Application Note

Food Testing &
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Confirmation of Sample Identity During Worklist Execution

Using the Agilent InfinityLab Sample ID Reader with Agilent MassHunter Software for measurement of pesticides in hemp products

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Abstract

With the release of the Agilent Infinity III LC series, the Agilent 1290 Infinity III Multisampler may be equipped with an optional Agilent InfinityLab Sample ID Reader. Sample IDs can be introduced with an external barcode reader, or just with spreadsheets containing appropriate sample barcode information. This application note shows how sample tracking to confirm the analysis of each scheduled sample is made possible by Agilent MassHunter Software for LC/MS sample measurements. This note highlights the benefits for the analytical workflow when an autosampler with the Sample ID Reader is used. This saves time, enables higher ease-of-use with fewer errors, and allows unequivocal mapping of sample identity with the analytical result. As an example, the determination of pesticides in edible hemp products will be demonstrated.

Introduction

With the increasing use of hemp products, the analysis of products like hemp leaf tea or hemp seed have recently gained attention. In addition to analyzing the natural ingredient profile, the analysis of contamination and residues such as pesticides is required. One regulation of note is the Health Canada Regulation for pesticides in hemp.[1] The accessibility of the quantification limits for regulated pesticides in hemp was demonstrated in another Agilent application note.[2] The typical reporting limits given by Health Canada are between 20 and 100 ppb, and require quantification limits in the ppt range. It was shown that this requirement could be met, with recoveries typically in the 100 ± 10% range by the method described in the Agilent application note.

Product recalls due to pesticide contamination have become a significant concern in the global food industry. In recent years, the frequency and scale of food recalls have surged, driven by stricter regulatory standards and increased consumer awareness. The financial impact of such recalls can be profound, encompassing direct costs such as product retrieval, disposal, and legal fees, as well as indirect costs including brand damage, loss of consumer trust, and market share decline.[3,4,5] The reputational damage from recalls can have long-lasting effects, making it imperative for companies to invest in preventive measures and advanced technologies to detect and mitigate contamination risks[5,6], as well as minimizing false positives and false negatives. In this application note, we will demonstrate the use of the Agilent InfinityLab Sample ID Reader, vials coded with a data matrix code on the bottom, and a software workflow to prevent sample mix-up. This helps to avoid time-consuming confirmatory measurements and prevents false positive and false negative reporting of pesticide residues in hemp products.

Experimental

Instrumentation

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Multisampler (G7167B) equipped with an Agilent InfinityLab Sample ID Reader (G4756A)
- Agilent 1290 Infinity III MCT (G7116B)
- Agilent Ultivo Triple Quadrupole LC/MS with Agilent Jet Stream source

Software

- Agilent MassHunter Acquisition Software (v. 12.2)
- Agilent MassHunter Qualitative Analysis Software (v. 12.0)
- Agilent MassHunter Quantitative Analysis Software (v. 12.1)

Column

Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7, µm (part number 695975-312)

Table 1. UHPLC method parameters.

Parameter	Value
Pump	
Flow Rate	0.5 mL/min
Solvents	A) 5 mM Ammonium formate + 0.1 % formic acid in water B) 0.1 % Formic acid in 90:10 methanol/acetonitrile
Gradient	Time (min) %B 0.00 50 1.00 50 8.00 95 9.00 100 10 100 Stop time: 10 min Post time: 2 min
Column	
Temperature	55 °C
Multisampler Needle Wash	3 sec acetonitrile

Table 2. MS method parameters.

Parameter	Value
Acquisition Mode	dMRM, all transitions with molecular weights, fragments, voltages and collision energies are given in another application note[1]
Polarity	Positive or Negative (compound-dependent)
Capillary Voltage	4,000 V in positive mode, 3,000 V in negative mode
Drying Gas Flow	10 L/min
Drying Gas Temperature	200 °C
Nebulizer Pressure	35 psi
Sheath Gas Temperature	200 °C
Sheath Gas Flow	10 L/min
Nozzle Voltage	300 V (either polarity)
Q1 and Q2 Resolution	Unit (0.7 amu), optimized by autotune
Delta EMV	0 V

Chemicals

In this study, 5 M ammonium formate solution (G1946-85021) and an amount of formic acid for LC/MS (G2453-85060) were used.

Standards

The standards used included the Canada Cannabis Pesticide Kit (2020), in 5 × 1 mL Submixes (part number PST-CBS-CAN).

Calibration

From a 1 ppm stock solution comprising all pesticides, the following concentrations were diluted for preparing calibration curves: 25, 10, 5.0, 2.5, 1.0, 0.75, 0.50, 0.25, and 0.10 ppb. As dilution solvent, a 50:50 (v/v) mixture of mobile phase A and B or clean matrix extract was used. The detailed dilution pattern is described in another application note.[1]

Samples

Hemp seeds and hemp leaf tea were both purchased from a local store.

Sample preparation[1]

1. Weigh 1.0 g of material into a 50 mL tube. Add two ceramic homogenizer pellets (part number 5982-9313).
2. Add 15 mL of ACN and shake for five minutes at high speed.
3. Decant the supernatant solvent into an unconditioned Agilent SampliQ C18 EC cartridge (part number 5982-1365). Keep the 50 mL tube with pellet for Step 4. Gravity elute into a clean 50 mL tube.
4. Add 5 mL of ACN and shake for five minutes at high speed.
5. Decant the supernatant solvent into the SampliQ C18 EC SPE cartridge used in Step 3. Gravity elute. Keep the 50 mL tube with pellet for Step 6.
6. Rinse the tube with 5 mL of ACN and pass the supernatant through to the same SPE cartridge.
7. Bring the collected eluent (extract) up to 25 mL with ACN (25-fold dilution).
8. Mix 50 µL of extract with 450 µL of 50:50 mobile phase A:mobile phase B (v/v) in a 1.5 mL tube (250-fold dilution). Vortex for 10 seconds, then centrifuge at 14,000 rpm for five minutes.
9. Transfer to a vial. Samples are ready for LC/MS/MS analysis.

Additional materials

- Vial, screw, amber, write-on with data matrix code, certified, 2 mL (part number 5182-0716-ID)
- Blue screw caps, PTFE/silicone septa (part number 5190-3156)
- Forty-vial sample container with bottom holes for data code reading (part number 5401-0068)
- Sample tray palette with open bottom for data code reading (G7167-60205)
- USB handheld barcode scanner (part number 5018-0003)

Solvents

- Agilent InfinityLab Methanol for LC/MS (part number 5191-5111-001)
- Agilent InfinityLab Acetonitrile for LC/MS (part number 5191-5101-001)
- Agilent InfinityLab Water for LC/MS (part number 5191-5121-001)

Results and discussion

For quantitative determination of pesticides in hemp products, a calibration for 120 pesticide compounds in the concentration range of 0.1 to 25 ppb was created as described in the experimental section. The linearity coefficients were typically > 0.999. An overlay of the MRM transitions for all compounds on a concentration level of 1 ppb is shown in Figure 3.

To demonstrate the confirmation workflow[7,8,9], including reporting analytical data in combination with mapping of sample identification via the Sample ID Reader, real samples from three different hemp seed and hemp leaf tea products were used. The samples were spiked with 50 ppb of the pesticide mixture and prepared as described in the experimental section with a final 250-fold dilution.

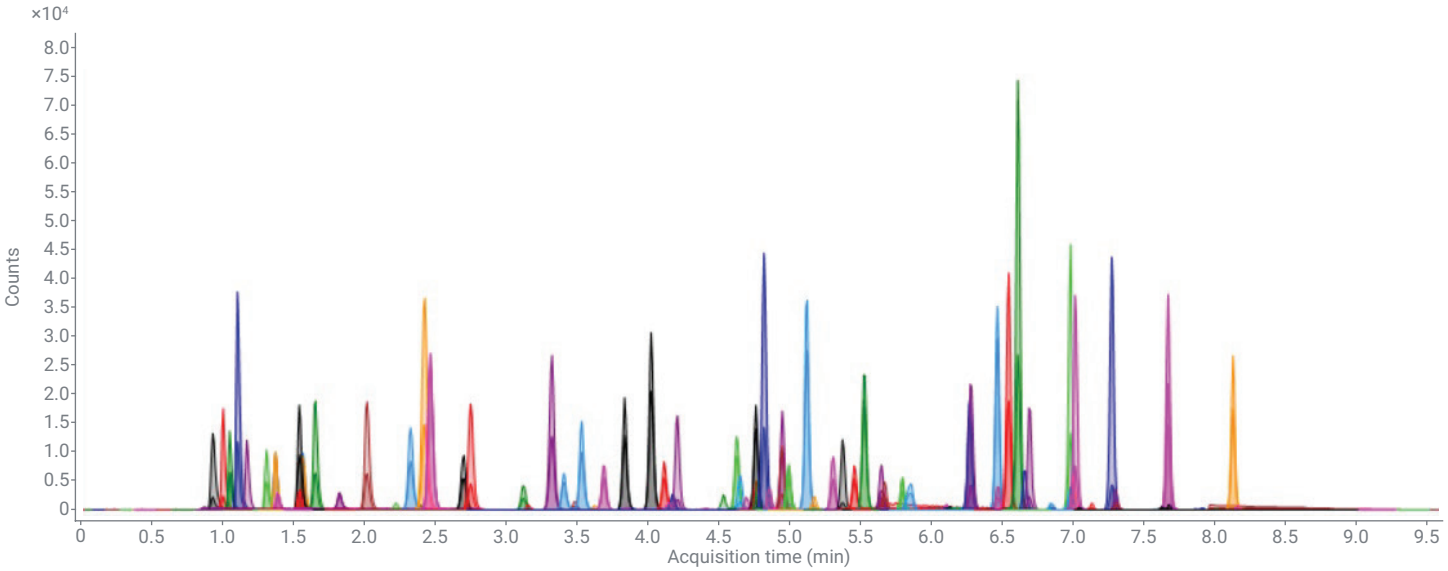

Figure 1. Overlay of all pesticide MRM transitions used in the described multimethod at a concentration of 1 ppb.

Before setting up the sample sequence table, the Sample ID Reader must be enabled for barcode verification in the Worklist Run Parameters window (Figure 2). In addition, the handling of samples with mismatching data codes can be chosen. With the insertion of the sample trays loaded with the coded sample vials, all vials will be simultaneously automatically scanned from the bottom, and information is saved for the next step.

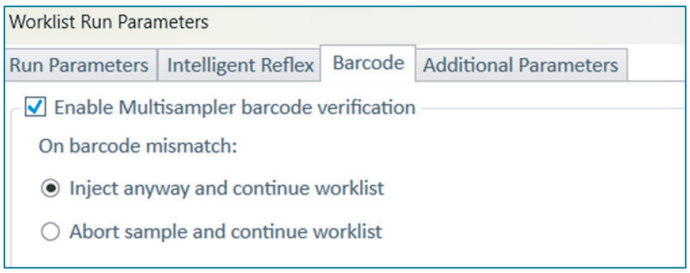

Figure 2. Agilent MassHunter run parameters for the Agilent InfinityLab Sample ID Reader installed with the Agilent 1290 Infinity III Multisampler.

In the sequence table, the sample name, acquisition method, data filenames, and the sample positions must be given. The expected barcode can be scanned into the respective cell using a handheld barcode reader. The vials must be placed in the sample rack according to their expected barcode and defined sample location (Figure 3A). The current status of each sample is given in an additional status column, and the cells for "Barcode status" and "Barcode (Actual)" remain empty. With this setup, the worklist in MassHunter software can be started. At the start of the run, the expected barcode of the respective vial is compared with the information acquired by the Sample ID Reader (actual barcode) and displayed in the worklist together with the status as "Matched" or "Not Matched" (Figures 3B and 4, respectively). In this experiment, after the worklist completed, all sample measurements were marked as completed and all actual barcodes matched with expected barcodes (Figure 3C).

A

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Pending	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-01.d	3613010532		
2	Pending	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-01.d	361301052K		
3	Pending	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-01.d	3613010525		
4	Pending	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-01.d	361301052U		
5	Pending	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-01.d	361301052P		
6	Pending	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-01.d	3613010512		

B

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1>	Acquiring	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-01.d	3613010532	Matched	3613010532
2	Pending	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-01.d	361301052K		
3	Pending	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-01.d	3613010525		
4	Pending	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-01.d	361301052U		
5	Pending	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-01.d	361301052P		
6	Pending	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-01.d	3613010512		

C

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Completed	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-01.d	3613010532	Matched	3613010532
2	Completed	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-01.d	361301052K	Matched	361301052K
3	Completed	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-01.d	3613010525	Matched	3613010525
4	Completed	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-01.d	361301052U	Matched	361301052U
5	Completed	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-01.d	361301052P	Matched	361301052P
6	Completed	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-01.d	3613010512	Matched	3613010512

Figure 3. Sample sequence table with sample and barcode status. (A) Sample sequence with scanned vial codes in the column "Barcode (Expected)"; cells for "Barcode Status" and "Barcode (Actual)" are still empty. (B) Start of acquisition of the sample in position A1 with confirmation of matched barcode in the column for "Barcode (Expected)" and "Barcode (Actual)". (C) End of sequence; all locations and sample IDs were confirmed and the expected barcodes matched with the actual barcodes.

In case a match between the expected barcode and the actual barcode fails, this will be displayed in the sequence table (Figure 4). Figures 4A and 4B show two possible scenarios. In Figure 4A, one sample acquisition was marked due to a vial present at the sample location with a nonmatching barcode. Figure 4B shows the case of two misplaced samples. Both samples were measured, but both barcodes were marked as not matching. This error can be resolved by comparing the actual barcodes with the expected barcodes. Since the samples were measured even with the mismatch, the data were acquired, and the correct data set can be assigned manually without loss of time for an additional acquisition.

A

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Completed	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-02.d	3613010532	Matched	3613010532
2	Completed	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-02.d	361301052K	Matched	361301052K
3	Completed	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-02.d	3613010525	Matched	3613010525
4	Acquisition Failed	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-02.d	361301052U	Not Matched	361301052I
5	Completed	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-02.d	361301052P	Matched	361301052P
6	Completed	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-02.d	3613010512	Matched	3613010512

B

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Completed	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-03.d	3613010532	Matched	3613010532
2	Completed	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-03.d	361301052K	Matched	361301052K
3	Completed	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-03.d	3613010525	Matched	3613010525
4	Acquisition Failed	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-03.d	361301052U	Not Matched	361301052P
5	Acquisition Failed	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-03.d	361301052P	Not Matched	361301052U
6	Completed	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-03.d	3613010512	Matched	3613010512

Figure 4. Sample sequence table with sample and barcode status. (A) One actual barcode is mismatched with the expected barcode due to an incorrectly placed coded vial, which is not part of the sequence. (B) Two actual barcodes are mismatched due to a misplacement of the vials. Comparison of actual barcode, expected barcode, and sample position identify the error.

Finally, a worklist report was generated for the cases shown in Figures 3C and 4B. The sample table in the respective worklist reports are shown in Figures 5A and 5B.

Figure 5A shows the worklist report table, confirming that all samples with expected barcodes were identified at the right position and were measured. Figure 5B shows a worklist table with two mismatching sample vials, where the expected barcode was not confirmed for the sample in the given position. Even so, the samples were measured according to the settings given in the worklist run parameters list (Figure 2).

A

Worklist Table

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Completed	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-01.d	3613010532	Matched	3613010532
2	Completed	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-01.d	361301052K	Matched	361301052K
3	Completed	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-01.d	3613010525	Matched	3613010525
4	Completed	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-01.d	361301052U	Matched	361301052U
5	Completed	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-01.d	361301052P	Matched	361301052P
6	Completed	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-01.d	3613010512	Matched	3613010512

B

Worklist Table

	Status	Sample Name	Sample Position	Method	Data File	Barcode (Expected)	Barcode Status	Barcode (Actual)
1	Completed	HempLeaf-1	P1-A1	Hemp_Pestizides-3.m	Hemp-Leaf-1-03.d	3613010532	Matched	3613010532
2	Completed	HempLeaf-2	P1-A2	Hemp_Pestizides-3.m	Hemp-Leaf-2-03.d	361301052K	Matched	361301052K
3	Completed	HempLeaf-3	P1-A3	Hemp_Pestizides-3.m	Hemp-Leaf-3-03.d	3613010525	Matched	3613010525
4	Acquisition Failed	HempSeed-1	P1-B1	Hemp_Pestizides-3.m	Hemp-Seed-1-03.d	361301052U	Not Matched	361301052P
5	Acquisition Failed	HempSeed-2	P1-B2	Hemp_Pestizides-3.m	Hemp-Seed-2-03.d	361301052P	Not Matched	361301052U
6	Completed	HempSeed-3	P1-B3	Hemp_Pestizides-3.m	Hemp-Seed-3-03.d	3613010512	Matched	3613010512

Figure 5. Worklist reports created from the scenarios shown in Figures 3C and 4B. (A) Worklist completed with all barcodes matching. (B) Worklist containing two samples with a barcode mismatch.

In the measured hemp tea leaf samples, the resulting MRM of a spike sample of 50 ppb Fipronil is shown in Figure 6. The Health Canada reporting limit for Fipronil is 60 ppb.[1,2] The measured concentration in hemp seeds and the hemp tea leaves was 0.1983 ppb and 0.1921 ppb, respectively. With the 250-fold dilution during sample preparation, the resulting values are 49.58 and 48.02 ppb, respectively, which is within the typically accepted window of –20 to +30% recovery. The respective R^2 value of the Fipronil calibration curve was 0.9998, and the calculated LOQ in hemp leaves was 4 ppb. The measurement of a blank hemp tea leaf matrix sample showed no residual Fipronil at the retention time of 5.181 minutes (Figure 6C).

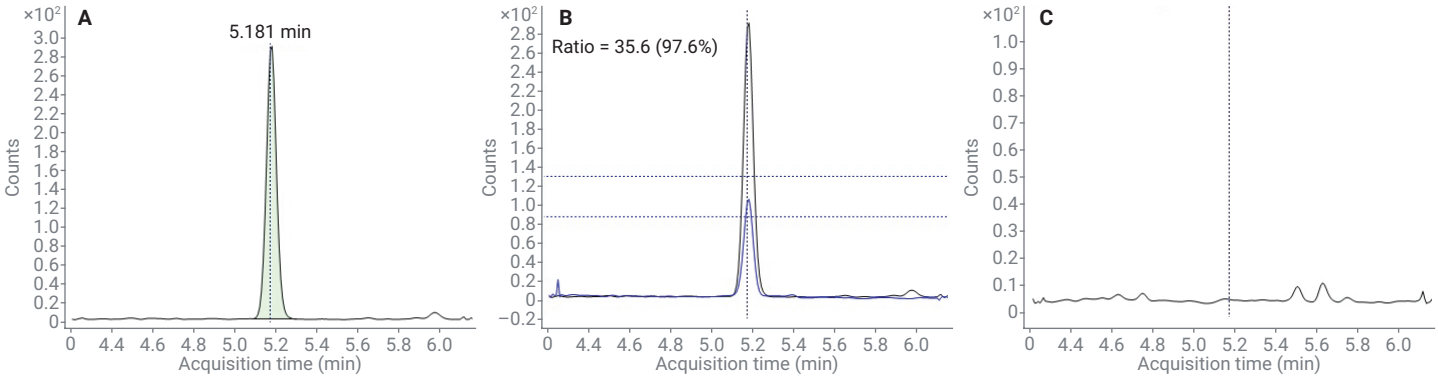

Figure 6. Spike sample of 50 ppb Fipronil in hemp tea leaves (MRM of Fipronil, retention time 5.181 minutes). (A) Quantifier transition. (B) Qualifier transition and quantifier/qualifier ratio. (C) Blank matrix of hemp tea leaves, quantifier transition.

Conclusion

This application note demonstrates the use of the Agilent Sample ID Reader in tandem with the Agilent 1290 Infinity III Multisampler for confirmation of sample identification. With this setup, vials with matrix data codes at their bottom can be used for confirmation of analytical data in combination with the expected sample ID at a given position in a sequence. For reporting, a worklist report can be generated. This avoids errors in sample handling and sequence setup, and saves time and money for confirmatory measurements, which significantly reduces the risk for false positive and false negative reporting. The measurement of the samples (hemp seed and hemp tea leaves) showed excellent recoveries typically within ± 10%. The linearity was typically greater than 0.9990, with limit of detection values less than 5 ppb in matrix.

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