

A Smart Clean-up Approach to Reduce Matrix Effects and Isobaric Interference for Multi-residue Pesticide Analysis, Based Upon LC-MS/MS, of the Difficult Matrices, Black Tea and Cocoa Beans

Waters™

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INTRODUCTION & OBJECTIVE

Reliable analytical methods are needed for detection, quantification, and identification of hundreds of pesticide residues in many different commodities. The analysis of the complex matrices, tea and cocoa, is challenging due to the high amounts of endogenous components such as fats, pigments, and other phytochemicals. These components are known to cause matrix effects and isobaric interference, which negatively impact detection and quantitation of pesticide residues. Reducing the level of co-extractives by using an efficient clean-up, such as a simple pass-through protocol using Oasis™ PRiME HLB, helps to obtain reliable results, maintain instrument robustness, and minimize cleaning needs.

The objective of this collaborative project was to establish the performance of a sample preparation method involving a pass-through clean-up after QuEChERS extraction, with matrix-matched calibration and determination using the ACQUITY™ UPLC™ I-Class System and Xevo™ TQ-XS Mass Spectrometer. The recovery and repeatability for 395 analytes were determined from the analysis of five replicates prepared at 0.01 and 0.1 mg/kg in each commodity.



METHOD PROTOCOL

Samples of tea and cocoa were extracted by GALAB using a modification of the QuEChERS CEN Method 156624, whereby the optional dSPE step was replaced by a pass-through SPE with Oasis PRiME HLB in the Short Plus format, optimized for this analysis.

QuEChERS extraction

Add 10 ml H₂O, internal standard, (triphenyl phosphate), 10 ml MeCN and CEN Method 156624 QuEChERS salts (P/N 186006813) to 10 g homogenized sample
Freeze at -20 °C

Pass-through clean-up

Pass 5 ml supernatant through Oasis PRiME HLB Short Plus (P/N 186008887)
Discard 1st 1 ml of eluent and collect next 2 ml into vial

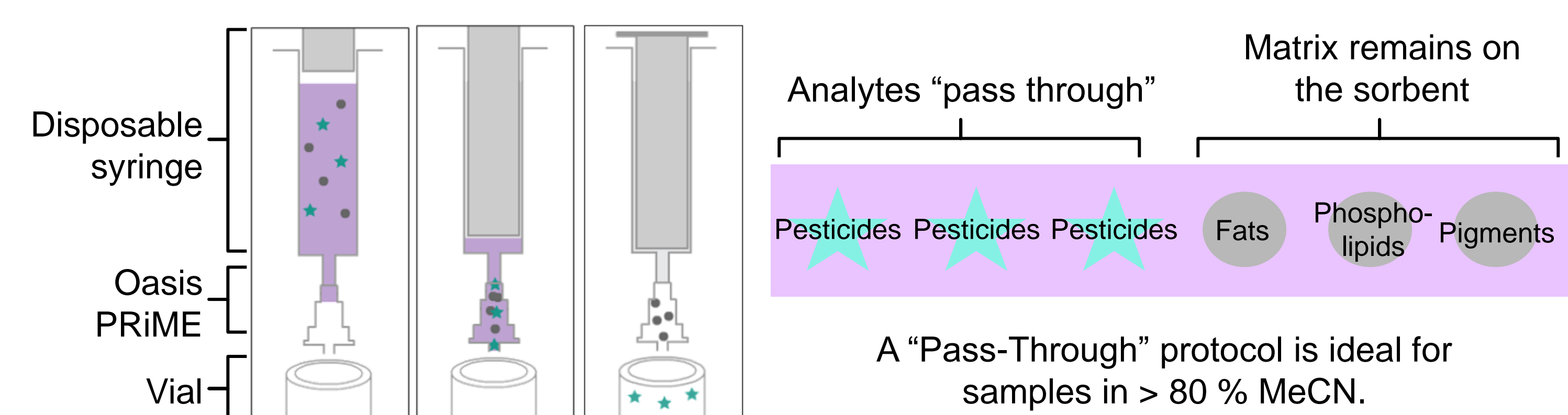
Determination by UPLC-MS/MS

ACQUITY I-Class and Xevo TQ-XS with polarity switching including 395 analytes



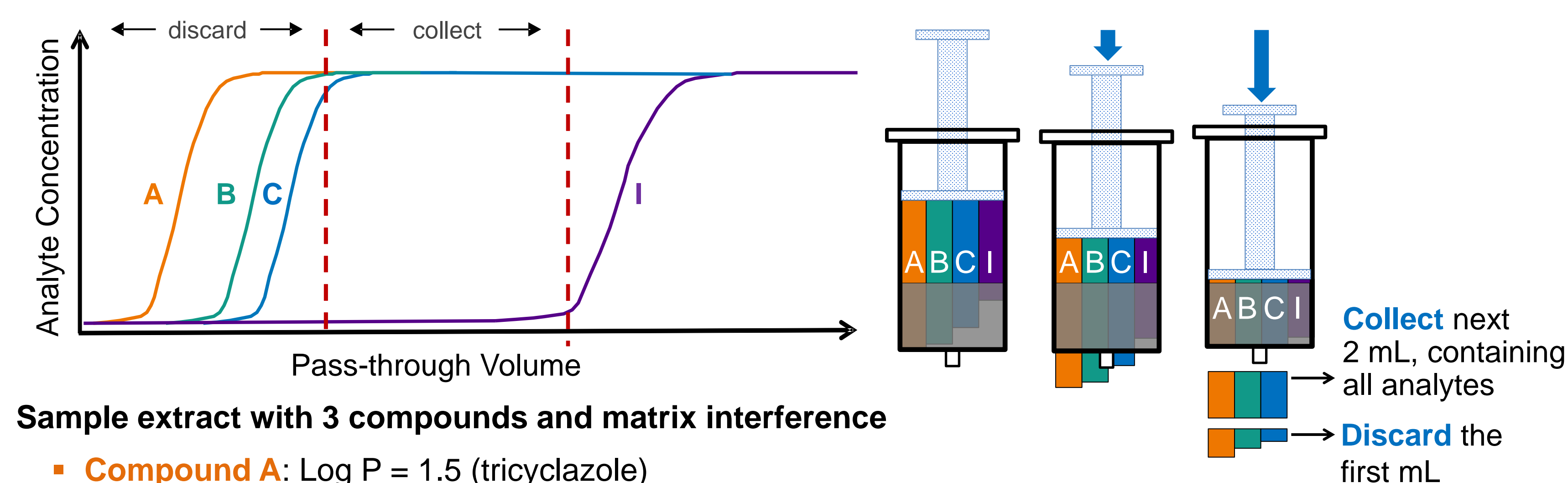
HOW DOES PASS-THRU SPE WORK?

Dispersive solid-phase extraction (dSPE) with multiple sorbents can be used to remove matrix co-extractives during QuEChERS but can lead to losses of certain pesticides. The “Pass-Thru” protocol inverts the classical SPE approach by passing through the analytes of interest and instead retaining co-extracted fats, phospholipids and pigments by the sorbent. The Short Plus format of the Oasis PRiME HLB cartridge allows for extracts to be pushed through the cartridge manually using a syringe. A clean eluate is achieved in only a few minutes ready for LC injection.



WHY DO WE DISCARD THE FIRST FRACTION?

The Oasis PRiME Short Plus cartridge behaves as a short chromatography column. Clean-up of the extracted sample can be optimized by evaluating the stage at which the analytes pass through the cartridge. Here, the first 1 mL that elutes was discarded to collect the majority of analytes with satisfactory recovery.



Sample extract with 3 compounds and matrix interference

- Compound A: Log P = 1.5 (tricyclazole)
- Compound B: Log P = 2.8 (prothioconazole)
- Compound C: Log P = 6.1 (doramectin)
- Matrix interference I: (fats, phospholipids with strong retention)

EVALUATION OF PERFORMANCE

The SANTE guidelines specifies an average recovery for each spike level tested to be between 70 and 120%. The results from analysis of the spikes @ 0.01 mg/kg in tea and cocoa are shown in Figure 1. The vast majority of the 395 analytes were within tolerance in both commodities. The identify of some of the outliers is annotated. Figure 2 shows the distribution of measured recoveries in both commodities at 0.01 and 0.1 mg/kg. Some analytes were not detected at 0.01 mg/kg. Almost all the compounds exhibiting recoveries between 30 and 140 %, showed consistent results with repeatability (RSDr) within tolerance of the SANTE guidelines, which state that RSDr for each spike level tested should be ≤ 20%. At both concentration, in both commodities, 99% of the analytes were within this tolerance (excluding the non-detects). Figure 3 shows the distribution of repeatability (one outlier removed).

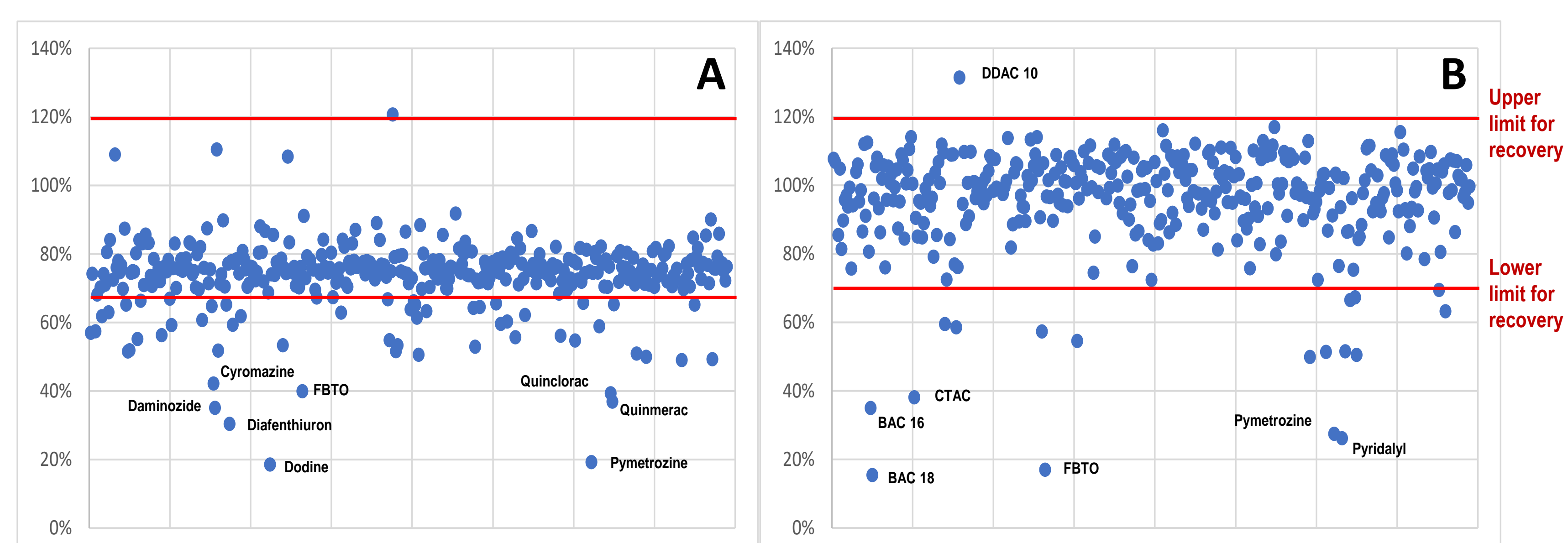


Figure 1. Recovery of pesticide residues spiked at 0.01 mg/kg in tea (A) and cocoa (B).

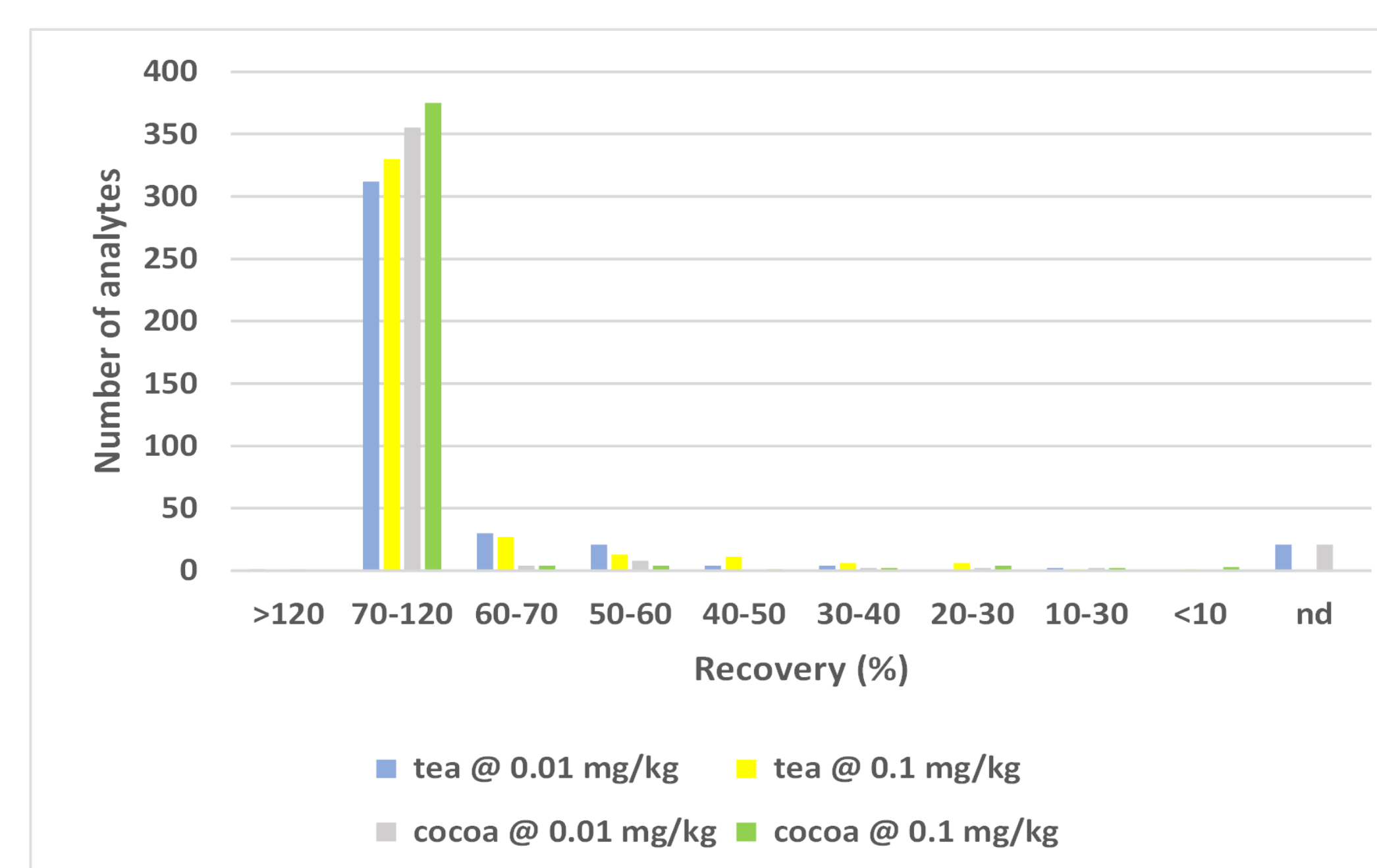


Figure 2. Distribution of measured recoveries.

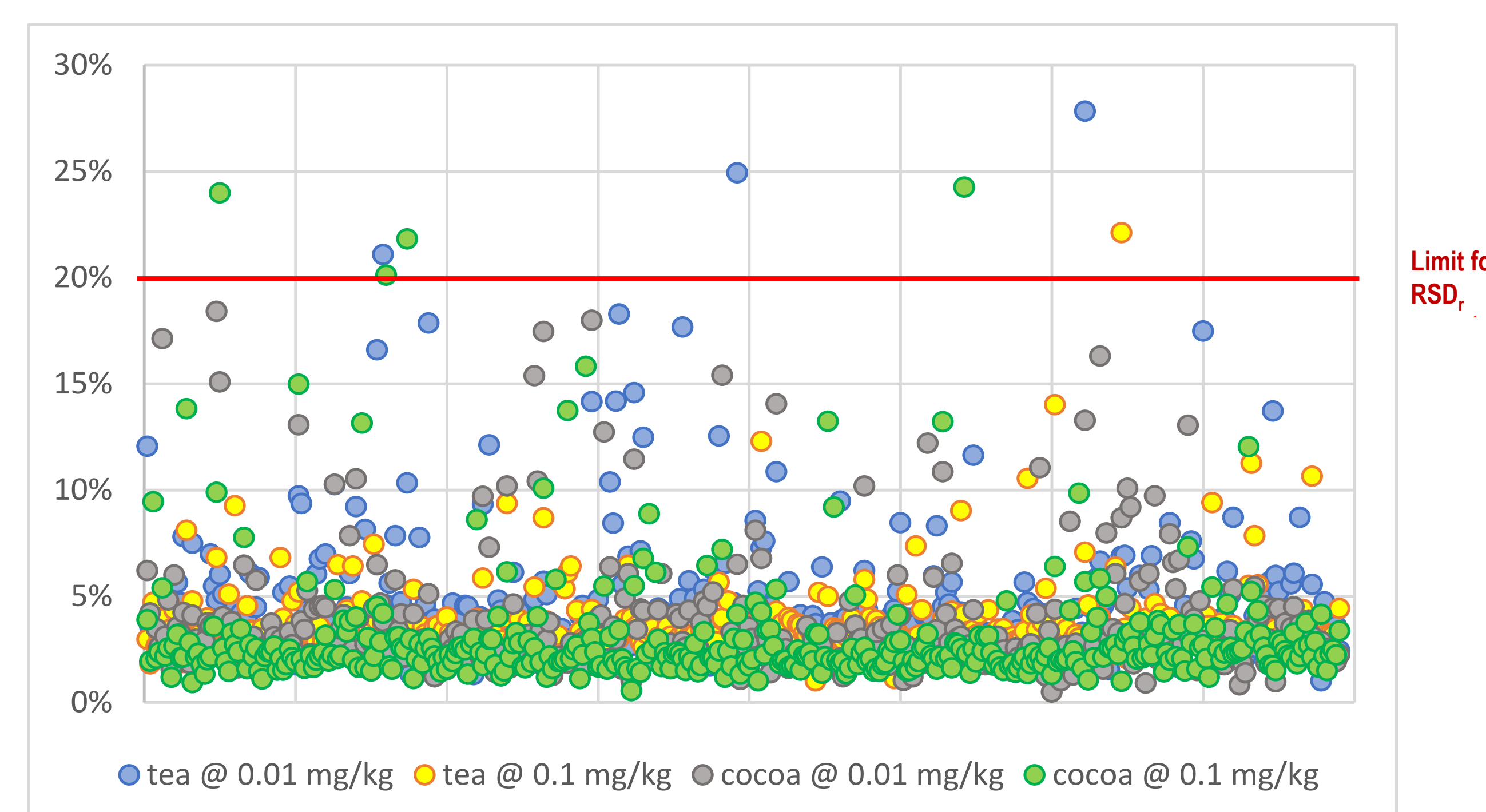


Figure 3. Distribution of repeatability.

CONCLUSIONS

- This poster describes a sensitive multiresidue method for the determination of pesticide residues in two complex matrices, tea and cocoa, using UPLC-MS/MS (ACQUITY UPLC I-Class System coupled with the Xevo TQ-XS Mass Spectrometer).
- “Pass through” SPE with Oasis PRiME HLB offers a quick and effective alternative to dilute and shoot or dSPE.
- The performance of the method has been successfully evaluated using the SANTE acceptance criteria.
- This method has been demonstrated as suitable for analysis of tea and cocoa, for checking compliance with MRLs and has the potential for determination at much lower concentrations.