189.0	39	245.1	28
Aldicarb sulfoxide	4.35	207.1	69.2	17	89.1	13
Atrazine	8.60	216.1	68.1	34	174.1	16
Bifenazate	10.90	301.1	170.1	18	198.1	6
Carbaryl	7.50	202.1	127.1	29	145.1	11
Chlorpyrifos	13.85	349.9	97.0	32	197.9	19
Cyprodinil	12.45	226.1	77.1	43	93.1	33
DEET	8.60	192.1	91.1	29	119.1	17
Dichlorvos	6.80	220.9	109.1	18	127.1	13
Dichrotophos	5.05	238.1	112.1	12	127.0	18
Dimethomorph	10.80	388.2	165.1	30	301.1	19
Etoxazole	14.00	360.3	113.1	54	141.1	30
Fenamiphos sulfone	7.30	336.1	188.0	26	266.0	19
Fenamiphos sulfoxide	7.45	320.1	108.1	40	233.0	24
Fenhexamid	11.20	302.2	216.2	27	270.2	19
Fenoxycarb	12.30	302.1	88.1	17	116.1	7
Flonicamid (ESI-)	4.50	228.1	81.1	13	146.0	22
Fludioxinil (ESI-)	10.30	247.1	126.1	32	180.1	29
Flutriafol	8.10	302.1	70.1	17	123.0	28
Imazilil	8.10	297.1	159.0	24	201.0	17
Imidacloprid	5.30	256.1	175.1	17	209.1	17
Malathion	9.80	331.0	99.0	25	127.0	12
Metamidophos	3.10	142.0	94.1	14	125.0	13
Myclobutanil	10.80	289.2	70.1	18	125.1	31
Ochratoxin A	10.80	404.0	102.1	63	239.0	22
Oxydemeton methyl	4.70	247.0	109.0	27	169.0	13
Paclobuterol	9.80	294.1	70.1	19	125.0	33
Piperonyl butoxide	13.75	356.3	119.1	31	177.1	12
Pymetrozine	4.50	218.1	105.1	20	176.1	17
Pyrazophos	13.20	374.1	194.1	31	222.1	20
Pyrethrin I (M+NH ₄)	10.95	346.2	170.1	22	198.1	12
Pyrethrin II (M+NH ₄)	10.80	390.2	165.1	29	303.1	19
Simazine	7.65	202.1	124.1	16	132.0	19
Spinetoram	13.85	748.6	98.0	37	142.1	29

Analyte	RT	Parent Ion	Product 1	CE 1	Product 2	CE 2
Spinosyn A	13.55	732.6	97.9	40	142.1	29
Spinosyn D	13.85	746.6	97.9	36	142.1	28
Spiromesifen	13.95	273.1	187.1	16	255.2	13
Spirotetramat	11.20	374.2	216.1	31	302.2	16
Tebuconazole	12.60	308.1	70.1	21	125.0	33
Tebuthiuron	7.60	229.1	116.0	26	172.1	16
Thiabendazole	5.70	202.0	131.1	31	175.1	25
Thiabendazole ¹³ C ₆ (IS)	5.70	208.0	137.1	32	181.1	25
Thiamethoxam	4.80	292.1	181.1	21	211.1	11
Triadimefon	10.20	294.1	69.1	20	197.1	14
Triethylphosphorothioate	13.70	199.0	125.0	16	143.0	14
Trifloxystrobin	13.30	409.2	145.0	41	186.1	17
Zoxamide	12.45	336.0	159.0	38	187.0	21

Table 5. Recovery and Precision Data for Pesticides and Mycotoxins in Cannabis

(n=4)	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
Mycotoxins								
Conc. in sample	20 ng/g		50 ng/g		100 ng/g		200 ng/g	
Conc. in extract	2 ng/mL		5 ng/mL		10 ng/mL		20 ng/mL	
Aflatoxin B1	67.6	1.92	73.8	1.39	72.4	1.11	79.3	1.23
Aflatoxin B2	67.4	2.26	77.0	2.26	75.3	2.70	81.0	1.55
Aflatoxin G1	69.5	5.37	76.6	1.78	75.1	2.06	80.0	1.71
Aflatoxin G2	75.3	3.72	77.5	1.31	73.3	1.91	79.4	2.42
Ochrotoxin A	22.6	29.38	47.0	5.82	48.6	2.08	52.7	3.19
Pesticides								
Conc. in sample	50 ng/g		100 ng/g		200 ng/g		500 ng/g	
Conc. in extract	5 ng/mL		10 ng/mL		20 ng/mL		50 ng/mL	
Abamectin	ND	ND	ND	ND	ND	ND	88.2	6.50
Acephate	44.9	4.09	65.4	3.72	67.3	3.99	75.7	2.60
Acetochlor	89.7	5.08	86.4	1.71	86.0	1.33	82.7	2.02
Aldicarb sulfoxide	< LOD	< LOD	52.9	5.85	67.2	4.89	72.6	3.19
Atrazine	91.4	1.33	91.1	3.09	88.8	3.13	86.3	2.13
Bifenazate	84.0	3.76	80.4	1.41	78.9	2.57	77.8	2.78
Carbaryl	78.7	2.56	76.0	6.54	89.2	2.04	80.6	0.55
Chlorpyrifos	< LOD	< LOD	79.7*	9.39*	79.7	3.71	85.0	2.60
DEET	92.6	2.38	88.2	3.92	92.0	4.02	84.2	2.13



Dichlorvos	83.4	8.99	81.2	4.44	83.3	3.94	81.7	2.45
Dichrotophos	81.4	2.83	81.0	3.18	85.3	3.35	81.1	2.05
Dimethomorph	85.4	2.98	81.6	3.87	85.0	2.73	81.7	2.03
Etoazole	74.3	3.05	72.6	1.40	72.7	3.25	72.1	1.42
Fenamiphos sulfone	86.2	5.54	84.2	5.35	89.1	2.74	84.1	1.28
Fenamiphos sulfoxide	81.5	2.65	79.4	3.57	83.0	2.68	78.3	0.96
Fenhexamid	84.3	1.22	82.4	5.55	83.6	2.13	79.4	1.61
Fenoxycarb	85.6	1.72	81.9	3.89	79.5	4.55	80.7	2.08
Flonicamid	82.6	2.74	87.5	3.00	83.8	4.95	80.2	1.79
Fludioxinil	77.8	6.43	76.1	2.87	78.4	3.32	74.6	1.61
Flutriafol	84.7	1.56	77.7	3.08	82.0	2.76	78.1	1.55
Imazilil	92.6	1.19	86.2	4.20	85.2	1.98	78.7	1.26
Imidacloprid	72.7	5.24	76.8	3.22	81.6	1.87	77.9	6.85
Malathion	90.2	4.82	85.0	4.94	98.8	10.72	90.2	6.05
Cyprodinil	75.7	6.88	70.8	3.63	67.8	7.86	69.6	2.77
Metamidophos	71.2	7.19	64.6	1.42	63.4	2.91	62.8	2.94
Myclobutanil	90.5	2.06	83.9	2.78	85.4	3.32	81.6	0.42
Oxydemeton methyl	78.7	5.72	78.5	2.37	82.0	1.90	77.4	2.42
Paclobuterol	80.2	3.71	81.0	4.10	96.5	2.98	100.6	1.75
Piperonyl butoxide	64.2	6.46	69.7	1.92	73.6	5.05	76.0	1.76
Pymetrozine	34.2	4.83	28.7	12.97	24.7	4.55	24.2	9.18
Pyrazophos	79.1	2.60	76.6	7.81	78.6	1.12	83.2	1.27
Pyrethrin I	< LOD	< LOD	< LOD	< LOD	64.7	5.69	81.5	4.27
Pyrethrin II	73.6	6.82	73.2	3.12	79.9	0.37	76.5	1.32
Simazine	61.2	8.96	81.1	1.39	92.3	3.19	83.6	1.30
Spinetoram	84.3	3.19	78.9	5.19	83.8	3.07	79.1	3.68
Spinosyn A	82.0	2.73	78.0	6.75	79.9	3.32	75.8	0.60
Spinosyn D	79.5	2.59	77.2	6.74	81.5	3.23	75.3	0.60
Spiromesifen	37.5	11.95	59.2	3.31	67.3	1.07	67.9	3.08
Spirotetramat	77.2	4.69	73.8	6.37	78.3	2.82	79.1	1.56
Tebuconazole	80.2	3.68	79.3	3.43	78.1	5.70	78.1	1.02
Tebuthiuron	81.7	3.54	76.9	2.86	80.0	3.45	77.1	1.76
Thiabendazole	97.2	3.40	95.8	4.79	100.4	2.44	99.6	1.82
Thiamethoxam	86.1	3.97	80.5	3.78	81.9	4.21	79.8	3.25
Triadimefon	88.4	3.51	86.3	0.58	87.6	2.96	90.5	1.15
Triethylphosphorothioate	< LOD	< LOD	100.1	9.02	89.2	4.40	82.9	2.26
Trifloxystrobin	93.1	1.52	87.4	2.82	83.2	7.31	85.8	0.83
Zoxamide	82.6	4.19	77.6	4.56	77.9	1.51	80.6	1.63
Overall average	77.1	4.62	77.3	4.05	79.3	3.35	78.7	2.31

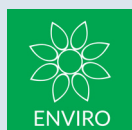
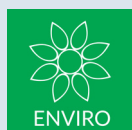


Table 6. Comparison of ChloroFiltr® vs GCB

Conc = 200 or 500 ng/g (n=4)	ChloroFiltr®		GCB	
	Recovery	RSD	Recovery	RSD
Aflatoxin B1	77.6	1.58	70.3	0.91
Aflatoxin B2	78.6	1.04	63.0	0.66
Aflatoxin G1	76.9	1.72	70.0	3.13
Aflatoxin G2	77.6	1.65	70.5	2.05
Ochrotoxin A	53.9	3.30	62.2	3.46
Abamectin	93.0	6.87	ND	ND
Acephate	75.4	3.93	74.8	3.53
Acetochlor	80.7	0.63	74.7	1.14
Aldicarb sulfoxide	70.0	6.09	70.7	2.49
Atrazine	76.6	0.67	62.0	2.55
Bifenazate	74.7	1.66	77.2	0.67
Carbaryl	79.8	0.96	86.3	2.99
Chlorpyrifos	77.1	7.63	41.0	16.75
DEET	77.3	1.49	69.1	1.05
Dichlorvos	78.3	1.68	73.7	1.38
Dichrotophos	79.4	0.72	75.0	0.96
Dimethomorph	78.5	3.06	70.0	1.31
Etoxazole	70.9	2.10	64.5	1.60
Fenamiphos sulfone	82.0	1.20	76.8	0.51
Fenamiphos sulfoxide	76.7	1.44	72.6	1.23
Fenhexamid	76.2	2.04	73.3	0.75
Fenoxycarb	80.0	1.19	77.9	2.08
Flonicamid	77.4	4.44	69.4	4.78
Fludioxinil	72.3	1.84	71.0	1.30
Flutriafol	76.1	0.83	72.5	1.66
Imazilil	76.1	0.30	70.2	0.70
Imidacloprid	78.0	7.86	70.3	7.13
Malathion	85.8	6.95	78.9	8.48
Cyprodinil	66.6	6.58	17.0	3.42
Metamidophos	64.1	9.16	61.2	5.18
Myclobutanil	80.1	2.61	74.7	1.58
Oxydemeton methyl	75.6	1.06	71.2	1.21
Paclobuterol	93.4	3.90	88.0	7.71
Piperonyl butoxide	76.6	1.40	68.2	5.44



(n=4)	Recovery	RSD	Recovery	RSD
Pymetrozine	21.5	28.47	12.9	10.36
Pyrazophos	79.7	2.89	69.2	2.49
Pyrethrin I	77.5	4.84	70.1	9.29
Pyrethrin II	74.0	2.27	69.6	1.10
Simazine	81.0	0.93	61.7	3.20
Spinetoram	77.4	2.70	61.6	1.73
Spinosyn A	73.9	0.56	63.6	2.30
Spinosyn D	73.4	0.56	63.8	2.99
Spiromesifen	66.0	2.08	65.8	2.20
Spirotetramat	75.5	0.59	71.1	0.81
Tebuconazole	76.7	2.32	72.8	1.87
Tebuthiuron	76.0	0.98	77.7	1.38
Thiabendazole (no IS)	60.0	2.67	19.8	2.92
Thiamethoxam	78.2	1.20	76.8	4.07
Triadimefon	83.2	3.43	76.8	1.84
Triethylphosphorothioate	82.4	2.77	79.2	6.79
Trifloxystrobin	82.5	2.77	69.6	2.60
Zoxamide	78.4	3.81	77.2	2.40
Overall average	75.6	3.18	67.6	3.14

Note: The sorbent combination was 150mg MgSO₄, 50mg PSA, 50mg C18, and either 50mg ChloroFiltr® or 7.5mg GCB. Significant variation in results are highlighted in red.

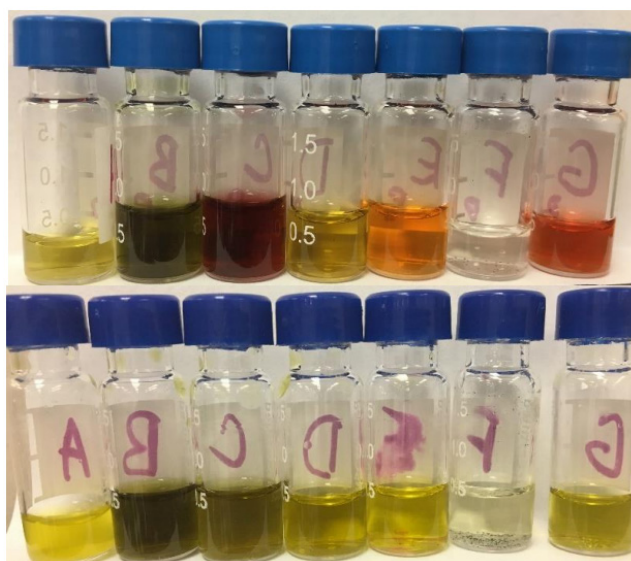


Figure 3. Extracts following various dSPE clean ups. Top: Grand Daddy Bottom: Tahoe OG

Discussion:

A combination of MgSO_4 , C18, PSA and ChloroFiltr® (Blend A) allowed for the most sample clean up, without loss of pesticides and mycotoxins, for all cannabis samples tested. Average recovery of the 48 pesticides and five mycotoxins using the selected dSPE blend was 75.6% were as the average recovery when including GCB instead of ChloroFiltr® was 67.6%. Regardless of the sample's original pigmentation, this blend successfully removed both chlorophyll and purple hues from all strains tested. The other six dSPE blends evaluated were unable to provide the sample clean up needed or had previously demonstrated to be detrimental to the recovery of pesticides routinely analyzed in cannabis (3).

Blends B, C and G provided minimal sample cleanup for all strains of cannabis tested. As shown in Figure 3, the Grand Daddy strain still contained chlorophyll after treatment with Blend B whereas Blends C and G were unable to remove its purple hue in part to not containing any PSA. While Blend E contained 25 mg of PSA, this proved to be an inadequate amount of sorbent, as shown in Figure 3.

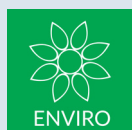
Blend F, which contained 50 mg of GCB, provided the clearest final extracts. Previous pesticide recovery studies have demonstrated that this large of volume of GCB leads to poor recovery of planar pesticides. As states across America begin to expand their required pesticide testing lists for both recreational and medicinal cannabis laboratories, it is critical that all classes of compounds have adequate recoveries.

Blend D demonstrated removal of some chlorophyll as well as the purple pigmentation. Using this blend (150 mg MgSO_4 , 50 mg PSA, 50 mg C18) and including a minimal amount of GCB (7.5 mg) is commonly used in cannabis testing labs. To determine whether GCB or ChloroFiltr® provided the best sample cleanup and extraction efficiency, Blend A was compared to this additional configuration. As shown in Table 6, higher recoveries are achieved when ChloroFiltr® is included in the dSPE blend, as opposed to GCB.

Following the use of unbuffered QuEChERS extraction salts, cannabis samples then require dSPE for optimal sample cleanup and analyte recovery. As additional cannabis strains become popular across the country, it is imperative that testing laboratories select sample preparation consumables that can remove not only chlorophyll but purple pigmentations as well. Using UCT's proprietary ChloroFiltr® in combination with PSA, both hues can be removed without comprising the recoveries of pesticides and mycotoxins.

Conclusion:

The sample preparation technique selected by a cannabis lab for their plant material must be capable of removing both chlorophyll and other hues of pigmentation. Using a dSPE blend that contains UCT's novel ChloroFiltr®, in conjunction with PSA, C18 and MgSO_4 , both green and purple coloration is successfully removed from various strains of cannabis. Recovery of both pesticides and mycotoxins are maximized using the above blend, without loss of planar pesticides. Compared to typical dSPE products that utilized GCB instead of ChloroFiltr®, the blend selected has proven to be optimal for troublesome compounds that are found on a variety of state monitoring lists.



References:

- [1] Recommended methods for the identification and analysis of cannabis and cannabis products, United Nations Office of Drugs and Crime (2009)
- [2] W. Ross, Newsweek, (2016)
- [3] Koesukwiwat, Urairat, et al. "High Throughput Analysis of 150 Pesticides in Fruits and Vegetables Using QuEChERS and Low-Pressure Gas Chromatography Time-of-Flight Mass Spectrometry." Journal of Chromatography A, vol. 1217, no. 43, 2010, pp. 6692–6703., doi:10.1016/j.chroma.2010.05.012.

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