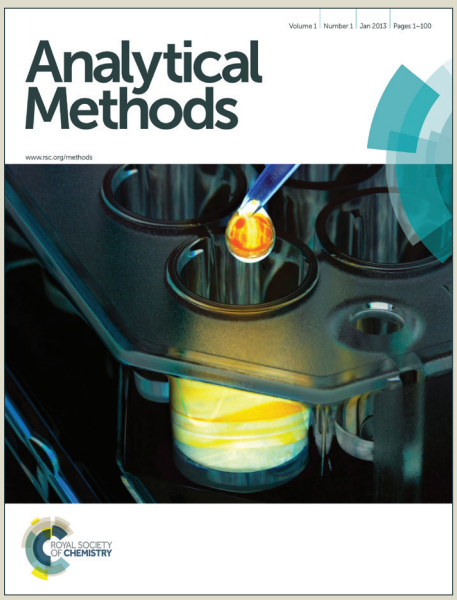
Analytical Methods

Accepted Manuscript



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Ammonium salting out extraction with analyte preconcentration for sub-part per billion quantitative analysis in surface, ground and drinking water by flow injection tandem mass spectrometry

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Keywords: Tandem mass spectrometry; flow injection analysis; pesticide residue analysis; water analysis; salting out extraction; ammonium chloride.

Abstract

A recently-reported extraction method that merged principles of QuEChERS and salting out liquid-liquid extraction (SALLE) by using ammonium salts (chloride, acetate, formate) has been improved for high-throughput quantitation of bioactive chemicals in water by flow injection tandem mass spectrometry (FI/MS/MS). The new method for water analysis uses the ratio of acetonitrile/water adjusted to yield extract preconcentration in one step: 7.0 mL water aliquot, 3.5 mL of acetonitrile extraction solvent and 3.0 g of NH_4Cl (s) resulted in post-extraction acetonitrile phase volume of 1.4 mL (extract preconcentration factor = 5). This preconcentration factor can be adjusted by changing the acetonitrile/water aliquot ratio to achieve the desired sensitivity while optimizing method performance. The acetonitrile/water partition coefficients (K_{aw}) of analytes in the acetonitrile/water/NH_4Cl ternary systems were measured during method development. K_{aw} values were used to predict analyte recoveries when the acetonitrile/water ratios were varied in the extraction procedure. The effect of aqueous system pH was evaluated and the results were used for extraction optimization. A validation study was successfully conducted in creek, pond, ground, and drinking water for the following pesticides: aminocyclopyrachlor methyl, azimsulfuron, chlorantraniliprole, chlorimuron ethyl, chlorsulfuron, cyantraniliprole, diuron, flupyrsulfuron methyl, hexazinone, oxamyl, methomyl, sulfometuron methyl, triflusulfuron methyl. The method met the stringent 0.1 μg/L (ppb) limit of quantitation (LOQ) specified by the European Union for 10 out of 13 pesticides tested. The LOQ for the 3 least responsive analytes, i.e. chlorsulfuron, oxamyl and methomyl, was 0.3 μg/L. Limits of detection (LODs) were between 10 and 100 ng/L (ppt). FI/MS/MS acquisition time was 30 seconds/injection. The correlation between analyte recoveries and publicly-available physicochemical properties, such as octanol/water partition coefficients (K_{ow}) and aqueous solubility, was also assessed, allowing the prediction of method applicability to other chemicals, such as pharmaceuticals and other pesticides not tested in the study.

Introduction

An important application of analytical chemistry is to monitor the environment for the presence of chemicals that result from human activity in order to assess environmental and wild life safety, and human exposure risks. For this purpose, natural bodies of water and drinking water are frequently studied to measure concentrations of bioactive chemicals,[1,2] such as those found in pharmaceuticals, personal-care products and pesticides, to ensure these products are being used responsibly and levels are within specified limits. Bioactive chemicals have also been monitored at water treatment plants to evaluate the concentration of contaminants in wastewater from a city or region and the effectiveness of treatment methods.[3] Water analytical methods must meet stringent performance requirements in order to provide useful data.[4,5] For example, the maximum concentration of pesticides permitted in ground water and drinking water by the European Union is 0.1 μg/L (parts per billion, ppb).[5] The requirement of measuring sub-ppb levels of chemicals in water severely limits the analytical techniques that are suitable for this purpose. State-of-the art combinations of chromatography and mass spectrometry (e.g. LC/MS, LC/MS/MS, GC/MS, GC/MS/MS) are the preferred instrumental analysis techniques because of their sensitivity and selectivity.[6]

A high-throughput analytical technique that is emerging as a reliable option for trace-level quantitative screening in complex matrices is flow injection tandem mass spectrometry (FI/MS/MS). Several methods that use FI/MS/MS have been developed recently, with applications to detect and quantify pesticides in food[7-9] and body fluids,[10] pharmaceuticals as adulterants in dietary supplements,[11,12] endogenous compounds in blood and urine,[13,14] as well as illicit drugs in forensic studies.[15] Advantages of FI/MS/MS over conventional techniques (e.g. HPLC/MS/MS or GC/MS) include faster instrumental analysis (typically <60 seconds)[16,17] and

method simplicity because of avoidance of chromatography.[18] On the other hand, FI/MS/MS has lower selectivity because it lacks one measurement dimension, i.e. retention time/analyte separation. Nevertheless, the selectivity of FI/MS/MS has proven to be adequate for several applications.[7-18] Another limitation of FI/MS/MS is that, unlike hyphenated chromatography/MS techniques, preconcentration of the analytes cannot be achieved upon injection; increasing the injection volume results in widening of the analyte peak and does not provide significant gain in sensitivity.[9] Consequently, preconcentration steps have been necessary, e.g. solid-phase extraction (SPE), to achieve adequate sensitivity at the expense of sample preparation throughput for quantitation of pesticides in water using FI/MS/MS.[9]

Improvements of FI/MS/MS methods have been possible because of new sample preparation approaches. For example, a simple and fast sample extraction method based ammonium salt-induced acetonitrile/water phase separation ("ammonium salting out") was recently developed and coupled with FI/MS/MS.[19] The salting out phenomenon occurs because dissolution of the salt changes the physicochemical properties of the system, such as vapor pressure and ionic strength of each solvent in the mixture, resulting in phase separation.[19,20] In a recent study,[19] three salts of ammonium were proven to be particularly effective to partition pesticides into the acetonitrile phase: ammonium chloride (NH_4Cl), ammonium formate (HCO_2NH_4) and ammonium acetate ($CH_3CO_2NH_4$). Ammonium chloride (NH_4Cl) was chosen in that study because it yielded the best recoveries for the analytes tested, and it is the lowest cost and safest material.[19] Ammonium acetate and ammonium formate have also been selected as preferred ammonium salting out agents in previously-reported analytical methods, including flow injection analysis/mass spectrometry with real-time infinite dilution,[21] salting-out assisted liquid/liquid extraction (SALLE) coupled to HPLC/MS/MS,[22,23] and a modified QuEChERS method coupled

to HPLC/MS/MS and GC/MS/MS.[24,25] Other ammonium salts, such as $(NH_4)_2SO_4$, have also been successfully used in salting out methods for trace-level analysis of pesticides and antibiotics by LC/UV.[26,27]

The consensus is that ammonium salts represent a mass spectrometry-friendly option,[22] because they evaporate and/or decompose in the ion source, which reduces the need of instrument maintenance due to residual salts.[19,25] Analyte recoveries obtained by ammonium salting out extraction are comparable to those obtained by salting out with the inorganic alternatives (e.g. NaCl, $MgSO_4$); but, ammonium salting out improves instrumental analysis ruggedness and performance.[19,25] The ammonium salting out extraction improves sensitivity and selectivity in FI/MS/MS by avoiding the formation of unwanted metal cation adducts, decreasing the magnitude of matrix effects and reducing the risk of matrix interferences.[19] The applicability of ammonium salting out extraction coupled to FI/MS/MS for pesticide residue analysis has already been demonstrated, with limits of quantitation (LOQs) down to 0.01 mg/kg in food, blood plasma and urine.[19] The sensitivity achieved by the previously-reported method is adequate for food analysis and bio-analysis, but not for water analysis. High-throughput sample preparation methods, such as ammonium salting out, need to be improved for use with FI/MS/MS to meet the stringent limits of quantitation (LOQs) necessary for water analysis. Therefore, in this study, we focused our effort in improving the ammonium salting out extraction and FI/MS/MS to achieve the required sensitivity for pesticide residue analysis in water, while maintaining high-throughput and method simplicity. The results of this study include: (i) the report of partition coefficients measured for pesticides in the acetonitrile/water/NH_4Cl ternary systems, their dependence on pH and considerations for method development; (ii) an improved sample extraction method that achieves analyte preconcentration by adjusting the acetonitrile/water

volume ratio, and a procedure for optimizing this extraction method based on mass spectrometer sensitivity and analyte recoveries; (iii) method validation in surface, drinking and ground waters; and (iv) an approach for predicting the performance of the extraction method for other chemicals (e.g. pharmaceuticals) based on publicly-available physicochemical properties, such as octanol/water partition coefficients, K_{ow}.

Experimental

Reagents and reference standards

The acetonitrile, water, and methanol used in this study were HPLC-grade solvents from EMD Chemicals (Gibbstown, New Jersey, U.S.A.). The salting out agent, ammonium chloride with purity >99%, was purchased from Sigma-Aldrich (Saint Louis, Missouri, U.S.A.). Concentrated formic acid (purity >98%), concentrated acetic acid (purity >99.7%), ammonium hydroxide (~30% aqueous solution), as well as standard buffer solutions of pH 1.67, 7.00, and 10.00 (used for pH-meter calibration) were obtained from EMD Chemicals (Gibbstown, New Jersey, U.S.A.). All reference standards of the pesticides tested in this study were synthesized by DuPont Crop Protection, Global Technology Division, E. I. du Pont de Nemours and Company. These were aminocyclopyrachlor methyl, azimsulfuron, chlorantraniliprole, chlorimuron ethyl, chlorsulfuron, cyantraniliprole, diuron, flupyrsulfuron methyl, hexazinone, methomyl, oxamyl, sulfometuron methyl, and triflusulfuron methyl. The chemical structures of these compounds appear in Figure S-1. Note that these pesticides were selected for this study because they represent diverse physicochemical properties, such as polarity, volatility, stability, aqueous solubility, pKa, and ionization efficiency under electrospray conditions. In addition, these analytes cover multiple pesticide active ingredient classes: pyrimidine carboxylic acid herbicides, anthranilic diamide

insecticides, sulfonylurea herbicides, triazinone herbicides, carbamate insecticides, and phenylurea herbicides. Note that aminocyclopyrachlor, the acid form of aminocyclopyrachlor methyl previously tested,[19] was only included in preliminary experiments in this study. Aminocyclopyrachlor is not amenable to salting out by the NH_4Cl extraction[19] or QuEChERS even under low pH conditions.[28] Therefore, the method proposed here is not expected to be applicable to that herbicide active ingredient.

Measurement of acetonitrile/water partition coefficients (K_{aw}) in NH_4Cl salting out

The experimental partition coefficient of analytes in biphasic acetonitrile/water upon salting out were determined by mixing acetonitrile, water, NH_4Cl and the compound of interest, allowing the system to equilibrate, and then analyzing an aliquot of each phase in a high-performance liquid chromatograph equipped with a diode array detector for ultraviolet absorbance measurements (HPLC/UV/DAD). Briefly, a total of 52 biphasic systems (13 pesticides x 4 pH conditions tested) were prepared in 50-mL propylene centrifuge tubes. Each system was prepared by weighing 5.0 g of NH_4Cl (intentionally in excess to achieve saturation) into each tube, followed by addition of 10 mL of deionized water. A 10 mL aliquot of acetonitrile that contained the individual pesticide of interest at a concentration of 0.50 mg/mL was then added. Formic acid (10 µL), acetic acid (10 µL) or ammonium hydroxide (100 µL) were added as pH modifiers to achieve pH values in the resulting saturated NH_4Cl aqueous phases of 2.20, 2.78, and 7.59, respectively. The fourth pH condition tested did not use a pH modifier, and had a pH of 4.08 in the saturated NH_4Cl aqueous phase. The systems were shaken vigorously for ~5 minutes, and then allowed to rest for ~5 minutes. This cycle was repeated several times until the solid NH_4Cl did not dissolve further (i.e. system saturation was achieved). The samples were

centrifuged. Aliquots of the top (acetonitrile-rich) and bottom (water-rich) phases were analyzed by HPLC/UV/DAD to quantify the amount of each pesticide that partition into each layer, allowing the partition coefficients (K_{aw}) to be calculated.

Control samples of representative water matrices

Representative control water samples from 4 different sources were used in this study. Ground water was obtained from a well located in Kemblesville, Pennsylvania, U.S.A. Surface waters were collected from Lums Pond State Park (Bear, Delaware, U.S.A.) and White Clay Creek State Park (Newark, Delaware, U.S.A.). Drinking (tap) water was collected at DuPont Stine-Haskell Research Center, Newark, Delaware, U.S.A. These control water samples were stored frozen (-20°C). Prior to each use, they were allowed to thaw completely and shaken well to ensure sample homogeneity.

NH_4Cl salting out extraction with analyte preconcentration for water analysis

An outcome of this study was a validated pesticide multi-residue method for water analysis. The sample preparation procedure is illustrated in Figure 1. Briefly, a volume of 3.5 mL of acetonitrile extraction solvent was added to 15-mL plastic centrifuge tubes (one tube per water sample), followed by addition of 70 µL of 10% formic acid (aq) solution. Water sample aliquots of 7.00 mL were then transferred into the corresponding plastic tubes. The salting out agent, 3.0 g (± 0.1 g) of solid NH_4Cl, was then added to each sample, and the tubes were capped and shaken by hand for 1 minute. The samples were allowed to rest for approximately 1 minute. Two phases formed readily, thus centrifugation was not necessary. In preparation for sample dilution, 750 µL of acetonitrile were added into autosampler vials. Then, a 250 µL aliquot of each

acetonitrile extract (top layer) was transferred into its corresponding autosampler vial, capped, and shaken by hand. Excess ammonium chloride that was present in the extract precipitated. Therefore, the autosampler vials were briefly centrifuged. The resulting samples were then analyzed by FI/MS/MS.

Note that the order of addition described above was designed to facilitate mixing of the liquids without the need for shaking or swirling. That is, acetonitrile added first, formic acid solution added second, followed by the water aliquot added last, mixed relatively well because of the density of each liquid, resulting in one homogeneous phase prior to addition of the solid salt. Also, the 3.0-g portions of NH_4Cl were pre-weighed prior to analysis for several analytical sets. This increased throughput/reduced sample preparation time.

Flow injection tandem mass spectrometry (FI/MS/MS) instrumental analysis

The flow injection tandem mass spectrometry instrument conditions used in this study were based on a recently-published FI/MS/MS method applied to analysis of food and biological samples.[19] Briefly, a triple quadrupole mass spectrometer model API-5000 from Applied Biosystems/Sciex (Foster City, California, U.S.A.) was employed. The instrument was equipped with electrospray ionization (ESI). An Agilent 1290 HPLC instrument (Agilent Technologies, Wilmington, Delaware, U.S.A.) was used in flow injection mode for sample introduction; that is, by connecting the autosampler and the ESI source with a 100 cm PEEK capillary (part number 0890-1915, Agilent Technologies, Wilmington, Delaware, U.S.A.). Methanol was used as the carrier solvent, flowing at 400 μL/min. The sample injection volume was 2.0 μL. Electrospray ionization conditions were set as follows: spray voltage 4.9 kV, temperature 550°C, GS1 30 arbitrary units, GS2 80 arbitrary units. Because the triple quadrupole mass spectrometer was

operated in multiple reaction monitoring (MRM) mode, each analyte was measured by recording two specific MS/MS experiments (parent-to-fragment ion transitions). The exact MS/MS conditions are provided in Table 1. These selected ion transitions were found to provide adequate selectivity for analyte screening in this method (Reference 9 and Reference 17 provide detailed experiments that can be performed to ensure adequate selectivity in FI/MS/MS methods). The tandem mass spectrometry data output were processed using a smoothing factor = 3 and a bunching factor = 3 to improve signal to noise with the electronic filter options available in the instrument manufacturer's software (Analyst 1.5.1).

Calibration for quantitative analysis was achieved by the matrix-matched external standard method with calibration standards ranging from 0.07 ng/mL to 10 ng/mL. Additional experimental details about the preparation of matrix-matched standards are provided in Table S-1 as Electronic Supplementary Information.

Results and discussion

Acetonitrile/water partition coefficients (K_{aw}) in NH_4Cl salting out

The efficiency of liquid-liquid extraction procedures can be determined by calculating the partition coefficients of the analytes of interest. The same principle could be applied to salting out extractions. An experiment was designed to measure the partition coefficients (K_{aw}) of the analytes of interest (based on Equation 1) when subjected to NH_4Cl salting out extraction.

$$K_{aw} = \frac{[A]_a}{[A]_w} \qquad \text{Equation 1}$$

Where K_{aw} corresponds to the acetonitrile/water partition coefficient of the analyte, $[A]_a$ is the analyte concentration in the acetonitrile-rich phase, and $[A]_w$ is the analyte concentration in the water-rich phase.

For effective analyte preconcentration, sample cleanup and acceptable recoveries, it is desired that the compounds of interest preferentially partition into the acetonitrile phase. The partition coefficients (K_{aw}) can vary for acidic or basic compounds depending on the pH of the matrix. For example, the previously-reported variability in chlorsulfuron recoveries across multiple matrices in the earlier version of the ammonium salting out method[19] was likely due to pH changes across the tested samples, because chlorsulfuron has acidic properties in solution (pKa = 3.4).[19] In that study, acidic media such as grapefruit resulted in the highest chlorsulfuron recoveries (104 ± 15); whereas recoveries for this analyte where much lower in neutral-to-basic media such as egg and blood plasma (59 ± 4 and 66 ± 11, respectively).[19] The partition coefficients of neutral compounds are expected to be independent of pH, but it is necessary to determine an acceptable pH when working with acidic or basic compounds to ensure the design of rugged analyte extraction methods that consistently yield acceptable recoveries.

Effect of pH on analyte K_{aw} upon salting out with NH_4Cl

The effect of pH on the partition coefficient of the analytes of interest was evaluated. Four water systems were tested: (i) formic acid added (pH = 2.20), (ii) acetic acid added (pH = 2.78), (iii) NH_4Cl 'as is' (pH = 4.08), and (iv) ammonium hydroxide added (pH = 7.59). The acetonitrile and water phases were both analyzed by HPLC/UV/DAD (see experimental section for details). Each K_{aw} was calculated based on Equation 1. Table 2 displays the K_{aw} obtained for each analyte upon acetonitrile/water phase separation. Note that the K_{aw} obtained for the analytes in the

salting out experiment without pH-adjusting additives (i.e. NH_4Cl "as is") demonstrate a strong preference for the acetonitrile-rich phase. Therefore, even without further acidification, the salting out method using NH_4Cl could provide acceptable recoveries for the analytes of interest. For the compounds with acidic properties, the addition of formic acid to the system (i.e. pH 2.20) generally resulted in the best partition into the acetonitrile phase (that is, greater K_{aw}). Azimsulfuron and chlorsulfuron are the two analytes with the lowest pKa values (most acidic). Therefore, in principle, the partition coefficient of these compounds should experience the largest pH dependence. The experimental results confirmed this hypothesis, as illustrated in Figure 2. The addition of formic acid (system pH= 2.20) was included as a step in the final (validated) method since the acidic conditions should maximize the partition of azimsulfuron and chlorsulfuron into the acetonitrile-rich phase and ensure consistent and rugged method performance for the most acidic analytes.

Instantaneous extract preconcentration by ammonium salting out

In order for FI/MS/MS to be fit for the purpose of ultratrace-level analysis of pesticides in water, limits of quantitation (LOQs) of 0.1 µg/L must be met to satisfy regulatory method sensitivity guidelines. Extract preconcentration should help achieve these LOQs. Altering the ratios of water and acetonitrile while the NH_4Cl(s) is added in excess could provide an instantaneous extract preconcentration and clean up during salting out. Therefore, an experiment was design to test this hypothesis and evaluate the extract preconcentration that could be achieved. The preconcentration factors were calculated as the ratio of the water aliquot volume divided by the acetonitrile-rich (top) layer volume, since the analytes are extracted from the water aliquot into the acetonitrile-rich layer during salting out. Note that this definition of extract preconcentration

factors assumes the exclusive partition of the analyte into the acetonitrile-rich phase; therefore, it represents the maximum analyte preconcentration possible.

The experiment consisted of adding 3.0 g of NH_4Cl (excess) and 7.0 mL of water into a 15-mL propylene centrifuge tube, followed by addition of acetonitrile in 0.50 mL increments, mixing/centrifuging the system after each addition of acetonitrile, and measuring the resulting volumes of the acetonitrile-rich and water-rich layers by reading the markings on the tube. Note that the amount of NH_4Cl and water in the overall system were kept constant in this experiment; only the extraction solvent volume (acetonitrile) was varied.

The initial 2.0 mL of acetonitrile added to the system did not result in phase separation. That is, 2.0 mL of acetonitrile were miscible with the NH_4Cl (aqueous, saturated) system. The next 0.5 mL addition of acetonitrile (2.5 mL added total) resulted in phase separation, with a top acetonitrile-rich layer volume equal to 0.40 mL. Therefore, saturation of the aqueous phase with acetonitrile occurred when the water/acetonitrile ratio was between 3.5 (i.e. 7.0 mL of H_2O/2.0 mL ACN) and 2.8 (i.e. 7.0 mL of H_2O/2.5 mL ACN) in the presence of an excess of NH_4Cl. Then, phase separation continued to be measurable for water/acetonitrile ratios ≤ 2.8. The results are shown in Figure 3a, which displays the measured extract preconcentration as a function of the water/acetonitrile ratio. An extract preconcentration value of 5, which was obtained with a water/acetonitrile ratio = 2 is exemplified in the photograph displayed in Figure 3b. The water/acetonitrile ratio = 2 (that is, 7.0-mL water aliquot and 3.5 mL of acetonitrile extraction solvent), was chosen for the validation of this method to achieve the sensitivity objective of 0.1 μg/L (ppb) for the majority of analytes (this is discussed further in the next two sections). However, note that as displayed in Figure 3a, greater or smaller preconcentration factors could be obtained and used to meet a range of sensitivity requirements, as appropriate.

Predicting analyte recoveries in preconcentrated extracts using experimental K_{aw}

The instantaneous extract preconcentration achieved during salting out described in the previous section should be practical and reliable for ultratrace-level quantitative analysis of a wide range of chemicals. However, as the extract preconcentration factor increases, the amount of analyte that remains in the water-rich phase (unextracted) is also expected to increase. It is possible to predict the analyte recoveries using the K_{aw} already determined (Table 2), together with the following definition of experimentally-measured analyte percent recovery:

$$R_{exp} = \left(\frac{A_M}{A_T}\right) \times 100 \qquad \text{Equation 2}$$

Where R_{exp} is the experimentally-measured analyte percent recovery, A_M is the analyte measured in the acetonitrile-rich phase and A_T is the total analyte added. Note that the analyte measured, expressed in units of mass or mole, can be defined as $A_M = [A]_a V_a$; where $[A]_a$ is the analyte concentration measured in the acetonitrile-rich phase, and V_a is the volume of the acetonitrile-rich phase. Similarly, the total analyte added can be defined as the sum of the analyte present in the acetonitrile-rich and water-rich phases; $A_T = [A]_a V_a + [A]_w V_w$. Therefore, the predicted analyte percent recovery, R_p, can be defined as follows:

$$R_p = \left(\frac{[A]_a V_a}{[A]_a V_a + [A]_w V_w}\right) \times 100 \qquad \text{Equation 3}$$

Note that, from Equation 1, $[A]_a = [A]_w K_{aw}$. Therefore:

$$R_p = \left(\frac{K_{aw}V_a}{K_{aw}V_a+V_w}\right) \times 100 \qquad \text{Equation 4}$$

Predicted analyte percent recoveries, R_p, have been calculated using Equation 4 for the analytes evaluated in this study. For most analytes, especially the least polar compounds, R_p decreased at a low rate as a function of preconcentration factor, and remained >90% even when preconcentration factors were >15. On the other hand, significantly polar compounds such as oxamyl and methomyl have a more pronounced decrease of their R_p as preconcentration factors are increased (see Figure 4). A preconcentration factor equal to 5 was chosen as the optimal condition that should allow acceptable recoveries for all analytes (oxamyl and methomyl included). This decision was based on regulatory guidelines[4] which set the lower acceptable recovery at 50% for analyte concentration measurements ≤ 1 µg/L, as well as the results displayed in Figure 4. The calculation of R_p serves as an excellent tool for salting out extraction optimization without the need for execution of extensive and time-consuming experiments. The accuracy of recoveries predicted using this approach (i.e. R_p) was subsequently evaluated during method validation by comparison to experimentally-measured analyte recoveries, R_{exp}.

NH_4Cl salting out method for water analysis by FI/MS/MS

Sensitivity optimization. The optimized analyte salting out extraction was coupled with flow injection tandem mass spectrometry (FI/MS/MS) to achieve a high-throughput method capable of measuring pesticides at ultratrace levels. FI/MS/MS had been optimized at DuPont as part of other studies;[19] thus, the FI/MS/MS conditions used here were based on previously reported acquisition methods (see experimental section) and were not optimized further. Instead, typical solvents used to dilute extracts immediately prior to FI/MS/MS analysis were evaluated to

maximize mass spectrometry sensitivity. In essence, the preconcentration achieved during salting out followed by dilution with the most appropriate solvent to maximize sensitivity, provides sample cleanup and "solvent exchange" in a high-throughput fashion; that is, without the need for time-consuming steps such as solid-phase extraction (SPE) or solvent evaporation. The following six dilution solvents were tested: (i) methanol, (ii) 1.5% ammonium hydroxide in methanol, (iii) acetonitrile, (iv) 10% water in acetonitrile, (v) 0.1% formic acid in acetonitrile, and (vi) 1.0% ammonium hydroxide in acetonitrile. Emphasis was made to optimize the electrospray ionization efficiency of chlorsulfuron, methomyl and oxamyl, since these were the least-responsive analytes. These three analytes were the limiting factor in this multi-residue method regarding the achievable LOQs. The results revealed that the best signal responses were obtained for these three analytes when acetonitrile, 0.1% formic acid in acetonitrile, and 1.0% ammonium hydroxide in acetonitrile were used as diluent solvents. Pure acetonitrile was selected to keep the method as simple as possible, since the use of formic acid or ammonium hydroxide additives in the acetonitrile diluent did not seem to provide a benefit. The overall result of the optimization experiments described above was the salting out extraction method profiled in the experimental section and Figure 1.

Method validation: accuracy, precision, selectivity, dynamic range. A validation study was designed to test the proposed method and profile its performance. Four validation sets using creek, tap, well, and pond water were analyzed. Samples were fortified at 0.1 µg/L, 0.3 µg/L, and 1.0 µg/L with five replicates at each concentration level. Table 3 displays the results obtained for the 13 pesticides tested in the various water matrices. The overall recoveries correspond to the average of 60 measurements for each analyte (15 per water type), except for chlorsulfuron,

oxamyl, and methomyl which had 40 measurements averaged (10 per water type), since the 0.1 µg/L fortification did not yield adequate signal-to-noise for those pesticides. The overall average R_{exp} were within the acceptable range (50-120%)[4] for all compounds tested. Moreover, the standard deviations obtained for the overall average R_{exp} ranged from 7% to 14% (Table 3), and the relative standard deviations (RSDs) ranged from 10% to 18%, which are excellent for the proposed use of the method, particularly since the acceptability criterion in regulatory guidelines is RSDs <35%.[4] Figure 5 shows representative FI/MS/MS chronograms obtained for sulfometuron methyl in creek, pond, tap and well waters.

Method selectivity was assessed by comparing FI/MS/MS ion chronograms of control samples to those obtained for fortified samples. Selectivity was adequate for quantitative analysis of the analytes tested in the representative water matrices included in this study, as exemplified in Figure 5. This high-throughput FI/MS/MS method has been designed for quantitative screening. Therefore, depending on the application, additional confirmatory analysis may be necessary when analytes are detected. Selectivity in FI/MS/MS methods can be improved by inclusion of more than two MS/MS ion transitions.[11,29] Extensive selectivity assessments of chromatography/mass spectrometry techniques have been conducted.[30] However, a comprehensive evaluation is still needed to profile the selectivity (e.g. false positive and false negative rates) of FI/MS/MS when used for pesticide residue analysis and environmental analysis.[29]

The method validation results were consistent across the four water types tested. The experimentally-measured analyte recoveries (R_{exp}) correlated well with the K_{aw} values previously calculated. The validation study allowed R_{exp} to be compared to the predicted recoveries (R_p). This comparison is shown in Table 3, where ΔR is introduced as $R_{exp} - R_p$ calculated from the

overall average R_{exp} values. The ΔR ranged from -12 (azimsulfuron) to +7 (oxamyl). This range is within the variability expected in an ultratrace-level analytical method. The negative ΔRs were generally of greater absolute value, and this resulted in an overall ΔR (averaged for the 13 analytes) of -3.8. This negative value can be attributed to analyte losses that can occur before, during or after salting out; for example, analyte degradation, which is not accounted in the predicted recovery (R_p) calculation. This hypothesis is supported by the fact that the most negative ΔR values were obtained for sulfonylurea herbicides, which can hydrolyze during sample preparation. Nevertheless, the method validation results met the criteria for analysis of pesticides in water.[4]

Matrix matched calibration standards were used to quantitate the test samples. Linear regression correlation coefficients obtained during method validation were >0.99. The concentration of the matrix matched calibration standards ranged from 0.07 ng/mL to 10 ng/mL, as exemplified in Figure S-2. This concentration range was appropriate for the following 10 analytes: aminocyclopyrachlor methyl, azimsulfuron, chlorantraniliprole, chlorimuron ethyl, cyantraniliprole, diuron, flupyrsulfuron methyl, hexazinone, sulfometuron methyl, and triflusulfuron methyl. The 0.07 ng/mL to 10 ng/mL concentration range in the calibration standards corresponds to analyte concentrations in water samples ranging from 0.056 µg/L to 8.0 µg/L.

The 0.07 ng/mL calibration standard did not yield acceptable signal-to-noise for chlorsulfuron, oxamyl and methomyl. Consequently, the calibration standard concentration range used for those analytes was 0.15 ng/mL to 10 ng/mL. This corresponds to concentrations of chlorsulfuron, oxamyl and methomyl in water samples ranging from 0.12 µg/L to 8.0 µg/L.

***Matrix effects*.** Matrix effects were also assessed during method validation. This was done by preparing a calibration standard curve using a reagent blank taken through the entire method (Figure 1). HPLC-grade water was used to prepare the reagent blank system. The measured percent matrix effects (%ME) was defined as 100 multiplied by the ratio of analyte response in matrix divided by the analyte response in reagent blank. That is, %ME <100% corresponds to matrix suppression, %ME >100% corresponds to matrix enhancement, and %ME = 100% indicates absence of matrix effects. The assessment showed that both matrix enhancement and matrix suppression were encountered. The average %ME observed for each analyte were between 90% and 120% in creek water, 88% and 137% in tap water, 91% and 126% in well water, and 92% and 114% in pond water. These results indicate that the salting out extraction method offers remarkable sample cleanup in the waters tested. Nevertheless, it has been demonstrated that FI/MS/MS can be highly susceptible to matrix effects.[16-19] Therefore, to ensure method ruggedness, a careful assessment of matrix effects is recommended when implementing this method, as well as the use of matrix-matching or other suitable matrix effect correction approach.

Limits of quantitation (LOQs) and limits of detection (LODs). The LODs and LOQs obtained during method validation are reported in Table 3. The limit of quantitation (LOQ) was defined as the lowest validated fortification level. The defined LOQ targeted a signal-to-noise ratio between 5-to-1 and 20-to-1 for the analytes tested. The limit of detection (LOD) was estimated by measuring the analyte response at the LOQ level and the average background response of all control water samples, and then extrapolating to determine the analyte concentration expected to produce a S/N = 3. The method achieved the target LOQ of 0.1 µg/L for 10 of the 13 analytes

tested. The least-responsive analytes (chlorsulfuron, methomyl and oxamyl) had LOQ = 0.3 µg/L and LOD = 0.1 µg/L. The LODs obtained for all other compounds ranged from 0.01 µg/L to 0.04 µg/L, and are listed in Table 3.

Correlation between analyte recovery and analyte physicochemical properties

Physicochemical properties of bioactive chemicals are widely available in the scientific literature. Aqueous solubility and octanol/water partition coefficients (K_{ow}) are examples of physicochemical properties determined during the development phase of new agricultural chemicals. Consequently, and unlike the acetonitrile/water partition coefficients (K_{aw}) determined in this study, aqueous solubility and K_{ow} are accessible for a wide range of active ingredients. The analyte recoveries (R_{exp} and R_p) were plotted as a function of aqueous solubility, ratio of acetone and water solubility, and K_{ow} of the six non-ionizable (neutral) compounds tested in this study.[31] This was done to correlate the suitability of the proposed salting out method to well-known physicochemical properties of pesticides and presumably other chemicals. The results of this assessment are shown in Figure 6, demonstrating a very good correlation between publicly-available analyte properties, R_{exp} and R_p values.

Oxamyl, the analyte with lowest R_{exp} (57%) and R_p (50%) highlighted in Figure 6, establishes physicochemical property thresholds for which this salting out method may be suitable: (i) aqueous solubility ≤ 280 g/kg, (ii) acetone/water solubility ratio ≥ 2.4, (iii) Log K_{ow} ≥ -0.44.

NH_4Cl salting out method applicability to other pesticides and pharmaceuticals

Physicochemical properties of widely used pharmaceutical active ingredients[32,33] and pesticide active ingredients[34,35] were sought in the scientific literature. Reports of aqueous solubility

appeared in several different units, and were often expressed as a qualitative observations (e.g. "insoluble", "very low solubility", etc.). On the other hand, Log K_{ow} was the most consistent parameter across the references and chemical classes researched. Consequently, Log K_{ow} was selected for this assessment.

Histograms of Log K_{ow} values obtained for bioactive ingredients (150 pharmaceuticals and 120 pesticides) appear in Figure 7. The assessment revealed that Log K_{ow} values for the common chemicals included in this sample are normally distributed with a mean of 2.605. Log K_{ow} was greater than -0.44 (oxamyl's Log K_{ow}) for a total of 247 out of 270 compounds (91%), suggestive of wide applicability of the proposed salting out method for analysis of pesticides and pharmaceuticals. This is consistent with recent reports of broad application of ammonium salting out for trace-level analysis. For example, salting out with ammonium chloride, ammonium formate and ammonium acetate was demonstrated to be effective in extracting dozens of pesticides from food matrices in a modified QuEChERS method.[25] In addition, ammonium formate salting out extraction was also proven effective for the analysis of pesticides, flame retardants, polychlorinated biphenyls and polycyclic aromatic hydrocarbons.[24] The assessment reported here (Figure 7) serves as an additional tool to forecast the potential application of extraction methods across chemical classes.

Conclusions

An improved ammonium salting out method was developed, which provides instantaneous extract preconcentration and cleanup in one step. The method was proven to be suitable for ultratrace-level analysis of pesticides in water, meeting the stringent European Union sensitivity requirement (LOQ = 0.1 µg/L) for 10 out of 13 analytes tested. It should be noted that these

LOQs were achieved with a triple quadrupole mass spectrometer instrument model that was approximately a decade old (Applied Biosystems/Sciex API-5000). Better method sensitivity should be expected with more advanced instruments. The proposed salting out method is highly adaptable and has several parameters that can be optimized and varied depending on the intended application, desired LOQs, and analytes of interest. The variable parameters include: water aliquot size, acetonitrile extraction solvent volume, amount and type of ammonium salting out agent (e.g. acetate, formate, chloride), additives or pH modifiers used during salting out, and diluent solvent used prior to instrumental analysis. Although this method has been developed for compatibility with FI/MS/MS, it should also be applicable to (and improve the performance of) other instrumental analysis techniques such as LC/MS^n and GC/MS^n. This method is also predicted to be suitable for ultratrace-level analysis of a wide range of chemicals (e.g. pesticides, pharmaceuticals) based on the correlation found between method performance (i.e. analyte recoveries) and publicly-available physicochemical properties of the analytes tested.[36]

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36 The correlation assessment (Figure 6) and the prediction of method applicability to other chemicals (Figure 7) were conducted to encourage broad application of this method, as well as research to further improve the technique.

TABLES

Table 1. Mass spectrometer settings used in this study for water analysis by FI/MS/MS. Two fragment ions were recorded for each analyte.[a,b]

Analyte	Precursor Ion	Q1 Isolated Precursor Ion (m/z)	Q3 Scanned Fragment Ion (m/z)	DP (V)	CE (V)
Aminocyclopyrachlor	$(M+H)^+$	214	68	110	34
		214	101	100	40
Aminocyclopyrachlor methyl	$(M+H)^+$	228	68	90	40
		228	41	90	40
Azimsulfuron	$(M+H)^+$	425	182	75	40
		425	156	75	40
Chlorantraniliprole[c]	$(M+H)^+$	484	286	110	30
		484	453	110	26
Chlorimuron ethyl	$(M+H)^+$	415	186	75	40
		415	121	75	40
Chlorsulfuron[c]	$(M+H)^+$	358	141	75	40
		358	167	75	40
Cyantraniliprole[c]	$(M+H)^+$	475	286	110	34
		475	444	100	30
Diuron	$(M+H)^+$	233	72	91	27
		233	46	91	33
Flupyrsulfuron methyl	$(M+H)^+$	466	182	75	40
		466	156	75	40
Hexazinone	$(M+H)^+$	253	171	116	23
		253	71	116	43
Methomyl[c]	$(M+H)^+$	163	88	50	10
		163	106	50	10
Oxamyl[c]	$(M+NH_4)^+$	237	72	85	30
		237	90	75	19
Sulfometuron methyl	$(M+H)^+$	365	150	100	25
		365	199	100	25
Triflusulfuron methyl	$(M+H)^+$	493	264	75	40
		493	238	75	40

[a] Abbreviations: Q1 = quadrupole 1; Q3 = quadrupole 3; CE = collision energy; DP = declustering potential

[b] These conditions are based on those described in Reference 19.

[c] For this analyte, the sum of the ion transitions listed was used for quantitative screening.

Table 2. Acetonitrile/water partition coefficients (K_{aw}) measured by HPLC/UV/DAD for the 13 analytes of interest upon salting out with NH_4Cl, and their corresponding logarithm (Log K_{aw}) values.[a]

Acidic and basic analytes (ionizable in aqueous solutions)											
Analyte	pKa[b]	K_{aw} pH 7.59	K_{aw} pH 4.08	K_{aw} pH 2.78	K_{aw} pH 2.20	Avg[c] K_{aw} all pHs	Log K_{aw} pH 7.59	Log K_{aw} pH 4.08	Log K_{aw} pH 2.78	Log K_{aw} pH 2.20	Avg[c] Log K_{aw} all pHs
Chlorsulfuron	3.4	1.17	109	305	399	N/A[e]	0.07	2.04	2.48	2.60	N/A
Azimsulfuron	3.6	1.47	217	786	1484	N/A[e]	0.17	2.34	2.90	3.17	N/A
Chlorimuron ethyl	4.2	6.91	3193	1886	2151	N/A[e]	0.84	3.50	3.28	3.33	N/A
Triflusulfuron Methyl	4.4	57.7	2840	7761	2405	N/A[e]	1.76	3.45	3.89	3.38	N/A
Flupyrsulfuron methyl	4.9	10.7	1445	2166	1947	N/A[e]	1.03	3.16	3.34	3.29	N/A
Sulfometuron Methyl	5.2	1.24	224	246	263	N/A[e]	0.09	2.35	2.39	2.42	N/A
Aminocyclopy-rachlor methyl	-[d]	77.4	75.8	57.2	31.5	N/A[e]	1.89	1.88	1.76	1.50	N/A
Neutral analytes (non-ionizable in aqueous solutions)											
Analyte	pKa[b]	K_{aw} pH 7.59	K_{aw} pH 4.08	K_{aw} pH 2.78	K_{aw} pH 2.20	Avg[c] K_{aw} all pHs	Log K_{aw} pH 7.59	Log K_{aw} pH 4.08	Log K_{aw} pH 2.78	Log K_{aw} pH 2.20	Avg[c] Log K_{aw} all pHs
Chlorantranili-prole	N/A[e]	681	795	695	744	729 ± 52	2.83	2.90	2.84	2.87	2.86 ± 0.03
Cyantranili-prole	N/A[e]	326	352	334	323	334 ± 13	2.51	2.55	2.52	2.51	2.52 ± 0.02
Diuron	N/A[e]	176	181	189	188	183 ± 6	2.25	2.26	2.28	2.27	2.26 ± 0.01
Hexazinone	N/A[e]	28.6	28.8	28.5	27.9	28.5 ± 0.4	1.46	1.46	1.46	1.45	1.45 ± 0.01
Methomyl	N/A[e]	10.7	10.7	10.3	11.1	10.7 ± 0.3	1.03	1.03	1.03	1.04	1.03 ± 0.01
Oxamyl	N/A[e]	7.32	7.47	7.36	7.48	7.41 ± 0.08	0.86	0.87	0.87	0.87	0.87 ± 0.01

[a] K_{aw} values were calculated based on Equation 1.

[b] Acid dissociation constants do not apply to (and were not available for) the non-ionizable analytes.

[c] The average K_{aw} and Log K_{aw} (± standard deviation) across the pHs tested is reported for non-ionizable analytes in order to provide a statistical assessment of the measurements, since K_{aw} was not dependent on pH for those compounds (as expected). On the other hand, the average K_{aw} was not calculated for acidic and basic compounds due to the observed pH dependence.

[d] The pKa of protonated aminocyclopyrachlor methyl was not found in the literature, but the K_{aw} of this compound showed a dependence on pH consistent with protonation of the amino functional group at the lower pHs.

[e] N/A = not applicable.

Table 3. Experimentally-measured analyte percent recoveries (R_{exp}) obtained during validation of the salting out extraction/preconcentration FI/MS/MS method for ultratrace-level analysis of pesticides in water, and comparison to the predicted analyte percent recoveries (R_p) calculated prior to method validation.[a]

Analyte	Fort. level µg/L[b]	R_{exp} creek water	R_{exp} tap water	R_{exp} well water	R_{exp} pond water	R_{exp} overall avg.[c]	R_p	ΔR, $R_{exp} - R_p$	LOQ[d] µg/L	LOD[e] µg/L
Aminocyclopyrachlor methyl	0.1	68 ± 9	87 ± 8	87 ± 15	96 ± 18	83 ± 11	81	+ 2	0.1	0.02
	0.3	83 ± 2	87 ± 6	75 ± 9	82 ± 10					
	1.0	89 ± 9	84 ± 4	82 ± 4	78 ± 13					
Azimsulfuron	0.1	94 ± 11	60 ± 12	76 ± 10	103 ± 14	88 ± 14	100	- 12	0.1	0.02
	0.3	100 ± 5	81 ± 5	85 ± 12	94 ± 6					
	1.0	103 ± 8	85 ± 5	84 ± 6	89 ± 5					
Chlorantraniliprole	0.1	77 ± 13	104 ± 11	95 ± 14	91 ± 20	93 ± 12	99	- 6	0.1	0.04
	0.3	99 ± 8	97 ± 14	91 ± 9	97 ± 7					
	1.0	94 ± 3	96 ± 6	88 ± 4	92 ± 4					
Chlorimuron ethyl	0.1	82 ± 20	97 ± 19	94 ± 7	113 ± 15	97 ± 14	100	- 3	0.1	0.04
	0.3	112 ± 10	90 ± 10	93 ± 13	101 ± 12					
	1.0	94 ± 10	93 ± 4	89 ± 9	100 ± 8					
Chlorsulfuron	0.1	<LOQ[f]	<LOQ[f]	<LOQ[f]	<LOQ[f]	99 ± 13	98	+ 1	0.3	0.1
	0.3	121 ± 8	102 ± 10	101 ± 9	89 ± 16					
	1.0	97 ± 5	97 ± 9	92 ± 10	89 ± 5					
Cyantraniliprole	0.1	103 ± 11	100 ± 12	83 ± 23	81 ± 15	94 ± 13	98	- 4	0.1	0.04
	0.3	105 ± 10	96 ± 4	81 ± 14	97 ± 8					
	1.0	98 ± 13	98 ± 7	90 ± 6	93 ± 7					
Diuron	0.1	80 ± 11	92 ± 11	97 ± 10	112 ± 7	94 ± 13	96	- 2	0.1	0.04
	0.3	96 ± 10	91 ± 4	84 ± 6	96 ± 16					
	1.0	107 ± 9	96 ± 9	82 ± 9	98 ± 8					
Flupyrsulfuron methyl	0.1	78 ± 2	86 ± 4	91 ± 12	89 ± 8	89 ± 9	100	- 11	0.1	0.01
	0.3	101 ± 4	93 ± 5	80 ± 6	90 ± 7					
	1.0	93 ± 6	101 ± 3	79 ± 7	92 ± 5					
Hexazinone	0.1	73 ± 11	71 ± 10	65 ± 10	74 ± 10	73 ± 8	79	- 6	0.1	0.03
	0.3	77 ± 5	71 ± 7	65 ± 9	75 ± 10					
	1.0	79 ± 1	74 ± 5	70 ± 5	76 ± 7					
Methomyl	0.1	<LOQ[f]	<LOQ[f]	<LOQ[f]	<LOQ[f]	60 ± 7	60	0	0.3	0.1
	0.3	66 ± 8	69 ± 4	56 ± 8	61 ± 5					
	1.0	56 ± 3	52 ± 3	59 ± 3	56 ± 6					
Oxamyl	0.1	<LOQ[f]	<LOQ[f]	<LOQ[f]	<LOQ[f]	57 ± 10	50	+ 7	0.3	0.1
	0.3	66 ± 6	58 ± 7	64 ± 6	64 ± 14					
	1.0	58 ± 2	51 ± 2	47 ± 6	47 ± 5					
Sulfometuron methyl	0.1	90 ± 13	84 ± 13	87 ± 11	101 ± 14	92 ± 11	97	- 5	0.1	0.02
	0.3	101 ± 11	94 ± 7	84 ± 12	94 ± 12					
	1.0	93 ± 7	99 ± 5	86 ± 5	91 ± 6					
Triflusulfuron methyl	0.1	84 ± 7	92 ± 10	100 ± 6	80 ± 4	90 ± 10	100	- 10	0.1	0.01
	0.3	100 ± 7	93 ± 4	82 ± 7	89 ± 5					
	1.0	100 ± 5	101 ± 2	76 ± 3	88 ± 6					

[a] Recovery results shown correspond to the average ± standard deviation.

[b] Samples were fortified at 0.10 µg/L, 0.30 µg/L and 1.0 µg/L; a total of 5 fortifications were made at each level.

[c] Overall recoveries correspond to the average of 60 measurements for each analyte (15 per water type), except for chlorsulfuron, oxamyl, and methomyl which had 40 measurements averaged (10 per water type).

[d] The limit of quantitation (LOQ) was defined as the lowest validated fortification level. The defined LOQ targeted a signal-to-noise ratio between 5-to-1 and 20-to-1 for the analytes tested.

[e] The limit of detection (LOD) was estimated by measuring the analyte response at the LOQ level and the average background response of all control water samples, and then extrapolating to determine the analyte concentration expected to produce a S/N = 3.

[f] The LOQ of the method for quantitation of chlorsulfuron, methomyl and oxamyl was 0.3 µg/L.

FIGURE CAPTIONS

Figure 1. Schematic description of the ammonium chloride salting out extraction method for ultratrace-level quantitative analysis of small bioactive molecules (e.g. pesticides) in water matrices. The reagents and amounts in parenthesis correspond to those used during method validation; these could be adjusted to optimize the procedure for the desired application.

Figure 2. (a) Effect of pH on the acetonitrile/water partition coefficients, K_{aw}, obtained experimentally for the analytes chlorsulfuron and azimsulfuron upon salting out with NH_4Cl. The pH was adjusted by using diluted acid or base aqueous solutions instead of water, as follows: 0.1% formic acid (aq) pH = 2.20, 0.1% acetic acid (aq) pH = 2.78, water (control test) pH = 4.08, 0.05% ammonium hydroxide (aq) pH = 6.42, 0.1% ammonium hydroxide (aq) pH = 6.74, 1.0% ammonium hydroxide (aq) pH = 7.59. The pHs shown correspond to that of the aqueous solvent after saturation with NH_4Cl. The graph in (b) shows the logarithm (base 10) of the partition coefficients (Log K_{aw}).

Figure 3. Extract preconcentration effect observed when performing salting out in acetonitrile/water mixtures with NH_4Cl in excess. (a) Preconcentration factors obtained experimentally by mixing water (7.0 mL) and NH_4Cl (3.0 g) with varying volumes of acetonitrile to create different water/acetonitrile ratios (abscissa). The extract preconcentration factors were calculated as the ratio of the water aliquot volume divided by the acetonitrile-rich (top) layer volume. (b) Photo showing the expected preconcentration effect when performing NH_4Cl salting out with a water/acetonitrile 2:1 mixture (validated method: 7.0 mL H_2O, 3.5 mL acetonitrile, 3.0 g NH_4Cl). A red dye was used for enhanced visibility.

Figure 4. Predicted analyte percent recoveries, R_p, calculated from Equation 4 and plotted as a function of preconcentration factor for the four most polar analytes. Note that, as expected, R_p decreases as the preconcentration factor is increased; and, R_p decreases at a greater rate as the analyte K_{aw} decreases: R_p of aminocyclopyrachlor methyl (Log K_{aw} = 1.50) > hexazinone (Log K_{aw} = 1.45) > methomyl (Log K_{aw} = 1.03) > oxamyl (Log K_{aw} = 0.87).

Figure 5. Example FI/MS/MS ion chronograms obtained for sulfometuron methyl during method validation. The FI/MS/MS chronograms shown correspond to ion transition m/z 365 → m/z 150. Chronograms of control water samples (a through d) are representative of the adequate method selectivity obtained. Chronograms from the low level 0.1 µg/L (e through h) and high level 1.0 µg/L (i through l) fortifications show comparable analyte response across the sample types tested, consistent with relatively weak or similar matrix effects encountered in creek, pond, tap and well water.

Figure 6. Correlation between analyte recovery and physicochemical properties for the non-ionic pesticides tested. The recovery data (R_{exp}, R_p) are plotted for chlorantraniliprole (93, 99.0), cyantraniliprole (94, 97.8), diuron (94, 96.2), hexazinone (73, 79.1), methomyl (60, 60.1) and oxamyl (57, 50.4), as a function of their (a) solubility in water, (b) acetone/water solubility ratio, and (c) Log K_{ow}. Note that, based on these data, oxamyl and its properties can define the method applicability threshold for analyte solubility in water (280 g/kg), acetone/water solubility ratio (2.4) and Log K_{ow} (– 0.44).

Figure 7. Log K_{ow} of commonly-used pharmaceutical and pesticide bioactive ingredients (obtained from References 32-35). (a) Overall histogram of Log K_{ow} values obtained for a sample of 270 bioactive compounds; that is, pharmaceuticals and pesticides combined. (b) Histograms obtained for the pharmaceuticals and pesticides, separately. The assessment revealed that 90% of the pharmaceuticals and 93% of the pesticides (91% combined) had Log $K_{ow} \geq$ -0.44 (i.e. oxamyl), suggestive of broad applicability of the ammonium salting out method reported in this study.

FIGURES

Figure 1.

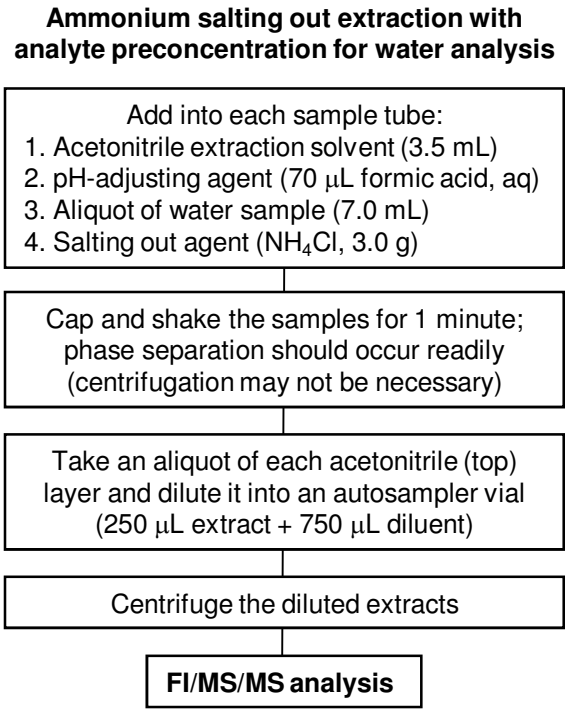

Figure 2.

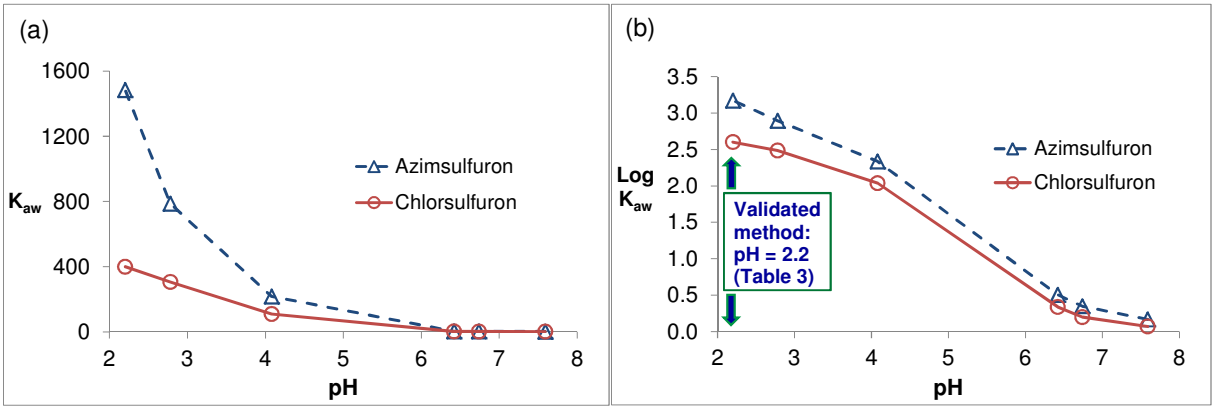

Figure 3.

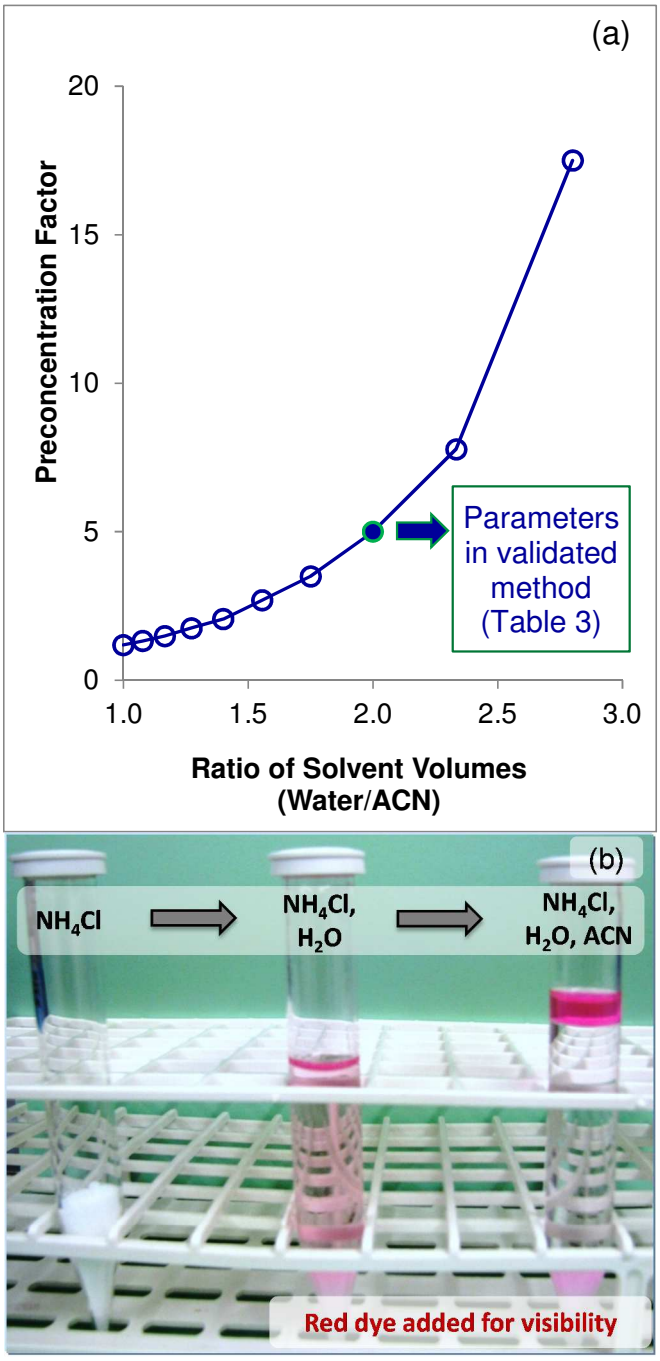

Figure 4.

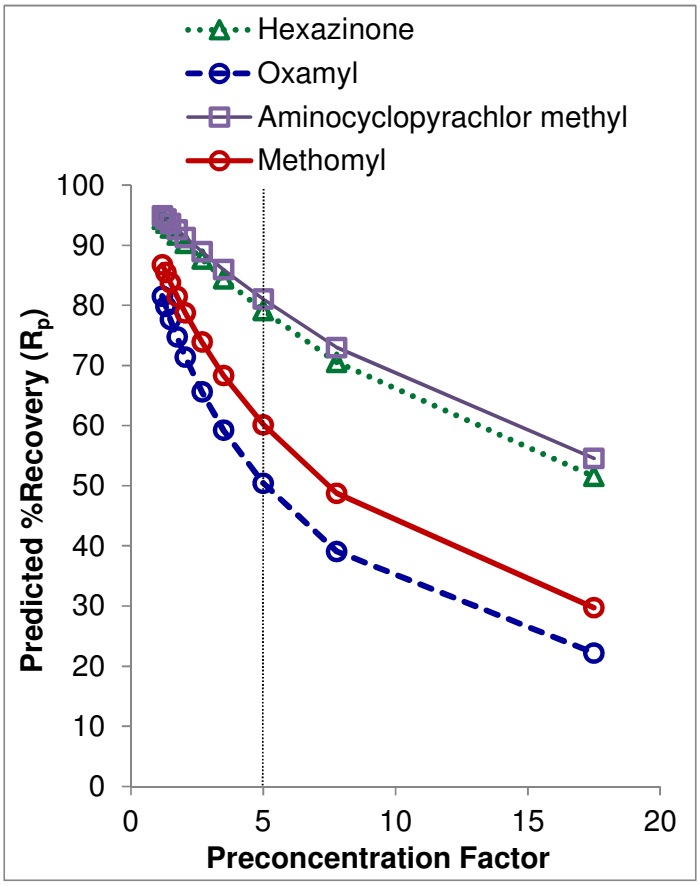

Figure 5.

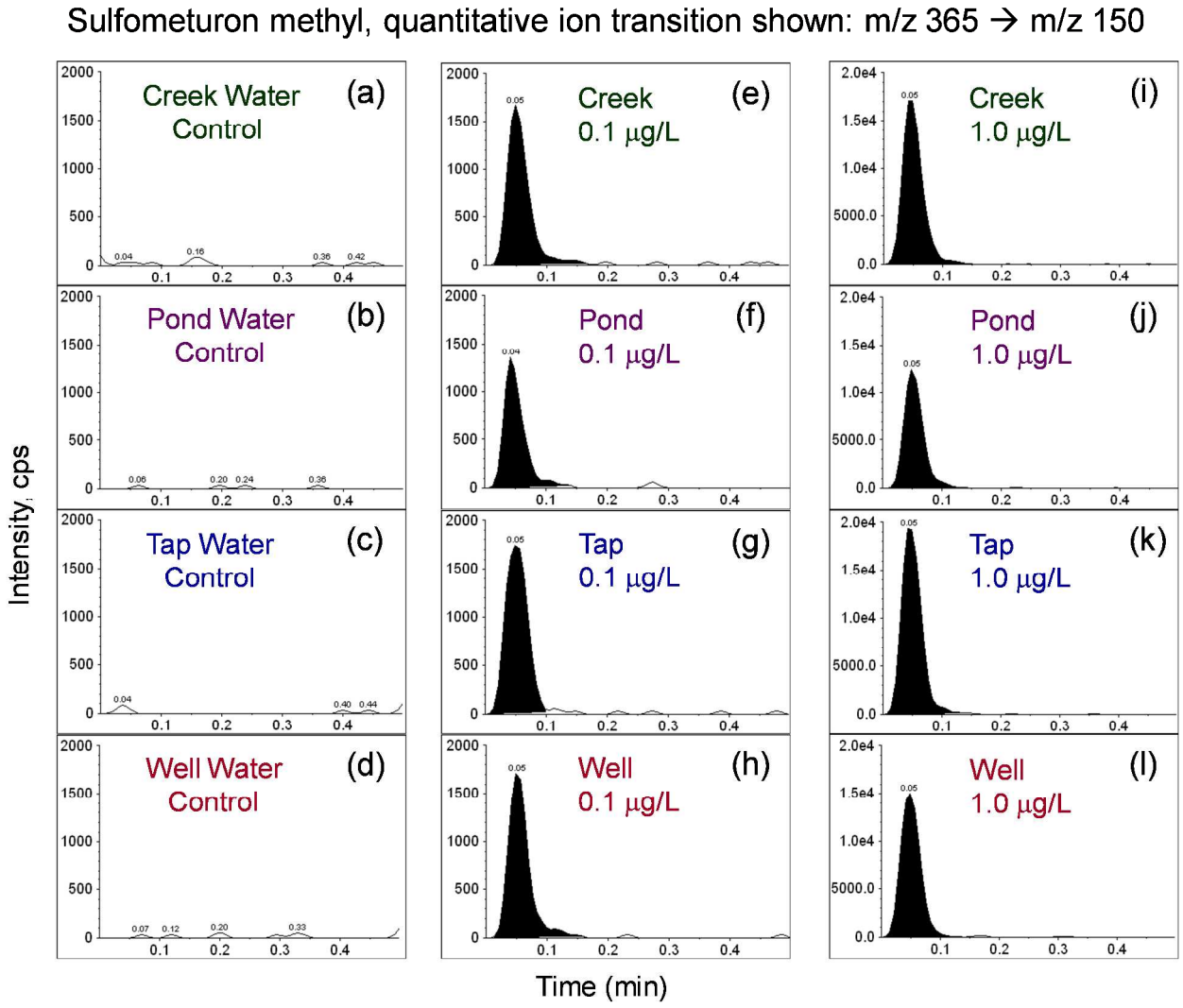

Figure 6.

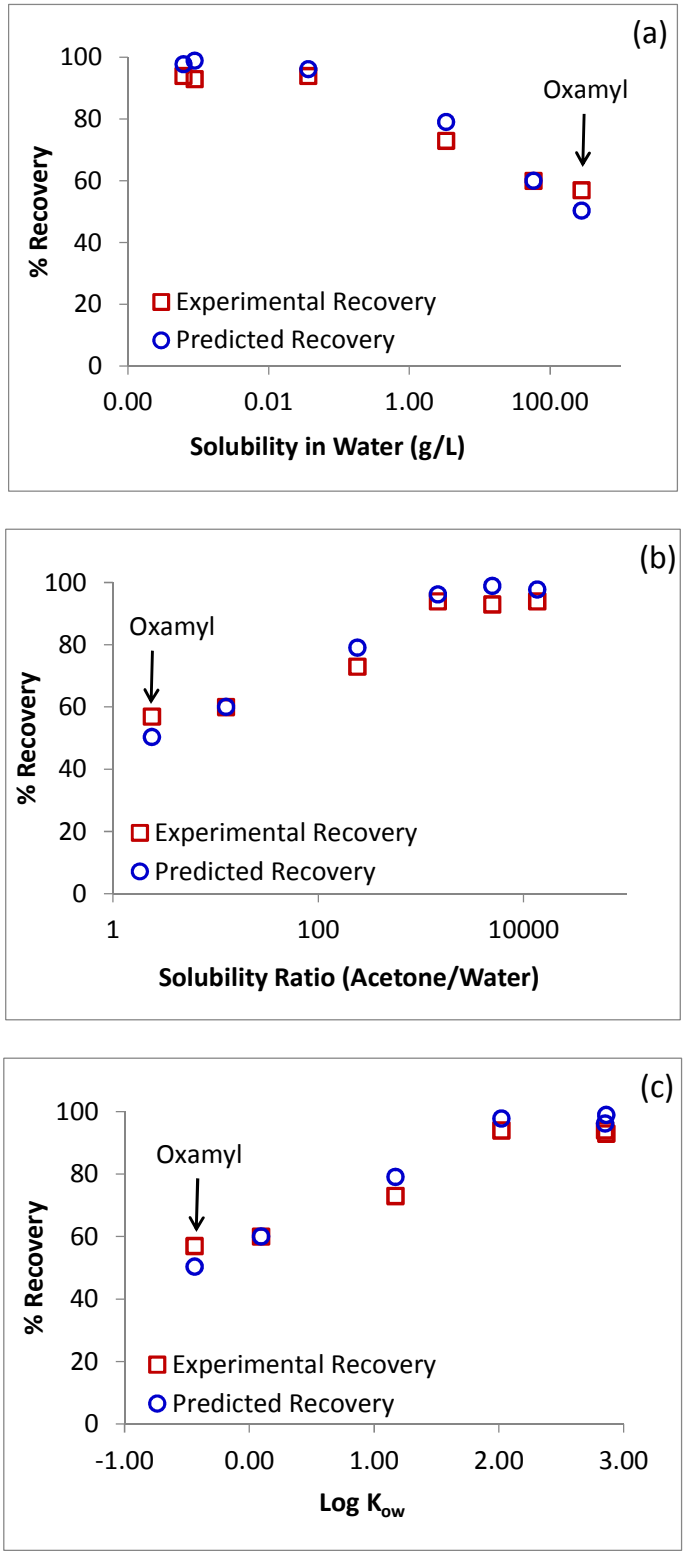

Figure 7.