RESIDUES AND TRACE ELEMENTS

The Use of Dispersive Pipet Extraction (DPX) Tips for the Sample Cleanup of Apples, Pears, and Oranges in the Analysis of Formetanate HCl

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The U.S. Environmental Protection Agency Analytical Chemistry Laboratory evaluated the effectiveness of dispersive pipet extraction (DPX) tip cleanup and compared the results with the Quick, Easy, Cheap, Effective, Rugged, and Safe dispersive tube cleanup for the sample preparation of three different fruit matrixes analyzed for formetanate HCl (FHCl). Using LC/MS/MS, the target LOD and the LOQ achieved for FHCl with dispersive tubes, 0.1 and 0.3 ng/g, respectively, were similar to the DPX tip sample cleanup. Recoveries at the LOQ ranged from 94 to 109%. A set of 20–40 samples could be prepared in one working day by one chemist.

For many reasons, the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach to sample preparation has become the method of choice for pesticide residue analysis. The increased speed in sample preparation has been welcomed by many laboratories that analyze thousands of food samples a year. Using a variation of the QuEChERS method, it was possible for two U.S. Environmental Protection Agency (EPA) chemists to analyze 4000 fruit samples for the *N*-methyl carbamate formetanate HCl (FHCl) over a period of less than 2.5 years. This rate of sample analysis would not have been possible without the QuEChERS approach to sample preparation. In the quest for even shorter sample preparation times and greater sample throughput, a technology was introduced that uses tips instead of tubes for sample cleanup. If $MgSO_4$, primary-secondary amine (PSA) sorbent, and graphitized carbon black (GCB) sorbent in a 15 mL tube provide a sufficient sample cleanup for many matrixes and many pesticide analytes, then $MgSO_4$, PSA sorbent, and GCB sorbent in a dispersive pipet extraction (DPX) tip should also provide a sufficient cleanup. This technique appeared to have the potential to further streamline sample preparation as well as further reduce solvent use.

The EPA Analytical Chemistry Branch analyzed 600 additional orange samples collected by the U.S. Department of Agriculture (USDA) Pesticide Data Program (PDP) in an ongoing dietary exposure study of FHCl. The analyses of the 600 samples needed to be completed in the 2 month timeframe by only one chemist. The method used to analyze the 4000 previous samples in the FHCl study was a version of the QuEChERS method, introduced by Anastassiades et al. in 2003 (1), and subsequently modified by Schenck et al. in 2007–2008 (2–4) and by Podhorniak et al. in 2010 (5). To complete the analyses of the 600 orange samples for publication in the PDP Annual Summary Calendar Year 2011 (6), the modified QuEChERS method selected needed to be further streamlined. The following method describes the use of a dispersive cleanup with tips instead of tubes for the sample preparation of 600 orange samples and additional validation with apples and pears.

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Experimental

Apparatus

(**a**) *Polypropylene centrifuge tubes.*—50 mL, No. 430828 (Corning Inc., Corning, NY).

(**b**) *Bottle top dispenser.*—2.5 to 25 mL or 5 to 50 mL, No. 4731351 (BrandTech Scientific, Inc., Essex, CT).

(**c**) *Grinder.*—SPEX Sample Prep Model 2000, SPEX CertiPrep Inc (Metuchen, NJ).

(**d**) *Grinding balls.*—Stainless steel 5/32 inch diameter, No. 2150 (SPEX CertiPrep Inc.).

(**e**) *6 g $MgSO_4$/1.5 g NaCl (prepackaged) in 50 mL centrifuge tube.*—No. ECMSSC50CTFS (UCT, Bristol, PA).

(**f**) *Centrifuge.*—No. C422, (Jouan Inc., Winchester, VA).

(**g**) *DPX extractor.*—Manual model (DPX Labs, LLC, www.DPXLabs.com).

(**h**) *Dispersive DPX tips.*—150 mg $MgSO_4$/50 mg PSA/25 mg GCB (prepackaged) in a 5 mL tip, No. Qg(25)–A 5 mL (DPX Labs, LLC).

(**i**) *Culture tubes.*—Disposable, borosilicate glass, 16 × 125 mm, 20 mL, KIMAX 51 No. 60825–630 (VWR International, LLC (Radnor, PA) or equivalent.

(**j**) *Nitrogen evaporator.*—12 sample nitrogen evaporator, 550–55EC, N–Evap–111 (Organomation Assoc., Inc., Berlin, MA) or equivalent.

(**k**) *Syringe filters.*—Acrodisc, 0.20 µm, 13 mm, PVDF (Pall Life Sciences, Ann Arbor, MI).

(**l**) *Syringes.*—Disposable, luer lock, 5 mL, No. 03–377–28 (Fisher Scientific, Pittsburgh, PA) or equivalent.

(**m**) *Pipets.*—Finnipipette, 1–5 mL, No. 21–377–244 (Fisher Scientific) or equivalent.

(**n**) *Pipet tips.*—Finntips, 1–5 mL, No. 21–377–304 (Fisher Scientific) or equivalent.

(**o**) *Vials with pre-slit caps.*—Screw top, wide opening, 2 mL, No. 186000307C (Waters Corp., Milford, MA) or equivalent.

(**p**) *Liquid chromatograph-mass spectrometer system.*—

Table 1. Comparison of recoveries of fortified fruit samples using DPX tip versus dispersive tube cleanup

	DPX tips recovery, % (RSD, %)			Dispersive tubes recovery, % (RSD, %)		
Level	Pears	Apples	Oranges	Pears	Apples	Oranges
LOQ – 0.3 ppb (three replicates)	102 (2.3)	96 (7.2)	114 (2.7)	106 (2.4)	96 (8.9)	109 (1.1)
2 × LOQ – 0.6 ppb (5–7 replicates)	90 (8.5)	99 (13.8)	98 (4.2)	103 (3.4)	89 (8.7)	106 (8.8)
5 × LOQ – 1.5 ppb (three replicates)	85 (3.8)	83 (5.4)	86 (4.8)	94 (2.8)	89 (5.1)	99 (7.1)
10 × LOQ 3.0 ppb (three replicates)	90 (7.7)	86 (5.4)	92 (7.6)	93 (3.5)	90 (1.3)	95 (5.3)

Acquity ultra-performance liquid chromatograph with a Quattro Premier tandem-quadrupole mass spectrometer (Waters Corp.) or equivalent.

(**q**) *Liquid chromatographic column.*—Acquity HSS–T3 (10 cm × 2.1 mm), 1.8 µm particle size (Waters Corp.) or equivalent.

(**r**) *Compressed N_2 gas*.—High purity (microbulk delivery Robert's Oxygen).

Reagents

(**a**) *Solvents*.—Acetonitrile (ACN), HPLC or GC Resolve (Burdick & Jackson, Muskegon, MI), or equivalent; toluene, HPLC grade (Fisher Scientific), or equivalent; methanol (MeOH), HPLC grade (Burdick & Jackson), or equivalent; chloroacetic acid buffer (Pickering Laboratories, Mountain View, CA), No. 1700–0132.

(**b**) *Reference standards.*—Obtained from the EPA National Pesticide Standard Repository located at the Environmental Science Center, 701 Mapes Rd, Ft. George G. Meade, MD 20755-5350.

(**c**) *Standard solutions preparation*.—(*1*) Stock solutions of FHCl prepared individually in 1% H_2O/ACN at 1.0 mg/mL. Stock solutions of propoxur prepared individually in MeOH at 1.0 mg/mL. (*2*) Fortification standard solutions prepared in ACN at 10.0 µg/mL. (*3*) Calibration standards prepared in either peach or nectarine matrix at 0.15 ng/mL (LOD), 0.45 ng/mL (LOQ), 0.9 ng/mL (2 × LOQ), and 4.5 ng/mL (10 × LOQ).

Control Sample Acquisition and Preparation

Apples, oranges, and pears were purchased from a local market for use as control samples and were stored at –80°C until analyzed. When organic produce was not available, samples found to contain no FHCl residues were used as a supplemental source of controls. Incurred samples were collected from several U.S. states including California, Ohio, Washington, and New York. The samples were comminuted and shipped frozen by the USDA-PDP.

Extraction

(**a**) Weigh 15.0 ± 0.1 g comminuted sample into a 50 mL centrifuge tube. Add the surrogate standard, propoxur, to each sample (excluding the reagent blank, the matrix blank, and the four matrix blank samples reserved for the matrix calibration standards). The matrix standards are labeled Cal 1–Cal 4. Add fortification standard, FHCl, to the matrix spike at this time. Add 15 mL ACN to the tubes using the bottle-top dispenser.

(**b**) Cap the tubes and shake for 1 min using the grinder set at 1000 strokes/min (spm). Add $MgSO_4$/NaCl (contained in a prepackaged, UCT 50 mL centrifuge tube) to a sample extract, add a metal grinding ball, immediately cap the tube, and shake vigorously by hand for about 10 s.

(**c**) When the $MgSO_4$/NaCl has been added to all tubes, shake vigorously using the grinder set at 1200 spm for 2 min, ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are sufficiently broken up during shaking.

(**d**) Centrifuge for 5 min at 2500 rpm. Proceed to the dispersive DPX tip cleanup.

DPX SPE Cleanup

Transfer 1.5 mL supernatant from each ACN extract to a corresponding 15 mL culture tube. Add 0.5 mL toluene to each culture tube. Using the DPX Extractor loaded with tips, slowly draw the ACN–toluene extract through the sorbent in the tips, hold 20 s and expel the extracts back to the culture tubes. Repeat 2–3 times. Move the frits that are located at the tops of the tips to the side. Any blunt dowel-like tool will work. Add 1.5 mL ACN–toluene (1 + 3, v/v) to the top of the tips to rinse the $MgSO_4$/CUPSA/CUCARB sorbent and collect the rinsate in the culture tubes with the sample extracts. Using a Turbovap (Zymark, Hopkinton, MA) with a water bath set to 40°C, evaporate the extracts just to dryness. Add appropriate volumes of fortification standard, FHCl, to the four matrix standard tubes labeled Cal 1, Cal 2, Cal 3, and Cal 4. Add the surrogate standard, propoxur, to the four matrix standard tubes labeled Cal 1, Cal 2, Cal 3, and Cal 4. Evaporate matrix standards to dryness using N_2 evaporation. Reconstitute all samples (matrix standards, matrix spike, matrix control, reagent blank, and samples) with 1.0 mL 0.01% ChlorAC buffer–MeOH (50 + 50, v/v), and vortex mix. Filter using a 0.2 µm PVDF syringe filter.

Total mg injected for orange, apple, and pear samples using ultra-performance LC (UPLC)/MS/MS is 7.5 mg with an injection volume of 5.0 µL. Total mg injected is determined with the following equation:

$$\text{mg sample equivalent} = \frac{\text{sample weight, g} \times \text{aliquot, mL} \times \text{injection vol., µL}}{\text{acetonitrile added, mL} \times \text{final vol., mL}}$$

where 15 g is the sample weight, 15 mL is the volume of ACN added, 1.5 mL is the aliquot of sample extract taken to dryness, and 1.0 mL is the final volume. Sample sets consisted of 20–40 samples, one matrix blank, one matrix spike, one reagent blank, and four matrix standards. Samples were quantitated using a four point linear curve of matrix standards

at concentrations equivalent to the LOD, LOQ, 2 × LOQ, and 10 × LOQ of both FHCl and propoxur.

Analysis

A tandem quadrupole mass spectrometer with an ultra-performance liquid chromatograph was used for the analysis of sample extracts with the following instrument parameters:

(**a**) *Column.*—HSS–T3 column (10 cm × 2.1 mm) of 1.8 µm particle size. Column temperature 40°C.

(**b**) *Mobile phase.*—RP: MeOH, H_2O, and 10 mM ammonium acetate prepared as two separate solutions: solvent A: 95% H_2O, 5% MeOH, and 10 mM ammonium acetate; solvent B: 95% MeOH, 5% H_2O, and 10 mM ammonium acetate. The initial conditions were set at 95 + 5 (solvent A + solvent B) and after 1 min at the initial conditions at 0.3 mL/min flow rate, a linear gradient was programmed within 7 min to 60 + 40 (solvent A + solvent B), followed by a 0.1 min step change to a flow rate of 0.4 mL/min, followed by a second linear gradient within 4.9 min to 100% solvent B, and then back to 95 + 5 (solvent A + solvent B) within 1 min using a linear gradient at 0.4 mL/min, and ending with a 1 min step change to 0.3 mL/min for a total analysis time of 16 min.

(**c**) *Injection volume*.—5 µL.

(**d**) *Flow rate.*—0.3 mL/min

(**e**) *Mass spectrometer operating conditions*.—Positive electrospray ionization multiple reagent monitoring (MRM) mode. The ion source temperature was 130°C, and the desolvation temperature was 450°C. The cone gas flow (nitrogen) was 50 L/h and the desolvation gas flow was 800 L/h of nitrogen gas. The collision cell gas was ultra high pure argon with a flow of 0.3 mL/min.

(**f**) *MRM transitions.*—Two precursor/product ion transitions were monitored for each compound, the more abundant ion transition was used for quantitation while the other ion transition was used for confirmation. The MRM transitions, cone voltages, and collision energies were as follows: for FHCl, 222.0 > 92.80 (25 V, 35 eV) and 222.0 > 164.9 (25 V, 15 eV), and for propoxur, 210.0 > 110.8 (17 V, 15 eV) and 210.0 > 167.9 (17 V, 7 eV). Since an external standard was used for quantitation, the retention times of the compound of interest in the standard and the same compound in the sample were ±0.1 min. Analytes were considered confirmed when MRM ion transition ratios were within ±20% (absolute) of the ratios in standards.

Results and Discussion

Sample Extraction and Cleanup

The EPA Analytical Branch tested DPX tips as an alternative to the dispersive tube (ECMPSCB15CT, UCT, Bristol, PA) cleanup for the QuEChERS multiresidue extraction (2–4). Although the 5 mL DPX tips contain 1/6 the amount of total dispersive sorbent in the QuEChERS tubes (225 mg tips versus 1350 mg tubes), the ratio of the sorbent components is the same. The tubes contain 900 mg $MgSO_4$/300 mg PSA/150 mg GCB, and the tips contain 150 mg $MgSO_4$/50 mg PSA/25 mg GCB for a ratio of 1 $MgSO_4$/1/3 PSA/1/6 GCB.

The smaller amount of dispersive sorbent and the smaller tip capacity necessitate a smaller volume of sample extract for cleanup. Instead of using 9 mL ACN extract and 3 mL toluene for the dispersive tube cleanup, 1.5 mL ACN extract and 0.5 mL toluene were used for the tips to maintain the same ratio of ACN to toluene. Using the dispersive tubes, a second aliquot of 4 mL cleaned, ACN–toluene extract was concentrated to 2 mL for a final sample concentration of 1.5 mg/mL solvent (5). Using the DPX tips, a second aliquot of sample extract was not needed. After the coextractants were removed by the sorbent in the tips, the 1.5 mL sample extract was concentrated to a final volume of 1 mL and the final sample concentration using the tips was also 1.5 mg sample/mL solvent.

Matrix coextractants were removed by drawing the sample through the dispersive sorbent in the tip and expelling the sample back into the culture tubes 2–3 times. Initially using this procedure, the recoveries of FHCl in fruit matrixes were approximately 70% across various levels such as the LOQ of 0.3 ng/g and 10 × LOQ, 3 ng/g. By adding a final solvent rinse of 1.5 mL ACN–toluene (1 + 3, v/v) to the top of the tips to flush the FHCl off the sorbent and collecting the rinsate in the culture tubes with the sample extracts, recoveries were improved to >90%. Without this step, recoveries were consistently 20% lower. The results of the analysis of 87 samples of apples, pears, and oranges fortified at four different levels are summarized in Table 1.

Method performance was further evaluated with samples that contained incurred residues of FHCl. Fifty apple samples were analyzed using the two cleanup methods, and the results were compared. For each sample, 9 mL of the 15 mL ACN extract was cleaned up with the dispersive tubes and 1.5 mL was cleaned up with the DPX tips. Using both cleanup methods, only eight of the 50 apple samples were found to contain incurred FHCl residue. The results summarized in Table 2 show good agreement between the dispersive tube and DPX tip cleanup of the same ACN sample extracts.

The UPLC/MS/MS chromatography of DPX tip sample extracts was consistent with the chromatography of fruit sample extracts obtained with dispersive tube cleanup. There was no significant loss in quality of chromatography of the analyte, FHCl, or the surrogate, propoxur. *See* Figures 1 and 2 for a comparison.

The DPX tip cleanup procedure was much faster than the dispersive tube procedure because there are fewer steps involved with tips than with tubes. For example, tubes must

Table 2. Comparison of analytical results of incurred FHCl obtained for apple sample extracts purified using DPX tip versus dispersive tube cleanup

Apples	Incurred FHCl level, ppb	
Sample	DPX tips	Dispersive tubes
1	4.95	4.84
3	8.439	6.69
4	36.92[a]	33.48[a]
8	9.38	9.03
21	0.510	0.361
29	0.609	0.666
32	0.776	0.546
47	0.383	0.265

[a] Peak areas outside that of the highest standard of the curve.

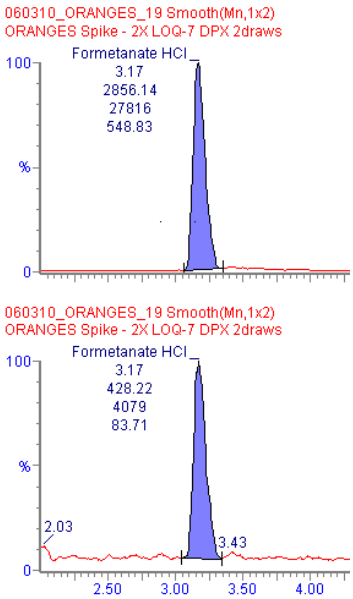

Figure 1. UPLC/MS/MS ion transitions monitored for FHCl in an orange spike prepared using dispersive tubes (fortified at 2 × LOQ of 0.6 ng/g).

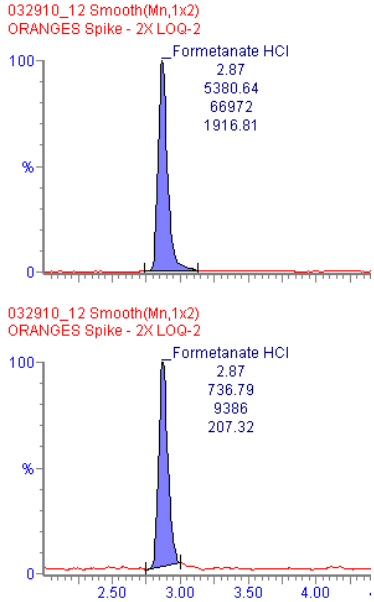

Figure 2. UPLC/MS/MS ion transitions monitored for FHCl in an orange spike prepared using DPX tips (fortified at 2 × LOQ of 0.6 ng/g).

be labeled, uncapped, and recapped; shaken either by hand or loaded and unloaded in an automated shaker; then centrifuged and uncapped again to obtain a second extract aliquot. Using tubes requires eight additional steps/sample set. All of these steps are eliminated with the use of the tips. In addition, the DPX Extractor expedites sample preparation by enabling the simultaneous extraction of 24 samples. One chemist can easily extract up to 40 samples/day using the DPX tip cleanup procedure.

Conclusions

The EPA Analytical Laboratory verified that DPX tips effectively recover FHCl from apples, oranges, and pears. The DPX tips eliminate many steps that are necessary when using QuEChERS tubes. If hundreds of samples a week are being analyzed, eliminating extra steps becomes significant. By adding a final solvent rinse to the top of the tips to flush the FHCl off the sorbent, recoveries using DPX tips are consistent with recoveries using dispersive tubes, and the chromatography is equally comparable. The use of DPX tips was found to be more advantageous for the analysis of FHCl in various fruit commodities because one chemist is capable of extracting 40 samples/day with no sacrifice of recovery or chromatography quality.

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