Evaluation of Automated Clean-up for Large-Scope Pesticide Multiresidue Analysis by Liquid Chromatography Coupled to Mass Spectrometry

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Overview

A clean-up step is often essential during the extraction process to remove undesired matrix components that may cause analytical interferences. However, this additional procedure entails time-consuming work. Moreover, it usually needs to be adapted to the different co-extractives from the matrix present in the samples. Therefore, the development of an automated and unified clean-up procedure means a significant reduction in the time and laboratory work needed for pesticide residue analysis.

In this study, different matrices (tomato, orange, rice, avocado and black tea) were extracted by manual dispersive clean-up (different procedures according to the matrix group) in parallel with an automated µSPE clean-up workflow, in both cases based on QuEChERTS extraction. The latter procedure employed clean-up cartridges containing a mixture of sorbent materials (anhydrous MgSO₄/PSA/C18/CarbonX) suitable for multiple matrices. All the samples were analysed by liquid chromatography and the results obtained from both procedures have been compared in terms of the extract cleanliness, recoveries, interferences, and sample workflow. At the levels studied, similar recoveries were achieved by both techniques (manual and automated), except for reactive compounds when PSA was used as the sorbent material, for which the µSPE recoveries were only between 70-120%. Furthermore, closer calibration line slopes were provided when µSPE was applied to the different matrices studied. It is important to note that up to 30 % more samples per day can be analysed using an automated µSPE compared to the manual method (which requires shaking, centrifuging, then taking the supernatant and adding formic acid in ACN; it also provides good repeatability - an RSD (%) < 10%). Consequently, this technique is a very useful option for routine analyses, greatly simplifying the work of analytical laboratories.

Methods

- **Extraction step**
  - Citrate QuEChERS

- **Clean up step**
  - Manual dSPE
  - µSPE Cartridge:
    - Anhydrous MgSO₄/PSA+C18+CarbonX

- **Analysis by LC-QqQ-MS/MS**

- **Triple Quadrupole**
  - TSQ Altis
  - Ion spray voltage (-): 3500 V
  - Ion spray voltage (L): 2500 V
  - Sweep gas 1 (arb. units): 325 ºC
  - Vaporiser temp.: 350 ºC
  - Working mode: SIM

  - Mobile phase A: Water (0.1 mM ammonium formate), 50% ACN (fluazifop),
  - Mobile phase B: Methanol (0.1% formic acid, 5 mM ammonium formate, 2% water)

- **Conclusions**

  For all the cases studied, the calibration curves had very close slopes offering good quantitative results for the different commodities; when µSPE was employed for the clean-up, a single calibration curve was used. The total amount of injected sample for the dSPE method was 0.5 µL whereas for the µSPE method, it was 0.15 µL. Injecting less matrix should result in less equipment maintenance and a lower signal suppression effect.

- **Results**

  - **TICs and extract appearance**
    - Sample: Avocado
    - Manual dSPE
    - Automated µSPE

  - **Linearly**
    - Manual dSPE
    - Automated µSPE

  - **Acidic compounds**
    - Steps:
      1. Condition µSPE cartridge (100 µL ACN)
      2. Elution cartridge step with sample (200 µL)
    - µSPE Cartridge:
      - Anhydrous MgSO₄/PSA+C18+CarbonX
    - Steps:
      1. Condition µSPE cartridge (100 µL ACN)
      2. Elution cartridge step with sample (200 µL)
      3. Elution cartridge with ACN (5% formic acid) (100 µL)

  - **Matrix Effect**
    - Manual dSPE
    - Automated µSPE

  - **Comparative chromatograms of µSPE of tomato sample spiked at 0.010 mg kg⁻¹ pesticide mix standard**

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Conclusions

The use of an automated µSPE clean-up reduces the laboratory workflow and allows increased sample throughput in routine analysis by eliminating tedious manual steps. Moreover, as only a single clean-up is employed equally for all commodities, greater homogeneity is typically obtained in the calibration curves. Instrument maintenance is also positively affected because, generally, cleaner extracts are obtained and so the lifespan of certain instrument parts (such as the ion source and columns) increase.

In general, the µSPE method provides recoveries that are very similar to those obtained with manual clean-up because the extraction step is the same for both approaches. However, some compounds are positively affected when using µSPE cartridges, such as acidic compounds (fluazifop, haloxifop and quizalofop), whereas, in general, recoveries < 50% are obtained when manual dispersive clean-up is used (they are retained by the PSA sorbent). The automated clean-up step can attain recoveries from 70 % -120 % because a final elution step of the cartridge (with acidified acetonitrile) was included. In conclusion, automatic µSPE avoids the qualitative and quantitative errors that are produced when dSPE is applied.