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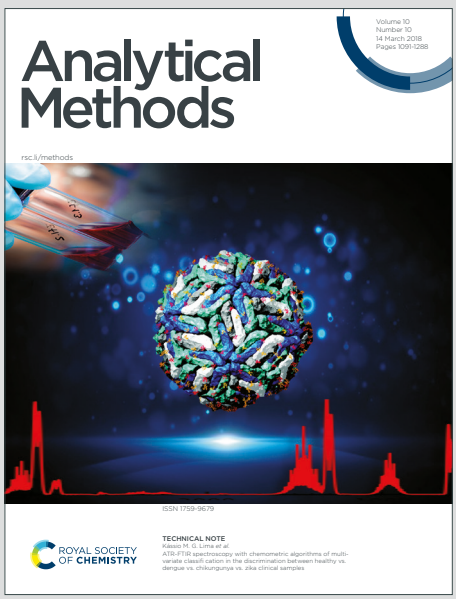

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QuEChERS: a simple extraction for monitoring quaternary ammonium biocide pollution in soils and antimicrobial resistance.

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Abbreviations

BAC-C12 – Benzyldimethyldodecylammonium chloride, BAC-C14 – Benzyldimethyltetradecylammonium chloride, d_7-BAC-C14 – d_7 - Benzyldimethyltetradecylammonium chloride, d_9-HDTMA - Hexadecyltrimethylammonium bromide-d_9, dSPE – Dispersive solid-phase extraction, HDTMA – Hexadecyltrimethylammonium chloride, $MgSO_4$ – Magnesium sulphate, NaOAc – Sodium acetate, QAC – Quaternary ammonium compound, QuEChERS – Quick, Easy, Cheap, Effective, Rugged and Safe

Keywords

Environmental solids, Liquid chromatography-mass spectrometry, Biocides, Antimicrobial resistance, QuEChERS

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Abstract

Quaternary ammonium compounds (QACs) are broad-spectrum disinfectants used in a range of everyday materials. Their high usage rates, limited regulation and reporting has meant their environmental release is largely uncontrolled and impact unknown. With links to antimicrobial resistance (AMR) and adsorption to wastewater solids (that are recycled), there is a need for more controlled disposal measures and monitoring. These environmental matrices are highly complex requiring methods that are often laborious and costly to undertake. Using a robust quantitative reversed-phase LC-MS/MS method, we have shown that an 'off the shelf' QuEChERS product can reliably extract (<10 %RSD) aromatic and aliphatic QACs anticipated within municipal, industrial and agricultural waste from water and soil, with reduced matrix effects of 95.7-104.4% for recoveries of up to 53% from soil when combined with extract dilution. Therefore, unlike current literature, this work has shown that, with minimal development, the QuEChERS product can provide a rapid, effective and low cost preparation for quantifying QAC pollution and monitoring AMR.

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Introduction

With a move to a Circular Economy the reuse of solid sludge waste from wastewater (WW) treatment is becoming increasingly popular, with the deposition of municipal waste on agricultural land as fertiliser (80%).[1] However, given many pollutants can remain within this organic material,[2,3] the analysis of these environmental solids and soil is becoming more important for environmental and public safety. Quaternary ammonium compounds (QACs) are a common broad-spectrum disinfecting agent and preservative within a range of everyday products[4-6] and industries,[7-9] used to inhibit microbial growth (at a minimum inhibitory concentration (MIC) of 0.5-5 mg/L) or cause cell death (minimum bactericidal concentration (MBC) of 10-50 mg/L).[5] Limited regulation governing the reporting levels in the majority of these products and poor efficacy of WW treatment for many chemicals,[10-12] along with their adsorption to environmental solids,[13] has meant that environmental exposure to these cationic surfactants through domestic and industrial WW has been largely uncontrolled, with a need to establish their fate and effects.[7,14] However, studies concerning the use of QAC detergents have shown an increase in biocide and multi-drug (antibiotic) resistance,[4,15-20] via the increased expression of genes for efflux pump proteins that actively remove biocides from the cell.[15] These antimicrobial resistance (AMR) genes have also been observed across species[4] and further add to concerns that the unrestricted environmental release of QACs can result in the over-exposure of bacteria to sub-MICs and AMR, highlighting the need for methods that monitor the abundance and impact of these compounds in environmental solids.

The molecular analysis of environmental solids is highly challenging due to their complexity and the sorption of trace material to more abundant (lipophilic) analytes in the sample (e.g. fulvic and humic acids).[2] To displace the analyte from the matrix multi-step sample preparations, that can take multiple hours per sample[3] and often with variable performance, are commonly used. Existing protocols to measure QAC biocides in environmental solids are an example of this, with more successful and highly cited methods needing more than an hour[21-24] per sample and/or do not report data concerning matrix effects.[21-23,25] The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method[26] offers significant flexibility for method development, and can facilitate screening of environmental samples for pollutants by targeting the removal of abundant hydrophobic interferences through selected dSPE materials (e.g. C18 or graphitised carbon black (GCB)). Recent work with QuEChERS has shown the potential for measuring surfactants[13,27-30] and extracting environmental solids with low matrix interference.[29,31] However, these protocols do not cover the breadth of surfactant biocides anticipated in environmental solids,[13,29-31] have used a bespoke extraction product,[29] require additional steps to the protocol,[13] or do not address samples, such as soil.[27,28] Given this, we believed QuEChERS could provide an alternative single protocol extraction for the range of common QAC disinfectants from soil

anticipated via exposure to municipal, industrial and agricultural waste, as a more rapid, cost effective method that exhibits low matrix interference. We have therefore investigated the usability of an 'off the shelf' QuEChERS product as part of a quantitative analytical workflow, to measure these highly important QACs within fortified soil, as a much-needed monitoring platform for AMR following WW contamination.

Experimental

Chemicals and reagents

Aromatic and aliphatic QACs commonly used in disinfectants and preservatives were selected for method evaluation. These included benzyldimethyldodecylammonium chloride (BAC-C12), benzyldimethyltetradecylammonium chloride (BAC-C14), benzyldimethylhexadecylammonium chloride (BAC-C16), didecyldimethylammonium bromide (DDMAB) and hexadecyltrimethylammonium chloride (HDTMA), obtained as solid standard reference materials from Sigma Aldrich (Poole, UK). While stearalkonium chloride (BAC-C18) was obtained from LGC (Teddington, UK) and the deuterated internal standards (ISs), benzyldimethyltetradecylammonium chloride-d_7 (d_7-BAC-C14) and hexadecyltrimethylammonium bromide-d_9 (d_9-HDTMA), were sourced from Toronto Research Chemicals (Ontario, Canada). All reference materials were hygroscopic and stored under argon gas in a vacuum desiccator. For the analysis and preparation of solutions and samples, oxygen-free nitrogen (OFN) was purchased from BOC gas (Port Talbot, UK), with acetonitrile (ACN), water (H_2O) and formic acid (FA) from Fisher Scientific (Loughborough, UK). QuEChERS materials were obtained from Biotage (Uppsala, Sweden) and included standard EN extraction and dSPE tubes of EN 'Waxed Fruit and Vegetables' (containing C18) and EN 'Pigmented Fruit and Vegetables' (containing graphitized carbon black (GCB) as detailed in the Supplementary material. As a test environmental matrix, garden topsoil was collected from an undisclosed location in West Wales, mixed and lyophilized to standardise the hydrophilic (water) content of the soil matrix in readiness for sample preparation.

Instrumentation

Sample separation was undertaken using a Thermo Scientific Surveyor Autosampler (AS) and MS PumpPlus LC system (Hemel Hempstead, UK) operated with a 3 µm 100 x 1 mm C18 Thermo Hypersil Gold column and a 5 µm 10 x 1 mm C18 Thermo Hypersil Gold guard cartridge (Runcorn, UK). Mass analysis was performed with a Thermo Scientific LCQ Classic ion trap (Hemel Hempstead, UK) operating with an electrospray ionization source in positive mode. Both instruments were controlled using Xcalibur 2.0.

LC-MS methodology

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Mobile phases for the LC separation consisted of 0.1% FA in H_2O (A) and 100% ACN (B) with an injection wash of 90% ACN and 10% H_2O (all 0.1% FA final concentration). Each sample (5 µL) was injected on column at a mobile phase flow rate of 50 µL/minute operating with a gradient elution; starting at 75% A:25% B, increasing to 100% B from 2-24 minutes, with a 20 minute wash prior to reconditioning at 75% A for 10 minutes. The mass spectrometer was operated using a spray voltage of 4.5 kV and capillary temperature of 200 °C. Following identification of the QAC precursor ion species, full mass scan (*m/z* 100-500) and multiple reaction monitoring (MRM) analyses were used for the four BAC compounds, DDMAB, and d_7-BAC-C14 (see Supplementary material for transitions and optimised collision energies (%CE)). For HDTMA and d_9-HDTMA a single ion monitoring (SIM) acquisition was used as a stable fragment ion could not be obtained. With a minimum number (≥10) of mass spectra for quantitation, the data was processed using Xcalibur 2.0 and Microsoft Excel 2010.

Stock and working solutions

Individual 1 mg/mL stock solutions were prepared in 100% ACN and stored at -20°C prior to use. For method evaluation a 'double blank' (S_b), a standard blank with IS (S_0), eight non-zero calibration standards (2-100 ng/mL), and four quality controls (8, 20, 60, 90 ng/mL) were prepared in 50:50 ACN:H_2O with relevant solutions containing an IS concentration of 30 ng/mL.

Sample preparation

The sample preparation procedure was tested using extracted quality controls (QCs) by comparing the analyte peak area spiked before and after extraction[32] (denoted SBE and SAE respectively), initially in water (total 4 mL) and then soil (2.5 g based on preliminary in-house screening). Performance figures of merit included percentage matrix effects (%ME), recovery (%REC) and process efficiency (%PE), with the respective precision given as %RSD (see Supplementary material). To confirm selectivity, additional solvent and matrix ('double') blanks were prepared for the relevant samples. All samples were prepared in triplicate and fortified by spiking with the QAC mixture and the IS sub-stock at an equivalent concentration of 60 and 30 ng/mL, respectively, with a further volume of H_2O (to equate a total volume of 4 mL) added to the soil sample prior to extraction. To establish the effect of extract dilution the spike concentration was increased proportionally for a 1:400 dilution, based on a mid-point estimated from preliminary screening data for relevant environmental samples. For SAE samples equivalent spike volumes of 50:50 ACN:H_2O and H_2O were added to the samples and vortexed. To extract the samples 10 mL ACN and the EN extraction tube were added, manually shaken for 1 minute and centrifuged at 4000 rpm for 5 minutes at room temperature. The resulting supernatant was transferred to the dSPE tube, vortexed for 1 minute and

centrifuged at the same conditions. The final supernatant was transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen. For undiluted samples, blanks and SBE extracts were reconstituted in 2 mL 50:50 ACN:H_2O, while the SAE extracts were reconstituted in an equivalent volume of ACN:H_2O, spiked with QAC and IS mixtures to achieve a concentration of 60 ng/mL and 30 ng/mL, respectively. However, for diluted blanks and SBEs, extracts were reconstituted as above but with a 1:400 reduction in concentration, while for the SAE, the extract was diluted in a volume of 50:50 ACN:H_2O containing 60 ng/mL and 30 ng/mL of QAC and IS mixtures, respectively.

Results and Discussion

Analytical method selectivity

During full scan analyses the base peak of each QAC showed a precursor ion consistent with the loss of the halide ion, representing the anticipated singly-charged molecular cation of these salt species ($[M\text{-}X]^+$). Fragmentation of the aromatic QACs at optimised %CE primarily generated product ion species related to the alkyl chain with the loss of the head group, and for DDMAB, the loss of a single alkyl chain. Unfortunately, stable fragment ions for HDTMA and d_9-HDTMA could not be obtained, and therefore SIM was used for quantitation (see Supplementary material). Once established, the LC method was developed for the mixture of standards based on initial in-house work; this employed a solvent gradient to achieve appropriate resolution with analyte separation according to hydrophobicity (as anticipated for reversed phase) and no clear evidence of aggregation. To minimise carryover, a compromise was required between chromatographic resolution and analysis time, with significant washing of the system needed. Following optimisation, the chromatographic selectivity was confirmed for the sample types and the stability of the chromatographic method was characterised, recording the mean, intra- and inter-precision (represented by %RSD and two-tailed F-test, respectively) of the relative retention time and peak area. Pleasingly, the separation showed good repeatability (<1.5 %RSD) and reproducibility, with a stable performance between the two days (see Table 1 and Supplementary material). The chromatographic peak area was also largely reproducible with only BAC-C14 showing a significant difference in precision between day 1 and day 2. However, given this remained <7% RSD the method was considered suitable for proceeding with further method evaluation.

Method evaluation for quantitation: Calibration homoscedascity, linearity, limit of detection (LOD), accuracy and precision

A calibration graph of the analyte peak area normalised to a relevant IS was constructed for the analytes over the anticipated quantitative range (e.g. 2-100 ng/mL, see Table 2). Given analytical measurements are often heteroscedastic, exhibiting unequal variance across the

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concentration range, linear and weighted regression relationships were assessed for each analyte, and selected according to the regression factor that provided the lowest relative error (%RErr) (see Supplementary material). A 1/x weighted regression typically provided the lowest %RErr, with analytes showing excellent linearity (R^2 >0.99) apart from BAC-C18, however this remained within acceptable levels at an R^2 >0.98. Interestingly, HDTMA displayed excellent linearity with either IS, showing the potential of this protocol to operate with a single IS if needed. As a measure of sensitivity, the LOD was determined using both statistical and empirical methods (see Supplementary material). Of these, the empirical approach was selected for method evaluation as those determined statistically were inconsistent with the required signal/noise (S/N >3) due to the heteroscedasticity of the data[33]. The empirical LOD showed sensitivity <1 ng/mL apart for HDTMA, however, this LOD remained sufficient at ~1 ng/mL, with either IS, for the anticipated target application based on in-house data, and is on par with recent studies that have quoted this metric[13,24]. For each analyte, the quantitative precision and accuracy were established using replicate QCs at four concentrations within the dynamic range (see Supplementary material and Table 2). Most pleasingly, all QCs showed good precision ≤12.6% regardless of concentration, including HDTMA with either IS, confirming that a single IS approach can be a viable quantitative method. Furthermore, good accuracy (<14.8 %) was determined for all compounds at each concentration, providing confidence that the method is capable of performing quantitative measurements.

Applicability of sample extraction for QAC biocides: fortified water and soil

An ideal preparative protocol should reliably extract analytes (<15 %RSD) with a low %ME (value of ~100%) and at high %REC. However, where signal enhancement or suppression is present, good precision is essential for accurate quantitation to enable a valid measure of analyte response by normalising the recovery (and overall quantified amount) and account for this change in signal. QuEChERS is a well-established protocol that exhibits these performance characteristics, using specific reagent blends for recognised standards (e.g. EN and AOAC) to extract acidic and basic pesticides[26]. However, the flexibility of QuEChERS facilitates the screening of environmental samples for other pollutants, by selecting appropriate dSPE material (e.g. PSA, C18 and GCB) to target the removal of abundant organic interferences, such as humic and fulvic acids. Therefore, in the interest of method accessibility, standard 'EN kits' containing hydrophobic dSPE sorbents were selected to target these lipophilic interferences, and tested with fortified water and soil to determine method viability for treated effluent and contaminated soil (and sludges), respectively.

Pleasingly, repeatable %ME were observed for both C18 and GCB sorbents however, this was in the form of significant signal enhancement that increased with analyte

hydrophobicity from 107.0 to 151.3 %ME (see Supplementary material). Given the biocides are spiked after extraction in the same solvent as the QC for this calculation, this result implies that co-extractives from the QuEChERS reagents have enhanced the analyte signal, potentially through limited (previously undetectable) aggregation of the biocide. Limited but repeatable recoveries were also observed for more hydrophobic analytes, although this was anticipated due to the more challenging measurement conditions (e.g. at lower recoveries) observed for aromatic BACs and the aliphatic QACs using GCB and C18 sorbents, respectively. However, given adsorption to the dSPE material is a competitive process, recoveries were expected to improve with matrices containing increased amounts of organic matrix (e.g. environmental solids). This was observed for the majority of analytes (apart from HDTMA) within soil, however, with a significantly lower proportional increase of %ME with analyte hydrophobicity (apart from BAC-C18), with values ranging from 106.1-152.6% regardless of dSPE sorbent (see Figure 1). This improved performance was also observed for the recovery precision, with most compounds showing RSDs <19%. An exception to this was the recovery of BAC-C18 using the GCB sorbent, where greater hydrophobicity and retention appears to result in a more variable interaction with the sorbent. However, given the positive overall performance, the products were explored further and optimised to reduce matrix enhancement.

Sample dilution can offer a quick and simple approach to reduce matrix effects,[34] although the success of this method is highly dependent on maintaining sufficient analyte within the extract for measurement. Given the high anticipated levels of QACs in environmental solids, we tested this approach by diluting the extract (rather than the initial sample), to offer greater flexibility in accommodating different dilution factors if further method optimisation was required. Based on a preliminary screen of relevant environmental solids carried out in-house, a dilution factor of 1/400 was to ensure the final concentration would reside in the middle of the analytical method dynamic range. This required a 400x adjustment of the fortification concentration of the soil samples, and these were extracted as per the 'undiluted' extracts. Disappointingly, this adversely affected the %ME precision for the GCB extraction however, experiments undertaken with C18 dSPE sorbent showed much improved matrix enhancement versus existing studies,[13,29] at 95.7-104.4 %ME for all analytes and a similar precision to undiluted extracts (see Figure 2). Pleasingly, this provides considerable confidence that the recovery measurements are representative of the amount of analyte extracted without the need for 'correction' or additional steps to the protocol. Again, recovery did decrease with hydrophobicity however, this loss was significantly lower (indicative of a competitive retention process), and equivalent to past work involving more rapid protocols,[24] including QuEChERS approaches that do not include C18 dSPE to remove organic interferences prone to environmental solids.[13,30] This data therefore, confirms a standard

QuEChERS product can extract, with reasonable and repeatable recoveries and minimal %ME, the range of aromatic and aliphatic QACs anticipated within environmental solids that have links to AMR,[15-20] as a quick and cheap 'off the shelf' method to determine their environmental distribution and impact, as part of a future monitoring programme for AMR.

Conclusion

There is an increasing need to establish exposure levels and sources of QAC pollution due to their high usage rates with limited regulation and reporting of biocide levels, sorption to environmental solids following WW treatment and links to bacterial cross- and co-resistance mechanisms. The extraction of QACs from environmental solids can require laborious preparative methods to achieve precise data with minimal %MEs and high recovery. Recent QuEChERS work has shown potential for a more limited selection of surfactant biocides anticipated in environmental solids (and those used in this study) however, these methods require additional steps to the protocol, have used a bespoke extraction product, or alternative matrices. Using a robust quantitative reversed-phase LC-MS/MS method, we have shown that an 'off the shelf' QuEChERS product can recover the range of anticipated QACs for municipal, industrial and agricultural waste, to values up to 53% and with <4% suppression and enhancement, as a repeatable single extraction for soil operating within <10 %RSD by using a simple extract dilution. With minimal method development, this provides a much needed rapid sample preparation method for quantifying the breadth of QAC pollution and monitoring the progression of AMR.

Conflicts of interest

The authors confirm that there are no conflicts to declare and ethical approval was not required for this work.

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References

1 Department for Environment Food and Rural Affairs, *Waste water treatment in the United Kingdom – 2012*, *Report No.: PB13811*, 2012.

2 Scotland and Northern Ireland Forum for Environmental Research, *Methodology for the analysis of selected pharmaceuticals and drugs of abuse in sediments and sludge, Report No.: ER09*, 2010.

3 Environmental Protection Agency, *Pharmaceuticals and personal care products in water, soil, sediment and biosolids by HPLC/MS/MS. Method 1694*, 2007.

4 A. J. McBain, R. G. Ledder, L. E. Moore, C. E. Catrenich and P. Gilbert, *Appl. Environ. Microbiol.*, 2004, **70**, 3449.

5 C. P. Gerba, *Appl. Environ. Microbiol.*, 2015, **81**, 464.

6 Mount Sinai Selikoff Centers for Occupational Health, Bellevue/NYU Occupational & Environmental Medicine Clinic, *Quaternary Ammonium Compounds in Cleaning Products: Health & Safety Information for Health Professionals*, 2015.

7 Scientific Committee on Emerging and Newly Identified Health Risks, *Assessment of the Antibiotic Resistance Effects of Biocides*, 2009.

8 G. A. Kahrilas, J. Blotevogel, P. S. Stewart and T. Borch. *Environ. Sci.*, 2015, **49**, 16.

9 K. Rajkowska, A. Koziróg, A. Otlewska, M. Piotrowska, P. Nowicka-Krawczyk, B. Brycki, A. Kunicka-Styczynska and B. Gutarowska, *Acta Biochim. Pol.*, 2016, **63**, 153.

10 The European Parliament, The Council of the European Union. *Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council*, 2008.

11 The European Parliament, The Council of the European Union. *Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy,* 2013.

12 European Union, *Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC*, OJEU. 2018, Report No.: L141/9.

13 A. Bergé, B. Giroud, L. Wiest, B. Domenjoud, A. Gonzalez-Ospina, and E. Vulliet, *J. Chromatogr. A*, 2016, **1450***,* 64.

14 European Parliament, The Council of the European Union. *Regulation (EU) no 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products*, 2012.

15 D. A. Rouch, D. S. Cram, D. DiBerardino, T. G. Littlejohn, and R. A. Skurray. *Mol. Microbiol*., 1990, **4**, 2051.

16 N. Akimitsu, H. Hamamoto, R. Inoue, M. Shoji, A. Akamine, K. Takemori, N. Hamasaki and K. Sekimizu. *Antimicrob. Agents Chemother*., 1999, **43**, 3042.

17 M. S. Sidhu, E. Heir, T. Leegaard, K. Wiger and A. Holck, *Antimicrob. Agents Chemother*., 2002, **46**, 2797.

18 S. E. Walsh, J. Y. Maillard, A. D. Russell, C. E. Catrenich, D. L. Charbonneau, and R. G. Bartolo, *J. Hosp. Infect*., 2003, **55**, 98.

19 M. Braoudaki and A. C. Hilton, *J. Clin. Microbiol.*, 2004, **42**, 73.

20 M. Zhang, M. M. O'Donoghue, T. Ito, K. Hiramatsu and M. V. Boost. *J. Hosp. Infect,.* 2011, **78**, 113.

21 X. Li and B. J. Brownawell, *Anal. Chem.,* 2009, **81***,* 7926.

22 T. Ruan, S. Song, T. Wang, R. Liu, Y. Lin, and G. Jiang, *Environ. Sci. Technol.,* 2014, **48**, 4289.

23 E. Martinez-Carballo, C. Gonzalez-Barreiro, A. Sitka, N. Kreuzinger, S. Scharf, and O. Gans. *Environ. Pollut.*, 2007, **146**, 543.

24 B. J. Heyde, A. Barthel, J. Siemens, and I. Mulder. *PLoS ONE,* 2020, **15**, e0237020.

25 F. Merino, S. Rubio, and D. Perez-Bendito., *J. Chromatogr. A*, 2003, **998**, 143.

26 M. Anastassiades, S. J. Lehotay, D. Štajnbaher and F. J. Schenck, *J. AOAC Int.,* 2003, **86**, 412.

27 Y. Xian, H. Dong, Y. Wu, X. Guo, X. Hou and B. Wang, *Food Chem.,* 2016, **212**, 96.

28 S. Mishima-Kimura, K. Yonemitsu, Y. Ohtsu, A. Sasao, H. Tsutsumi, S. Furukawa and Y. Nishitani, *Legal Med.,* 2018, **32**, 48.

29 R. Townsend, G. Van Keulen, C. Desbrow, and A. R. Godfrey, *Anal. Sci. Adv.*, 2020, **1**.

30 A. Bergé, A. Buleté, A. Fildier and E. Vulliet, *Anal. Chem.,* 2017, **89**, 9685.

31 R. Townsend, C. Desbrow and A. R. Godfrey, Biotage, 2014.

32 B. K. Matuszewski, M. L. Constanzer and C. M. Chavez-Eng, *Anal. Chem.,* 2003, **75**, 3019.

33 J. N. Miller and J. C. Miller, *Statistics and chemometrics for analytical chemistry.* Fourth Edition., Prentice Hall, London, 2000.

34 T. G. Hall, I. Smukste, K. R. Bresciano, Y. Wang, D. McKearn and R. E. Savage, *Tandem Mass Spectrometry - Applications and Principles*, InTech, Rijeka, 2012.

Figures and Tables

Table 1: Summary of physiochemical data and chromatographic reproducibility of adjusted retention times for each analyte (day 1: n=11, day 2: n=7). Chromatographic repeatability is represented by %RSD and reproducibility by two-tailed F- test; F-stat were $F_{(10,6)}$ 5.461^, $F_{(6,10)}$ 4.072§.

Analyte	Molecular Formula	logP	*m/z* Precursor (SRM fragment)	Chromatographic Stability		
				Repeatability		Reproducibility
				Day 1	Day 2	
BAC-C12	$C_{21}H_{38}N$	1.69	304 (212)	1.49	1.20	1.50
BAC-C14	$C_{23}H_{42}N$	2.55	332 (240)	0.87	1.21	1.96
BAC-C16	$C_{25}H_{46}N$	3.42	360 (268)	0.58	0.95	2.72
BAC-C18	$C_{27}H_{50}N$	4.28	388 (296)	0.59	0.91	2.43
DDMA	$C_{22}H_{48}N$	2.51	326 (186)	0.72	1.07	2.28
HDTMA	$C_{19}H_{42}N$	2.40	284 (n/a)	0.88	1.05	1.45
BAC-C14-d_7	$C_{23}H_{35}D_7N$	2.55	339 (240)	0.85	1.21	2.05
HDTMA-d_9	$C_{19}H_{33}D_9N$	2.40	294 (n/a)	0.90	0.96	1.16

Table 2: Summary table of the quantitative performance of the weighted (1/x) regression with linearity represented by the coefficient of determination (R^2) limit of detection (LOD), mean percentage accuracy and precision of quality control (QC) samples at 8, 20, 60 and 80 ng/mL for each analyte (n=5). Interestingly, the latter compound was also assessed using d_7-BAC-C14 to scope the possibility of using a single IS and has showed good linearity, accuracy and precision results for quantitation.

Analyte	MS Data (*m/z*)	Regression Function	Internal standard	Linearity (R^2)	LOD (ng/mL)	QC concentration (ng/mL)							
						Accuracy (%)				Precision (%)			
						8	20	60	90	8	20	60	90
BAC-C12	304>212	1/x	**d_7-BAC-C14**	0.995	0.06	-7.3	6.9	0.2	-5.2	5.5	8.0	3.1	7.7
BAC-C14	332>240	1/x		0.994	0.83	4.0	7.1	-3.6	-0.5	12.4	5.4	2.7	6.3
BAC-C16	360>268	1/x		0.991	0.21	-3.5	2.3	3.6	-1.0	8.0	9.1	3.0	7.1
BAC-C18	388>296	1/x		0.980	0.38	-7.9	-12.2	-2.8	-0.4	12.4	12.6	9.2	7.3
DDMA	326>186	1/x		0.993	0.23	1.8	6.9	4.6	0.5	8.8	6.1	5.5	4.6
HDTMA	284	1/x		0.994	1.02	-14.8	-3.8	1.6	2.5	7.1	9.2	4.8	5.4
HDTMA		1/x	**d_9-HDTMA**	0.996	1.51	-7.7	3.8	-1.7	-1.0	8.1	7.7	6.0	2.7

Figure 1: Mean percentage matrix effects and recovery of each analyte and internal standard (with standard error bars) for spiked soil samples following QuEChERS extraction (n=3) with C18 and GCB dSPE sorbent.

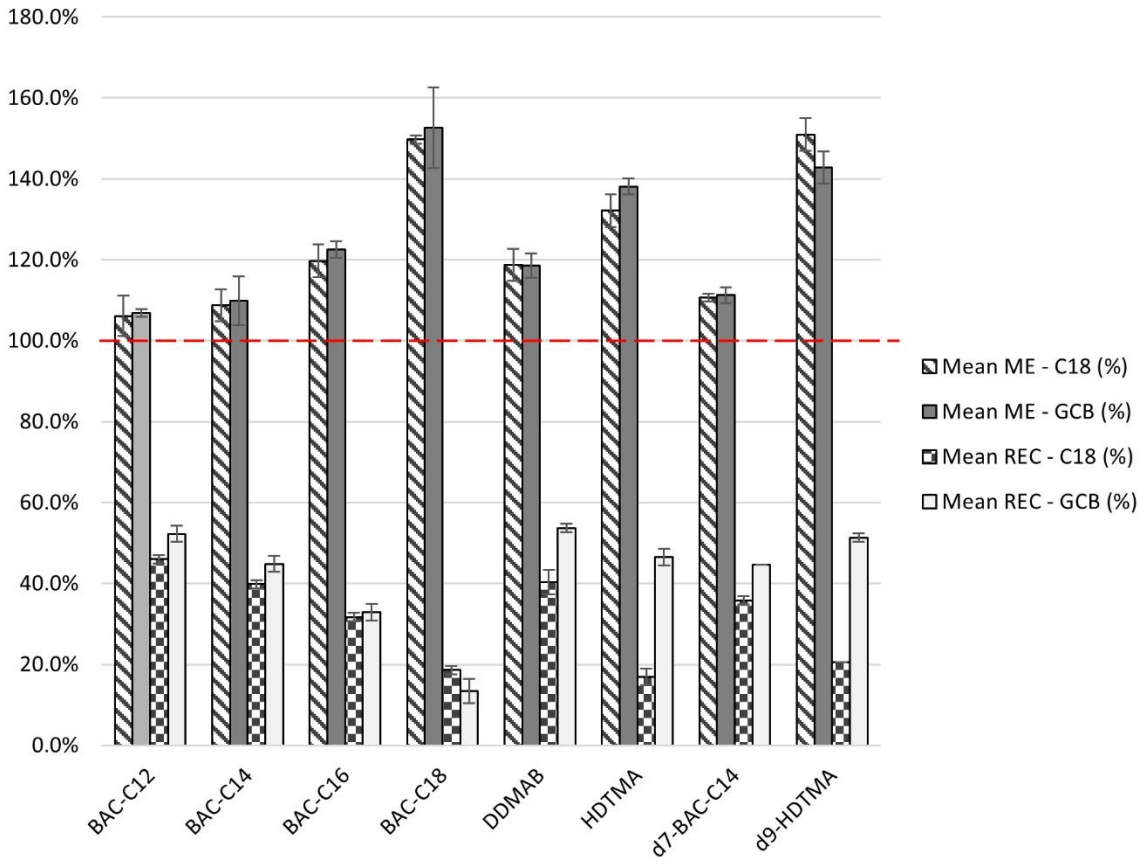
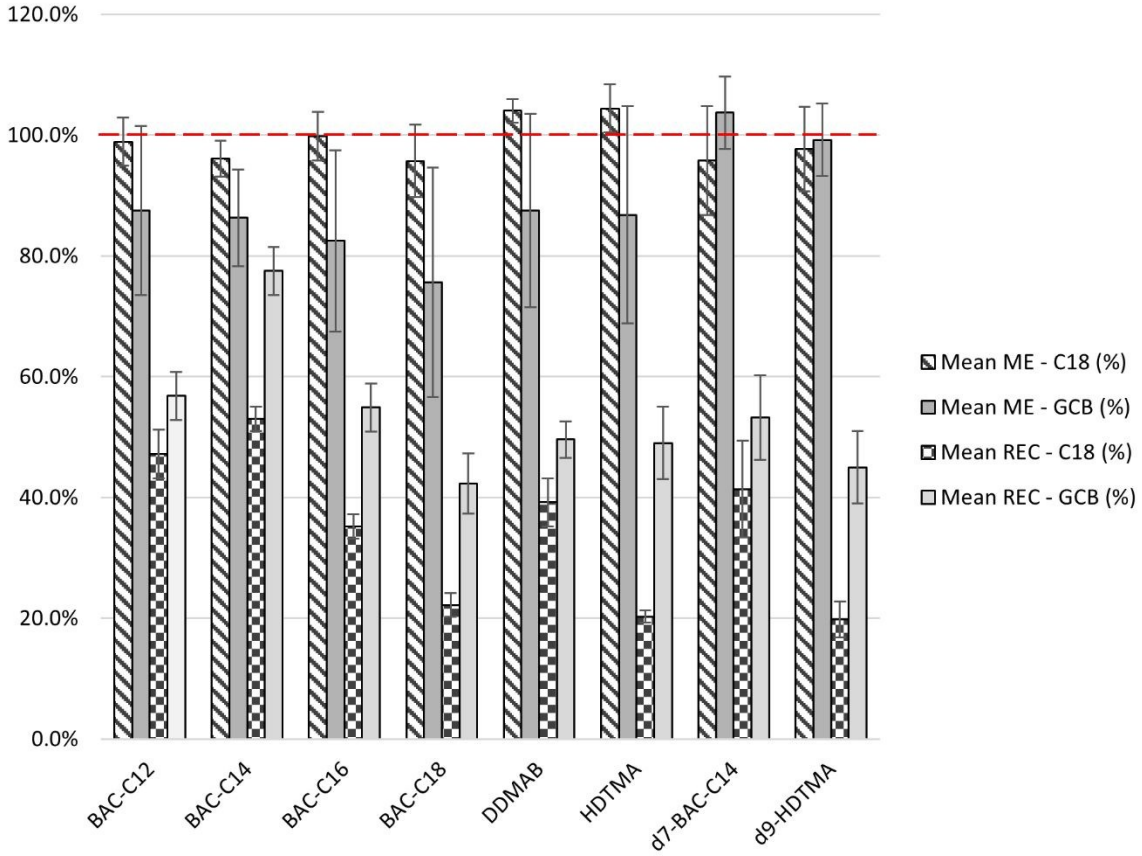


Figure 2: Mean percentage matrix effects and recovery of each analyte and internal standard (with standard error bars) for spiked soil samples following QuEChERS extraction (n=3) with C18 and GCB dSPE sorbent and subsequent extract dilution (1:400).



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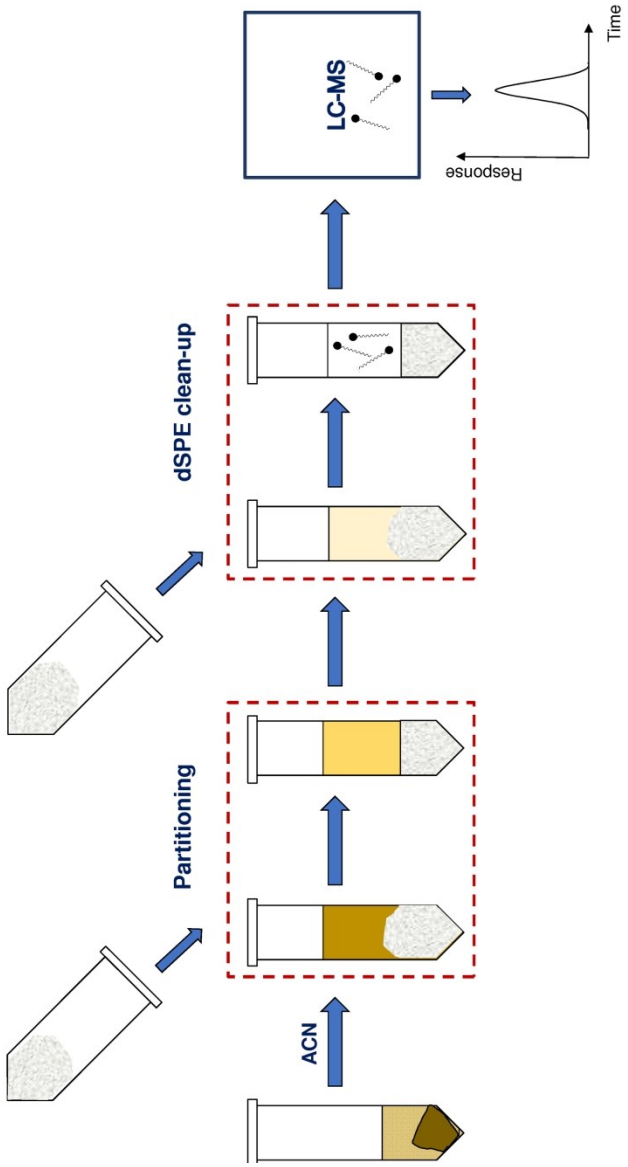

A rapid, robust 'off-the-shelf' preparation for extracting quaternary ammonium biocides from soil with low matrix interference.

209x297mm (300 x 300 DPI)