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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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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 dispersive-Solid Phase Extraction (dSPE) clean up versus UCT's dSPE clean up in 96-well plate format using Hamilton's [MPE]^2 — automated positive pressure extraction and evaporation module — for the analysis of 47 pesticides in marijuana. This analysis demonstrates that for most compounds investigated, the high-throughput cleanup method exhibits comparable results to traditional dSPE cleanup.

Materials and Methods

Marijuana samples were ground into a fine powder using a SPEX 6770 freezer mill. One gram of the homogenous marijuana powder was then added to a 50 mL centrifuge tube containing internal standard and 10 mL of deionized water. Samples were then vortexed and hydrated for 15 minutes. Following hydration, 10 mL of 2% formic acid in acetonitrile was added to the centrifuge tube along with UCT QuEChERS extraction salts (P/N ECMSSC-MP). Salt agglomerates were broken up by vortexing the tubes for 10 seconds. The tubes were then shaken for 1 minute at 1000 strokes/min using a SPEX Geno/Grinder and then placed in a centrifuge and spun for 5 minutes at 3000 RCF.

Pesticide analysis was performed by transferring 1 mL of the above supernatants to either UCT's traditional dSPE cleanup tubes (P/N ECQUUS142CT) or to UCT's dSPE cleanup, 96-well plate (P/N WSHECQUUS14-LD) (Figure 1).

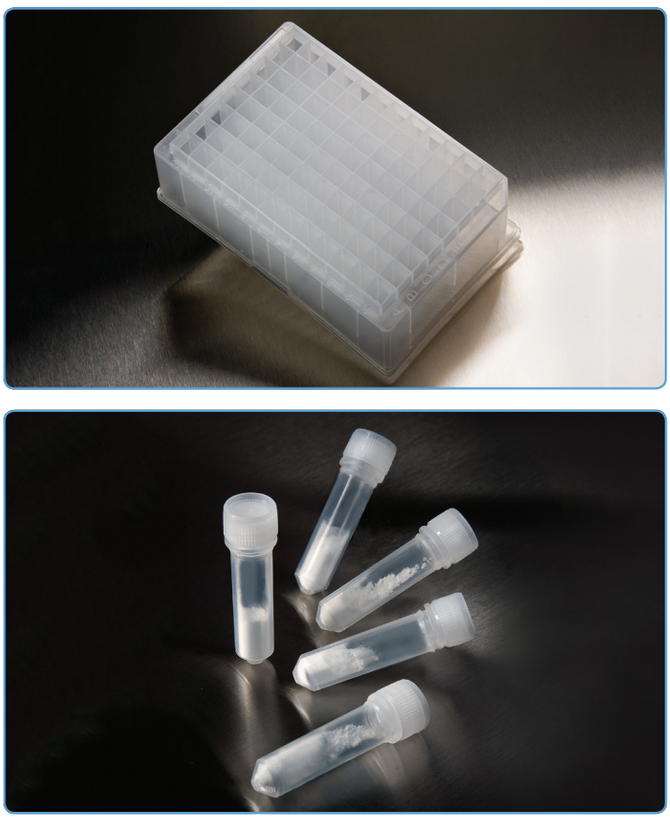


Figure 1:
dSPE plate and tubes

The traditional dSPE tubes were vortexed and then spun down for 2 minutes at 3000 RCF. Clean up via 96-well plate was executed by aliquoting 1 mL of the supernatant to the dSPE cleanup, 96-well plate. Hamilton's [MPE]^2 (Figure 2) was used to apply positive pressure to filter the extracts to allow them to flow through the plate at a rate of 1 mL/minute. Extracts were eluted directly into a 96-well collection plate, which was then transferred to the LC/MS for analysis. Quantitation was performed against a 6-point matrix-matched calibration curve prepared in unspiked marijuana extract.

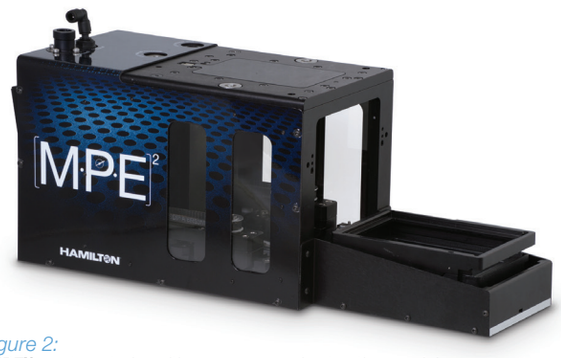


Figure 2:
[MPE]^2 - automated positive pressure and extraction module

Extracts were then analyzed for overall recovery at 3 varying concentration levels. Samples were analyzed 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. All samples were run in replicates of 5 for reproducibility studies.

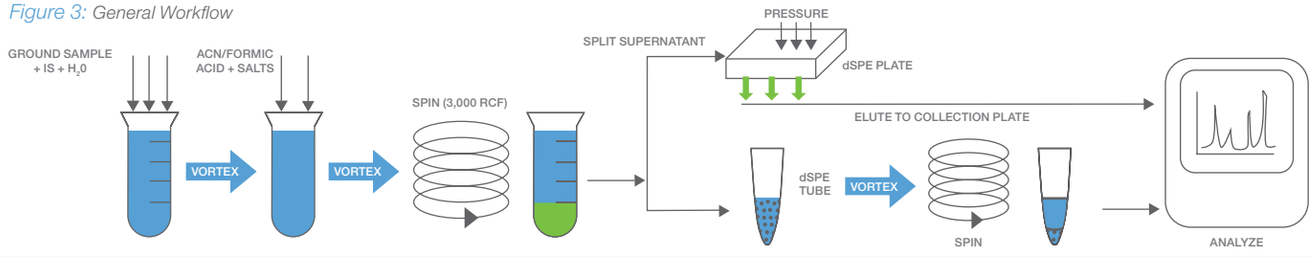
Results and Discussion

Due to the various regulations between states, a wide panel of commonly encountered pesticides was selected for this study (Table 1).

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 96-well plate dSPE were 85.0%, 88.9%, and 89.1%. Therefore, there was typically approximately 10-11% absolute difference in recovery between the two methods (Figure 4), which can be corrected 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. 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. It should also be noted that most compounds exhibited accurate reproducibility by both methods with %RSD values ranging from 2-4%. A few compounds exhibited significantly higher %RSD values with the 96-well plate dSPE method; namely, abamectin, chlorpyrifos, cyprodinil and pyrethrin I NH9 (Figure 5). However, abamectin also exhibited significantly higher %RSD values with the traditional dSPE method too, which indicates that analysis of this pesticide in particular may require more extensive method development.

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 cleanup, the supernatant must undergo an additional vortex and spin step and an additional transfer of the supernatant to a vial. In the laboratory with hand pipetting, it is estimated the dSPE plate method saves roughly 45-60 minutes on a 96 sample basis. With the replacement of hand pipetting by an automated liquid handling workstation, 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.

Figure 3: General Workflow



Conclusion

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 an additional clean up using either traditional dSPE or dSPE in a 96-well plate 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 within the method. Recoveries for the 96-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 processes.

Figure 4: Pesticide recoveries and differences between the two dSPE methods

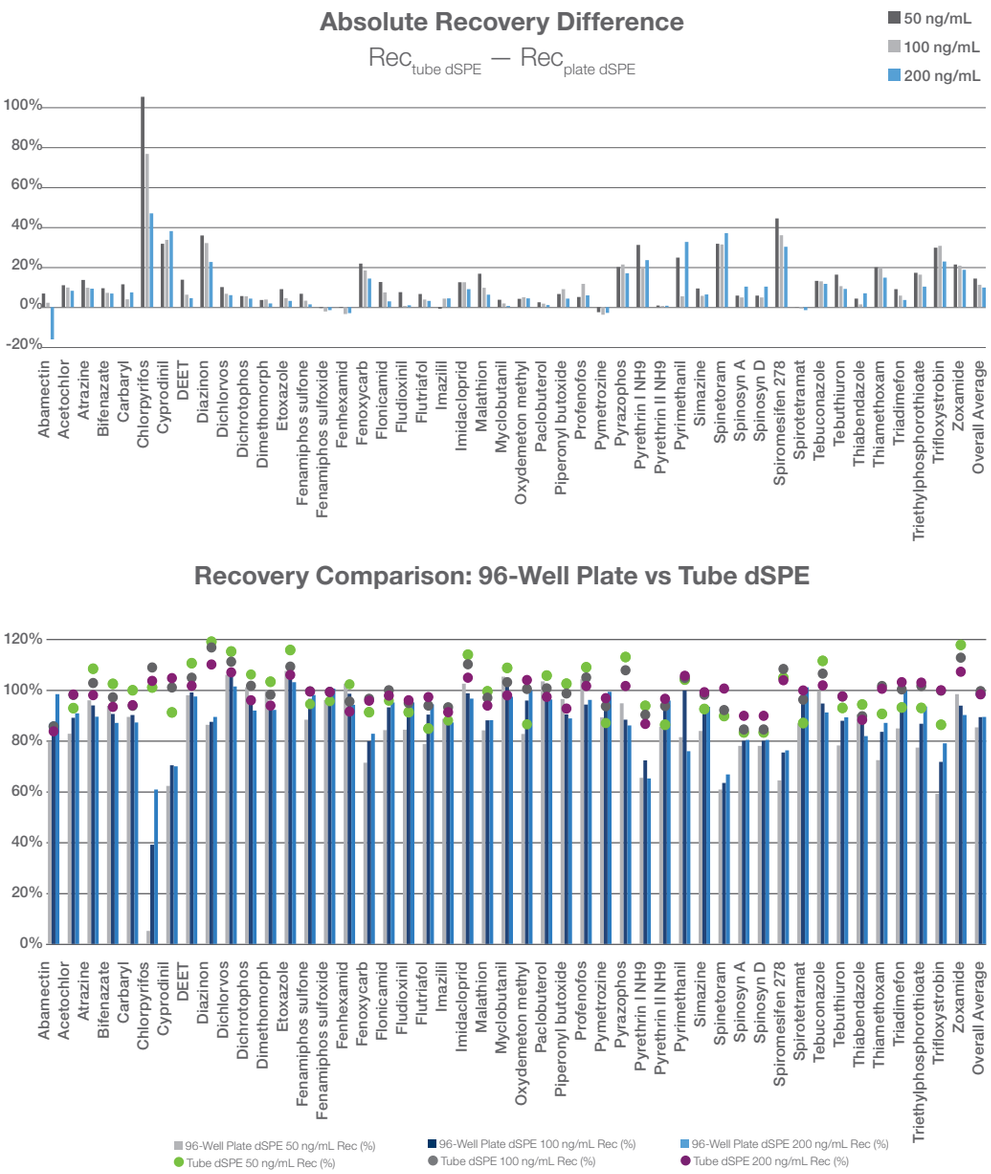
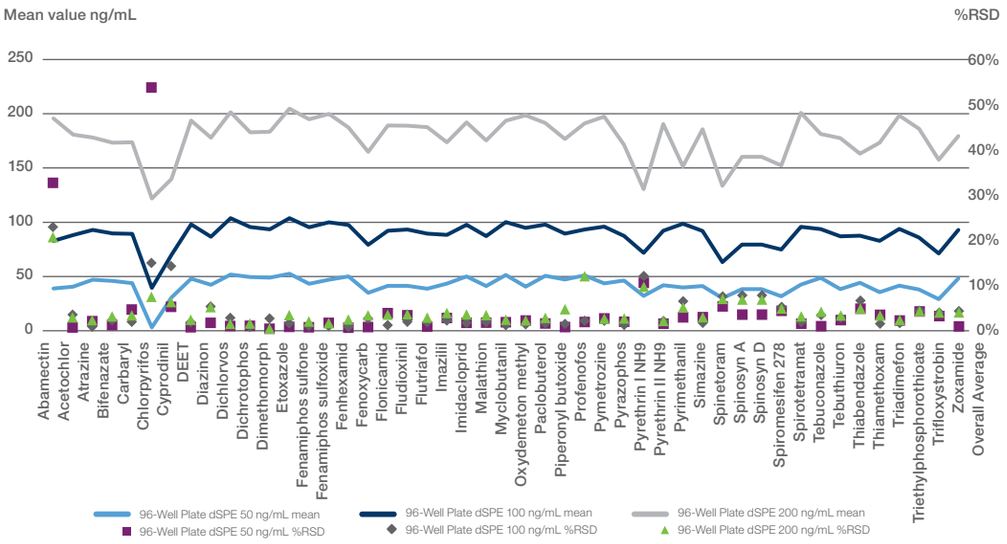
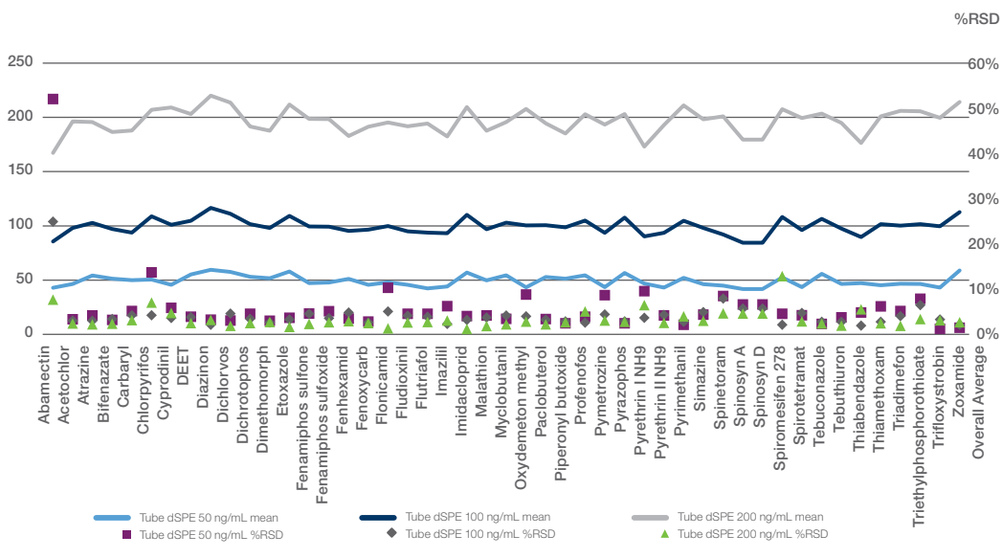


Table 1

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

Figure 5: Measured pesticide concentrations and %RSD for 96-well plate and tube dSPE methods





Key Words:

Marijuana, QuEChERS, pesticides, UCT, Hamilton Robotics, Cannabis, positive pressure SPE, automated liquid handling

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