Introduction

Demand for quick turnaround time in cannabis pesticide and mycotoxin testing laboratories has never been higher as cannabis and hemp markets mature. To remain competitive in a growing market, laboratories must continue to optimize their workflows to reduce their turn around time for their clients.

Due to the complexity of the reagents and consumables required for cannabis and hemp pesticide residue testing significant time is currently required for method development, procuring reagents and consumables, storing multiple shipments, and preparing calibration and QC standards. This can be especially true in high-volume laboratories running hundreds, if not thousands, of samples per month.

To help laboratories reduce their turn around time, and improve their overall efficiency and data quality, PerkinElmer has developed the ONE Pesticide(TM) ISO 17034 Reagent Kit (Part Number: COP-001KT) for labs that operate in accordance with the California, Oregon, or equivalent regulatory requirements. This comprehensive kit supplies laboratories with pre- aliquoted pesticide and mycotoxin calibration reagents specifically created with ease-of-use in mind. It also includes isotopically labeled internal standards and quality control standards. In this note, we investigate the amount of time saved following the ONE Pesticide(TM) ISO 17034 Reagent Kit workflow in contrast to the existing analytical workflow.
Results and Discussion

Saving Preparation Time with PerkinElmer’s ONE Pesticide®420™ Reagent Kit

In this study, multiple pesticide and mycotoxin testing laboratories compared the amount of time to analysis using their existing calibration and QC reagent preparation protocol to using the ONE Pesticide®420™ Reagent Kit. On average, laboratories reported up to 3X faster calibration standard preparation protocol when using PerkinElmer’s preconfigured Kit than when using traditional workflows. Remarkably, some laboratories reported that it took only three minutes to prepare the eight calibration vials for LC-MS/MS analysis. Figure 1 highlights the workflow typically carried out for calibration standard preparation in comparison to the workflow necessary using the ONE Pesticide®420™ Reagent Kit.

Outside the scope of simple calibration standard and QC sample preparation, time and effort required to procure separate analytical standards, internal standards, solvents and consumables is significantly minimized using the ONE Pesticide®420™ Reagent and Consumable Kit, seen below in Figure 2. Rather than taking the time to source these supplies from multiple vendors, the ONE Pesticide®420™ Reagent Kit provides standards and, internal standards consumables for sample pesticide and mycotoxin quantitation. The ONE Pesticide®420™ Reagent Kit saves time and enables laboratories to allocate valuable employee resources toward activities other than ordering and preparing reagents.

Figure 1. Workflow comparison showing calibration standard preparation time using existing workflows and PerkinElmer’s ONE Pesticide®420™ Kit. Existing workflows require sourcing materials and reagents from multiple vendors while using the ONE Pesticide®420™ Kit only requires coordination with one vendor.

Figure 2. The components of PerkinElmer ONE Pesticide®420™ reagent and consumable kit.
Generating Calibration Curves Using ONE Pesticide® Reagent Kit

To demonstrate the performance of the ONE Pesticide® Reagent Kit, calibration curves for each pesticide and mycotoxin standard were prepared using LC-MS/MS. The linearity is displayed below and shows R² values greater than 0.99 for all analytes. For reference, the California Bureau Cannabis Control (BCC) requires R² values to be greater than 0.99. This is achieved and shown below for the ONE Pesticide® Reagent Kit.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>0.9965</td>
</tr>
<tr>
<td>Acephate</td>
<td>0.9980</td>
</tr>
<tr>
<td>Acequinocyl</td>
<td>0.9984</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>0.9988</td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>0.99944</td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>0.99977</td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>0.99986</td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>0.99986</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>0.9996</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.9996</td>
</tr>
<tr>
<td>Bifenazate</td>
<td>0.9987</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.9990</td>
</tr>
<tr>
<td>Boscalid</td>
<td>0.9992</td>
</tr>
<tr>
<td>Captan</td>
<td>0.9932</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.9996</td>
</tr>
<tr>
<td>Carbafuran</td>
<td>0.9991</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>0.9992</td>
</tr>
<tr>
<td>Chlorfenapy</td>
<td>0.9998</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.9995</td>
</tr>
<tr>
<td>Cinerin-I</td>
<td>0.9996</td>
</tr>
<tr>
<td>Clofentezine</td>
<td>0.9992</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>0.9971</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

Table 1. Observed R² values for pesticides and mycotoxins generated using the eight point calibration curve provided in the ONE Pesticide® Reagent Kit. Calibration curve levels are at 1, 5, 10, 25, 50, 100, 150 and 250 ppb.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Observ (ppb)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>79.39</td>
<td>105.9</td>
</tr>
<tr>
<td>Acephate</td>
<td>75.75</td>
<td>101.0</td>
</tr>
<tr>
<td>Acequinocyl</td>
<td>76.41</td>
<td>101.9</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>74.71</td>
<td>99.6</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>74.59</td>
<td>99.5</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>75.43</td>
<td>100.6</td>
</tr>
<tr>
<td>Bifenazate</td>
<td>71.57</td>
<td>95.4</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>77.62</td>
<td>103.5</td>
</tr>
<tr>
<td>Boscalid</td>
<td>75.07</td>
<td>100.1</td>
</tr>
<tr>
<td>Captan</td>
<td>79.7</td>
<td>106.3</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>74.31</td>
<td>99.1</td>
</tr>
<tr>
<td>Carbafuran</td>
<td>74.46</td>
<td>99.3</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>73.92</td>
<td>98.6</td>
</tr>
<tr>
<td>Chlorfenapy</td>
<td>81.74</td>
<td>109.0</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>73.47</td>
<td>98.0</td>
</tr>
<tr>
<td>Cinerin-I</td>
<td>76.10</td>
<td>101.5</td>
</tr>
<tr>
<td>Clofentezine</td>
<td>76.24</td>
<td>101.7</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>79.50</td>
<td>106.0</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>77.42</td>
<td>103.2</td>
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<tr>
<td>Daminzoxide</td>
<td>72.69</td>
<td>96.9</td>
</tr>
<tr>
<td>Diazinon</td>
<td>78.50</td>
<td>104.7</td>
</tr>
<tr>
<td>Daminzoxide</td>
<td>78.50</td>
<td>104.7</td>
</tr>
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<tr>
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<tr>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Daminzoxide</td>
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</tr>
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</tr>
<tr>
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<td>104.7</td>
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<tr>
<td>Daminzoxide</td>
<td>78.50</td>
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<td>Diazinon</td>
<td>78.50</td>
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</tr>
<tr>
<td>Daminzoxide</td>
<td>78.50</td>
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<td>104.7</td>
</tr>
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<tr>
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<td>78.50</td>
<td>104.7</td>
</tr>
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<td>78.50</td>
<td>104.7</td>
</tr>
<tr>
<td>Daminzoxide</td>
<td>78.50</td>
<td>104.7</td>
</tr>
</tbody>
</table>

Accuracy of quantitation was investigated by comparing quality control samples at a known concentration of 75 ppb from generated calibration curves of each analyte (Table 2). The strict criterion of maximum deviation of 30% was met for the observed quality control samples.

Table 2. Accuracy of quality control sample at 75 ppb of each analyte. To calculate accuracy, the observed ppb value was divided by the theoretical ppb value at 75 ppb and multiplied by 100.
Using Internal Standards Provides Analytical Advantages

Due to matrix effects observed in complex cannabis samples, 30 internal standards are included in the ONE Pesticide\textsuperscript{420 TM} Reagent Kit. Inclusion of these 30 internal standards aims to improve quantitative analysis and overall recovery. Possible analyte loss during sample preparation is also addressed through the use of internal standards.

Experimental

Aiming to improve laboratory efficiency and consistency, the PerkinElmer ONE Pesticide\textsuperscript{420 TM} ISO 17034 Reagent Kit (Part Number: COP-001KT) includes calibration curve and QC standards as follows:

8 x Calibrator Vials consisting of 77 pesticide standards, 5 Mycotoxin standards, and 30 Internal Standards from 1ng/mL to 250 ng/mL

1 x Intermediate standard mix at 1µg/mL for Laboratory control samples (LCS) Preparation

3 x Quality Control Standard Vials
- 75 ng/mL Initial calibration verification (ICV) standard prepared from a second Lot
- 15 and 175 ng/mL Continuing Calibration Verification (CCV) Standards

1 x Internal Standard vial containing 30 Internal Standards

The included 77 certified pesticide reference standards support a laboratory’s efforts to meet the pesticide testing requirements in states that include regulated analytes found in California and Oregon as of March 2021. Please check with current regulations in your state for additional requirements.

Contribution substantially to ease-of-use, isotopically labelled reference internal standards are included to allow for the accurate quantitation of pesticides and mycotoxins in a cannabis or hemp matrix.

PerkinElmer also developed a Consumable Kit (Part Number: COP-001CT) to accompany the Reagent Kit which includes all the necessary consumables to prepare 500 cannabis or hemp samples. The Kit includes the following:

- 200 x autosampler LC-MSMS vials for calibrators and quality controls
- 700 x autosampler LC-MSMS vial caps
- 500 x 2 mL amber vials
- 500 x nylon syringe filter
- 500 x disposable syringe
- 500 x 50 mL graduate conical tube

Using ONE Pesticide\textsuperscript{420 TM} Reagent Kit

1. Bring calibrator solutions (8 vials) to room temperature and vortex for 30 seconds to mix.

2. Transfer 200 µL from each calibrator solution by pipetting into an empty LC-MS autosampler vial for each calibrator solution, resulting in 8 separate autosampler vials for each calibrator solution.

3. Transfer autosampler vials to Mass Spectrometer for final analysis.

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Quantity Included in Kit</th>
<th>Description</th>
<th>Concentration of Analytes (ng/mL or ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COP-IntCal</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Initial Calibrator - 1 µg/mL in 2 mL ACN with 0.1% formic acid</td>
<td>1000</td>
</tr>
<tr>
<td>COP-Cal-01</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 1 - 1 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>1</td>
</tr>
<tr>
<td>COP-Cal-02</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 2 - 5 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>5</td>
</tr>
<tr>
<td>COP-Cal-03</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 3 - 10 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>10</td>
</tr>
<tr>
<td>COP-Cal-04</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 4 - 25 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>25</td>
</tr>
<tr>
<td>COP-Cal-05</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 5 - 50 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>50</td>
</tr>
<tr>
<td>COP-Cal-06</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 6 - 100 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>100</td>
</tr>
<tr>
<td>COP-Cal-07</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 7 - 150 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>150</td>
</tr>
<tr>
<td>COP-Cal-08</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Calibrator 8 - 250 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>250</td>
</tr>
<tr>
<td>COP-QC-01</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Quality Control - 01 - 15 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>15</td>
</tr>
<tr>
<td>COP-QC-02</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Quality Control - 02 - 75 ng/mL in 4 mL ACN with 0.1% formic acid (second)</td>
<td>75</td>
</tr>
<tr>
<td>COP-QC-03</td>
<td>1</td>
<td>ONE Pesticide\textsuperscript{420 TM} - Quality Control - 03 - 75 ng/mL in 4 mL ACN with 0.1% formic acid</td>
<td>175</td>
</tr>
<tr>
<td>COP-IntStd</td>
<td>2</td>
<td>ONE Pesticide\textsuperscript{420 TM} Internal Standard - 4 mL ACN with 0.1% formic acid</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Hardware/Software
Chromatographic separation was conducted on a PerkinElmer LC-MS/MS® LX50 UHPLC system. Detection of analytes was executed using a PerkinElmer Q-Sight® 420 MS/MS detector with a dual ionization ESI and APCI source, which operate independently with two separate inlets into the mass spectrometer. Instrument operation, data acquisition and data processing was performed using the Simplicity 3Q™ software platform.

LC Method and MS Source Conditions
The LC method and MS source parameters are shown in Tables 4 and 5.

Conclusion
This study outlines how the ONE Pesticide® Reagent and Consumable Kit saves testing labs time in their procurement, reagent preparation and handling of consumables. As demonstrated by the calibration curves and quality control samples, this Kit allows rapid, and reliable quantitation of up to 77 pesticides and five mycotoxins residues in cannabis samples with mass spectrometry.

Highlights of the ONE Pesticide® Reagent Kit
• Provides laboratories a single source for all the pesticide, mycotoxin, internal standard reagents, quality control reagents and laboratory consumable hardware (vials, filters, etc)
• Ease of use – preconfigured ISO 17034 CRM standards and reagents improve data quality and allow more stringent compliance to ISO 17025 requirements
• Increase productivity - Prepare Calibration and QC Standards in minutes. Test more samples in less time than existing solutions.

Table 4. LC method conditions for ESI and APCI.

<table>
<thead>
<tr>
<th><strong>LC Conditions</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LC Column</strong> PerkinElmer Quasar SPP Pesticides (4.6 × 100 mm, 2.7 µm)</td>
</tr>
<tr>
<td><strong>Column Oven Temperature</strong> 30 ºC</td>
</tr>
<tr>
<td><strong>Auto sampler Temperature</strong> 20 ºC</td>
</tr>
</tbody>
</table>

| **Mobile Phase A** 2 mM ammonium formate + 0.1% formic acid in LCMS grade water |
| **Mobile Phase B** 2 mM ammonium formate + 0.1% formic acid in LCMS grade methanol |
| **Mobile Phase Gradient** A 18.5 min LC-MS/MS method with optimized gradient using ESI source was used for separation and analysis of 62 out of 66 pesticides and five mycotoxins residues regulated by California state in different cannabis matrices. |
| **Injection Volume** 3.0 µL |

Table 5. MS source conditions for both ESI and APCI methods.

<table>
<thead>
<tr>
<th><strong>ESI Method</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source Temperature</strong> 315 ºC</td>
</tr>
<tr>
<td><strong>HSID Temperature</strong> 200 ºC</td>
</tr>
<tr>
<td><strong>ESI Voltage (Positive)</strong> +5100 V</td>
</tr>
<tr>
<td><strong>ESI Voltage (Negative)</strong> -4200 V</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>APCI Method</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APCI Corona Discharge</strong> -3 µA</td>
</tr>
<tr>
<td><strong>Source Temperature</strong> 250 ºC</td>
</tr>
<tr>
<td><strong>HSID Temperature</strong> 180 ºC</td>
</tr>
</tbody>
</table>