Analysis of 47 Pesticides in Cannabis for High-Throughput Analysis: Traditional dSPE vs. Positive Pressure dSPE in a 96 Well Plate

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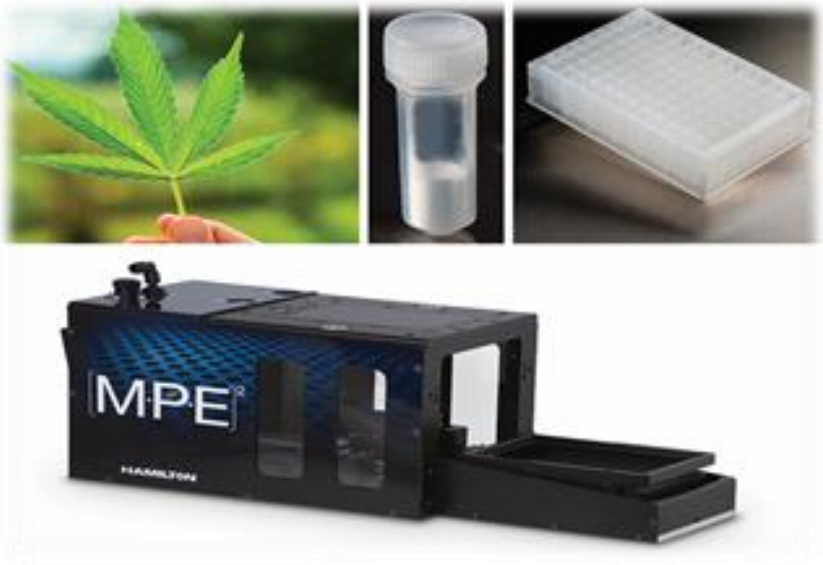

UCT Part Numbers

ECMSSC-MP
4g MgSO4/1g NaCl, Mylar Pack

ECQUUS142CT
Dispersive SPE Sorbent Blend for Pesticide Testing in Edibles
2 mL Centrifuge Tubes Included

WSHECQUUS14-LD
Dispersive SPE Sorbent Blend for Pesticide Testing in Edibles
96 Wellplate Format

WSH96CP
96 Well Collection Plate

SLAQ100ID21-3UM
Selectra® Aqueous C18 HPLC Column
100 X 2.1 mm, 3 µm

SLAQGDC20-3UM
Selectra® DA Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR
Guard Column Holder

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Summary:

An increasing number of jurisdictions within the United States have legalized the use of medicinal marijuana, along with several states that have also legalized it for recreational sale. Cannabis markets are relatively new and vary significantly by state when it comes to the regulation of pesticides and mycotoxins, as well as uniform testing methods for potency. Quality control methods are necessary to ensure product safety and appropriate cannabinoid profiling. While several methods are being investigated to determine the best way to evaluate these compounds of interest, it is important to keep in mind that these methods need to be scalable and also able to be used for high throughput analyses. This study examines using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction approach coupled with either traditional dSPE clean-up versus UCT's dSPE clean-up in well-plate format and Hamilton's MPE^2 Positive Pressure Extraction/Evaporation Module for the analysis of 47 pesticides in marijuana. We demonstrate that for most compounds investigated, the high throughput clean-up method exhibits comparable results to traditional dSPE clean-up.

Sample Pretreatment:

Grind marijuana sample to fine powder using a SPEX 6770 freezer mill.

QuEChERS Procedure (Figure 1):

1. **QuEChERS Extraction**
    a) Weigh 1 g of the pre-treated marijuana into 50-mL centrifuge tubes, add internal standard, and 10 mL of D.I H_2O, and vortex and hydrate for 15min.
    b) Add 10 mL of acetonitrile (MeCN) with 2% formic acid.
    c) Add QuEChERS extraction salts from pouches (ECMSSC-MP), and vortex for 10 sec to break up salt agglomerates.
    d) Shake for 1 min at 1000 stroke/min using a SPEX Geno/Grinder.
    e) Centrifuge at 3000 rcf for 5 min.

2. **dSPE Cleanup for Pesticide Residue Analysis**
    a) Transfer 1 mL of the supernatants to 2-mL dSPE tube (ECQUUS142CT) or to UCT's dSPE clean-up in well plate format (WSHECQUUS14-LD).
    b) Vortex traditional dSPE tubes for 1 min at 1000 stroke/min using the SPEX Geno/Grinder and then centrifuge at 3000 rcf for 5 min.
    c) Transfer 200 µL extract to the 2-mL auto-sampler vials.
    d) For clean-up via well plate, apply positive pressure utilizing Hamilton's [MPE]^2 at a rate of 1mL/min to filter the extracts through the plate. Elute extracts directly into a 96-well collection plate and transfer directly to the instrument for analysis.
    e) Analyze samples by LC-MS/MS (Thermo Scientific UltiMate 3000 LC system coupled to TSQ Vantage tandem MS) equipped with UCT's Selectra® Aqueous C18 HPLC column.

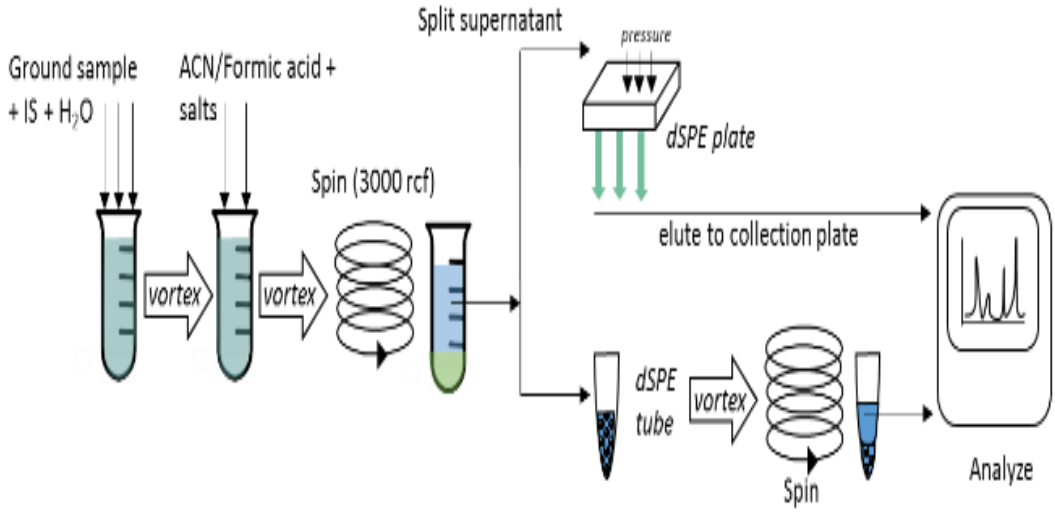

Figure 1: General Workflow

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Results/Discussion:

Due to the various regulations between states, a wide panel of commonly encountered pesticides was selected for this study (Table 1). Quantitation was performed against a 6-point matrix-matched calibration curve prepared in unspiked marijuana extract. Extracts were then analyzed for overall recovery at 3 varying concentration levels. All samples were run in replicates of 5 for reproducibility studies.

For most compounds, the recovery was greater than 65% for both methods of dSPE. The mean recoveries for traditional dSPE were 98.0%, 99.2% and 97.9% at pesticide concentrations of 50 ng/mL, 100 ng/mL and 200 ng/mL, respectively. For comparison, the mean recoveries at the same concentrations for well plate dSPE were 85.0%, 88.9% and 89.1%. Therefore, there was typically about a 10-11% absolute difference in recovery between the two methods (Figure 2), which can be corrected for by implementing the use of internal standards. When comparing the recovery differences between the two methods, there are six compounds with noticeably larger discrepancies across all three concentrations, namely: chlorpyrifos, cyprodinil, diazinon, spinetoram, spiromesifen 278 and trifloxystrobin (Figure 3). If these data sets are excluded, then the average absolute differences in recovery between the two methods decrease to 8.8%, 6.4% and 5.8% for concentrations of 50 ng/mL, 100 ng/mL and 200 ng/mL, respectively.

Resource allocation is an important factor to consider for each method. Figure 3 demonstrates the dSPE plate method has two fewer preparation steps compared to the dSPE tube method. In the plate format, once the initial supernatant is eluted into the collection plate, it is ready for analysis via LC/MS. For dSPE tube clean up, the supernatant must undergo an additional vortex and spin step and an additional transfer of the supernatant to a vial. By our estimates in the laboratory with hand pipetting, the dSPE plate method saves roughly 45-60 minutes on a 96 sample basis. With the replacement of hand pipetting by a liquid handling robot, the time savings could potentially double as all of the primary supernatant transfers to the dSPE plate could be automated. This fully automated option could free up a significant amount of laboratory technician time while also increasing accuracy and precision.

Tables:

Pesticides Analyzed			
Abamectin	Etoxazole	Oxydemeton methyl	Spinosyn D
Acetochlor	Fenamiphos sulfone	Paclobuterol	Spiromesifen 278
Atrazine	Fenamiphos sulfoxide	Piperonyl butoxide	Spirotetramat
Bifenazate	Fenhexamid	Profenofos	Tebuconazole
Carbaryl	Fenoxycarb	Pymetrozine	Tebuthiuron
Chlorpyrifos	Flonicamid	Pyrazophos	Thiabendazole
Cyprodinil	Fludioxinil	Pyrethrin I NH9	Thiamethoxam
DEET	Flutriafol	Pyrethrin II NH9	Triadimefon
Diazinon	Imazilil	Pyrimethanil	Triethylphosphorothioate
Dichlorvos	Imidacloprid	Simazine	Trifloxystrobin
Dichrotophos	Malathion	Spinetoram	Zoxamide
Dimethomorph	Myclobutanil	Spinosyn A	

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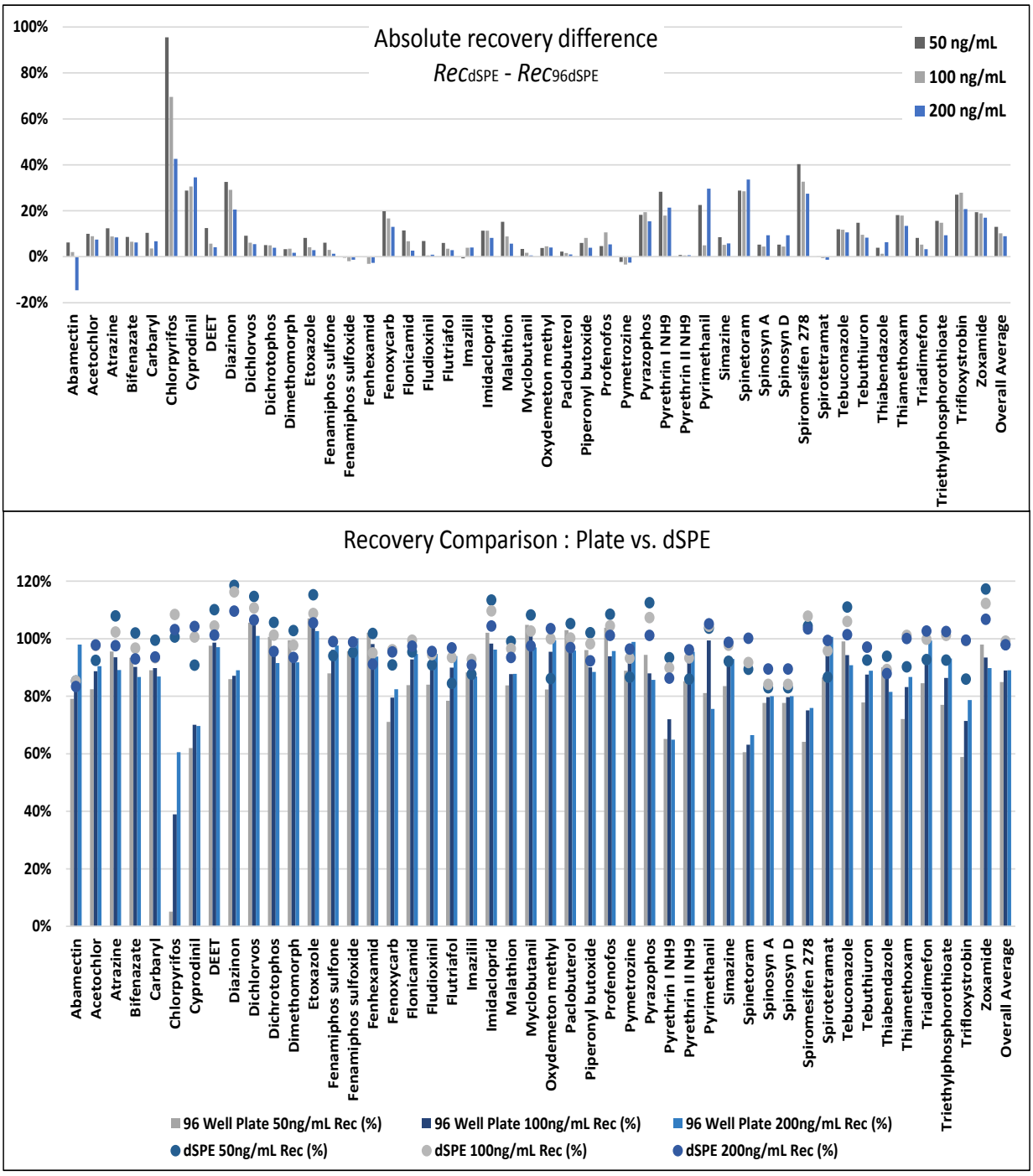

Figure 2: Pesticide recoveries and differences between the two dSPE methods

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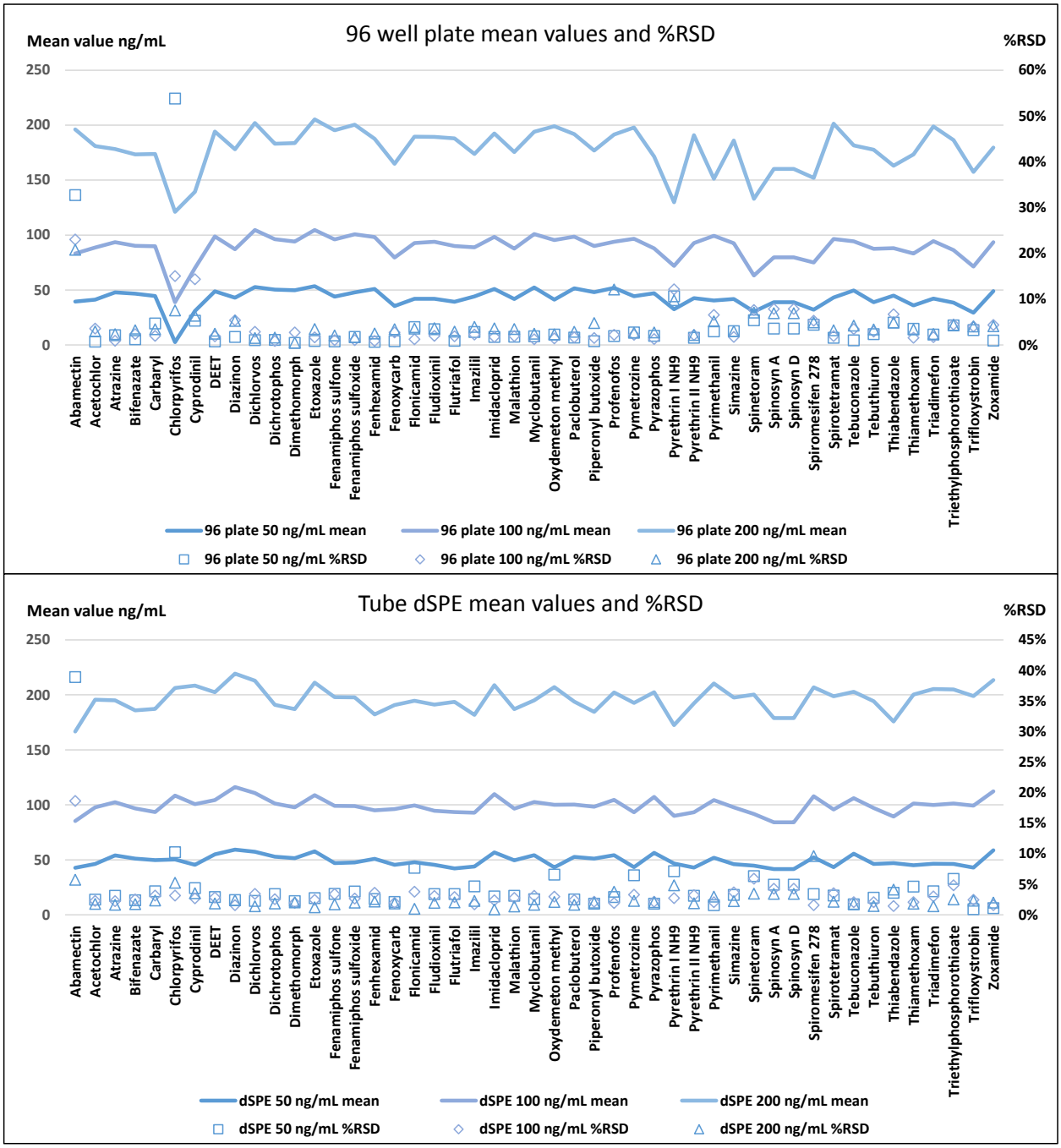

Figure 3: Measured pesticide concentrations and %RSD for well plate and tube dSPE methods

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Conclusions:

A fast and effective method was developed for the determination of 47 pesticide residues in marijuana samples. All analytes of interest were extracted using the QuEChERS approach, followed by either an additional cleanup using either traditional dSPE or dSPE in a well plate filtration format. Analysis of the samples was performed by LC-MS/MS utilizing a Selectra® Aqueous C18 HPLC column which allowed for improved retention of the more polar pesticides included in the method. Recoveries for the well plate dSPE method compared to the traditional dSPE were within 10% on average for most pesticide compounds. With the exception of a few compounds analyzed, %RSD values were ≤ 5% based on sets of 5 replicates. With the widespread legalization of marijuana, this simple method will prove beneficial for implementing high throughput regulatory testing and allowing for further automated platforms.

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