

Analysis of Terpenes in Cannabis Flower via LI-Syringe-GC-MS

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Abstract

The cannabis market in the United States has been growing exponentially as well as starting to emerge in other countries across the globe. Due to expanding legalization, testing demands have greatly increased along with the need for new analytical methodologies. The analysis of terpenes in a variety of commodities has become one of the most popular tests for cannabis laboratories. The results discussed in this research focused on the development of a simple workflow solution for laboratories conducting this analysis. From this research, it was found that a standard liquid injection by syringe (LI-syringe) paired with gas chromatography-mass spectrometry (GC-MS) proved to be a straightforward and robust method for sample introduction and analysis when investigating terpenes. The LI-syringe-GC-MS method achieved a calibration working range of $0.04-5.12 \,\mu\text{g/mL}$; r² values of 0.988-0.996(0.993 average); limit of quantification values of 0.017-0.129 µg/mL (0.047 average); analytical precisions of 0.55-9.64% RSD (1.56 average); overall LI-syringe-GC-MS method precisions of 1.73-14.6% RSD (4.97 average); and recoveries of 84.6-98.9% (90.2 average) for the 23 terpenes of interest. Sample workflow and results are discussed.

Introduction

Terpenes are a naturally occurring class of compounds that are found in different species of plants and are typically strong in odor [1]. Previous research has shown that over 100 terpenes have been identified in different cannabis chemical varieties (chemovars) [2]. The analysis of terpenes in cannabis has classically been done via headspace (HS)-GC-FID/MS, often using a full evaporative technique (FET) approach that uses small sample volumes. This works well for monoterpenes, which are smaller, volatile compounds. However, calibrating sesquiterpenes when using HS is more difficult because they are larger and less volatile.

To provide an improved method for sesquiterpenes in cannabis flower, we developed and

Terpenes standard 1 (cat.# 34095) Terpenes standard 2 (cat.# 34096) Terpenes MegaMix standard 1 (cat.#

Terpenes MegaMix standard 2 (cat.# 34143)

Related Products

(cat.# 23305)

34142)

1.4 µm (cat.# 13868)

0.25 µm (cat.# 16094)

Naphthalene-d8 internal standard (cat.# 31043)

Rxi-624Sil MS, 30 m x 0.25 mm x

Rxi-1301Sil MS, 30 m x 0.25 mm x

Topaz 4.0 mm Precision liner w/ wool

- ASE extraction cell (cat.# 25995)
- Norm-Ject syringe (cat.# 22773)
- PTFE syringe filter (cat.# 26142)
- 2 mL autosampler vial (cat.# 21142)
- 1 mL gas-tight syringe (cat.# 24575)

validated the following workflow using a traditional GC-MS liquid injection approach with a simple accelerated solvent extraction (ASE) sample preparation [3]. A unique aspect of this application is the use of a terpene-free surrogate matrix for matrix-matched calibrations. The advantages of matrix-matched calibrations include, but are not limited to, reducing inaccuracies in reporting and accounting for signal enhancement/suppression caused by matrix interferences.

Experimental

The experiments conducted in this study were designed to develop a fully validated (i.e., validated with the California Bureau of Cannabis Control [BCC]) terpenes method for cannabis flower.

Sample Matrices

Hop pellets were utilized as a terpene-free surrogate matrix for calibration curves; laboratory control samples (LCS); continuing calibration verification (CCV) samples; detection limits; and analytical precision samples. Cannabis shake was used for the LI-syringe-GC-MS method precision and accuracy experiments. Cannabis flower was used for chemovar testing. All matrices were homogenized on a sheet pan with a rolling pin prior to extraction.



Removal of Terpenes from the Hops Surrogate Matrix

Following homogenization, terpenes were removed from the hops surrogate matrix using a proprietary solvent cleaning process that was performed by Verity Analytics. To remove terpenes, homogenized hops can be extracted using either a manual shakeout method or an ASE 350 system. Previous work on the analysis of terpenes in cannabis demonstrated that there was no statistical difference between these procedures for most terpenes extracted from cannabis flower (except for linalool and camphene) [3]. Because the ASE 350 method produces similar results and reduces both labor and the amount of nonreusable materials that are consumed, it was used for the experiments presented here. Once prepared, the extracted hops were placed in an oven to evaporate any remaining solvent. Dried hops were then stored in an airtight container for later use.

Accelerated Solvent Extractor (ASE) Sample Preparation

For all experiments, 0.5 g samples of hops surrogate matrix, cannabis flower, or shake was weighed out and placed in a 10 mL ASE 350 stainless-steel extraction cell (cat.# 25995). The remaining volume in the cell was filled with diatomaceous earth. Samples were extracted using isopropyl alcohol (IPA) using the parameters outlined in Table I. After the samples were extracted, which typically produced 10–11 mL of extract, the extracts were brought to a consistent final volume of 12 mL. Then, 3 mL aliquots of extract were filtered using a Norm-Ject syringe (3 mL Luer Lock tip) (cat.# 22773) with a 0.22 µm PTFE filter (cat.# 26142). For LI-syringe-GC-MS method validation experiments, 500 µL of the filtered extract was added to a 2 mL autosampler vial (cat.# 21142) using a 1 mL gas-tight syringe (cat.# 24575) and then spiked with 5 µL of a 10 µg/mL naphthalene-d8 internal standard (ISTD) solution (cat.# 31043).

Table I: Dionex ASE 350 Extraction Parameters for the Analysis of Terpenes in Cannabis

Temperature	75 ℃
Pressure	1500 psi
Extraction Solvent	Isopropanol (IPA)
Static Time	5 min
Purge Time	90 sec
Heat Time	5 min
Cycles	1

Terpene Calibration and ISTD Solution Preparation

Preparation steps for the terpene and ISTD solutions for all experiments are outlined in Table II. After the calibration stock solution was prepared, serial dilutions were made according to Table III. Once calibration levels were prepared, they were spiked with an ISTD solution. For calibration levels 2-10, 5 µL of the 10 µg/mL ISTD were added. For calibration level 1, 10 µL of 10 µg/mL ISTD were added because the final volume of this calibration level was double that of the other levels.

Step	Terpene Calibration Solution Preparation (Starting from 2500 μg/mL Cannabis Terpene Standards 1 & 2 [cat.# 34095 & 34096])	ISTD Solution Preparation (Starting from 2000 µg/mL Naphthalene-d8 Standard [cat.# 31043])
1.	Add 400 μL of each terpene standard to 200 μL of IPA (1000 μg/mL stock solution).	Add 50 µL of Naphthalene-d8 standard (ISTD) to 9.95 mL of IPA (final concentration = 10 µg/mL).
2.	Add 61.4 μL of terpene stock solution to cleaned hops in ASE cell.	
3.	Extract using ASE 350 (see Table I).	
4.	Bring extract volume to 12 mL using IPA (final concentration = 5.12μ g/mL).	
5.	Prepare calibration standards by making the serial dilutions outlined in Table III from this extract.	



Table III: Serial Dilutions for Terpene Calibration

Calibration Level	Concentration (µg/mL)	Amount of Previous Standard (µL)	Amount of IPA (µL)	Final Amount (µL)*
10	5.12			500
9	2.56	500	500	500
8	1.28	500	500	500
7	0.64	500	500	500
6	0.32	500	500	500
5	0.16	500	500	500
4	0.08	500	500	500
3	0.04	500	500	500
2	0.02	500	500	500
1	0.01	500	500	1000

LI-Syringe-GC-MS Calibration and Method Validation

Method validation was completed by analyzing samples to determine terpene calibration; method detection limit (MDL)/limit of quantification (LOQ); analytical precision; method precision; and percent recovery. MDL/LOQ was determined from seven replicate low-level calibration points. LCS were made from the cleaned hop extracts at a concentration equivalent to calibration level 6 ($0.32 \mu g/mL$) and run to evaluate analytical precision and percent recovery. LI-syringe-GC-MS method precision was determined from seven different aliquots of cannabis shake extract and three different cannabis flower chemovars were tested to determine terpene content.

GC-MS Method Conditions for the Analysis of Terpenes in Cannabis

See Table IV and Table V for GC-MS selected ion monitoring (SIM) conditions.

Table IV: GC-MS Instrument Parameters

Thermo Scientific Trace 1310/TSQ 90	DO Parameters
Column	Rxi-624Sil MS, 30 m x 0.25 mm x 1.4 μm (cat.# 13868)
Injection	Liquid injection (LI-syringe)
Inj. Vol.	1 µL
Mode	Split (20:1)
Liner	Topaz 4.0 mm Precision liner w/ wool (cat.# 23305)
Inj. Temp.	280 °C
Purge Flow	5 mL/min
Oven	80 °C to 130 °C at 20 °C/min (hold 4 min) to 275 °C at 17 °C/min (hold 0.47 min)
Carrier Gas	He, constant flow
Flow Rate	1.5 mL/min
Detector/Mode	Single quad/SIM
Transfer Line Temp.	275 °C
Ion Source Temp.	275 °C



Table V: MS SIM Parameters

#	Name	Classification	Retention Time (min)	Masses
1	lpha-Pinene	monoterpenoid	4.52	91, 92.1, 93.1
2	Camphene	monoterpenoid	4.88	79.1, 93.1, 121
3	β-Myrcene	monoterpenoid	5.2	93.1
4	β -Pinene	monoterpenoid	5.39	79.1, 93.1
5	Carene	monoterpenoid	5.81	77, 93.1
6	α -Terpinene	monoterpenoid	5.99	77, 93.1
7	trans-Ocimene	monoterpenoid	6.12	93.1
8	D-Limonene	monoterpenoid	6.23	93.1
9	<i>p</i> -Cymene	monoterpenoid	6.33	119
10	cis-Ocimene	monoterpenoid	6.44	93.1
11	Eucalyptol (1,8-cineole)	monoterpenoid	6.57	81
12	γ-Terpinene	monoterpenoid	6.87	91, 93.1
13	Terpinolene	monoterpenoid	7.49	91, 93.1
14	Linalool	monoterpenoid	8.16	91, 92.1, 93.1
15	Isopulegol	monoterpenoid	9.19	67, 69.1
16	Naphthalene-d8 (ISTD)	NA	9.83	136
17	Geraniol	monoterpenoid	10.51	67.1, 69.1
18	β -Caryophyllene	sesquiterpenoid	12.19	91, 93.1
19	lpha-Humulene	sesquiterpenoid	12.56	91, 92.1, 93.1
20	cis-Nerolidol	sesquiterpenoid	13.15	79.1, 91, 93.1
21	trans-Nerolidol	sesquiterpenoid	13.42	79.1, 91, 93.1
22	Guaiol	sesquiterpenoid	13.95	93.1, 161
23	(-)-Caryophyllene oxide	sesquiterpenoid oxide	14.12	69.1, 79.1, 93.1
24	lpha-Bisabolol	sesquiterpenoid	14.52	93.1, 119

Results and Discussion

LI-Syringe-GS-MS Method Verification

As shown in Figure 1, all 23 target compounds were separated on an Rxi-624Sil MS column using the method developed here for the analysis of terpenes in cannabis. Method performance can be seen in Table VI. Over the calibration range of 0.04–5.12 μ g/mL, an average r² value of 0.993 was achieved for the 23 terpenes of interest. Two terpenes (i.e., α -bisabolol and β -caryophyllene) had the shortest calibration range spanning from 0.16–5.12 μ g/mL, but maintained good r² values of 0.990 and 0.992, respectively. An average LOQ of 0.047 μ g/mL was achieved as well as average analytical and method precision %RSDs of 1.56% and 4.97% respectively. Percent recoveries for all 23 terpenes met acceptance criteria (±30%) and ranged from 84.6% to 98.9% with an average of 90.2%.





Table VI: LI-Syringe-GC-MS Method Validation

Compound	Retention Time (min)	Working Range (µg/mL)	r²	LOQ (µg/mL)	Analytical Precision (%RSD)	Method Precision (%RSD)	Recovery (%)
α -Pinene	4.263	0.08 - 5.12	0.994	0.041	1.36	8.52	93.6
Camphene	4.581	0.04 - 5.12	0.996	0.038	1.25	5.17	93.6
β-Myrcene	4.928	0.08 - 5.12	0.992	0.046	1.51	3.17	91.6
β-Pinene	5.020	0.08 - 5.12	0.998	0.042	1.40	6.20	88.8
Carene	5.456	0.08 - 5.12	0.995	0.057	1.90	4.84	91.2
α -Terpinene	5.629	0.04 - 5.12	0.992	0.036	1.19	5.56	88.0
trans-Ocimene	5.736	0.04 - 5.12	0.995	0.022	0.71	3.35	88.8
D-Limonene	5.851	0.04 - 5.12	0.994	0.027	0.91	5.25	88.8
<i>p</i> -Cymene	5.908	0.04 - 5.12	0.994	0.040	1.32	ND	88.9
cis-Ocimene	6.053	0.02 - 5.12	0.993	0.017	0.55	3.04	88.4
Eucalyptol (1,8-Cineole)	6.142	0.04 - 5.12	0.996	0.032	1.07	9.04	91.2
γ-Terpinene	6.440	0.04 - 5.12	0.993	0.033	1.09	6.51	88.7
Terpinolene	7.112	0.04 - 5.12	0.993	0.035	1.16	4.35	90.1
Linalool	7.795	0.08 - 5.12	0.991	0.076	2.53	2.99	90.2
Isopulegol	8.848	0.04 - 5.12	0.993	0.029	0.95	3.31	90.2
Naphthalene-d8 (ISTD)	9.531	NA	NA	NA	NA	NA	NA
Geraniol	10.218	0.04 - 5.12	0.988	0.039	1.29	3.31	88.2
β -Caryophyllene	12.042	0.16 - 5.12	0.992	0.129	4.27	3.90	91.6
lpha-Humulene	12.384	0.04 - 5.12	0.991	0.027	0.91	3.88	86.7
cis-Nerolidol	12.940	0.08 - 5.12	0.994	0.080	2.67	14.57	89.3
trans-Nerolidol	13.192	0.08 - 5.12	0.989	0.050	1.65	2.58	98.9
Guaiol	13.720	0.08 - 5.12	0.993	0.060	1.98	3.27	85.9
(-)-Caryophyllene Oxide	13.816	0.04 - 5.12	0.993	0.030	0.98	1.73	84.6
α -Bisabolol	14.296	0.16 - 5.12	0.990	0.094	3.11	4.82	96.1
		Average	0.993	0.047	1.56	4.97	90.2

ND – Not Detected NA – Not Applicable

Analysis of Terpenes in Cannabis Flower

Once the LI-syringe-GC-MS method was established, the cannabis chemovar "mint chocolate chip" was tested for terpene content. The aforementioned workflow was applied to this cannabis flower sample, and the results, corrected for ASE extraction and dilution, are shown in Table VII. Of the 23 terpenes analyzed, 22 were found in the mint chocolate chip chemovar. p-Cymene was not detected (ND) in the sample, and two terpenes of interest (carene and guaiol) were reported at concentrations that fell outside of their calibration ranges.



Table VII: Mint Chocolate Chip Chemovar Terpene Results

#	Compound	LI-Syringe-GC-MS (µg/g)	
1	α -Pinene	32.2	
2	Camphene	3.84	
3	β-Myrcene	8.24	
4	β -Pinene	25.7	
5	Carene*	1.59	
6	α -Terpinene	1.95	
7	trans-Ocimene	2.02	
8	D-Limonene	52.1	
9	p-Cymene	ND	
10	cis-Ocimene	4.55	
11	Eucalyptol (1,8-Cineole)	1.46	
12	γ-Terpinene	1.97	
13	Terpinolene	2.21	
14	Linalool	106	
15	Isopulegol	3.96	
16	Naphthalene-d8 (ISTD)	NA	
17	Geraniol	4.45	
18	β -Caryophyllene	45.6	
19	α -Humulene	14.8	
20	<i>cis</i> -Nerolidol	25.4	
21	trans-Nerolidol	9.46	
22	Guaiol*	1.61	
23	(-)-Caryophyllene Oxide	2.97	
24	α-Bisabolol	14.7	
	Average	16.7	
*Concentra	tion outside of calibration range for LI-Syringe-	GC-MS	



Figure 2 is a graphical representation of the most abundant terpenes found in the mint chocolate chip chemovar. Linalool was reported with the highest concentration (106 μ g/g) making up 29% of the total terpene content. Other terpenes at higher percentages include D-limonene (14%), β -caryophyllene (12%), α -pinene (9%), and β -pinene (7%).



Conclusion

A method was developed for the analysis of terpenes in cannabis flower via LI-syringe-GC-MS using SIM. This workflow provided good results for both monoterpenes and the more challenging sesquiterpenes as demonstrated by an average r^2 value of 0.993; LOQ of 0.047 µg/mL; analytical precision of 1.56 %RSD; method precision of 4.97 %RSD; and recovery of 90.2%. It should be noted that this workflow used accelerated solvent extraction to reduce labor and materials, but previous work demonstrated that hand-shakeout extractions showed negligible differences and can also be applied to this method [3]. Additionally, this work included the analysis of terpenes in cannabis flower extracts because there is great interest in this area. Finally, when testing cannabis products, careful consideration should be given to utilizing matrix-matched calibrations as was done here to mitigate signal enhancement/suppression caused by matrix effects.

Additional Considerations

Since the development of this workflow, additional work was done to expand the number of terpenes that could be tested in cannabis and similar matrices. The current sample preparation workflow is anticipated to be suitable for the expanded terpenes list, but interested labs should verify this independently and note that the analytical methods use different GC columns and conditions. The expanded terpenes analysis uses an Rxi-1301Sil MS column (cat.# 16094) and terpene MegaMix #1 & 2 standards (cat.# 34142 & 34143). Chromatographic separation and conditions for the expanded list are shown in Figure 3.







References

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Rxi-624Sil MS Column

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Catalog No.	df (Film Thickness)	Internal Diameter (ID)	Length	Units
13868	1.4 µm	0.25 mm	30 m	ea.

Rxi-1301Sil MS Column

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Cannabis Terpenes Standard #1

(19 components)

(-)- α -Bisabolol (23089-26-1) Camphene (79-92-5) δ -3-Carene (13466-78-9) β -Caryophyllene (87-44-5) Geraniol (106-24-1) (-)-Guaiol (489-86-1) α -Humulene (6753-98-6) p-Isopropyltoluene (p-cymene) (99-87-6) (-)-Isopulegol (89-79-2) d-Limonene (5989-27-5) Linalool (78-70-6) β-Myrcene (123-35-3) Nerolidol (7212-44-4) Ocimene (13877-91-3) $\begin{array}{l} \alpha \text{-Pinene (80-56-8)} \\ (-)-\beta \text{-Pinene (18172-67-3)} \\ \alpha \text{-Terpinene (99-86-5)} \\ \gamma \text{-Terpinene (99-85-4)} \\ \text{Terpinolene (586-62-9)} \end{array}$

Catalog No.	Concentration	Solvent	Volume	Unit
34095	2500 μg/mL	Isopropanol	1 mL/ampul	ea.

Cannabis Terpenes Standard #2

(2 components)

(-)-Caryophyllene oxide (1139-30-6) 1,8-Cineole (Eucalyptol) (470-82-6)

Catalog No.	Concentration	Solvent	Volume	Unit
34096	2500 µg/mL	Isopropanol	1 mL/ampul	ea.

Terpenes MegaMix Standard #1

(43 components)

$\begin{array}{l} (-)-\alpha-\text{Bisabolol} (23089-26-1) \\ (+)-Borneol (464-43-7) \\ L(-)-Borneol (464-45-9) \\ Camphene (79-92-5) \\ 3-Carrene (498-15-7) \\ Carvacrol (499-75-2) \\ \beta-Caryophyllene (87-44-5) \\ (-)-a-Cedrene (469-61-4) \\ Cedrol (77-53-2) \\ 1,8-Cineole (Eucalyptol) (470-82-6) \\ Citronellol (106-22-9) \\ trans-B-Farnesene (18794-84-8) \\ Farnesol (4602-84-0) \\ (1R)-endo-(+)-Fenchyl alcohol (2217-02-9) \\ Geraniol (106-24-1) \end{array}$	$\begin{array}{l} (-)-\text{Guaiol} (489-86-1) \\ \alpha-\text{Humulene} (6753-98-6) \\ \text{Isoborneol} (124-76-5) \\ m-\text{Isopropyltoluene} (535-77-3) \\ o-\text{Isopropyltoluene} (527-84-4) \\ p-\text{Isopropyltoluene} (p-Cymene) (99-87-6) \\ (-)-\text{Isopulegol} (89-79-2) \\ d-\text{Limonene} (5989-27-5) \\ \text{Linalool} (78-70-6) \\ \text{Menthol} (89-78-1) \\ \beta-\text{Myrcene} (123-35-3) \\ \text{Nerol} (106-25-2) \\ \text{Nerolidol} (7212-44-4) \\ \text{Ocimene} (13877-91-3) \\ \text{R}-(-)-\alpha-\text{Phellandrene} (4221-98-1) \end{array}$	Phytane (2,6,10,14-Tetramethylhexadecane) (638-36-8) α -Pinene (80-56-8) (-)- β -Pinene (18172-67-3) Sabinene (1387-41-5) Sabinene Hydrate (546-79-2) Squalene (111-02-4) Terpinen- (4-01 (562-74-3) α -Terpinene (99-86-5) γ -Terpinene (99-85-4) a-Terpinel (98-85-5) Terpinolene (586-62-9) Thymol (89-83-8) Valencene (4630-07-3)
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Catalog No.	Concentration	Solvent	Volume	Unit
34142	2500 μg/mL	Isopropanol	1 mL/ampul	ea.

Note: Terpenes MegaMix Standard #1 (cat.# 34142) and Terpenes MegaMix Standard #2 (cat.# 34143) have been formulated in different but compatible GC-amenable solvents for specific product stability purposes.

Terpenes MegaMix Standard #2

(16 components)

Camphor (76-22-2) (S)-(+)-Carvone (2244-16-8) (-)-Caryophyllene oxide (1139-30-6) Citral (5392-40-5) (1R)-(-)-Fenchone (7787-20-4) Geranyl acetate (105-87-3)	Isoborny Menthon (+)-Noot Octyl ace Piperiton (R)-(+)-P	acetate (125-12-2) e (10458-14-7) katone (4674-50-4) :tate (112-14-1) e (89-81-6) ulegone (89-82-7)	Safranal (116-26-7) (-)-α-Thujone (546 d-Valerolactam (67! (-)-Verbenone (119)	-80-5) 5-20-7) 6-01-6)	
Catalog No.	Concentration	Solvent	Volume	Unit	
34143	2500 µg/mL	Toluene	1 mL/ampul	ea.	

 34143
 2500 µg/mL
 Toluene
 1 mL/ampul
 ea.

 Note: Terpenes MegaMix Standard #1 (cat.# 34142) and Terpenes MegaMix Standard #2 (cat.# 34143) have been formulated in different but compatible GC-amenable solvents for specific product stability purposes.
 ea.

Naphthalene-d8				
Concentration	Solvent	Volume	Unit	
2000 µg/mL	Methylene chloride	1 mL/ampul	ea.	
	d8 Concentration 2000 µg/mL	d8 <u>Concentration</u> Solvent 2000 µg/mL Methylene chloride	Concentration Solvent Volume 2000 µg/mL Methylene chloride 1 mL/ampul	







Extraction Cell Body, 10 mL, for ASE 150/350

Catalog No.	Product Name	Units
25995	Extraction Cell Body, 10 mL, for ASE 150/350	ea.



Norm-Ject Plastic Syringe, 3 mL Luer Lock Tip

Catalog No.	Product Name	Units
22773	Norm-Ject Plastic Syringe, 3 mL Luer Lock Tip	100-pk.



13 mm Syringe Filter, 0.22 um, PTFE, White

Catalog No.	Product Name	Units
26142	13 mm Syringe Filter, 0.22 µm, PTFE	100-pk.



2.0 mL, 9 mm Short-Cap, Screw-Thread Vials (vial only)

	•	
Catalog No.	Product Name	Units
21142	Short-Cap Vial with Grad Marking Spot, 9-425 Screw-Thread, 2.0 mL, 9 mm, 12 x 32 (vial only), Amber	100-pk.



Syringe, Hamilton 1001, PTFE Tip, Gas-Tight

Catalog No.	Product Name	Units
24575	Syringe, Hamilton 1001 (1 mL/LTN/22/2"/2pt), PTFE Tip, Gas-Tight	ea.



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