RAFA 2019, 2019-11-06, L55

# Multi-plug Filtration Clean-up (m-PFC) Method and Automated Device for Analysis of Pesticide and Veterinary Drug Residues

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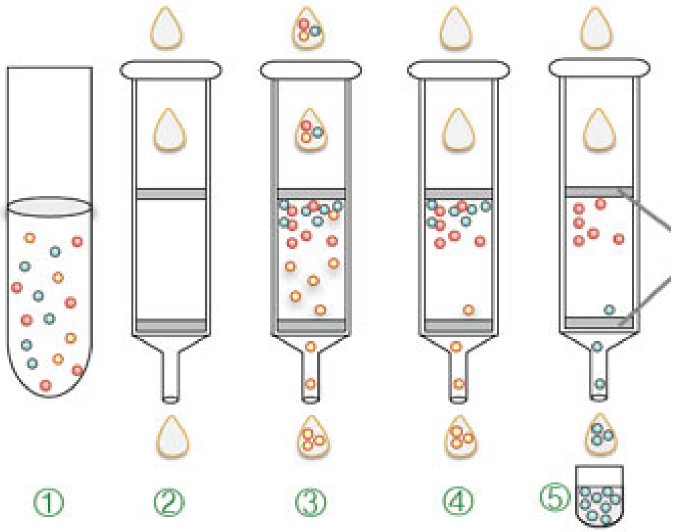
中国農業大學
China Agricultural University

# Contents

# Pesticide Residue Cleanup Methods




# I. d-SPE method based on Carbon Nanotubes -Optimization on QuEChERS method

QuEChERS: by Steve Lehotay, Anastassiades et al. since 2003：

from SPE to r-DSE

Methods: AOAC: 2007.01

EU: EN15662

China： GB 23200.113-2018 etc.

sample +acetonitrile

↓ vortex

add $MgSO_4$ and NaCl

↓ vortex、centrifugation

1ml supernatant， add $MgSO_4$ and PSA

↓ vortex、centrifugation 、filtration

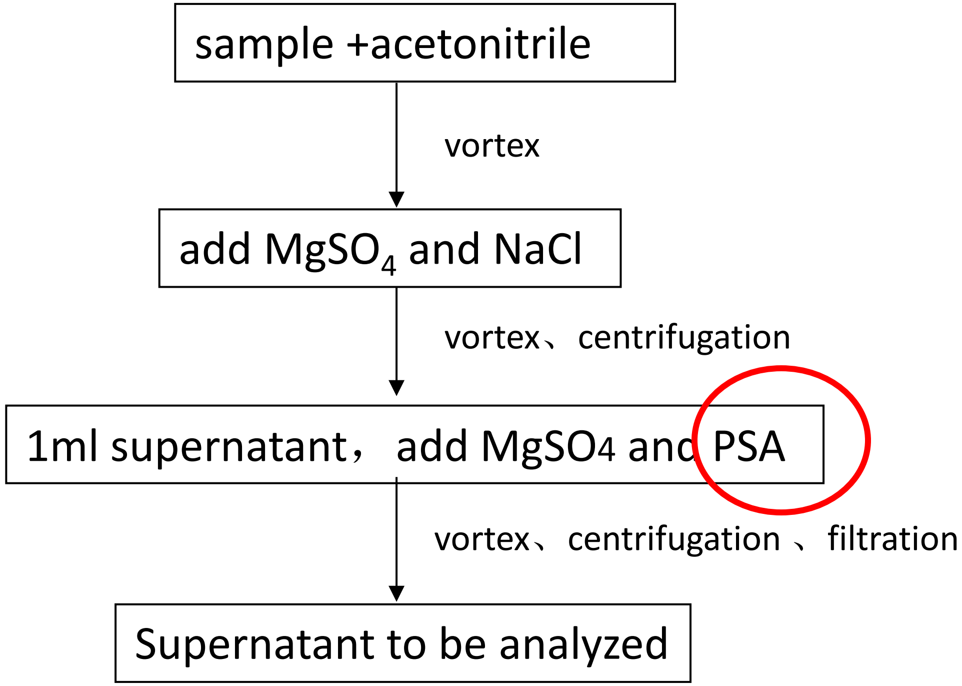
Supernatant to be analyzed by GCMS, HPLCMS etc

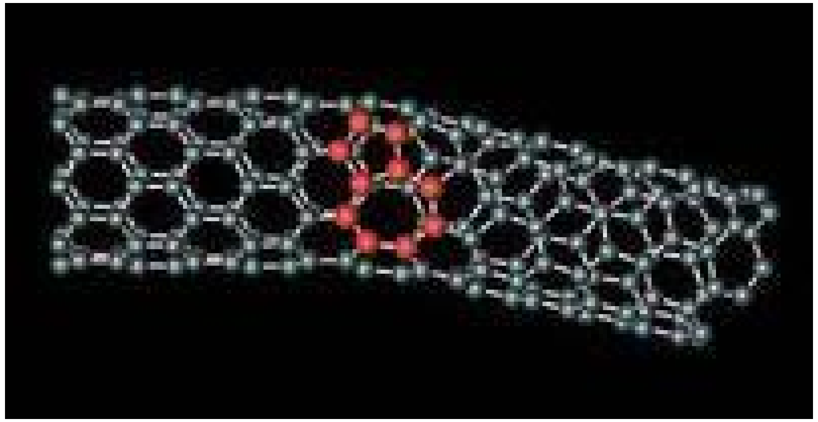
Advantage: Quick

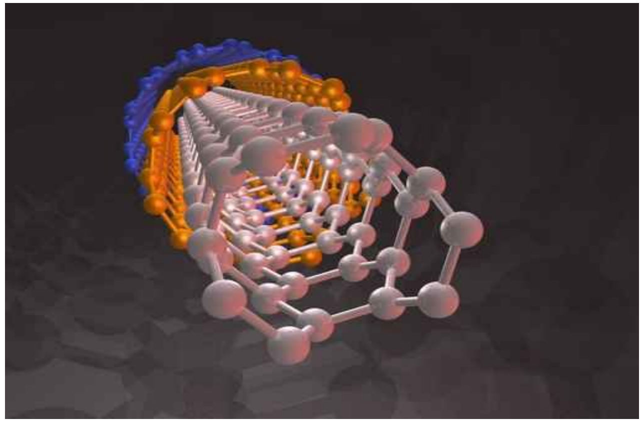
Disadvantage: cleanup pe

# I. d-SPE method optimization based on MWCNTs

## Finding other absorbents and combinations





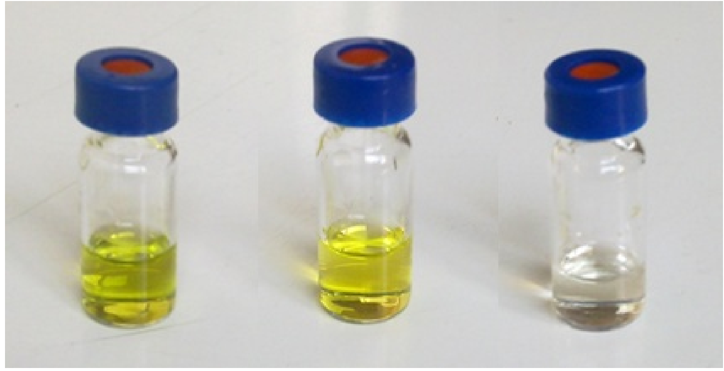
MWCNTs, amino-modified CNTs;
N-enriched Carbon

mixed with PSA/GCB/C18/PSA etc

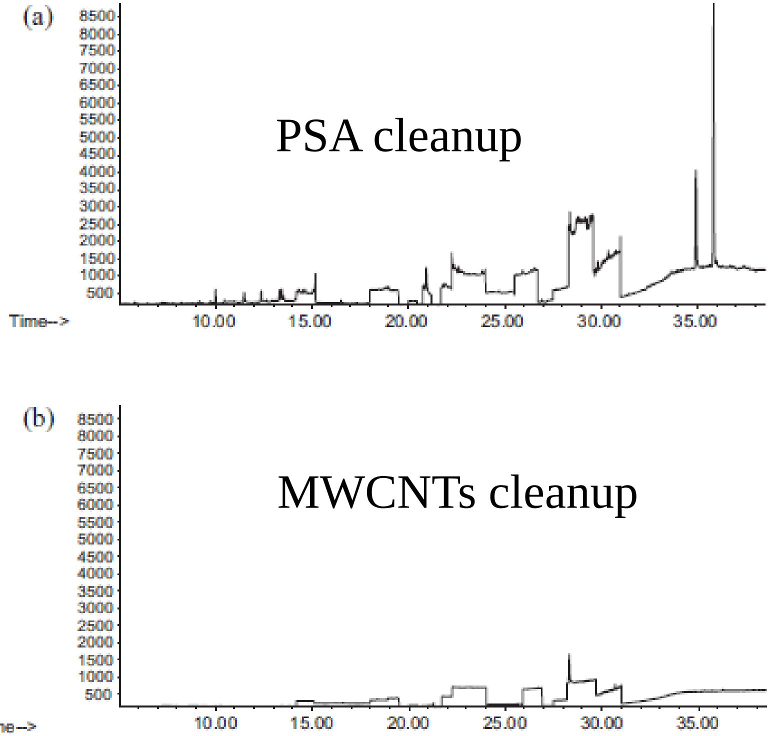
Anastassiades M., Lehotay S.J., Štajnbaher D., et al. *Journal of AOAC international*, 2003, 86(2): 412-431

# I. d-SPE method optimization

- **MWCNTs as r-DSPE cleanup material for cabbage, spinach, orange  and grape**



**Without cleanup** | **r-dSPE (PSA)** | **r-dSPE (MWCNTs)**

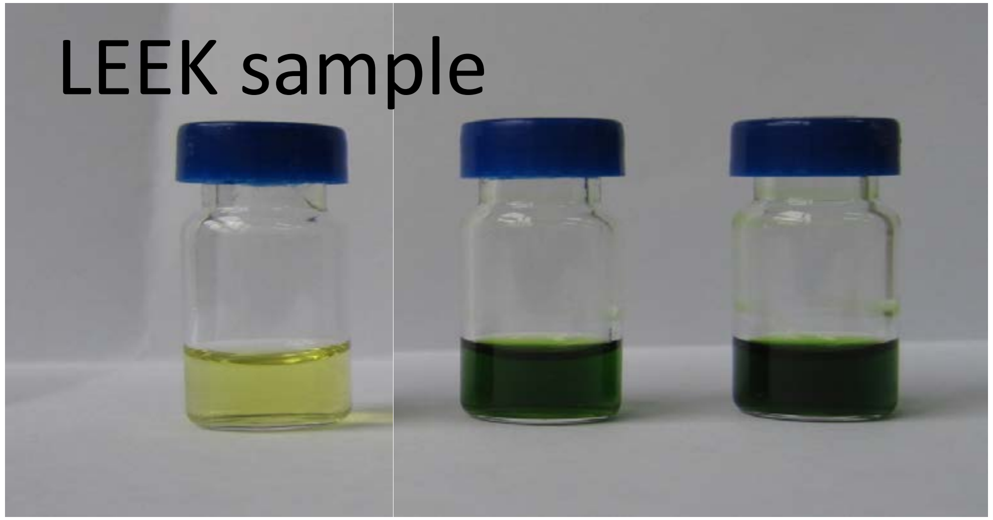



**MWCNTs, better cleanup performance**
**fewer interference peaks in the chromatogram**
**Method performances meet the creteria**

Zhao, P., Wang, L., Zhou, L., Zhang, F., Kang, S., **& Pan, C**. ***Journal of Chromatography A***, 2012, 1225(1588), 17-25.

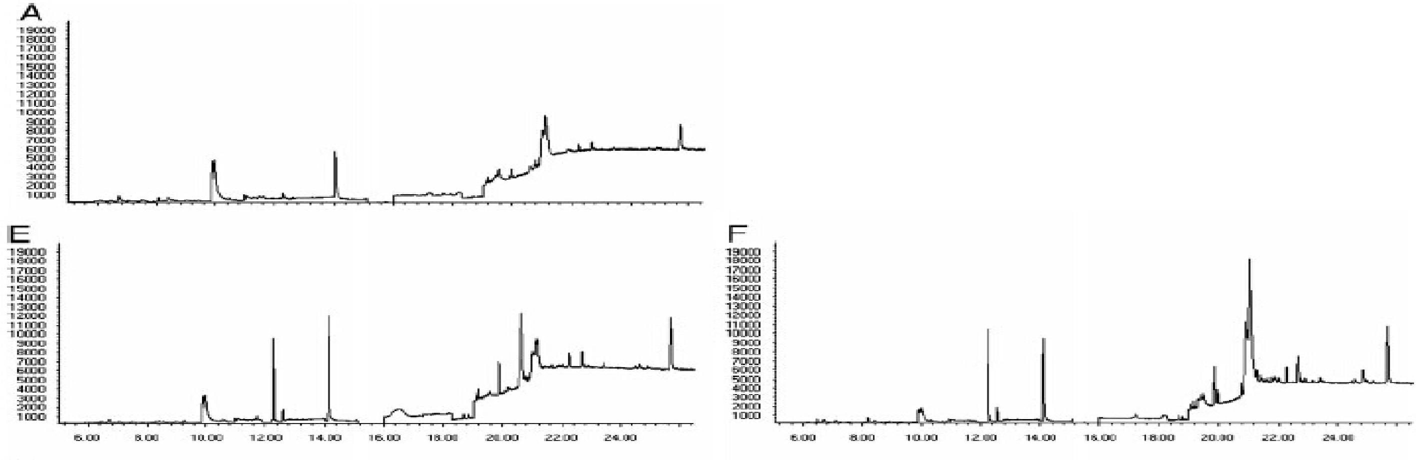
# I. d-SPE method optimization

- **MWCNTs as r-DSPE cleanup material for complex matrices: leek, onion, ginger and garlic**




A: MWCNTS E:PSA F:without

Agilent GC 6890 with mass spectrometric detector




**Chromatogram for blank leek extract after different r-DSPE sorbents with GCMS:**
**(A) MWCNTs;**
**(E) PSA;**
**(F) without r-DSPE**

*Published in Journal of Separation Science,* 2011

# II. m-PFC method development

## ———Multi-residue analysis –GCMS in Soybeans by DPX method

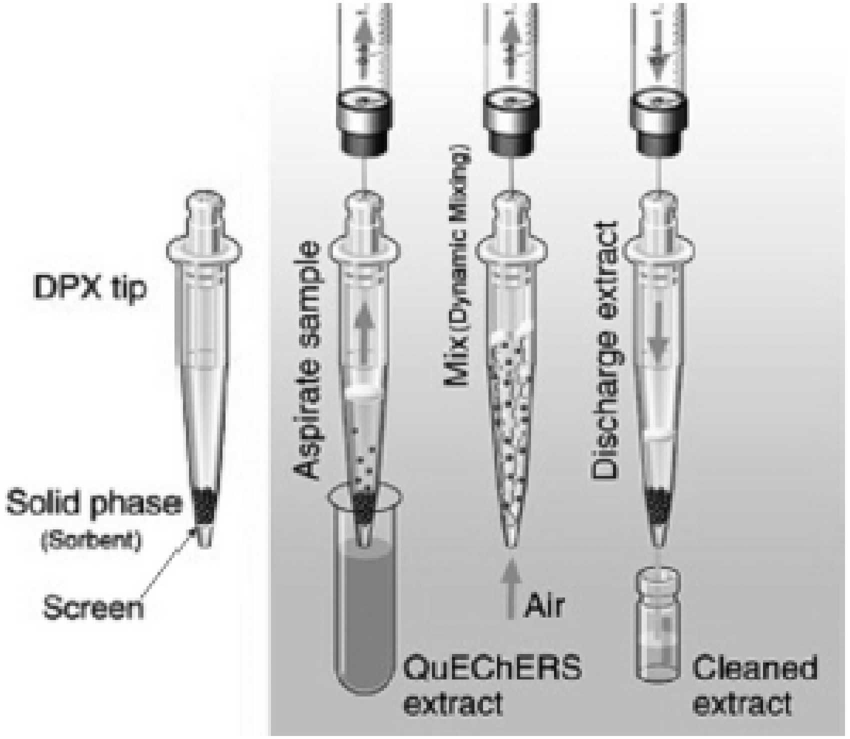
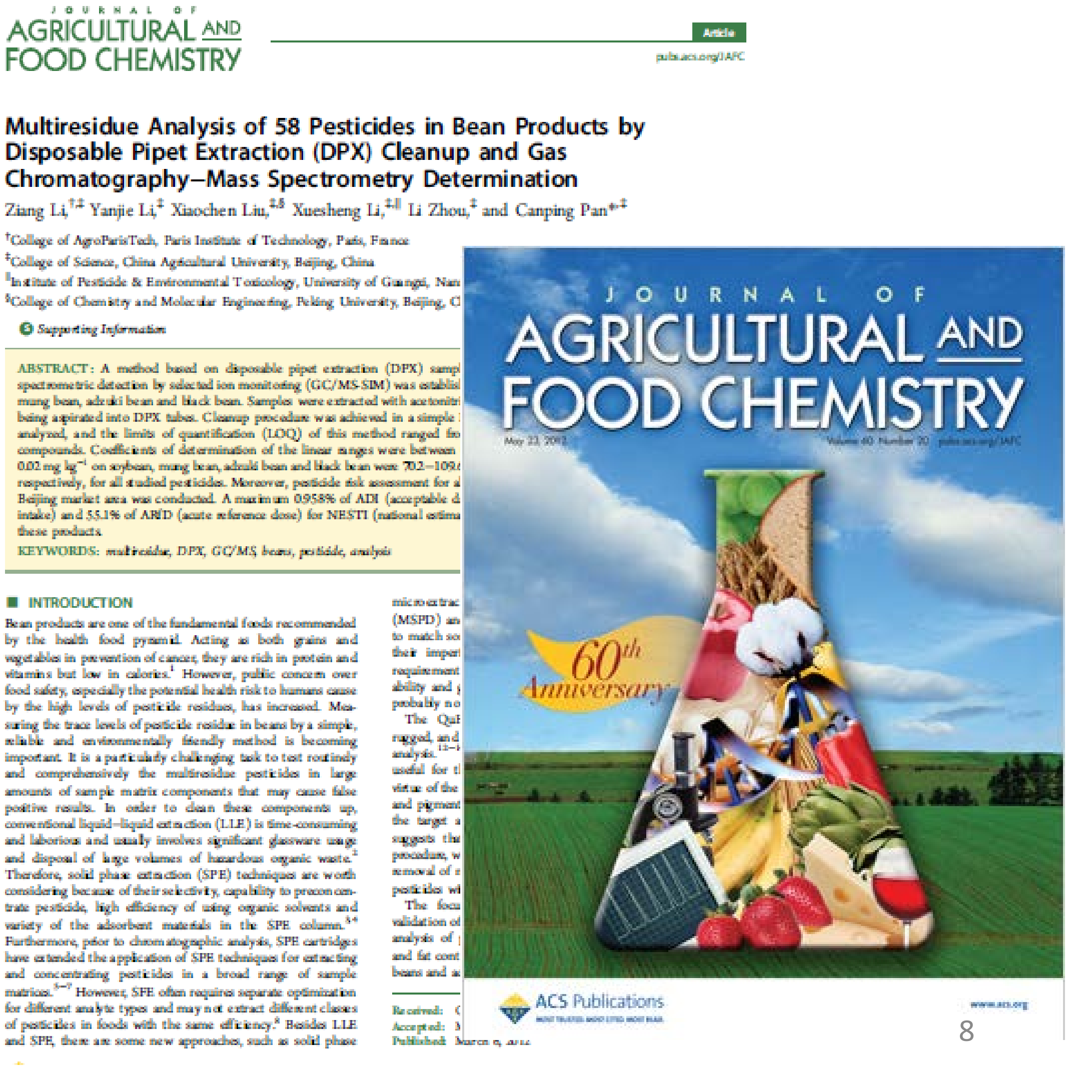


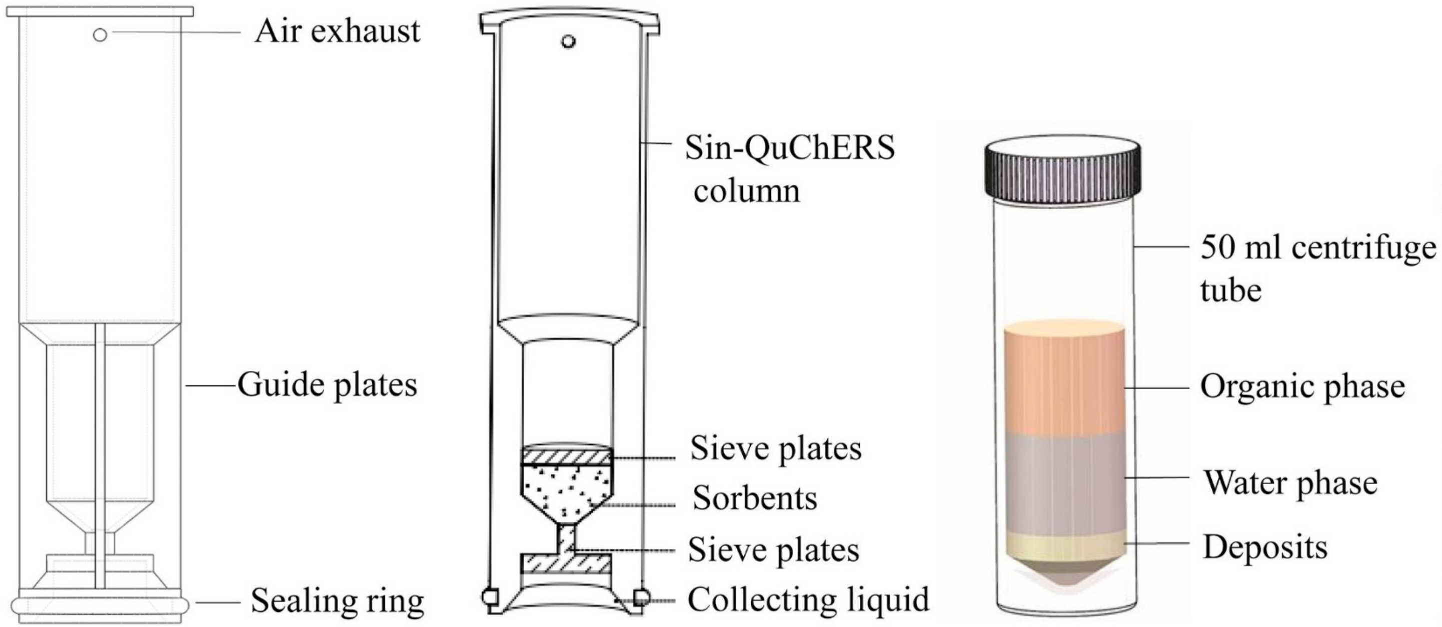

Figure 2. Schematic diagram of a DPX cleanup. Modified schematic diagram adapted from the online publication of GERSTEL, http://www.gerstel.de/pdf/p-gc-an-2009-01.pdf (Guan, H.; Brewer, W. E.; Morgan, S. L.; Stuff, J. R.; Whitecavage, J. A.; Foster, F. D. Automated Multi-Residue Pesticide Analysis in Fruits and Vegetables by Disposable Pipette Extraction (DPX) and Gas Chromatography/Mass Spectrometry. 2009, AN/2009, 1–7).

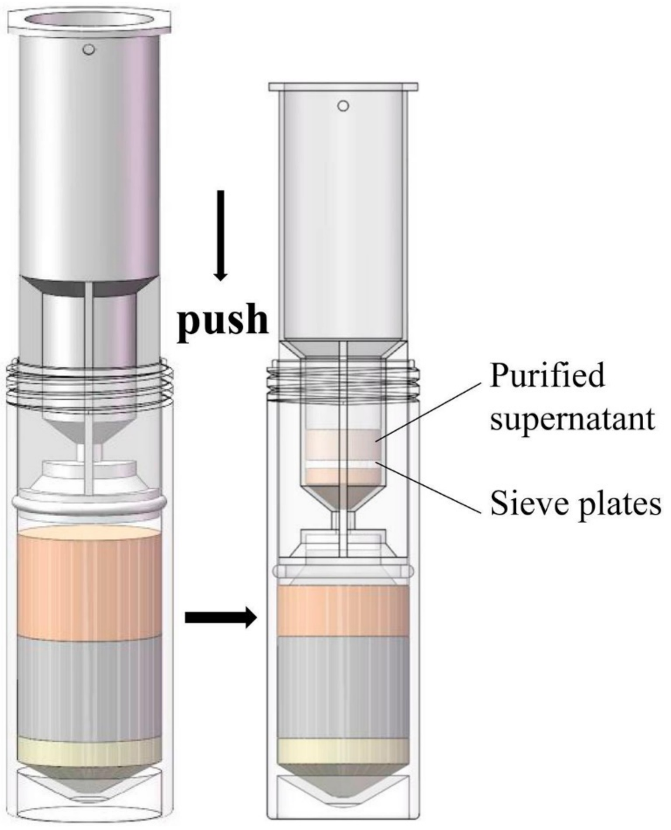


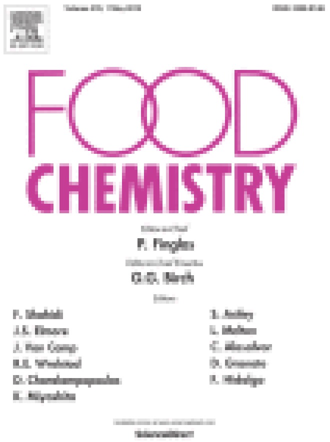

Ziang Li, PAN C et al, J Agric Food Chem. 2012, 16;60(19):4788-98.

# Sin-QuEChERS

Sin-QuEChERS diagram



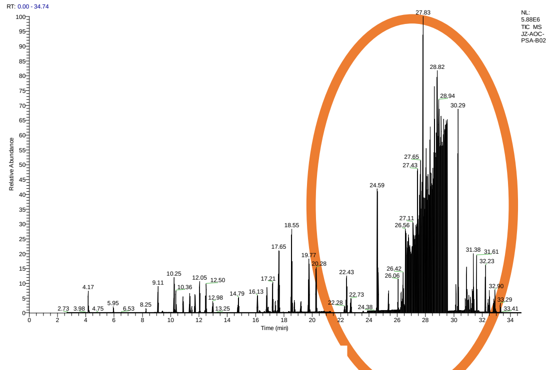




Absorbents: MWCNTS/PSA etc

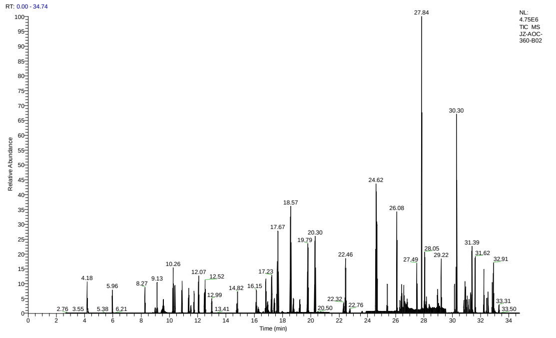
Pesticides residues in Peppers and processed products

https://doi.org/10.1016/j.foodchem.2018.12.017

# Method specificity check comparison

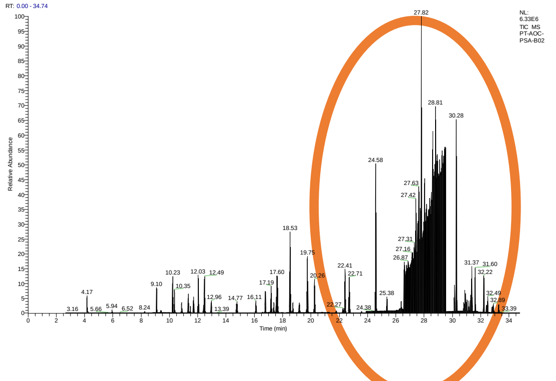


a

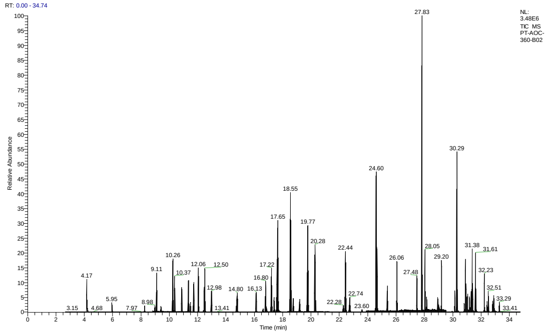


b

Citrus Sample



a

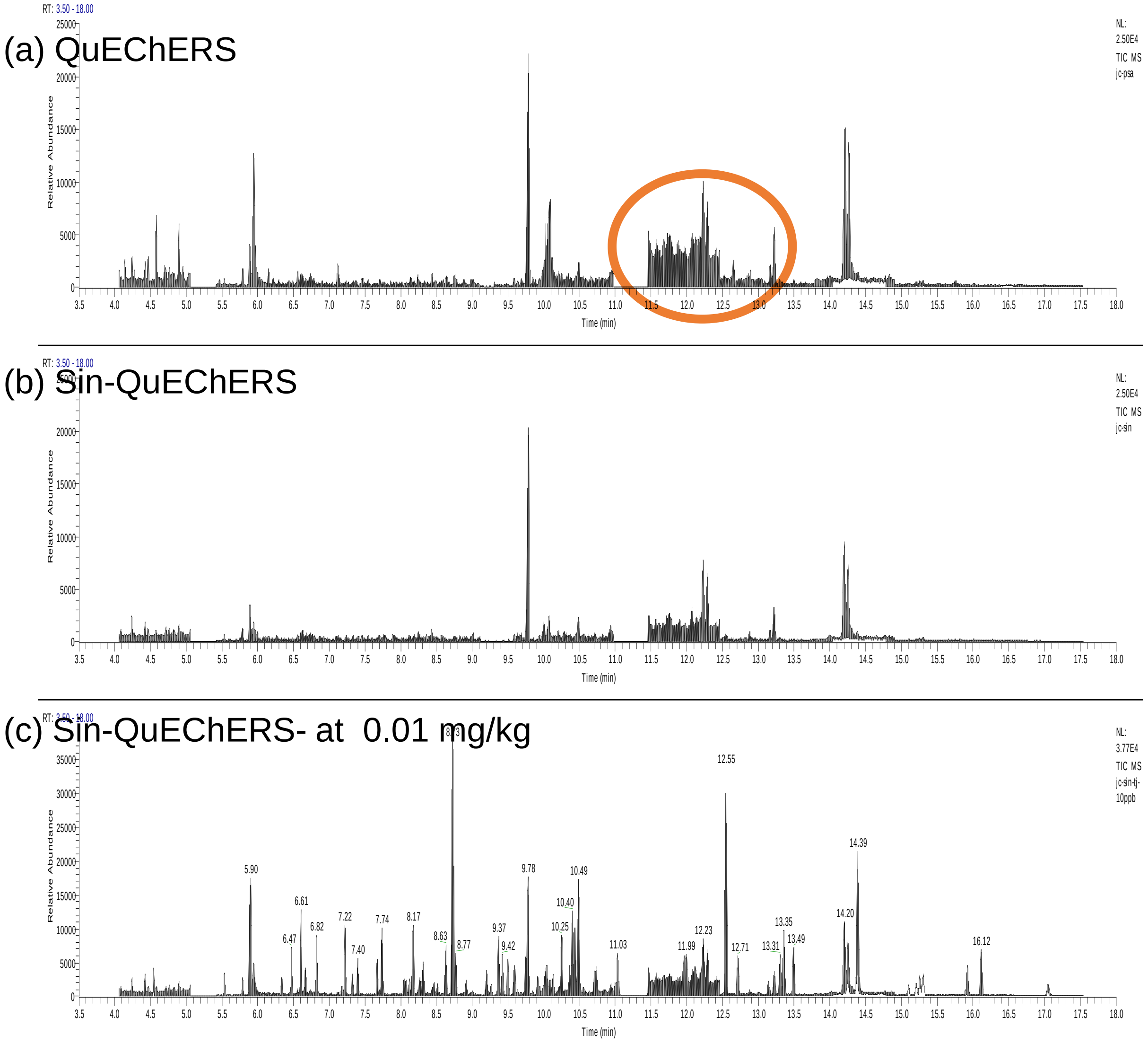


b

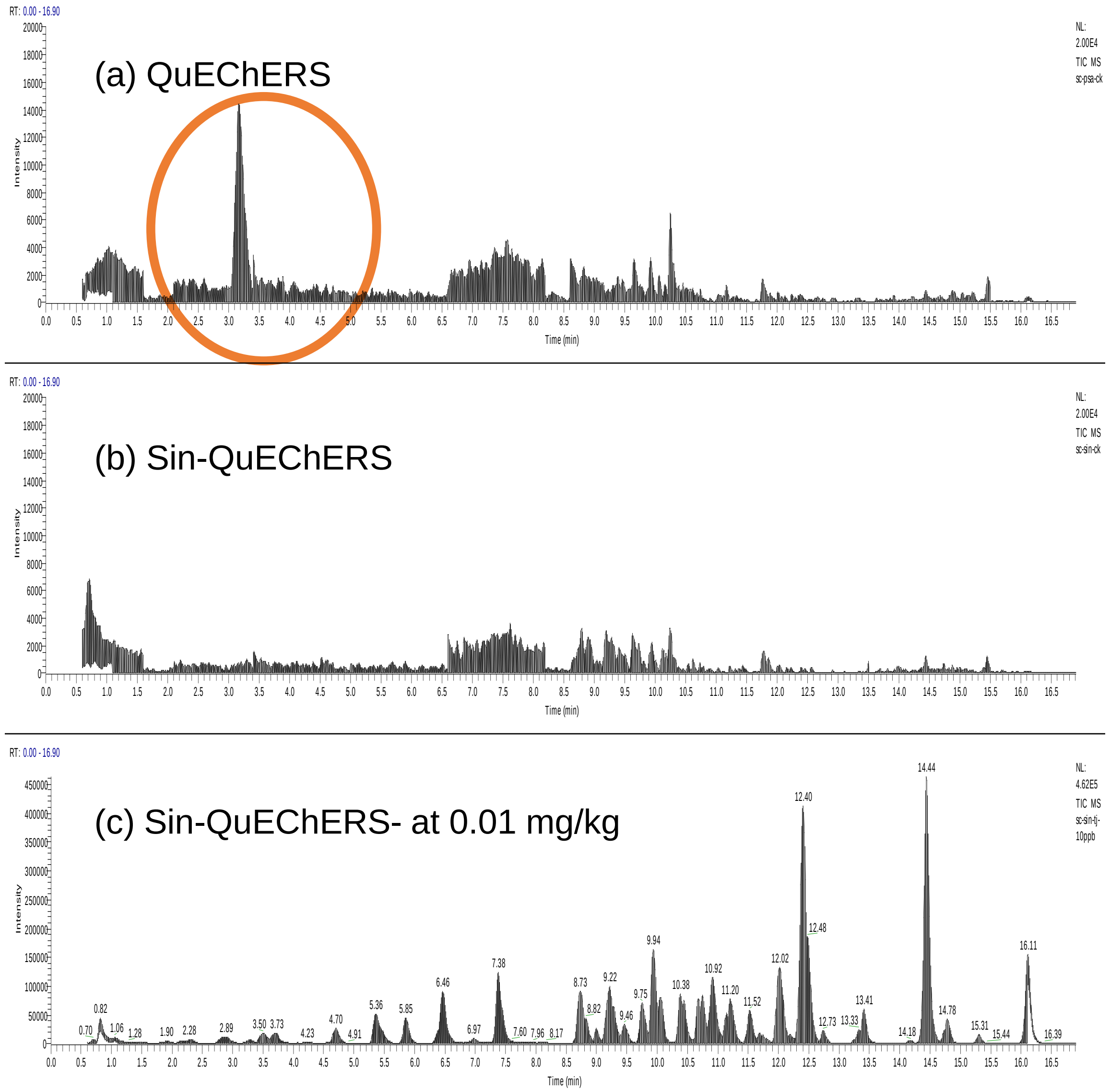
Grape Sample

a QuEChERS          b Sin-QuEChERS-Nano

# GC-MS/MS Leek

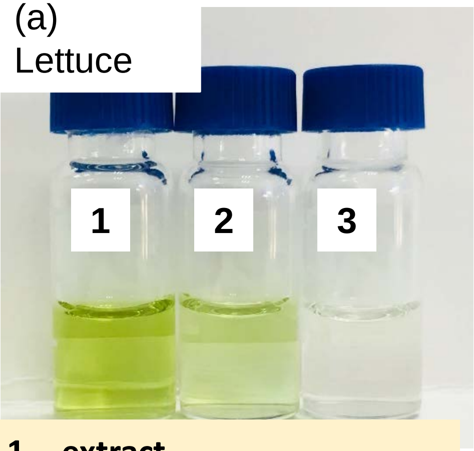



# LC-MS/MS Lettuce



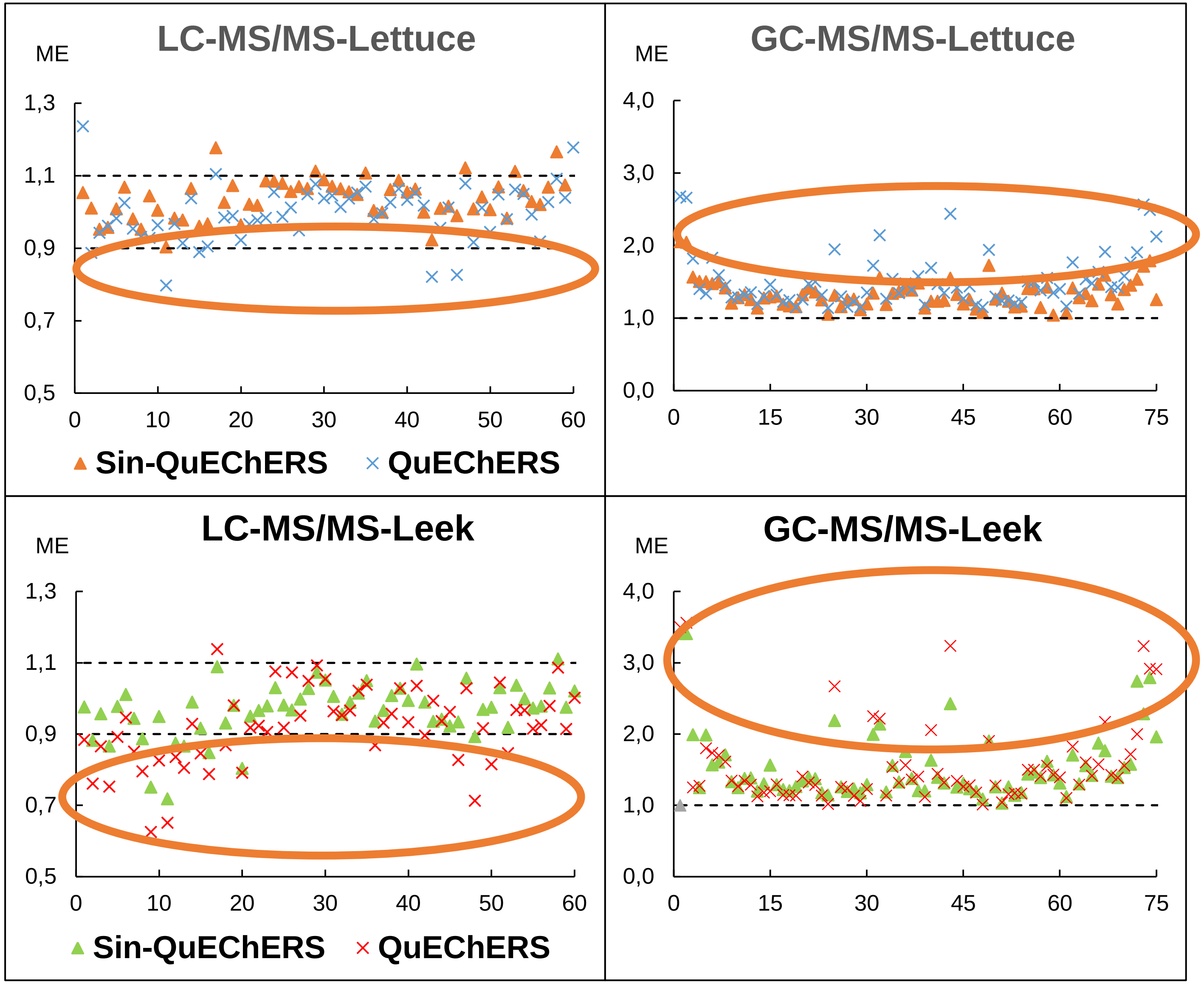
# Matrices Effects comparison on Sin-QuEChERS –conventional QuEChERS

(a)
Lettuce




1、extract
2、QuEChERS（PSA）
3、Sin-QuEChERS

(b)
Leek


**GC-MS/MS: 75 pesticides**

**LC-MS/MS: 60 pesticides**

# 88 pesticides in Hemp Plant by Sin-Q; lcmsms

**Sin-QuEChERS： 15mg MWCNTs+90mg PSA+80mg C18+80mg GCB+0.6g $MgSO_4$ + 2g $NaSO_4$**

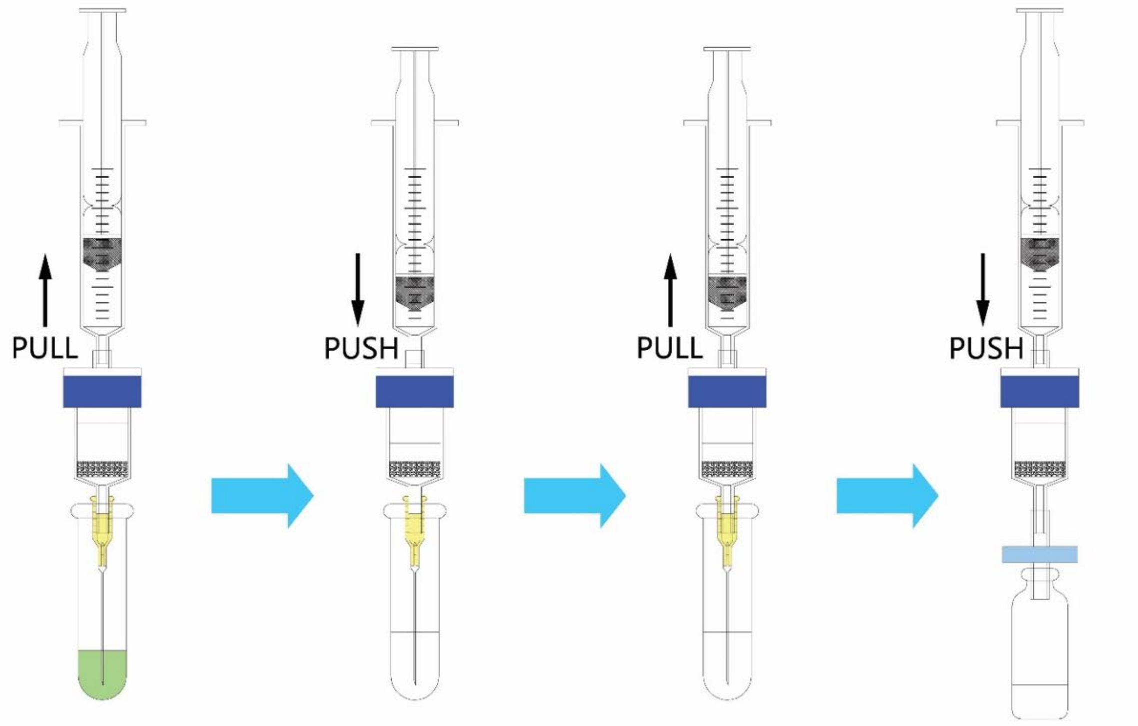
1 2 3 4 5 6 7 8

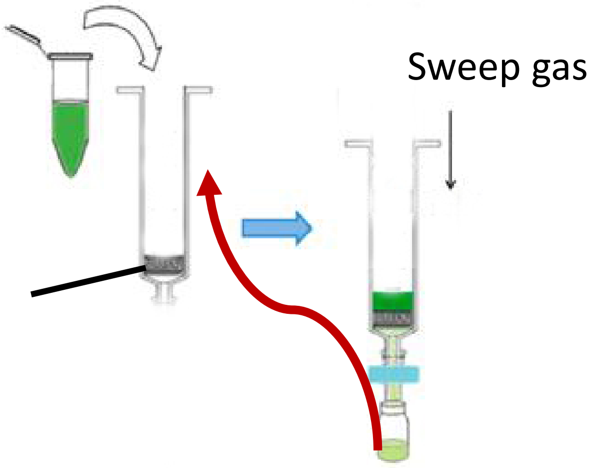
**Recovery on each mL elution**

| | ＜70% | 70%-140% | ＞140% |
|---|---|---|---|
| 1 mL | 51 | 37 | 0 |
| 2 mL | 28 | 45 | 15 |
| 3 mL | 17 | 21 | 50 |
| 4 mL | 2 | 28 | 58 |
| 5 mL | 1 | 30 | 57 |
| 6 mL | 1 | 37 | 50 |
| 7 mL | 1 | 31 | 56 |
| 8 mL | 3 | 33 | 52 |

# II. m-PFC method development

## Concept of multi- competition equilibration of Matrix VS Analytes



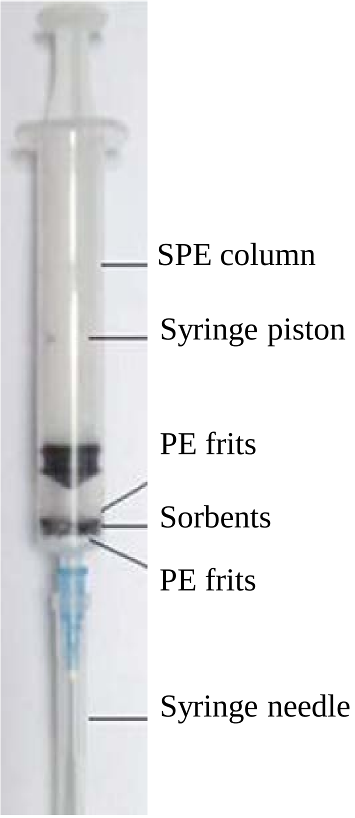



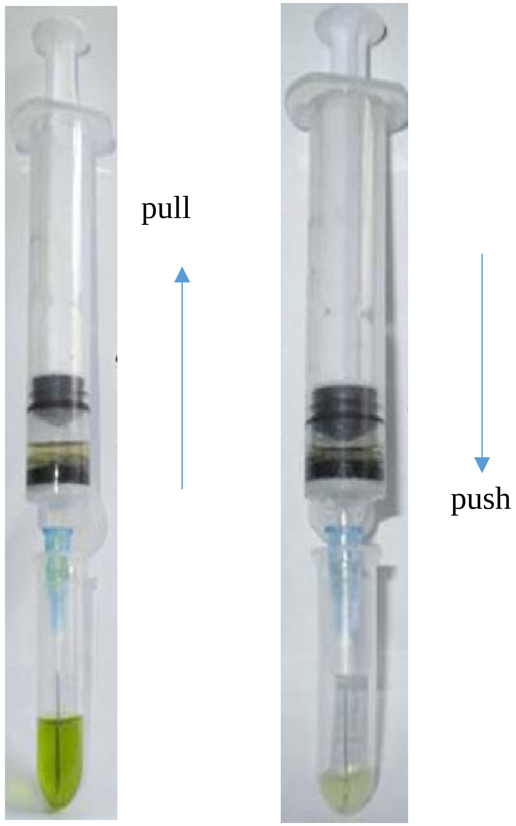

m-PFC: **Multi-plug Filtration Clean-up**

Multi-Filtration Cleanup : m-FC

# II. m-PFC method development

Multi-plug filtration cleanup (m-PFC) for analysis of pesticide residues.




1-4 cycles

**<u>M-PFC procedure</u>**

Packed optimized d-SPE sorbents in columns, connect to syringe. Pull and push the piston to let extracts through the sorbents.

**<u>Advantages</u>**:

without solvent evaporation, vortex , centrifugation

Absorbent active sites;

multiple cycles : multiple equilibration

Improve method performance and efficacy

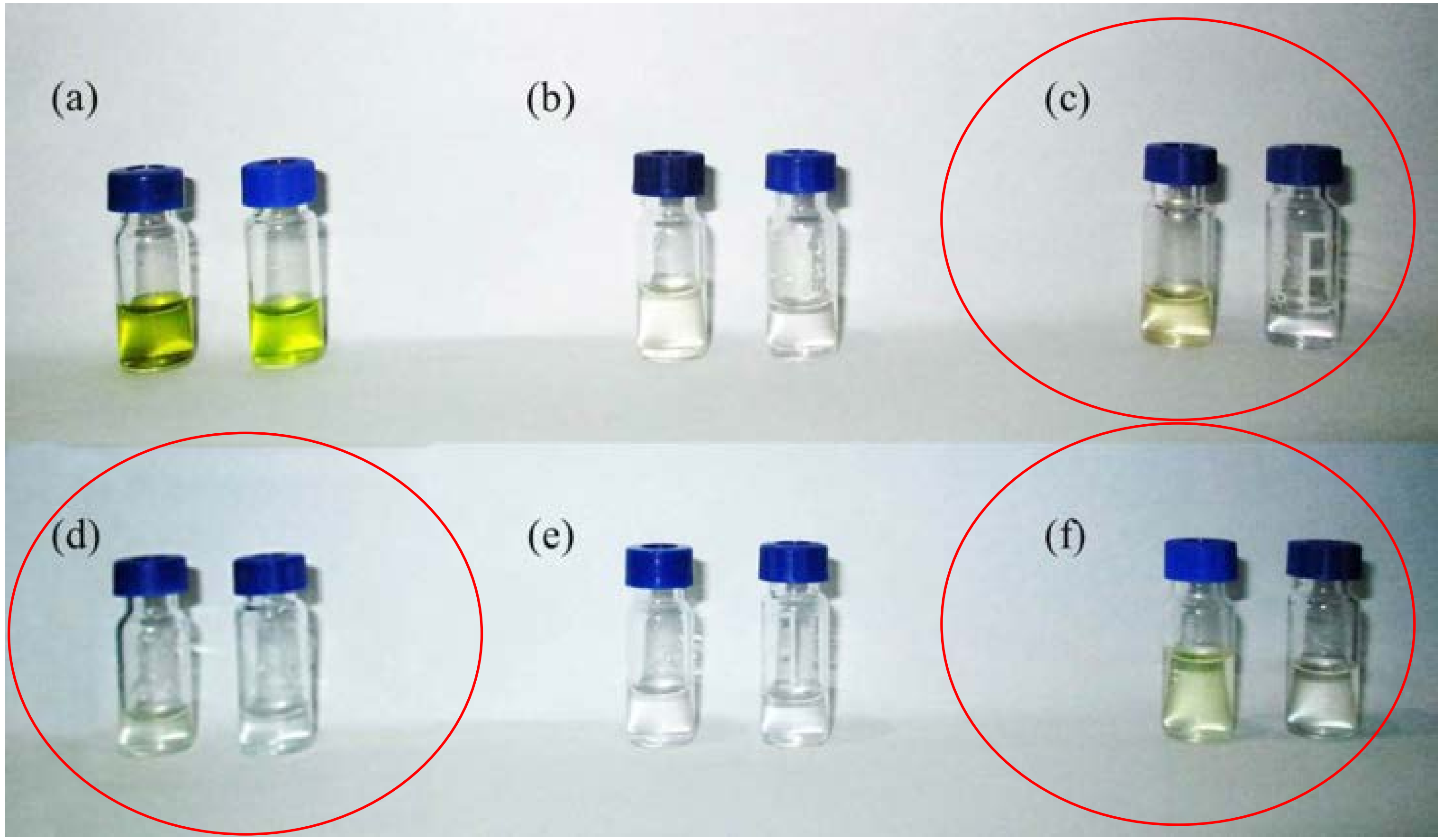
# m-PFC method developemnt

**Comparation**: m-PFC and d-SPE were compared for：

- 25 typical **pesticides**
- in **6 matrices** (wheat, spinach, carrot, apple, citrus and peanut)
- by LC-MS/MS detection

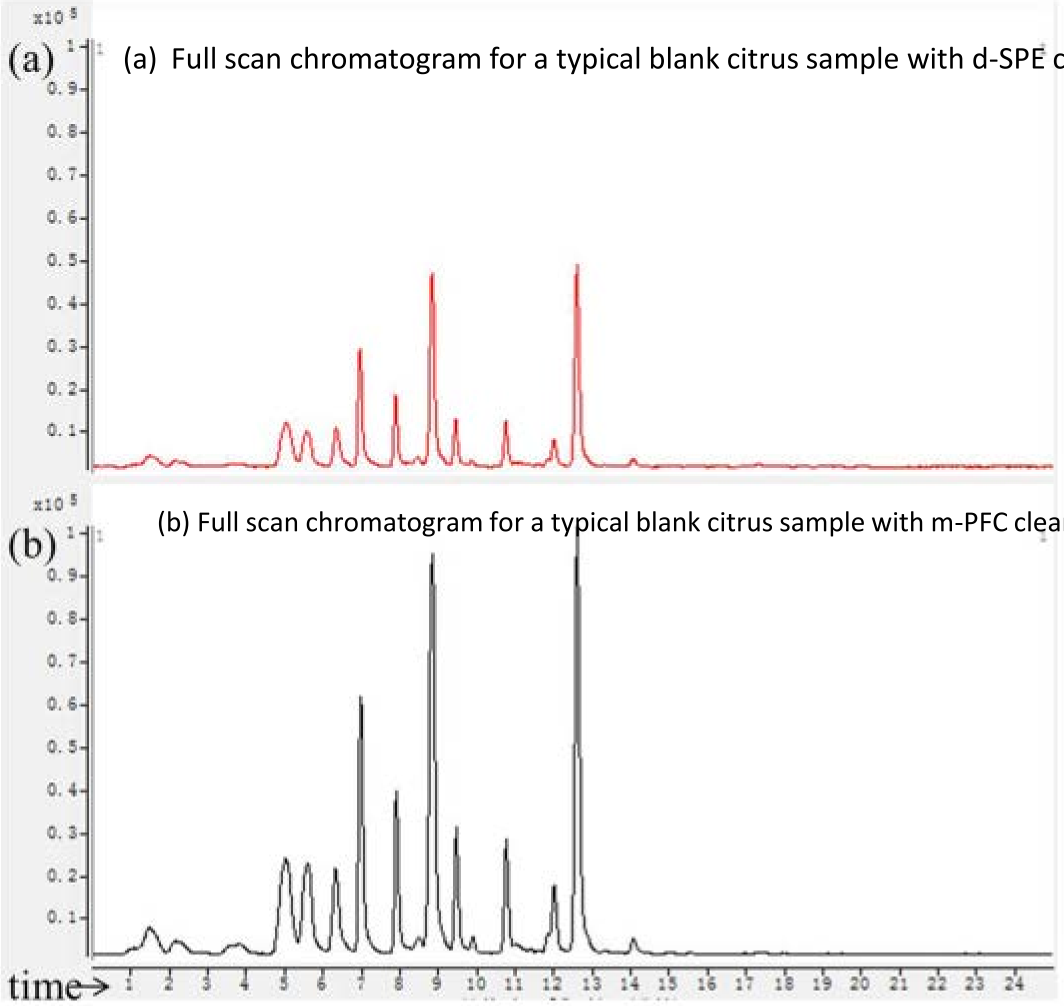


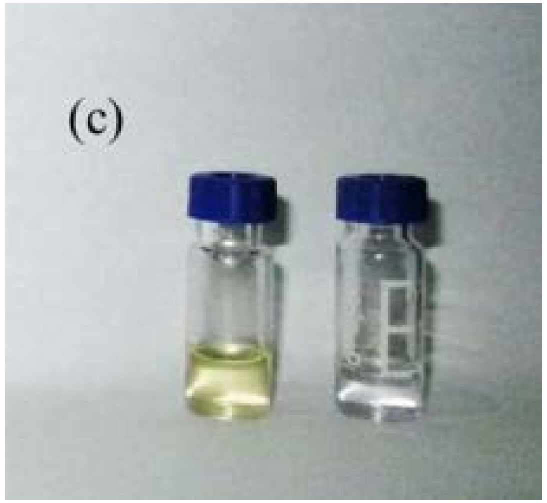
# Comparison between d-SPE and m-PFC:
# same amount of absorbents




Comparison of visual check (left : d-SPE, right :m-PFC):
(a): spinach sample; (b): wheat sample; **(c): citrus sample;**
**(d): peanut sample;** (e): apple sample; **(f): carrot sample.**

# Comparison between d-SPE and m-PFC



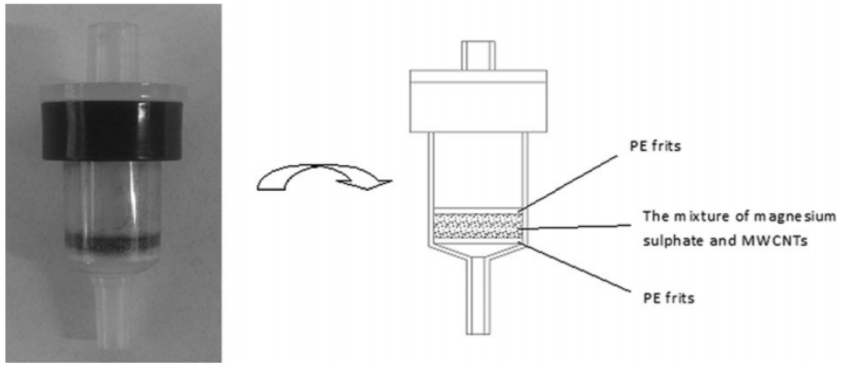

the chromatographic response intensity (Y-axis) of m-PFC method could be 2 times as of less matrix interferences

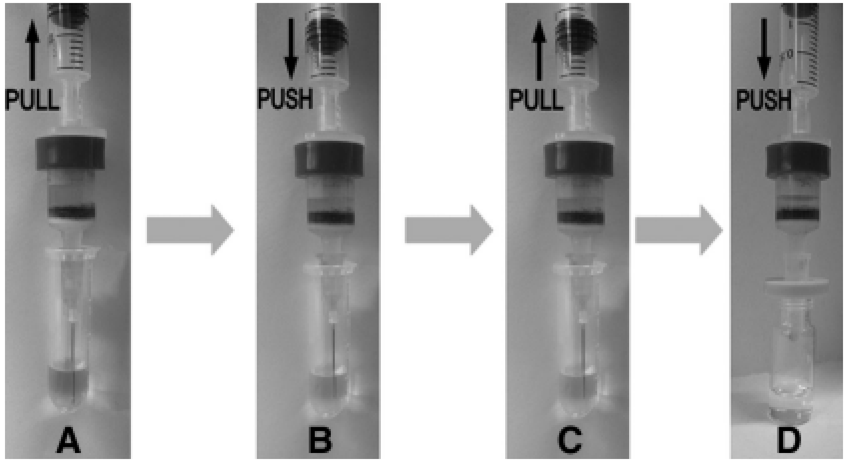
Better cleanup performance

Qin, Y., Zhao, P., Fan, S., Han, Y., Li, Y., **& Pan, C**. ***Journal of Chromatography A***, 2015, 1385, 1-11.

# II. m-PFC method application

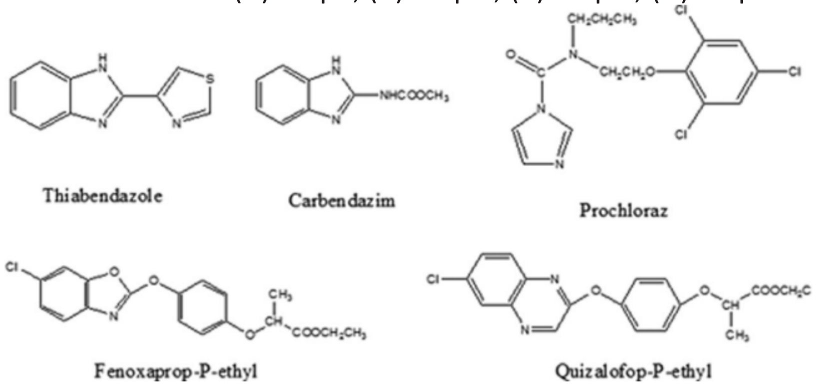
Analysis of 40 typical pesticide residues in apple, cabbage, and potato




Schematic diagram of the rapid m-PFC method. (A) Step1; (B) Step 2; (C) Step 3; (D) Step 4.

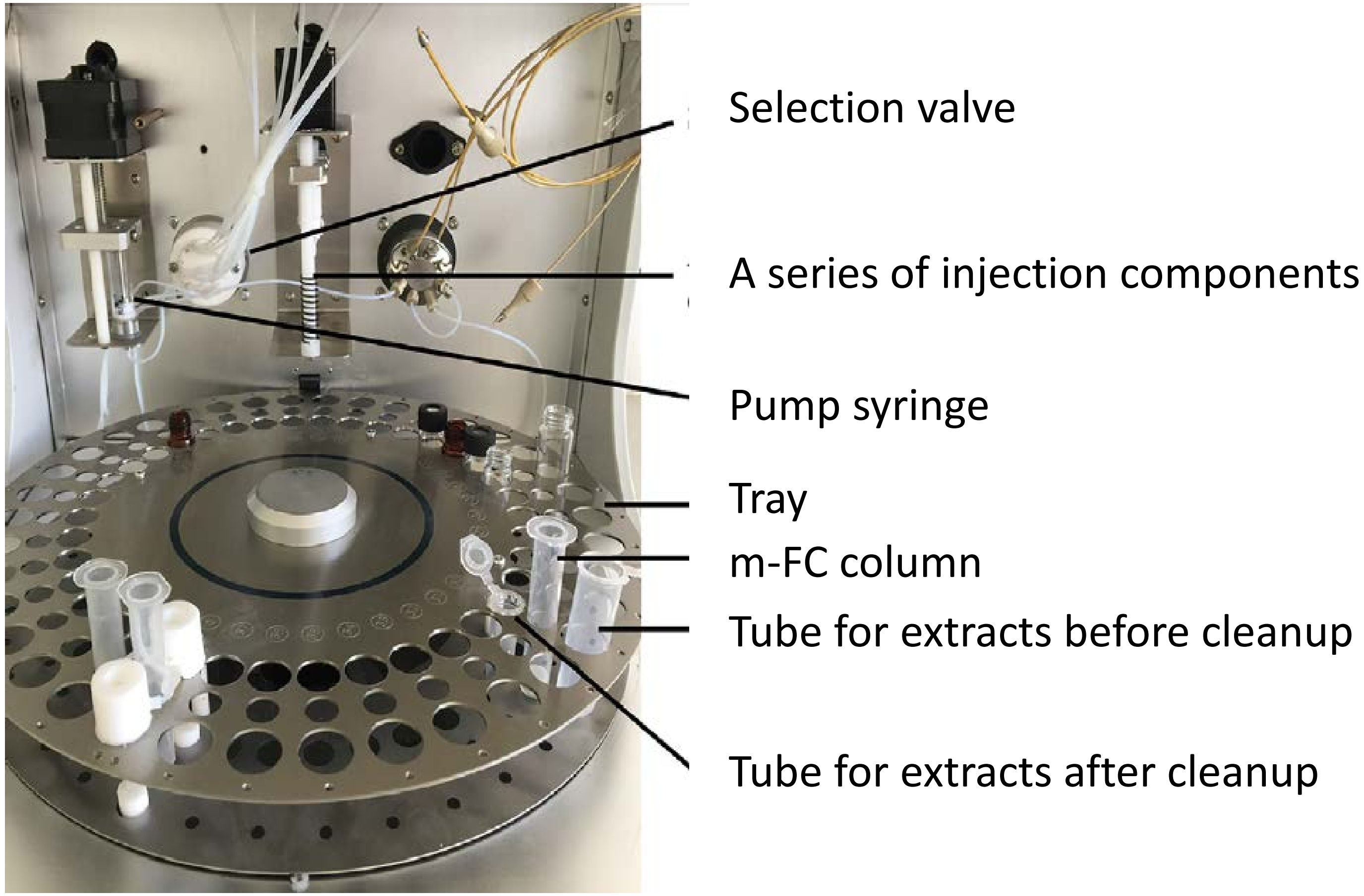
Recoveries： 71 %- 117 %（ except the benzo-heterocyclic compounds ）

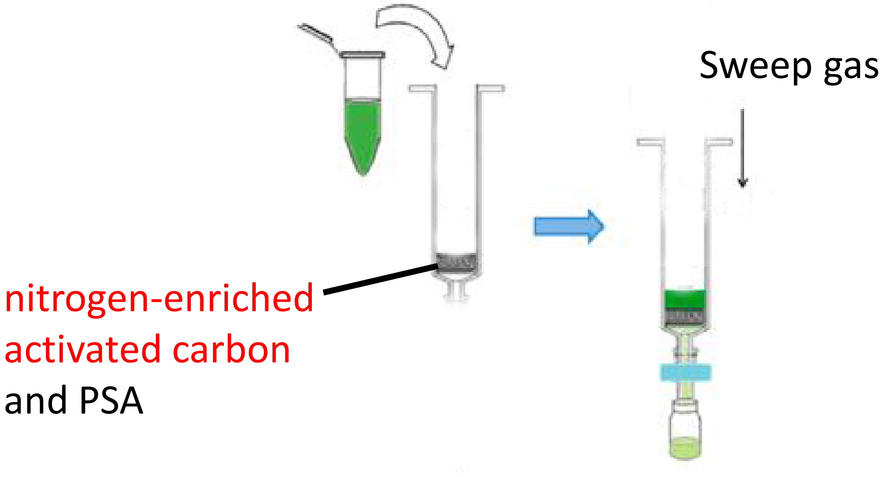



RSDs：＜15 %

Zhao, P.; Fan, S.; Yu, C.; Zhang, J.; Pan, C., *Journal of Separation Science* **2013**, *36* (20), 3379-3386.

# m-FC automatic device and evaluation
# ——Semi-automatic m-FC

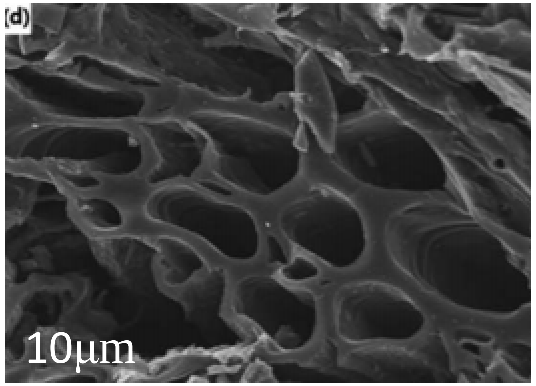


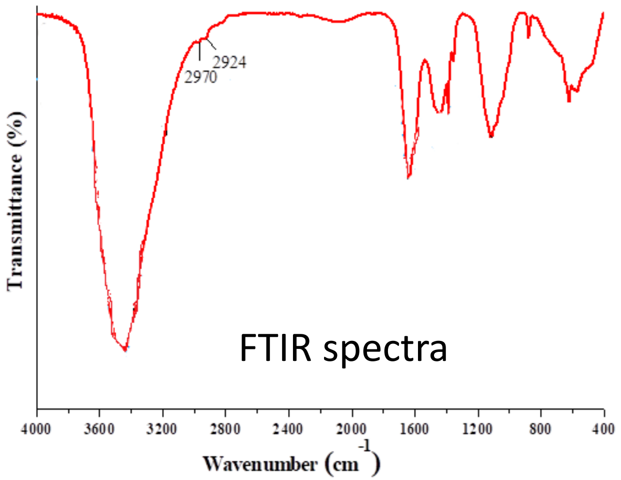




- Optimization of m-FC Cycles
- Optimization of m-FC volume
- Optimization of flow rate
- With nitrogen-enriched activated carb

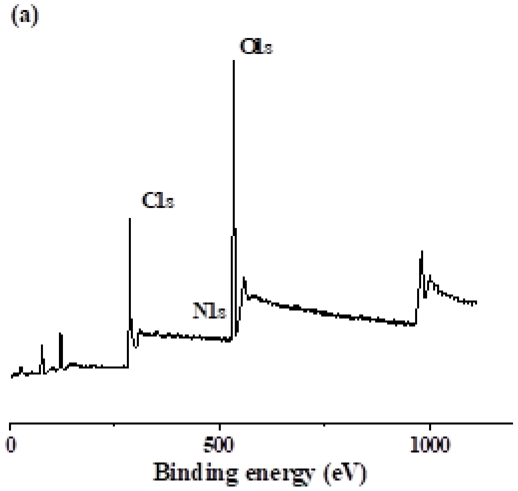
**Components diagram of the Auto m-FC equipment**

# m- FC automatic device and evaluation
## ——Semi-automatic m-FC

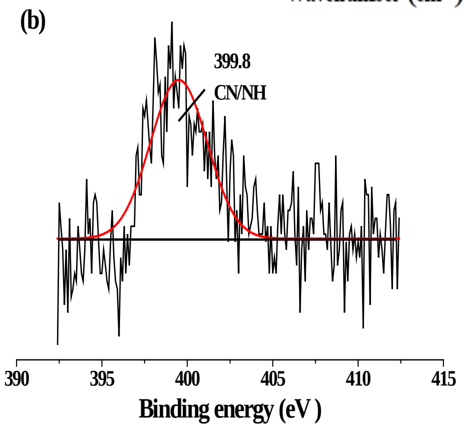



FTIR spectra




XPS wide scan




N1s core-level spectra

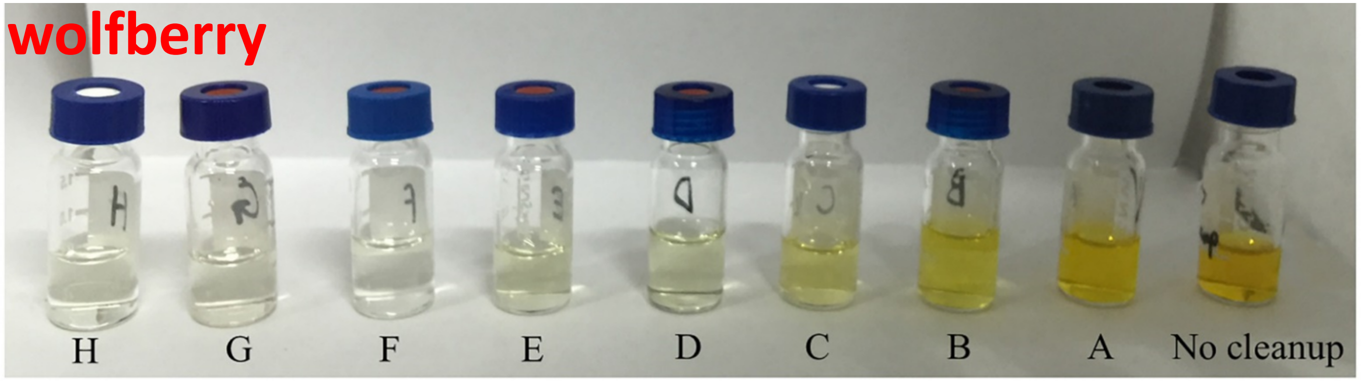
nitrogen-enriched activated carbon

The BET surface area： 1868.36($m^2$/g)

Eco-friendly material prepared from the wasted medium density fiberboard

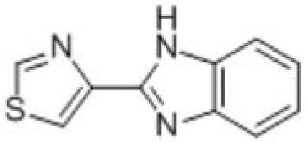
# III. m- FC automatic device and evaluation
## ——Semi-automatic m-FC

nitrogen-enriched activated carb: adsorb interfering substances in matrices, rather than the analytes

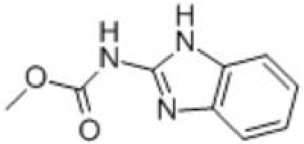



A: 5 mg MWCNTs+ 30 mg PSA + 150 mg $MgSO_4$
B: 5 mg MWCNTs+ 50 mg PSA + 150 mg $MgSO_4$
C: 5 mg MWCNTs+ 30 mg PSA + 5 mg GCB+ 150 mg $MgSO_4$
D: 5 mg MWCNTs+ 50 mg PSA + 5 mg GCB+ 150 mg $MgSO_4$
E: 10 mg commercial activated carbon+ 150 mg $MgSO_4$
F: 10 mg commercial activated carbon+ 50 mg PSA+ 150 mg $MgSO_4$
G: 10 mg nitrogen-enriched activated carbon+ 150 mg $MgSO_4$
H: 10 mg nitrogen-enriched activated carbon+ 50 mg PSA+ 150 mg $MgSO_4$

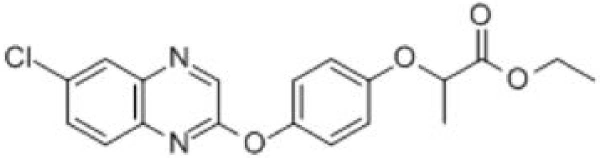
MWCNTs: somebenzo-heterocyclic compounds loss



Thiabendazole



Carbendazim



Quizalofop-p-ethyl

| | apple | citrus | peanut | wheat | tea | spinach |
|---|---|---|---|---|---|---|
| Thiabendazole | 89(6) | 93(4) | 93(4) | 104(2) | 84(6) | 95(1) |
| Quizalofop-p-ethyl | 93(4) | 96(3) | 92(3) | 93(6) | 93(2) | 94(6) |
| Carbendazim | 101(3) | 89(3) | 87(6) | 89(3) | 96(7) | 88(4) |

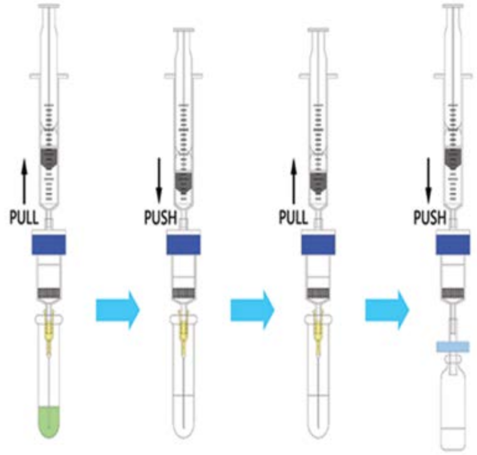
Recoveries and RSDs at 100μg/kg in six matrices

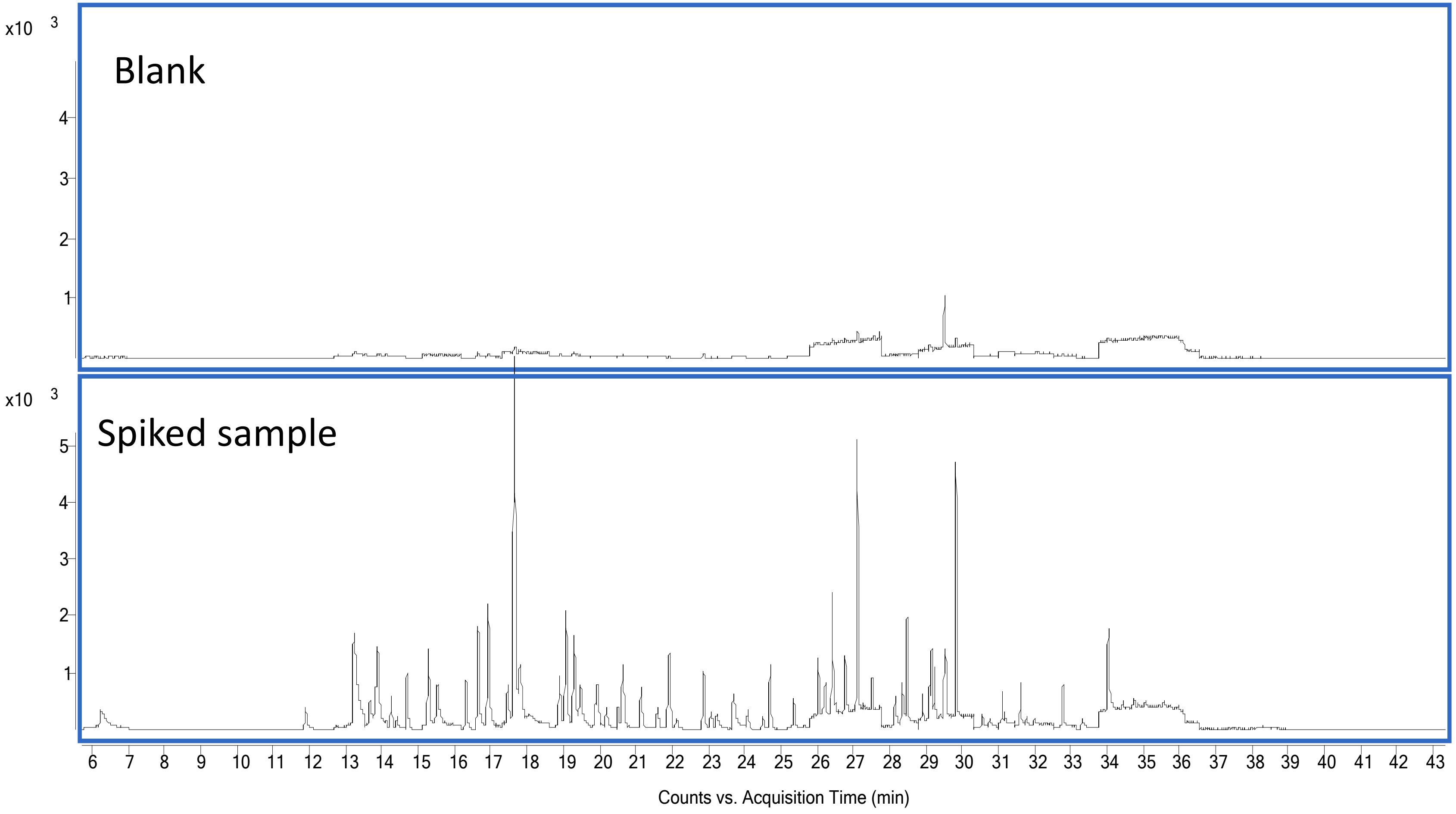


Qin, Y., Zhang J., Li, Y., Wang, Q., Wu Y., Xu L., Jin x., & **Pan, C**. ***Journal of Chromatography A***, 2017, 1515:62-68.

# II. m-PFC method application

- Analysis of 186 pesticide residues in tomato and tomato products

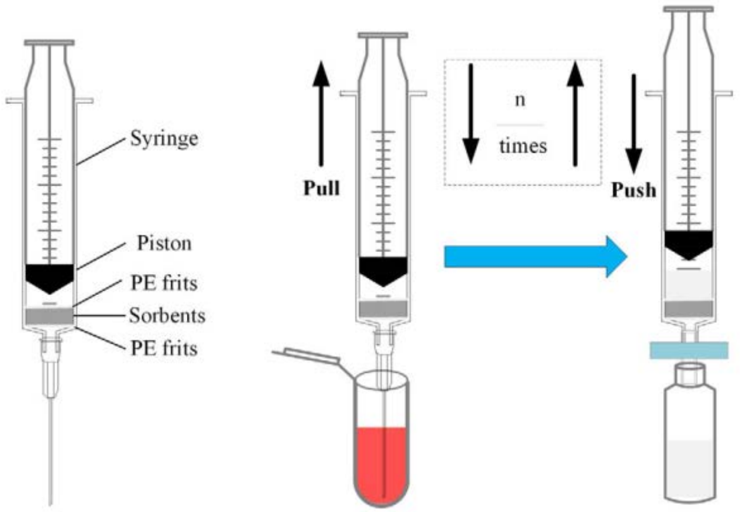


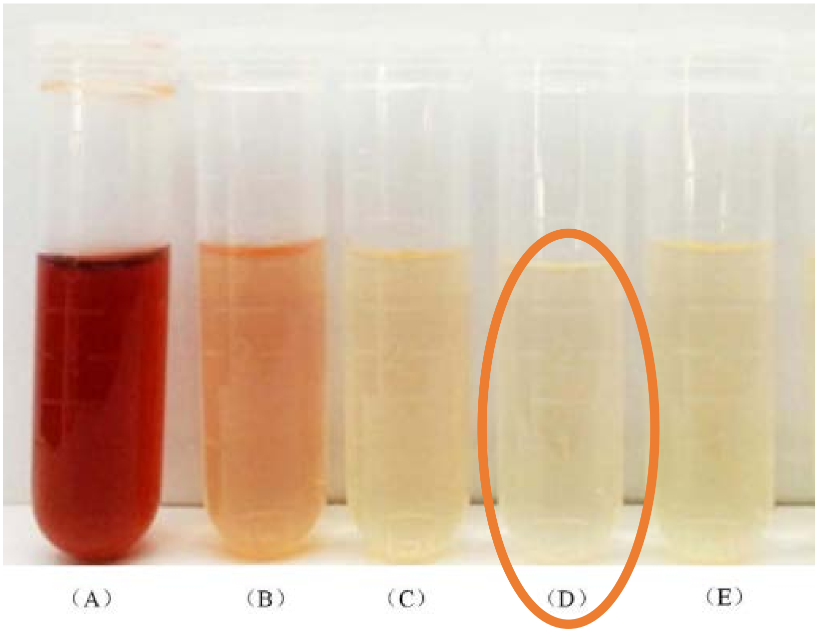




Zhao, P.; Huang, B.; Li, Y.; Han, Y.; Zou, N.; Gu, K.; Li, X.; Pan, C., Journal of agricultural and food chemistry 2014, 62 (17), 3710-3725.

# II. m-PFC method application

- Analysis of 142 pesticide residues in Chinese liquor (Moutai etc) and liquor-making raw materials (sorghum and rice hull)




(A)without cleanup,

(B)1 cycle,

(C)2 cycles,

(D)3 cycles,

(E)4 cycles.

Method validation: n=2-3

Recoveries： 71 %- 121 %

RSDs：＜16.8 %

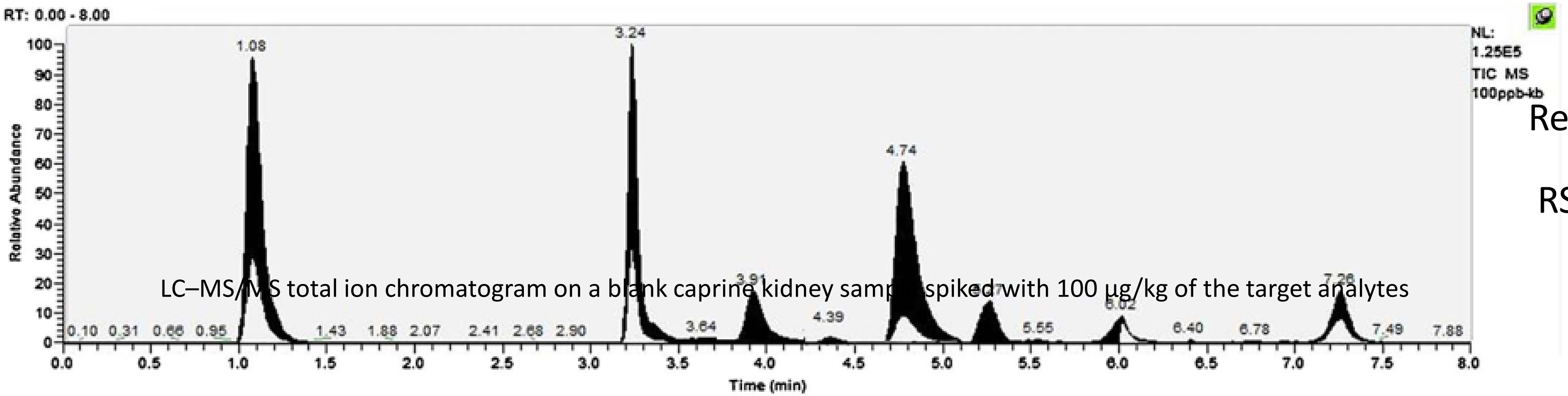
LOQs： 0.1~5μg/kg

LODs： 0.03~1.5μg/kg

Han Y, Song L, PAN C, et al. Food Chemistry, 2018:258-267.

# II. m-PFC method application

- Analysis of veterinary drug residues in typical animal matrices: meat, kidney, milk:
- involved low-temperature cleanup to remove lipid, and further by m-PFC with MWCNTs and other




LC–MS/MS total ion chromatogram on a blank caprine kidney sample spiked with 100 μg/kg of the target analytes

Recoveries： 82-107%

RSDs： < 15%

| Analyte | bovine meat (μg/kg) | | | caprine meat (μg/kg) | | | swine meat (μg/kg) | | | bovine kidney (μg/kg) | | caprine kidney (μg/kg) | | swine kidney (μg/kg) | | milk (μg/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CC_\alpha$ | $CC_\beta$ | MRL | $CC_\alpha$ | $CC_\beta$ | MRL | $CC_\alpha$ | $CC_\beta$ | MRL | $CC_\alpha$ | $CC_\beta$ | $CC_\alpha$ | $CC_\beta$ | $CC_\alpha$ | $CC_\beta$ | $CC_\alpha$ | $CC_\beta$ |
| *cis*-Tilmicosin | 50.1 | 50.1 | 50.0 | 50.1 | 50.3 | 50.0 | 50.2 | 50.4 | 50.0 | 1.00 | 1.26 | 1.00 | 1.18 | 1.00 | 1.25 | 0.20 | 0.36 |
| *trans*-Tilmicosin | 50.1 | 50.3 | | 50.2 | 50.5 | | 50.2 | 50.5 | | 1.00 | 1.25 | 1.00 | 1.25 | 1.00 | 1.23 | 0.20 | 0.43 |
| Sulfamethazine | 100 | 100 | 100 | 100 | 101 | 100 | 100 | 101 | 100 | 2.00 | 2.30 | 2.00 | 2.26 | 2.00 | 2.26 | 1.00 | 1.20 |
| Sulfamethoxazole | 100 | 101 | 100 | 100 | 101 | 100 | 100 | 100 | 100 | 2.00 | 2.15 | 2.00 | 2.20 | 2.00 | 2.15 | 1.00 | 1.15 |
| Sulfamonomethoxine | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 2.00 | 2.25 | 2.00 | 2.28 | 2.00 | 2.25 | 1.00 | 1.13 |
| Abamectin | 20.1 | 20.2 | 20.0 | 2.00 | 2.13 | – | 2.00 | 2.28 | – | 2.00 | 2.30 | 2.00 | 2.30 | 1.00 | 1.28 | 2.00 | 2.25 |
| Doramectin | 20.1 | 20.2 | 20.0 | 2.00 | 2.25 | – | 2.00 | 2.18 | – | 2.00 | 2.26 | 2.00 | 2.26 | 1.00 | 1.18 | 2.00 | 2.21 |
| Ivermectin | 20.1 | 20.2 | 20.0 | 2.00 | 2.23 | – | 2.00 | 2.33 | – | 2.00 | 2.33 | 2.00 | 2.15 | 1.00 | 1.20 | 2.00 | 2.20 |

CCαs and CCβs of the analytes in each matrix

Qin Y , Jatamunua F , PAN C, et al. Journal of Chromatography B, 2017, 1053:27-33.

# II. m-PFC method application

- Sample preparation- with IMS and Raman rapid detection

10 g of homogenized apple sample

↓

Add 10 mL Acetonitrile

↓ Shake vigorously 1 min

Add 5 g NaCl

↓ Shake 1 min and Centrifuge

Clean up with m-PFC → DART-MS; ASAP-MS/MS

↓ Raman Spectr.

↓ Ion Mobility Spectroscopy

# II. m-PFC method application

- Ion Mobility Spectrometry(IMS) for rapid analysis

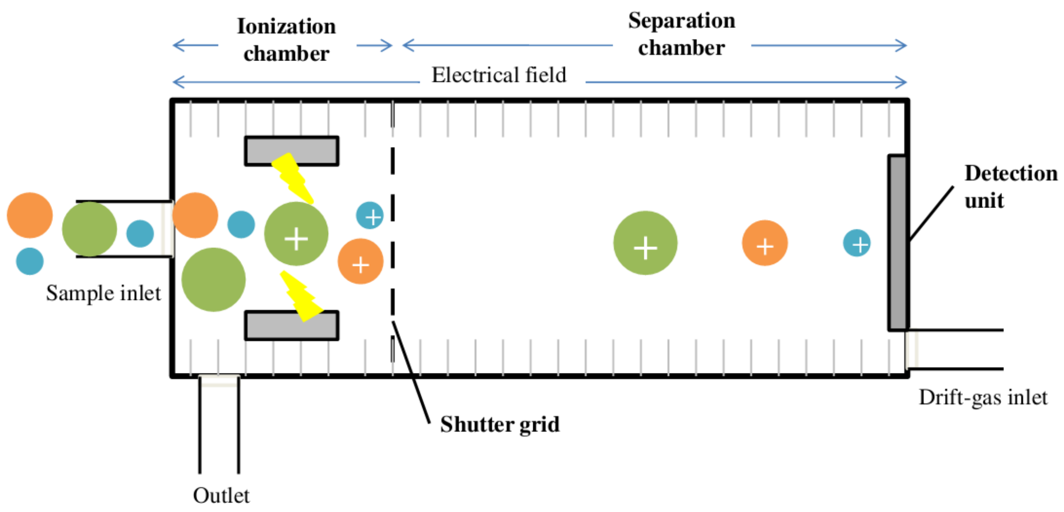
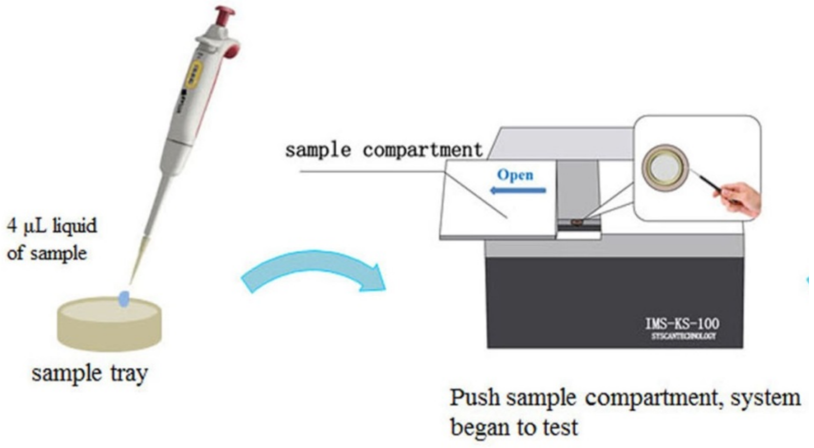
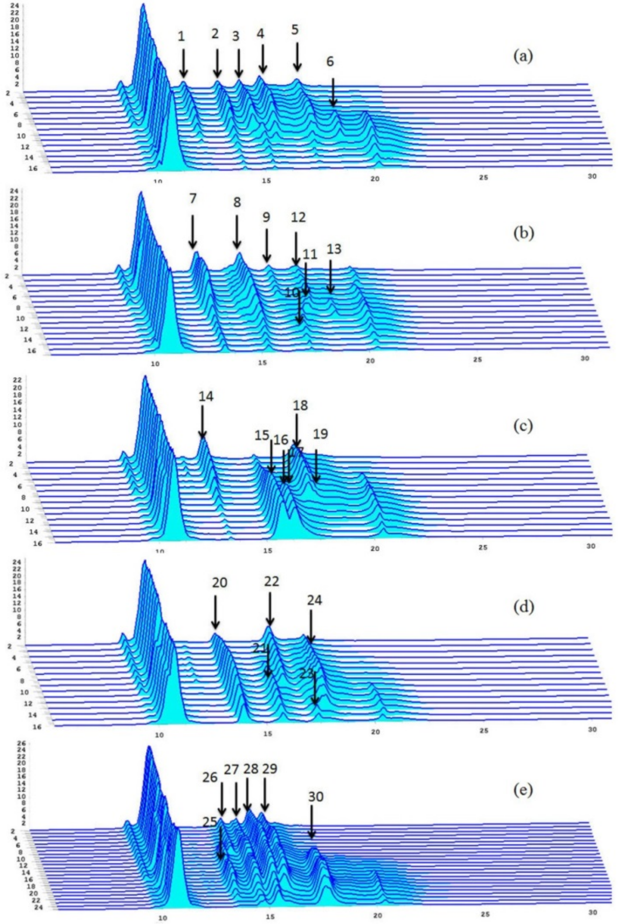
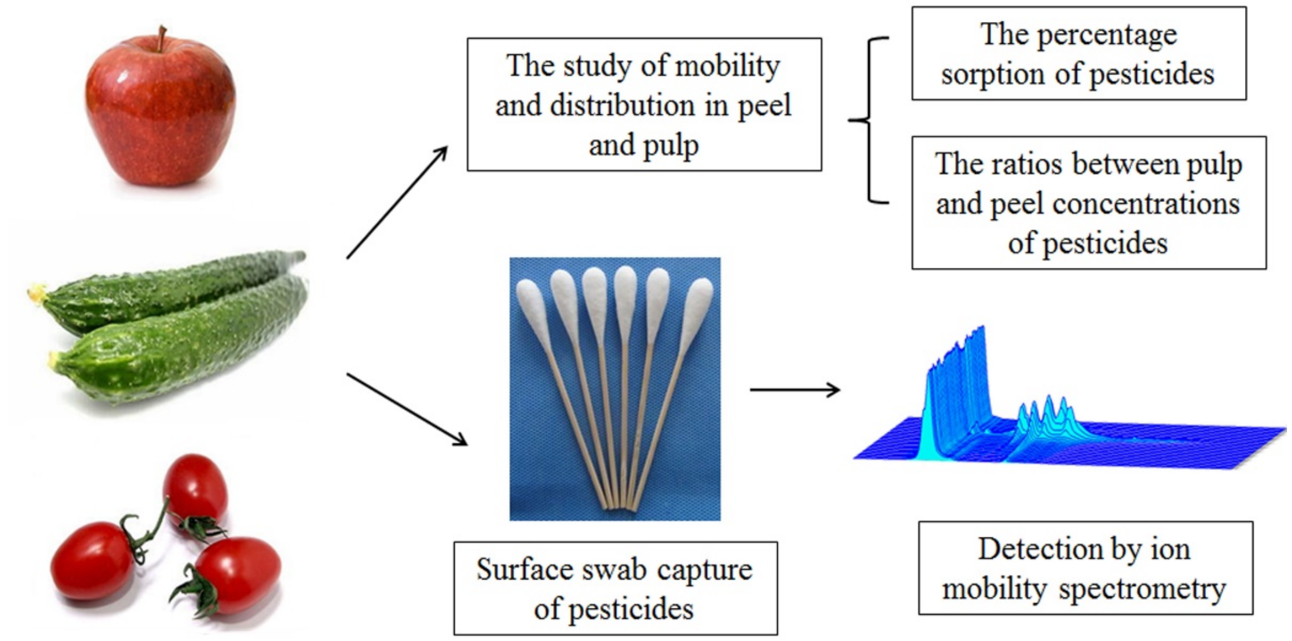


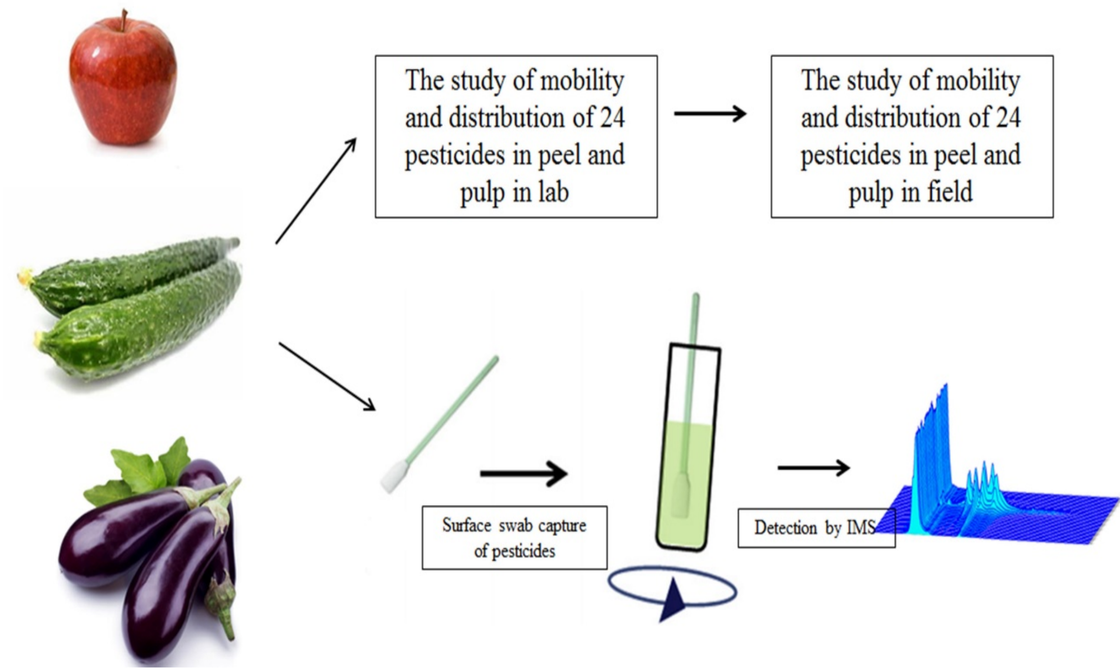

**Figure 2.** A conventional ion-mobility spectrometry (IMS) device.

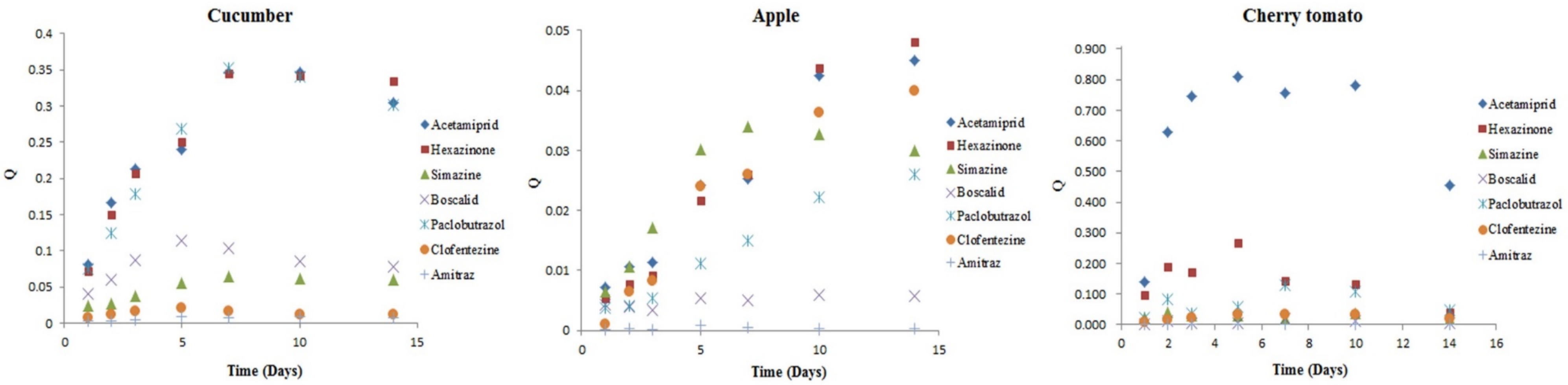



**(a)** 1 Methamidophos, 2 Omethoate, 3 Monocrotophos, 4 Isocarbophos, 5 Phosphamidon, 6 Triadimenol;
**(b)** 7 Methomyl, 8 Carbaryl, 9 Isoprocarb,
10 Metolachlor, 11 Chlorbenzuron , 12 Uniconazole, 13 Triadimefon;
**(c)** 14 Acephate, 15 Acetamiprid, 16 Thiacloprid, 17 Nitenpyram, 18 Paclobutrazol, 19 Propiconazol;
**(d)** 20 Tricyclazole, 21 Dimethomorph,
22 Propyzamide, 23 Tebuconazole, 24 Myclobutani;
**(e)** 25 Diazinon, 26 Simazine, 27 Atrazine,
28 Ametryn, 29 Alachlor, 30 RH-5849.






J. Agric. Food Chem. 2017, 65, 182−189.

# II. m-PFC method application

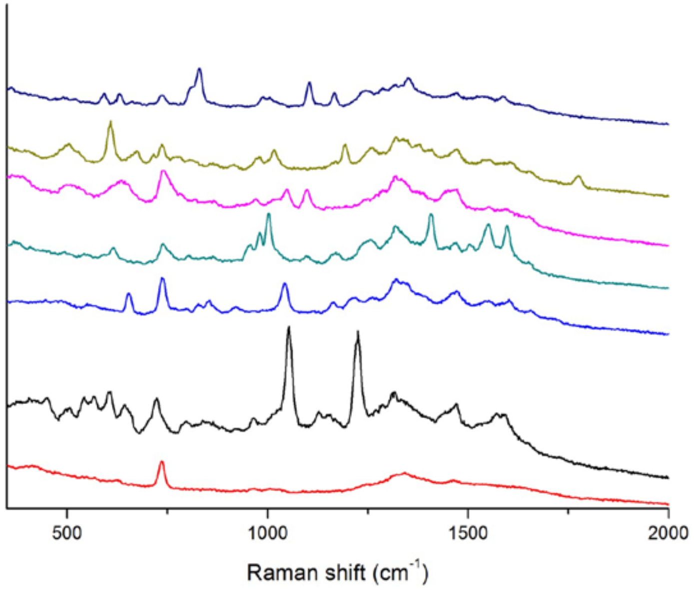
- Raman for rapid analysis for HHPs


**Traditional methods**

- Long time
- Hard to real-time detection

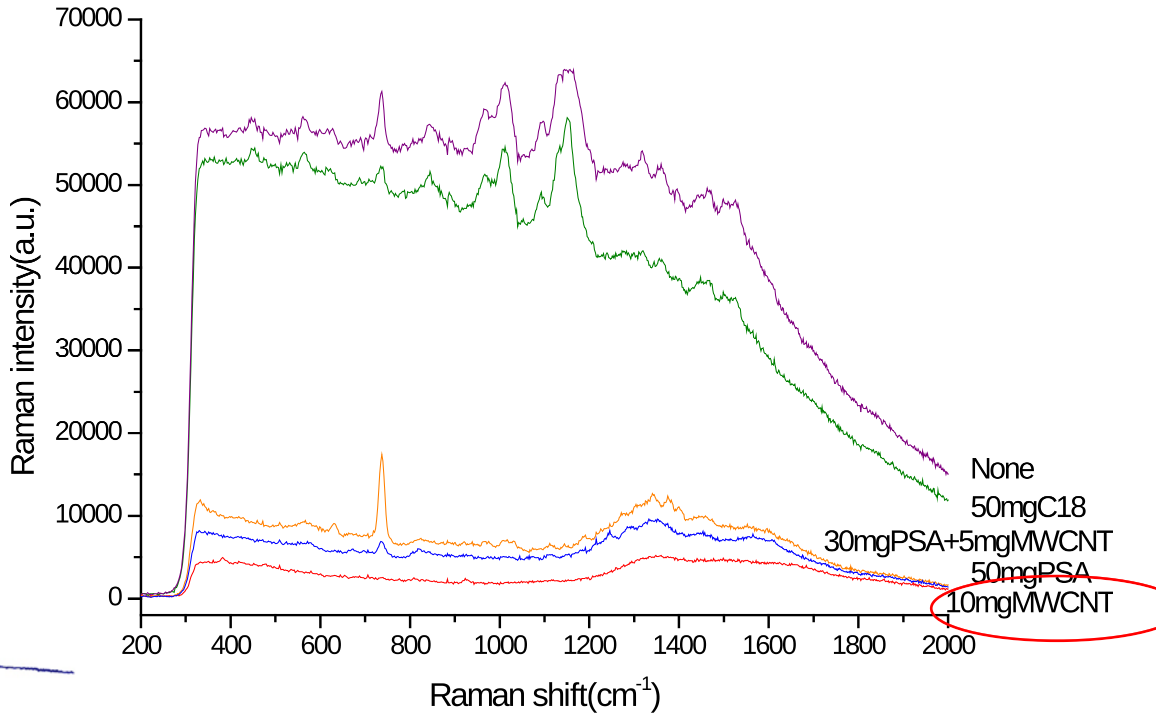
**Raman spectroscopy**

- Detect in seconds
- Small and portable



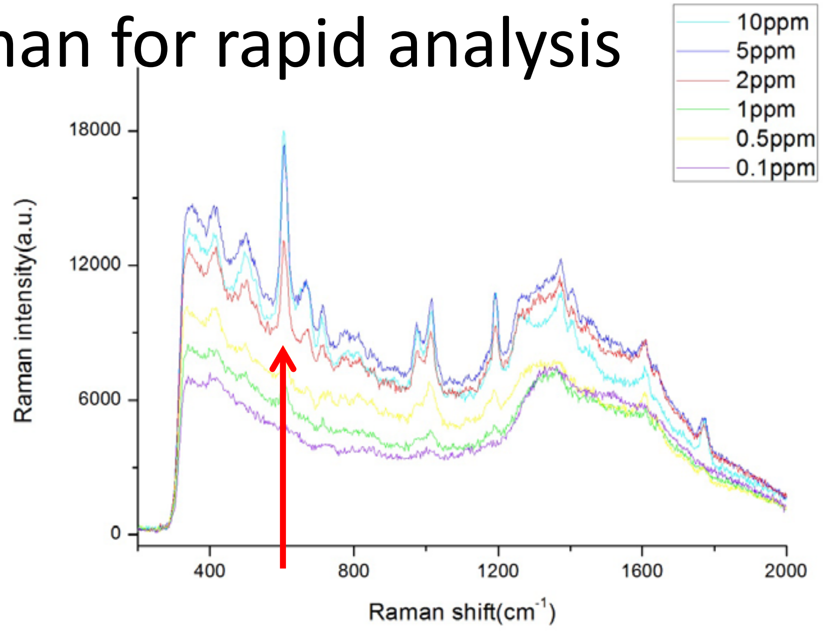

blank

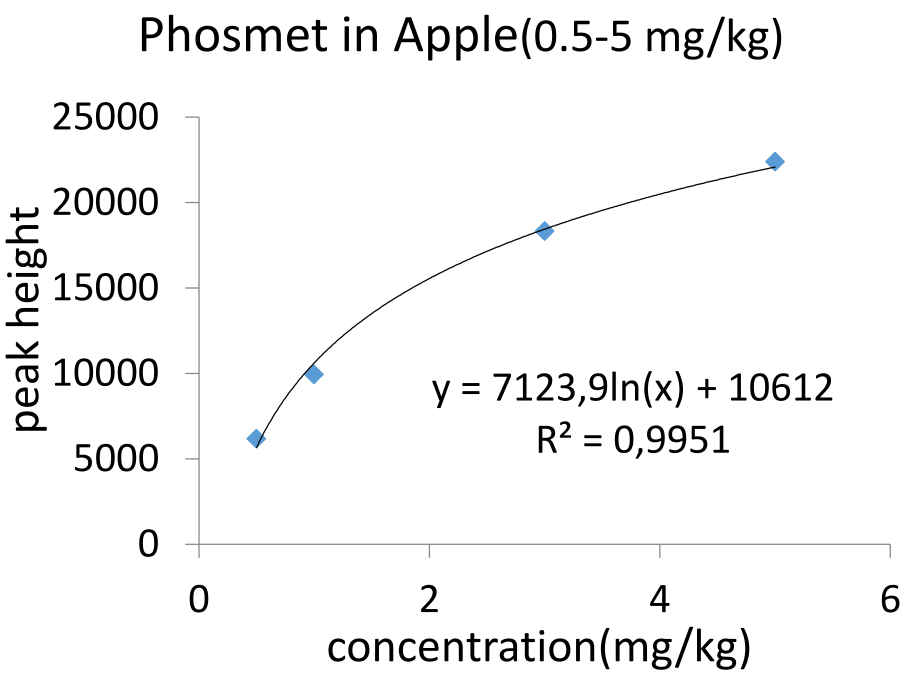
Different cleanup methods in a blank apple sample



# II. m-PFC method application

- Raman for rapid analysis



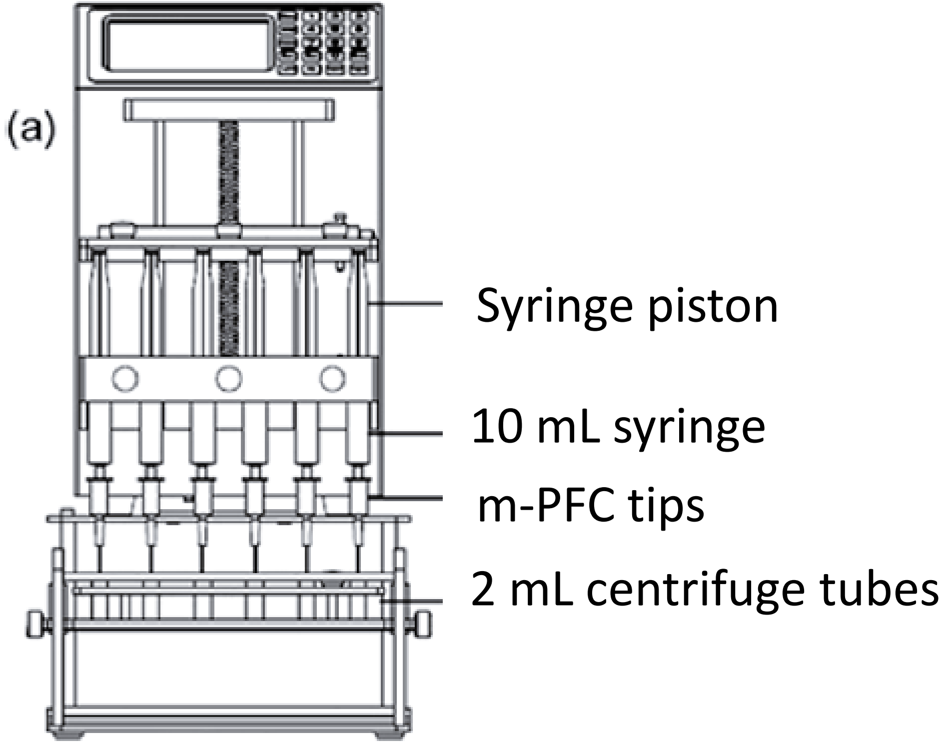

**Quantification**

**Method Recoveries**

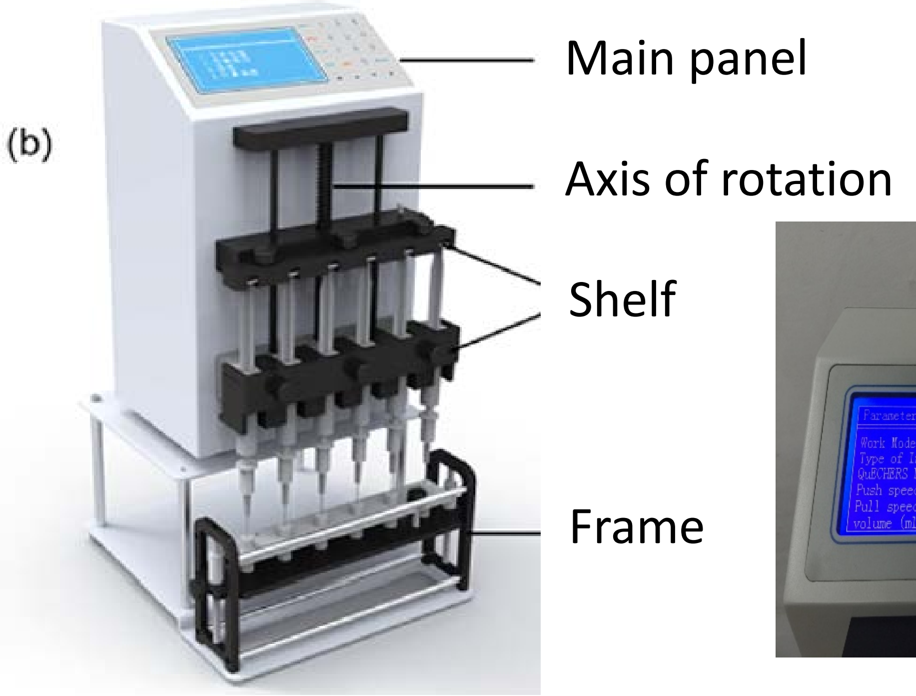
| concentration（mg/kg） | recovery %（n=4） 1 | 2 | 3 | 4 | average recovery % | RSD% |
|---|---|---|---|---|---|---|
| 0.5 | 83.1% | 101.4% | 101.1% | 100.4% | 96.5% | 9.3 |
| 1 | 73.3% | 73.1% | 93.3% | 93.5% | 83.3% | 14 |
| 2 | 71.8% | 84.1% | 77.3% | 75.8% | 77.3% | 6.6 |

*Jiang Li, PAN C, et al, Rapid detection of pesticide residues in fruits by surface-enhanced Raman scattering , SN Applied Sciences, 2019*

# III. m-PFC automatic device and evaluation
# ——Semi-automatic m-PFC




**(a) schematic diagram of equipment**

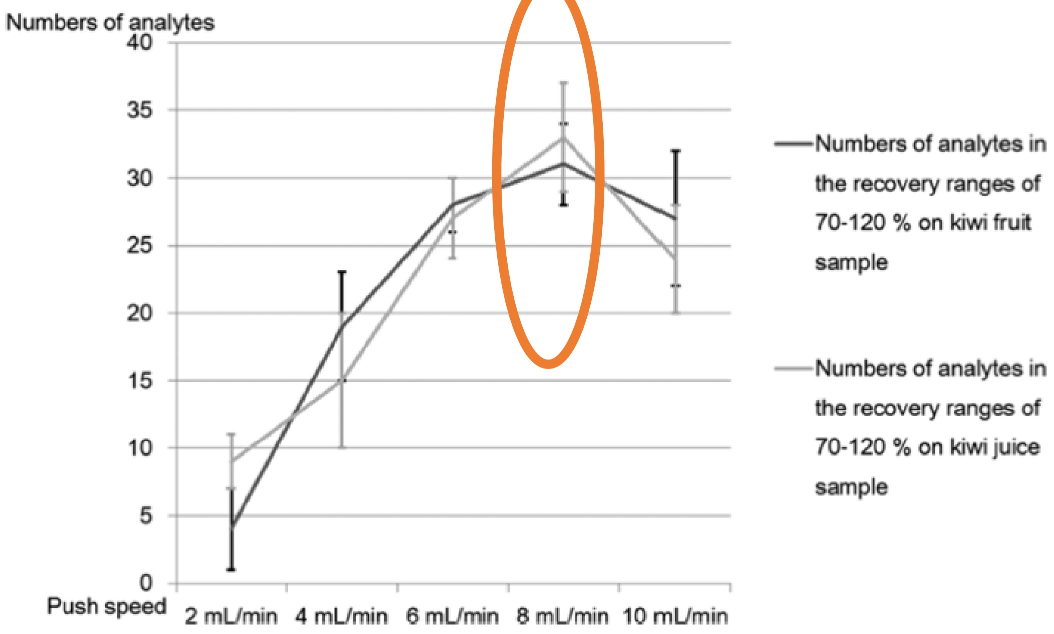


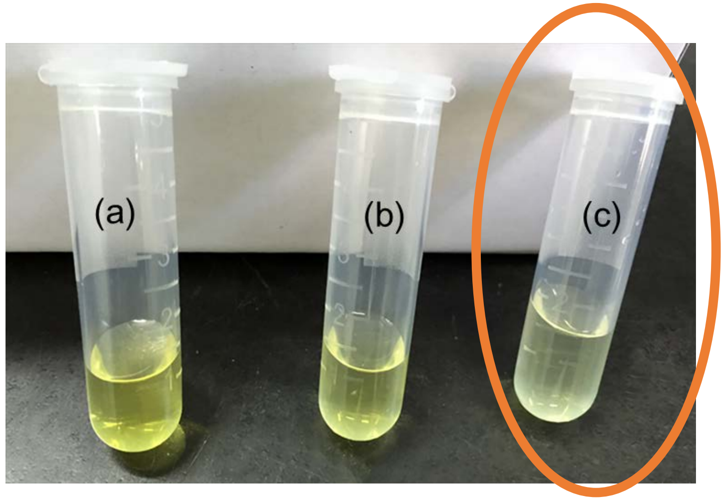

**Method Editor**

**(b) components of automated m-PFC equipment**

# III. m-PFC automatic device and evaluation
# ——Semi-automatic m-PFC

- Optimization of m-PFC Cycles
- Optimization of automated m-PFC Volume
- Optimization of pushing speed
- Optimization of pulling speed



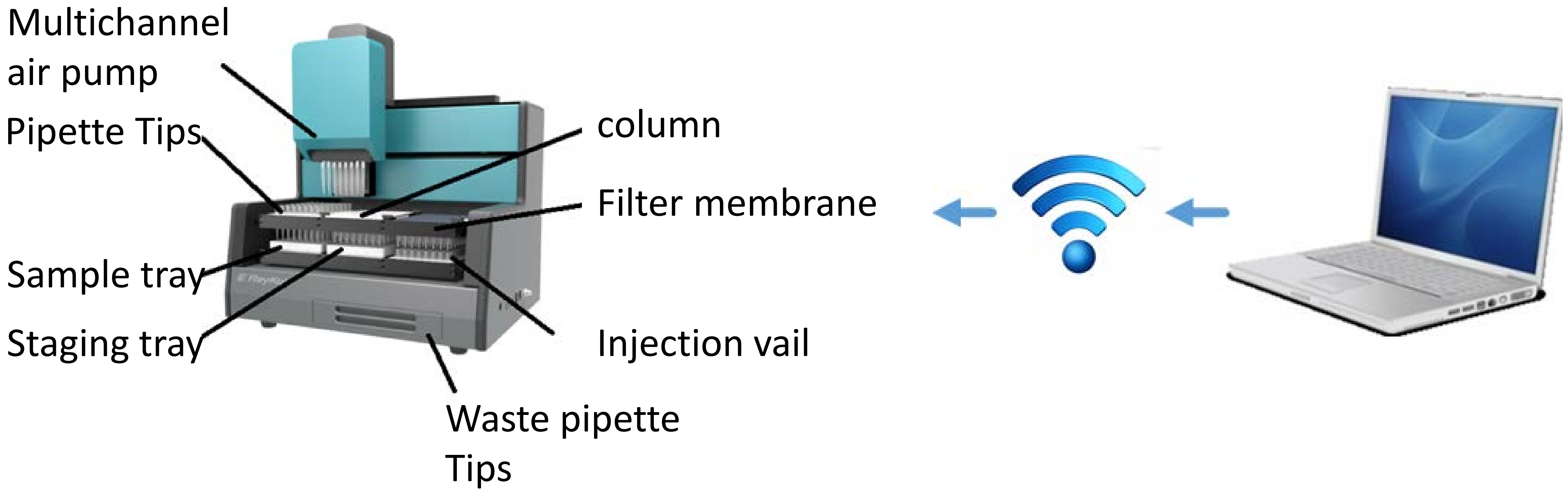

Comparison at different pushing speeds on the kiwi fruit sample spiked with the pesticides at 0.1 mg/kg:

(a)4 mL/min; (b) 6 mL/min; (c) 8 mL/min.

Qin, Y., Zhang J, He, Y, Han, Y., & **Pan, C**. ***Analytical and Bioanalytical Chemistry***, 2016, 408:5801-5809

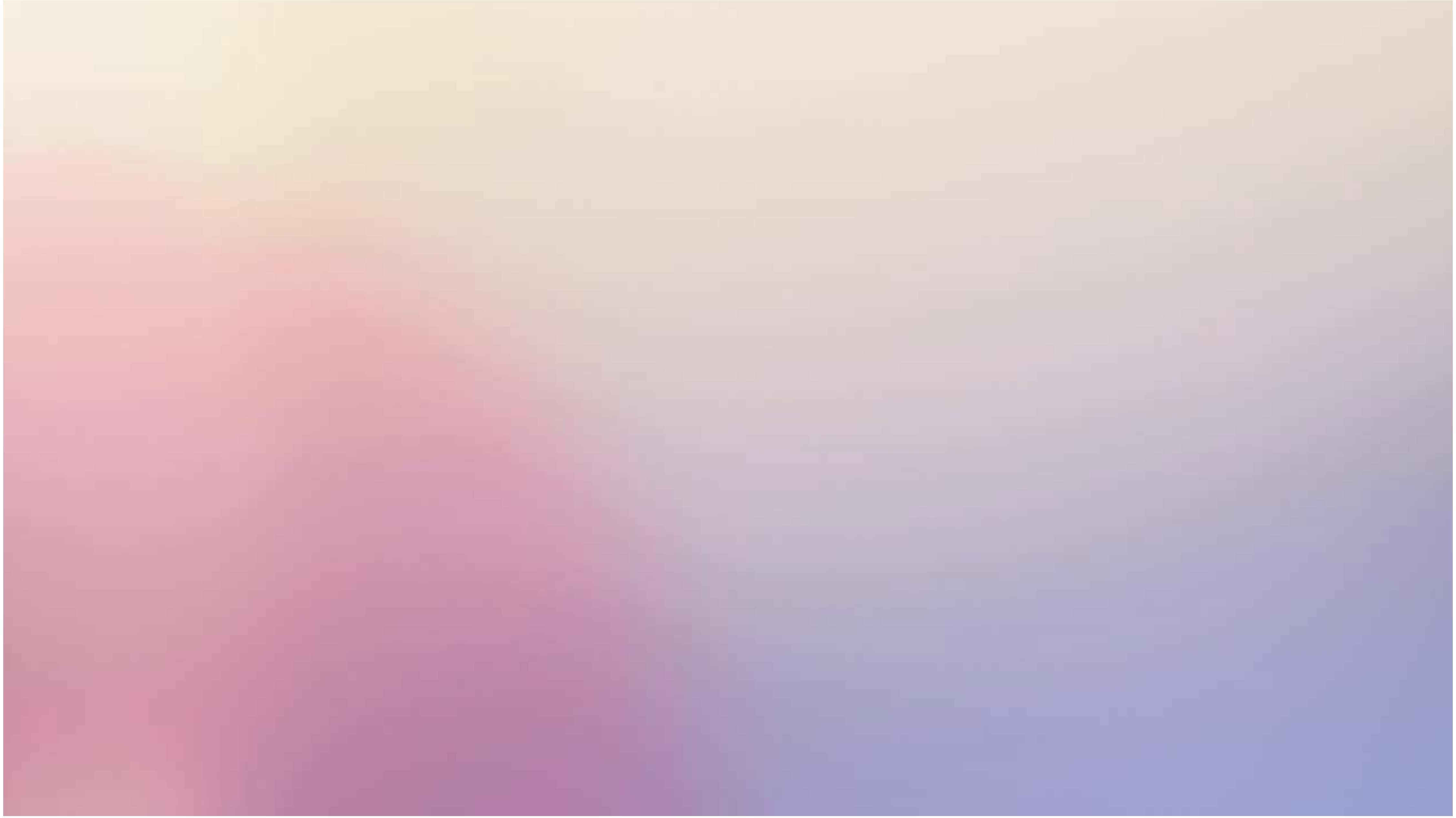
# III. m-PFC automatic device and evaluation

## Multi-channel m-PFC /FC device




Sample → (add) → Acetonitrile → (add, vortex) → NaCl+MgSO4 → (vortex, centrifugation) → Supernatant

Pull the piston → Push the piston → Repeat 1-4 cycles → vail



Comparison of the proposed method with mean recoveries and RSD in celery at 0.05 mg/kg

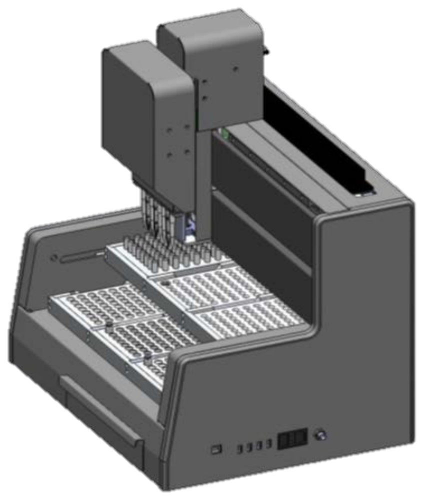
| m-PFC | automatic one-step m-PFC | | manual m-PFC | |
|---|---|---|---|---|
| | Average recovery | RSD(n=3) | Average recovery | RSD(n=3) |
| methomyl | 96% | 1.1% | 92% | 2.5% |
| clothianidin | 98% | 2.5% | 97% | 2.9% |
| imidacloprid | 94% | 1.6% | 93% | 6.4% |
| acetamiprid | 95% | 1.0% | 94% | 2.8% |
| acephate | 86% | 2.4% | 84% | 2.7% |
| imazalil | 91% | 2.1% | 84% | 2.2% |
| pyrimethanil | 77% | 1.3% | 76% | 3.3% |
| thiophonate-methyl | 92% | 0.6% | 85% | 2.0% |
| flutriafol | 99% | 1.7% | 96% | 1.9% |
| metalaxyl | 101% | 1.8% | 96% | 2.9% |
| chlorantraniliprole | 88% | 2.2% | 87% | 2.3% |
| kresoxim-methyl | 101% | 1.5% | 94% | 2.7% |
| boscalid | 85% | 1.1% | 85% | 3.0% |
| tebuconazole | 97% | 1.7% | 91% | 1.5% |
| tebufenozide | 98% | 3.4% | 95% | 2.6% |
| difenoconazole | 98% | 0.7% | 96% | 2.5% |
| pyraclostrobin | 86% | 1.3% | 86% | 1.4% |
| trifloxystrobin | 93% | 1.7% | 91% | 3.2% |
| hexythiazox | 96% | 1.7% | 99% | 3.0% |
| pyridaben | 101% | 1.1% | 102% | 2.4% |

**massive determination high efficiency**

**robust and repro-ducible**

**high-throughput**

**Automatic m-PFC process and filtration with filter membrane**

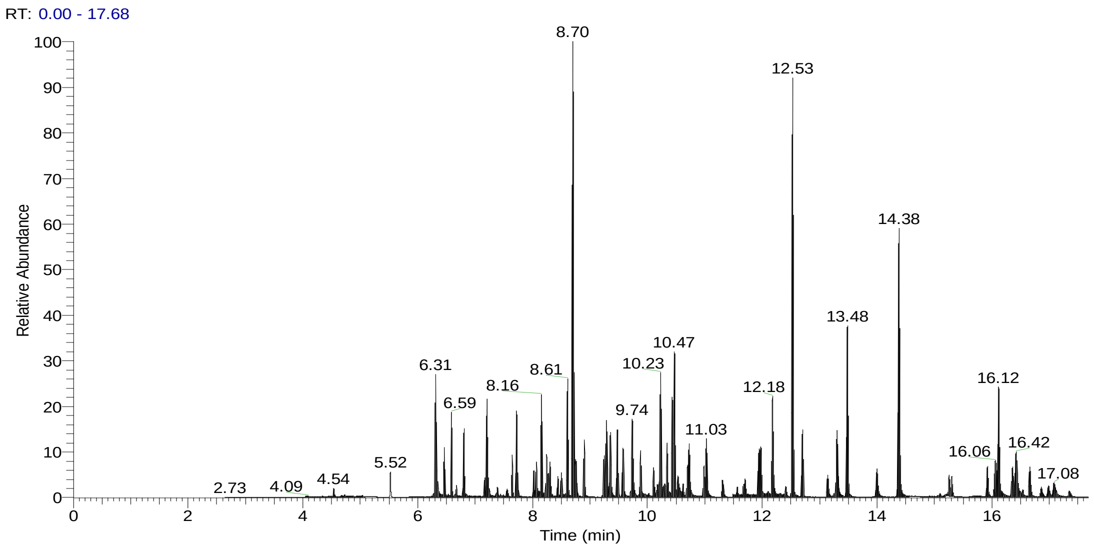


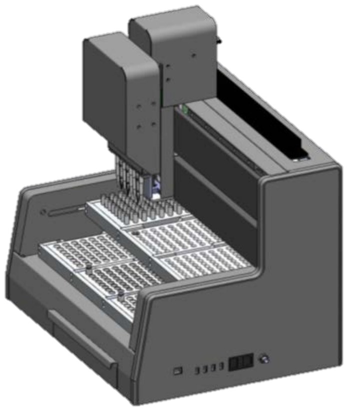
# Application of automatic multi-channel m-PFC device

Evaluations: 48 pesticide residues in green tea

82 pesticide residues in mulberry leaves and processed tea

Recovery √ | Matrix effect √
RSD √ | LOD √
Linearity √ | LOQ √



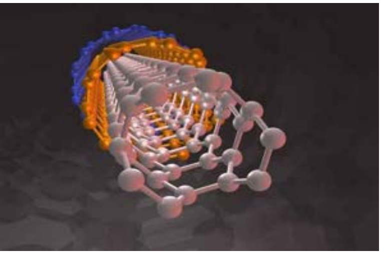



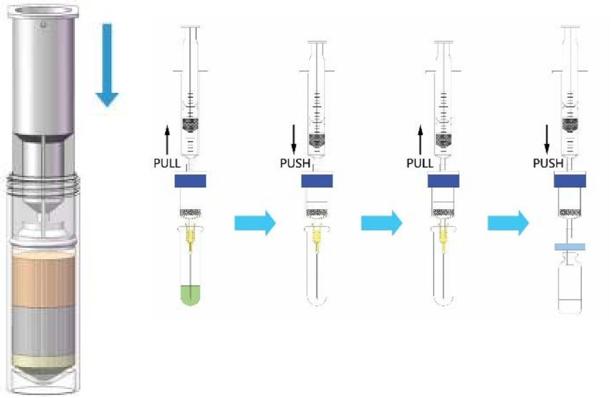
Comparison of the proposed method with other automatic m-PFC methods in mulberry processed tea

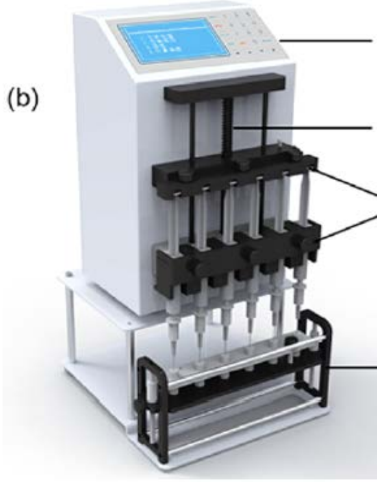
| Method | Recoveries (%) | RSD (%) | Sample number of batch process | Cleanup time cost per sample (min) | Automated filter |
|---|---|---|---|---|---|
| Automatic multi-channel | 73-116 | 2-13 | 48-64 | 0.8 | Yes |
| Automatic m-FC | 76-118 | 5-17 | 24 | 2-3 | No |
| Semi-automatic m-PFC | 72-124 | 1-19 | 6 | 2 | No |

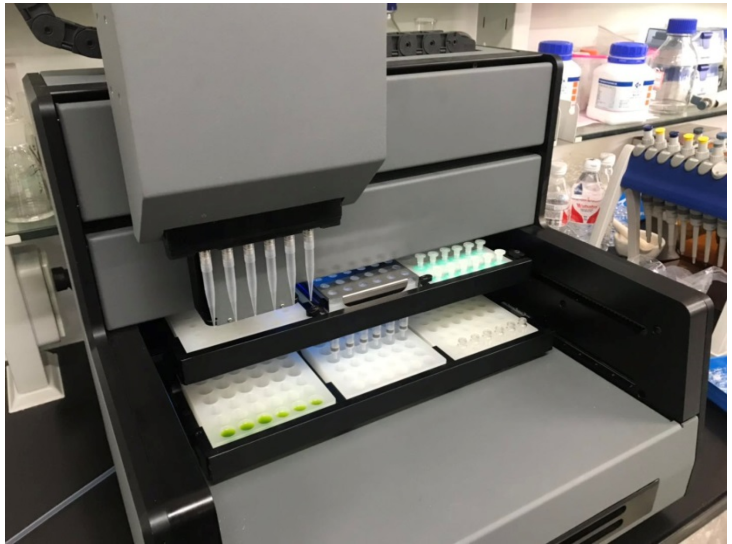
# Summary & Discussion

- 1. **MWCNTs, Nitrogen-enriched Carbon** : effective d-SPE materials
- 2. **m-PFC/FC**: rapid, potential high-throughput analysis
- 3. m-PFC automatic devices : speed, high efficiency, labor-saving , easy, robust and more reproducible



# Thank you for your attention！

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