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Reuse of consumable pipette tips for large-scale trace analysis of contaminants of emerging concern in wastewater

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Scientific laboratories generate substantial plastic waste, particularly from single-use pipette tips, especially in high-throughput analysis. This study explores the feasibility of reusing pipette tips through green solvent washing within a large-scale environmental monitoring program analysing >100 contaminants of emerging concern (CECs) in water, including complex matrices like wastewater. Eleven cleaning solvents were screened for their ability to reduce chemical carryover, with four selected for further evaluation based on analytical performance and environmental impact using AGREEprep scores (*i.e.*, acetonitrile (MeCN), acetone, ethanol:water (EtOH : H_2O, 50 : 50 v/v) and 1% nitric acid (NA) aq)). Solvent effectiveness varied with analyte hydrophobicity and tip material. A four-wash protocol (W4) was required to achieve >98% reduction in carryover. Tests using wastewater and up to 40 reuse cycles (*i.e.*, W160) confirmed additional challenges due to matrix complexity but showed consistent solvent performance trends. Tip integrity was assessed through scanning electron microscopy (SEM) and gravimetric analysis; some solvent-tip combinations (*e.g.*, 1% NA (aq) with capillary piston tips) showed some degradation. Life cycle assessment (LCA) indicated that although MeCN provided high cleaning efficacy, its high global warming potential (GWP) limited its sustainability in repeated use. EtOH : H_2O (50 : 50 v/v) offered the best overall balance of cleaning performance, low GWP, and minimal tip damage. A compound-specific removal profile and a practical selection tool were developed to guide solvent choice and reuse strategies. This is the first comprehensive study demonstrating solvent-based pipette tip reuse as a viable, environmentally sustainable approach for trace-level chemical analysis in complex environmental monitoring workflows.

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Sustainability spotlight

The sustainability of scientific research is a growing environmental concern, with laboratories generating significant single-use plastic waste. This study systematically assesses pipette tip reuse for the analysis of >100 chemicals of emerging concern at ng L^{-1} concentrations in complex water samples and its feasibility in high-throughput, large-scale analysis. This research enables laboratories to better understand the opportunities to implement such sustainable practices, by reducing plastic pipette tip consumption and how it may affect analytical performance. This work aligns with UN Sustainable Development Goals 12 (responsible consumption and production) and 13 (climate action) by promoting resource efficiency and waste reduction in scientific research. Implementing such strategies is critical for minimising the research laboratory environmental impact and fostering a more sustainable research ecosystem.

1 Introduction

The triple planetary crisis is one of the greatest challenges for current and future generations,[1] where pollution, climate change and biodiversity loss are the three main interlinked issues.[2] Currently, plastic is one of the largest sources of pollution in the environment due to its non-degradable nature and increased consumption and production.[3] Beyond ecological damage, growing evidence links plastic exposure, including microplastics, to potential human health impacts,[4,5] affecting respiratory, reproductive, and digestive systems,[6] as well as neurological functions,[7,8] for example. In 2020, 367 million tons of virgin plastic were produced across the world, not including recycled plastic production. Of this, only 29.5 million tons were collected as general public consumer waste, where 42.0% was sent to energy recovery operations (*e.g.*, incineration), 23.4% to landfills and only 34.6% to recycling facilities, raising concerns.[9] This waste can cause environmental issues, such as harming wildlife[10] and contributing to climate change.[11] As of

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2023, plastic waste has now increased to over 413 million tons, highlighting this issue as a growing concern.[12]

Even environmental scientific research can contribute to contamination, and research laboratories can have a high environmental impact due to high energy consumption, waste generation and resource depletion, with most practices being considered outdated.[13–15] Therefore, new sustainable procedures that are in line with the UN Sustainable Development Goals (SDGs) are required.[16,17] A clear example recently reported that laboratories were estimated to use four and ten times more water and energy, respectively, than office buildings.[18] This meant they were ranked 40th for carbon dioxide (CO_2) emissions if they were classified as a country in themselves.[19] Furthermore, it is well known that most research laboratories generate a high quantity of plastic waste, producing almost 15 times the amount of an average individual.[20] These plastics are mainly single-use consumables due to their convenience, affordability and sterility, including pipette tips, centrifuge tubes, vials, weighing boats, and culture dishes,[21] with 87% of this waste attributed to 10 common types of plasticware.[22] It has been estimated that ∼5.5 million tons of laboratory plastic waste from 20 500 research institutions across the world were disposed of in 2014,[20] comparable to >1 million UK residents' CO_2 emissions and 2% of the total world's plastic.[1]

At the same time, interest within the scientific community to improve laboratory 'greenness' and reach net-zero targets is on the rise, with the number of articles published featuring the term 'green chemistry' increasing by almost three-fold, and 'sustainable lab' by approximately four-fold between 2013 and 2023 (Fig. S1, SI 1). Programs like 'My Green Lab Certification', 'Laboratory Efficiency Assessment Framework (LEAF)', 'Labconscious' and 'Laboratory Efficiency Action Network (LEAN)' are now also available.[14,23] Work has been done previously to make laboratories more sustainable by reducing water and energy consumption.[24] However, further research must be done to minimise, reuse and recycle single-use plastics. A decrease between 50–74% in lifetime CO_2 emissions could be achieved by just switching from incinerating to recycling recyclable plastic products in the laboratory,[25] and plastic pollution could decrease by 40% compared to business-as-usual if further recycling strategies are implemented.[22] However, many recycling facilities do not take laboratory plastics due to their contamination risk,[26] meaning they are instead incinerated, with those of the highest contamination risk incinerated at temperatures above 1000 °C for several hours.[27,28] This highlights the need for more environmentally-friendly initiatives and the introduction of new opportunities to demand management for reducing and reusing plastic as an alternative to recycling.[29] Nevertheless, this represents an enormous challenge when it comes to, for example, biological, medical, and environmental research, especially regarding cross-contamination and ensuring experiments and the resulting data are reliable, reproducible and robust. Switching to glass containers and other tools might not always be possible, for reasons such as higher initial costs and additional safety concerns surrounding breakage,[30] leaving the reuse of plastic as a realistic option.

Our laboratory focuses on environmental monitoring of water samples at large spatiotemporal scale, necessitating efficient, high-throughput analytical methods.[31] One such analytical method has eliminated the need for laborious sample preparation steps (*e.g.*, solid-phase extraction (SPE)) in favour of a direct-injection liquid chromatography tandem mass spectrometry (LC-MS/MS) approach,[32,33] but it still produces a sufficiently large amount of single-use plastic waste. During the sample preparation stage, a minimum of two pipette tips are used per sample, totalling ∼20 000 tips annually, per instrument. Although pipette tips are the most ubiquitous single-use plastic across all labs, they are just one of many plastic consumables used during sample processing; others include sampling bottles, centrifuge tubes, and Falcon tubes. This underscores an urgent need for sustainable alternatives. The aim of this study was to evaluate the impact of plastic pipette tip reuse on performance for a large-scale water monitoring programme of trace chemical residues generally present in samples at the ng L^{-1} concentration level, with a particular focus on wastewater. The objectives included: (i) to identify commonly used plastic pipette tip types and evaluate chemical residue carryover from >100 selected contaminants of emerging concern (CECs) following various washing regimes of single-use tips, (ii) to investigate any additional matrix effects arising from the washing procedure when it is applied to reusing tips for wastewater sample analysis using both targeted and untargeted approaches (*e.g.*, by examining extracted ion chromatograms (EICs) for the presence or absence of peaks), (iii) to evaluate the 'green' metrics for the washing regimes through a scoring system (*i.e.*, the analytical greenness metric for sample preparation (AGREEprep)), (iv) to evaluate if cleaning plasticware impacts its physical integrity using Scanning Electron Microscopy (SEM) measurement and gravimetric analysis, and lastly, (v) to calculate the carbon footprints of washing regimes effective at removing carryover and compare them to the footprint of single-use tips. This paper represents the first comprehensive assessment of pipette tip 'reuse' using a green chemistry approach for trace quantitative chemical analysis for large-scale application in complex water samples.

2 Materials and methods

2.1 Reagents, chemicals and wastewater matrix

For wash solvents, Optima® LC-MS formic acid (FA), Supelco ultrapure nitric acid (NA) and HPLC grade butan-1-ol (BuOH) were supplied from Fisher Scientific (Loughborough, Leicestershire, UK); HPLC grade methanol (MeOH), absolute ethanol (EtOH) and LC/MS grade acetonitrile (MeCN) were acquired from VWR Avantor (Lutterworth, UK); HPLC grade ethyl acetate (EtOAc) and ACS reagent grade propan-2-ol (IPA) were purchased from Merck Life Science (Gillingham, UK) and LC/MS grade acetone was supplied by Honeywell (Seelze, Germany). Ultrapure (UP) water (H_2O) was dispensed from an 18.2 MΩ cm at 25 °C Millipore Milli-Q water purification system (MilliporeSigma, Massachusetts, USA).

A total of 100 reference standards containing pharmaceuticals, illicit drugs and metabolites, and 33 stable isotopically labelled internal standards (SIL-IS) were mainly purchased from Sigma Aldrich and QMX (Essex, UK) and used for targeted analysis. Additionally, 12 reference standards and 5 SIL-IS, including pesticides, were used when investigating the maximum reuse cycle. When using suspect screening analysis, an extra 35 compounds were used for confirmation, totalling 185 chemicals. All standards had ≥98% purity, and further information can be found in the SI 1 Section S1. Physicochemical properties of all compounds can be found in Table S1, SI 2. Stocks were prepared at a concentration of 1 mg mL^{-1} in MeOH and stored in the dark in silanised amber vials at −20 °C. Working solutions were prepared in MeOH by diluting the stocks and stored under the same conditions.

2.2 Plasticware selection

Both vacuum-based and positive displacement pipette tips are commonly used in laboratories with 100, 200 and 1000 μL dispenser volumes. Pipette tips dispensing 10 μL or lower volumes were not included in this study due to their small volume, which was insufficient to study carryover effects with the LC-MS/MS method. OneTip Blue and Yellow graduated pipette tips (100–1000 μL and 10–200 μL, respectively) were purchased from Starlab (UK) Ltd (Milton Keynes, UK) and are referred to as 'blue' and 'yellow' tips, respectively, in this study. Both tips are made of polypropylene (PP). Capillary Piston (CP) positive displacement tips (10–100 μL and 100–1000 μL) were purchased from Gilson UK (Dunstable, UK) and are referred to as CP100 and CP1000, respectively. Both tips' capillaries are made from PP, while pistons are made of high-density polyethylene (HDPE) for CP100 and polyacetal (POM) for CP1000.

2.3 Wash solvent chemical selection

One of the best methods to reduce the environmental impact in laboratories is selecting greener chemical solvents for experimentation and adopting a green chemistry approach. Therefore, the CHEM21 solvents guide was used to select the solvents needed for washing in this study, where they are categorised as recommended, problematic, hazardous (H) and highly hazardous (HH).[34] A total of nine solvents were selected from the list based on their laboratory availability, family (*i.e.*, minimum of one per family) and lowest possible score (*i.e.*, low/medium environmental, safety and health scores): UP H_2O, MeOH, EtOH, IPA, BuOH, Acetone, EtOAc, MeCN, and 1% FA (aq). Due to their H and HH categories, chemical solvent families such as esters and hydrocarbons were not chosen. Furthermore, even though NA does not appear in the list, this acid is commonly used in cleaning plasticware in the laboratory,[35,36] and therefore was also selected as a 1% NA (aq) solution. A mixture of the two greenest solvents as EtOH : H_2O (50 : 50, v/v), was also investigated in order to solubilise chemicals from a wider range of polarities. This resulted in a total of 11 solvents being tested.

2.4 Environmental samples

Influent wastewater was collected as 24-h composite samples in a wastewater treatment plant (WWTP) in the UK for 30 days (representing a full month) using refrigerated ISCO Glacier portable water samplers set at 4 °C (Teledyne ISCO, Lincoln, NE, USA) located after the fine screen and before the primary clarifier. A total of 30 sub-samples were collected for this study from the autosamplers using 30 mL Nalgene bottles. Bottles were prerinsed thrice each with MeOH and UP H_2O prior to deployment to avoid potential contamination and stored at −20 °C at WWTPs after collection. At the end of the collection period, all samples were shipped to the laboratory at 4 °C and subsequently stored at −20 °C.

To prepare matrix effects working solutions, samples were defrosted and pooled in equal volumes, ensuring consistency across all experiments in the study. Samples were handled in appropriate hoods and in small volumes to minimise the biohazard risk from wastewater.

2.5 Sample preparation

To investigate CEC residue carryover contamination after the washing of plasticware, a tip was first rinsed using the maximum volume capacity (*e.g.*, 1000 μL for blue tips) three times to replicate standard good practice of wetting the pipette tip before aspirating and dispensing[37,38] with UP H_2O spiked with 100 CECs at five concentration levels between 500 ng L^{-1} and 15 000 ng L^{-1}, and 33 SIL-IS at 500 ng L^{-1}. These concentrations were selected to represent the expected range found in previously tested influent wastewater samples, where SIL-IS is added at a constant concentration of 500 ng L^{-1} for quantification purposes.[31,33] After exposing the plasticware to CECs, it was rinsed with one of 11 solvent wash chemicals between one (W1) and four (W4) times. Finally, the evaluated volume of UP H_2O was drawn up into the plasticware before being transferred into an amber glass LC-MS snap top vial (Agilent, Cheshire, UK) and stored at −20 °C until analysis. These carryover solutions were prepared for analysis in triplicate at each CEC concentration level for each number of washes ($n = 1$ to 4 washes). Peak area reductions from the initial Quality Control (QC) concentrations were used to investigate the carryover. A schematic showing the procedure is shown in Fig. S2 (SI 1). To address the worst-case scenario for carryover, a detectable peak area was defined as a signal intensity greater than or equal to three times the signal-to-noise ratio, which was classified as a true peak and thus considered potential contamination.

To test for potential matrix effects, a similar procedure was applied using wastewater as a matrix. Plasticware was first rinsed three times with influent pooled wastewater spiked with 133 CECs between 500 ng L^{-1} and 15 000 ng L^{-1} and SIL-IS at 500 ng L^{-1} before being washed 1–4 times (*i.e.*, W1–W4). UP H_2O (*i.e.*, carryover solution) was added to the plasticware and then transferred into centrifuge filter vials (centrifugation at 15 °C, 9000 rpm for 10 min) to remove solid particulates prior to freezer storage and/or analysis. To account for the impact of filtration, all QC samples were filtered using the same filters. For plasticware with a volume capacity >200 μL, 0.2 μm PTFE Thermo Scientific™ 2 mL nonsterile centrifugal filters (Thermo Scientific™, Loughborough, UK) were used, and for plasticware with a capacity <200 μL, 0.2 μm PTFE Single Step™ nano filter vials (Thames Restek UK, Buckinghamshire, UK) were used.

This experimental design resulted in the carryover evaluation of a total of 15 samples prepared in UP H_2O and 15 similarly spiked wastewater samples per wash and per solvent, for each of the four types of plasticware assessed ($n = \sim1635$ carryover solutions). For each batch of carryover solutions analysed, five QC solutions were prepared in 2.5% MeOH in UP H_2O or 2.5% MeOH in wastewater using a standard reference solution containing the same compounds as the carryover study at the same concentrations used for pipette tip exposure (500–15 000 ng L^{-1}) and an internal standard at a constant 500 ng L^{-1}. QCs were across the sequence, approximately every 15 samples. In order to compare data from different batches, QCs were used to normalise the peak areas obtained. No background subtraction was required in UP H_2O samples, as blank UP H_2O was analysed, and the carryover solutions in both matrices across batches showed no CECs present.

Lastly, to determine the maximum reuse potential of pipette tips, a carryover experiment was conducted over 40 repeated washing cycles (*i.e.*, $W = 4$ for $n = 40$), resulting in 160 washes. This equalled a total chemical wash volume of 160 mL for blue and CP1000, 32 mL for yellow, and 16 mL for CP100 tips. Each cycle involved pipette aspiration of a wastewater sample spiked at a concentration of 500 ng L^{-1} (both standards and SIL-IS), followed by the optimised washing regime using three different solvents.

2.6 Instrumental analysis

Analysis was performed following a previously developed quantitative LC-MS/MS method for 133 CECs in different water matrices (*e.g.*, surface water, effluent, and influent).[31,33] The method was successfully validated in influent wastewater following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.[39] A Shimadzu LCMS-8060NX (Shimadzu Corporation, Kyoto, Japan) was used with a Raptor biphenyl analytical column (30 × 3.0 mm, 2.7 μm) fitted with a guard column (5 × 3.0 mm, 2.7 μm) (Thames Restek, Saunderton, UK) and a 10 μL sample injection volume. Mobile phases were 0.1% (v/v) FA in UP H_2O (A) and 0.1% (v/v) FA in MeCN : MeOH (1 : 1, v/v) (B) at a flow rate of 0.5 mL min^{-1}. Details of the gradient elution conditions can be found in SI 1. Multiple reaction monitoring (MRM) was performed with positive-negative ionisation polarity switching using electrospray ionisation (ESI), where two MRM transitions were used for confirmation (one for SIL-IS), using the most intense ion for quantification purposes (Table S1, SI 1). LabSolutions™ (v. 5.99 SP2, Shimadzu, Kyoto, Japan) and LabSolutions Insight (v. 3.7, Shimadzu, Kyoto, Japan) were used to acquire and process chromatographic data. MS conditions (Table S2, SI 1) and further details of the method can be found in SI 1. Details on limits of detection and quantification (LODs and LOQs) can be found in Table S3, SI 1, as per previous published methodology.[31,33]

High-resolution chemical screening was performed using a Shimadzu LCMS-9030 LC-QTOF-MS instrument (Shimadzu Corporation, Kyoto, Japan) following a previous study.[32] This approach was used to expand the number of compounds detected under the most effective washing procedures and to assess potential differences in peak presence when evaluating the maximum number of pipette tip reuses, by comparing EIC-derived peak areas between blank samples and reused tips. Separations were performed on a Shim-pack Velox 2.1 × 100 mm, 2.7 μm biphenyl column (Shimadzu Corporation, Kyoto, Japan) held at 40 °C throughout the analysis with an injection volume of 40 μL. A 17-min LC gradient was used with the following phases: 2 mM ammonium formate with 0.002% FA either in H_2O (A) and in MeOH (B), and 0.5% acetic acid in MeCN : MeOH : H_2O : IPA (1 : 1:1 : 1, v/v/v/v) as wash. ESI-HRMS was run in both positive (100–920 Da) and negative (50–920 Da) ionisation modes. Fragmentation was performed in data-independent analysis (DIA) mode with a variable isolation width and collision energy of 30 ± 25 V and 25 ± 15 V, and a scan time of 25 and 28 ms, for positive and negative modes, respectively. LabSolutions Insight Explorer Library Screening software v. 3.8 SP1 was used for suspect screening analysis against a library of ∼1200 compounds containing retention time (t_R), MS1 and MS2 data. Confidence Level 2(a) was assigned following the Schymanski framework.[40] Details of the gradient elution and suspect screening points of confirmation can be found in the SI 1.

2.7 AGREEprep calculator

The AGREEprep calculator created by Wojnowski *et al.* (2022) was adapted and applied to the washing process to determine which cleaning method was greenest.[41] This calculator uses 10 weighted criteria (SI 1) to calculate an overall greenness score from 0 to 1 for laboratory preparation methods, with 1 determined as the 'greenest' and 0 the least green. AGREEprep accounts for aspects such as operational safety, chemical greenness, resource use, and method optimisation. The details of the rules adopted for this calculator in the context of manual tip washing are listed in SI 1.

2.8 Tip physical degradation investigation

The wash chemicals tested can affect the strength, flexibility, surface appearance, colour, dimensions or weight of plastics once in contact.[42] Therefore, gravimetric pipette calibrations were conducted to evaluate whether the volumetric accuracy of pipette tips was affected by the washing procedure. The wash regime was applied to the tips before aspirating deionised water at three volumes, as recommended in the pipette manufacturer's guidelines (*i.e.*, 10, 50 and 100% of volume capacity).[43] The mass of water aspirated at each volume was measured using a Fisher PS 60 balance capable of reading down to 0.0001 g (Fisher Scientific, Loughborough, Leicestershire, UK), allowing 99% accuracy at the 10 mg level as required for gravimetric measurements of 10 μL of water. This process was repeated eight times across two pipettes and two users for each tip type, with gravimetric measurements for new unwashed tips (*i.e.*, blanks) included for comparison. A two-tailed *t*-test was performed to determine if the water masses for washed tips were significantly different from the blanks ($p < 0.05$). Tips showing no significant difference were deemed suitable for reuse. Additionally, a pass/fail rate was determined for each tip based on compliance with ISO 8655–2 mechanical pipette standards, which consider the standard deviation, relative system error (μL), absolute system error (%), and random error (CV) of masses (Table S4, SI 1). The pass/fail rates of washed tips were also compared to those of the unwashed blanks for validation. Additional degradation routes, such as photodegradation, were not considered but were likely to be negligible as pipette tips were stored away from light and under standard laboratory conditions. Photodegradation would have also been accounted for through using blanks from the same pack as the washed tips.

Additionally, microscopy techniques were employed to examine the potential surface degradation of pipette tips following exposure to chemical solvents. For this, pipette tips were submerged for ~72 hours in a beaker containing the chemical solvent being investigated. Light microscopy was conducted using an Olympus BX53M microscope, with images captured by an Olympus SC50 5-megapixel camera to document surface features (Olympus, Tokyo, Japan). For the maximum reuse cycle investigation, higher resolution analysis using SEM was performed with a Zeiss LEO Gemini 1525 microscope (Zeiss, Oberkochen, Germany) operating at 5 kV, employing the secondary electron detector to capture the tip surface morphology. To image the inner surface of the pipette tip and check for morphology changes, tips were carefully cut in half. Two transverse cuts, each 5 mm apart, were made along the tip. Imaging was performed at the centre of each section to avoid artefacts from the cutting process. Sectioned pipette tips were affixed onto carbon tape-mounted microscopy stubs to ensure stability during imaging. Prior to SEM analysis, the samples were sputter-coated with a 15 nm chromium layer to enhance conductivity and imaging clarity. This combination of light and electron microscopy provided a comprehensive assessment of surface changes and potential degradation caused by solvent exposure.

2.9 Life-cycle assessment (LCA)

A LCA literature search was conducted for wash chemicals and pipette tips to compare the global warming potential (GWP), expressed as $kgCO_2$ equivalent ($kgCO_2e$), of cleaning and reusing tips *versus* using new ones. For the review, a 'cradle-to-gate' approach was adopted, covering the GWP from raw ingredient extraction to basic material production (Fig. S3, SI 1). In the case of plasticware, this included up to the production of the polymer but not the formation of the tips, and for wash chemicals, this included the production of the basic material with no refinement for laboratory-grade work. This method was selected as emissions caused by user actions are likely to be highly dependent on the degree of automation of the work, and specific experiments in which the tips are used. It was assumed that tip washing would be performed immediately before use, therefore extra considerations around PPE use were also not considered. Additionally, <1% of consumables emissions are thought to be due to disposal *via* incineration,[29] and Ragazzi *et al.* (2023) suggests that the transportation of materials contributes ≤6% to overall consumable emissions,[25] and so these factors were not considered. A life-cycle inventory (LCI) summarising the breadth of the LCA is defined in Table S5, SI 1.

Whenever possible, GWP data was sourced from Ragazzi *et al.* (2023) to ensure consistency of production values. Where data was not available, either an average cradle-to-gate value across similar products was used, or data was sourced from alternative literature. Preference was given to literature using EcoInvent, which is considered a well-established data source for LCAs.[44] All GWP values and their sources can be found in Table S5, SI 1. Based on the GWP of the pipette tips and wash chemicals found in literature, a calculator was created to establish how much $kgCO_2e$ was produced during each washing step and compared to using fresh plasticware, shown in eqn (S1) (SI 1) and in Table S2 (SI 2). The data collected for this LCA is secondary, based on literature. Therefore, it is limited as the production pathways and GWP are not specific to the polymers and wash chemicals purchased in this study, which have been produced at varying manufacturing sites and methods, information on which is not always available through suppliers. The LCA calculations have been categorised as 'level 2'.[44]

2.10 Statistical and data analysis

Microsoft® Office Excel (WA, USA), R (v. 4.4.2) and RStudio (v. 2024.09.0) were utilised for statistical and data analysis. **Flourish** and **BioRender** were used for visualisation purposes. For the generation of molecular descriptors and physicochemical properties, ACD Labs Percepta (Advanced Chemistry Development Laboratories, ON, Canada) and Dragon version 7.0 (Kode Chemoinformatics, Pisa, Italy) were used. Correlations between the logarithm of the octanol–water partition coefficient ($\log P$) and normalised peak areas were evaluated using linear coefficient of determination (R^2), with trend lines fit at 95% confidence interval; where strengths of correlations classify as not significant (0.00–0.199), weak (0.20–0399), medium (0.40–0.599), strong (0.60–0.799) and very strong (0.80–1.00). For investigating differences on performance across solvents and tips, principal component analysis (PCA) was performed using R. To investigate outliers from reusing tips using 40 washing cycles, plots of peak heights were performed with a 95% of confidence interval of the locally estimated scatterplot smoothing (LOESS) regression, and outliers were identified as values above $Q3$ + 1.5-fold of the IQR or below Q1 − 1.5-fold of the IQR, where Q is quartile and IQR is interquartile range. For high resolution analysis, raw files were converted to mzML format using MSConvertGUI (v. 3.0.25204-2ad5a87), from Proteo Wizard. Then, files were analysed by MS-DIAL (v. 5.5.250627) to process extracted EIC data, including chromatographic alignment and compound identification using MS/MS spectral libraries derived from authentic standards. Analyses were performed in both positive and negative ionisation modes, referencing libraries containing 16 232 and 8887 compounds, respectively (*i.e.*, MSMS-Public_all-pos-VS19.msp and MSMS-

Public_all-neg-VS19.msp, August 2024). Detailed MS-DIAL parameters are provided in Table S6, SI 1.

3 Results and discussion

3.1 Solvent selection shortlisting

A preliminary test was performed for the 11 cleaning solvents using blue tips, UP H_2O spiked with CECs and one wash. From the initial 133 CECs, the number of compounds detected in the carryover solutions after the wash was reduced by between 44–97%, depending on the chemical solvent used and across the five concentrations investigated. IPA resulted in the highest number of compounds still detected after the wash ($n = 74$), whereas MeCN had the lowest ($n = 4$) (Fig. 1(a)). A total of 45 CECs were not detected after washing using any of the chemical solvents. This could be due to substantive removal or that they were too strongly bound to the plastic material to be effectively washed off and remained adsorbed onto the tips. Only 11% of the compounds detected in the carryover solution were in the lowest third of log P values (ranging from −0.42 to 2.00), with 42% ranging between 2.06 to 3.11, and 47% from 3.12 to 5.67; the higher the log P, the higher the carryover that was detected. On the other hand, one compound, the illicit drug cocaine, was not removed completely by any of the wash solvents (average % removal by peak area across the 11 solvents = 99.7 ± 0.33%), but blanks run throughout the batches were negative, showing the UP H_2O itself was not a cause for positive detection. Individual data can be found in Table S3 of SI 2. Regarding peak area reduction of the compounds detected in the carryover solutions, across all QC concentrations tested and the 11 solvents, an average of 98.4 ± 3.28% reduction was observed after the chemical solvent wash (range: 88.5 ± 17.2% to 99.8 ± 0.07% across different chemical solvents). The highest removal was observed using a wash with acetone. This was followed by EtOAc, 1% NA (aq), MeOH, EtOH : H_2O (50 : 50, v/v), H_2O, EtOH, BuOH, 1% FA (aq), IPA and MeCN (Table 1). The overall reduction and number of compounds detected can be observed in Fig. 1(b), with solvents showing significant differences in performance between them. Therefore, the average values of % peak area reduction and the number of compounds removed were calculated, and the top five solvents were selected for further investigation (by descending rank order): MeCN, EtOH : H_2O (50 : 50, v/v), 1% NA (aq), EtOAc and acetone. Due to EtOH being one of the greenest solvents, it was also included as a single solvent despite ranking ninth.

To further reduce carryover, the number of washes was increased from W1 to W4 using the six selected chemical solvents. Overall, results obtained showed an additional reduction in the number of compounds detected (Fig. 1(c)) as well as peak areas after every wash tested (Fig. S4, SI 1); the

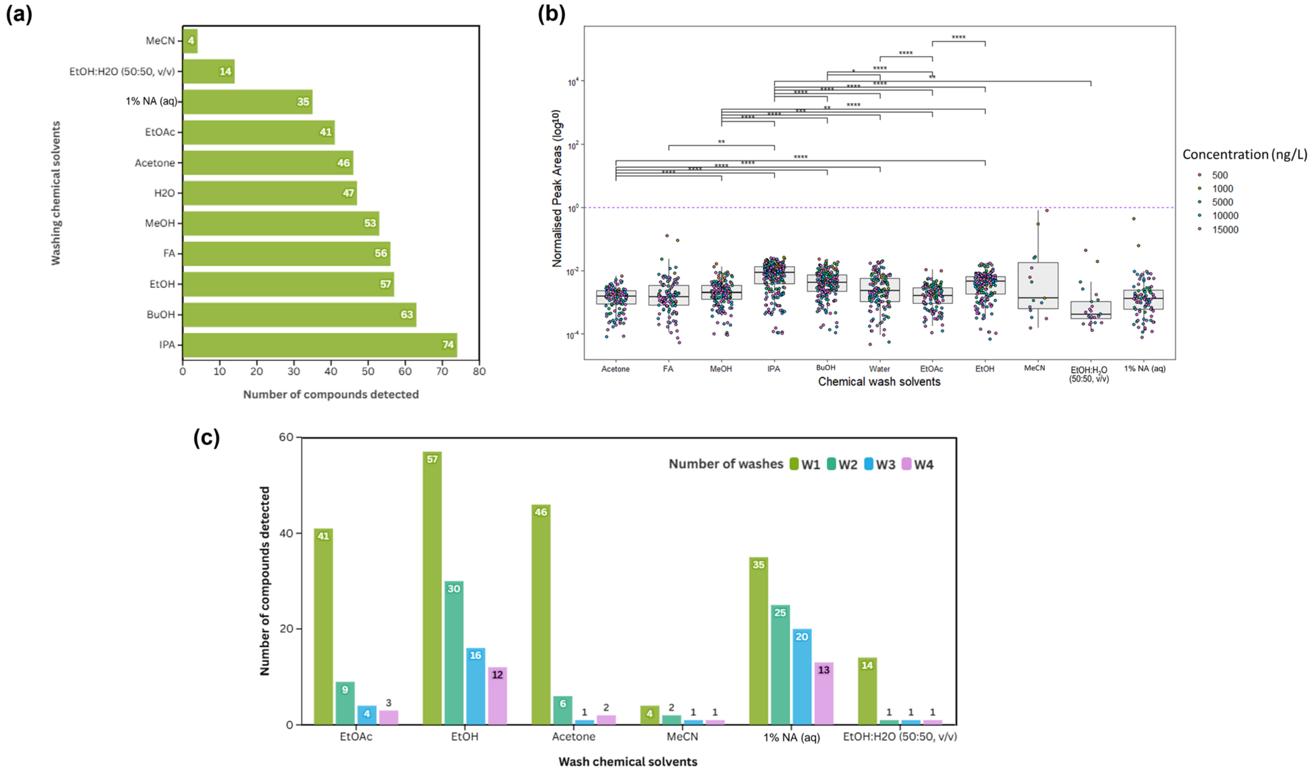
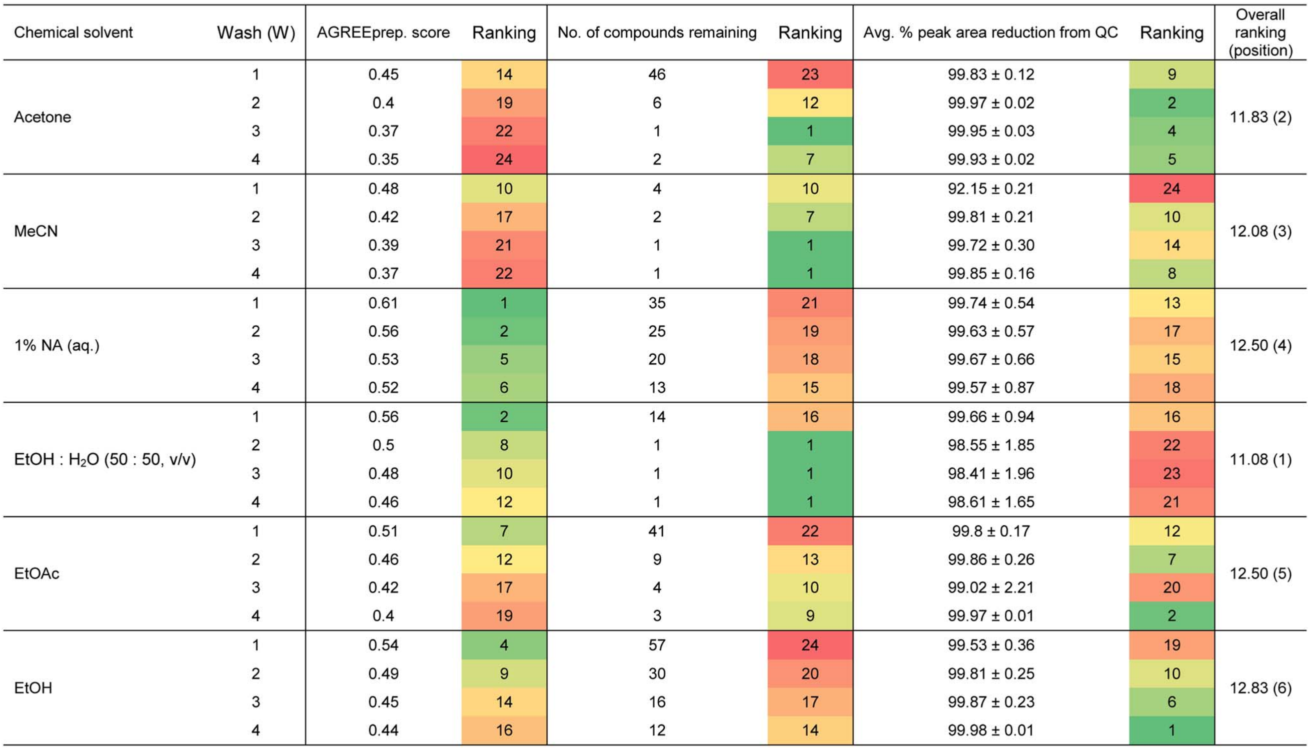


Fig. 1 (a) Number of compounds detected across the five concentrations investigated after washing blue tips ($n = 3$ per solvent) using 11 different chemical solvents with one wash (W1). (b) Boxplot graph showing normalised peak areas by the 11 different chemical solvents investigated using blue tips after a single wash cycle, showing the different compounds detected by concentration by different colours and a logarithmic scale. (c) Number of compounds detected per wash (W1–W4) and chemical solvent ($n = 6$) investigated across the five concentrations investigated.

Table 1 Overall washing regime ranking of the six chemical solvents selected, based on the AGREEprep ranking, the number of compounds detected after each wash (W1–W4) using ultrapure water and the average (avg) % of peak area reduction of the analytes detected



Chemical solvent	Wash (W)	AGREEprep. score	Ranking	No. of compounds remaining	Ranking	Avg. % peak area reduction from QC	Ranking	Overall ranking (position)
Acetone	1	0.45	14	46	23	99.83 ± 0.12	9	11.83 (2)
	2	0.4	19	6	12	99.97 ± 0.02	2	
	3	0.37	22	1	1	99.95 ± 0.03	4	
	4	0.35	24	2	7	99.93 ± 0.02	5	
MeCN	1	0.48	10	4	10	92.15 ± 0.21	24	12.08 (3)
	2	0.42	17	2	7	99.81 ± 0.21	10	
	3	0.39	21	1	1	99.72 ± 0.30	14	
	4	0.37	22	1	1	99.85 ± 0.16	8	
1% NA (aq.)	1	0.61	1	35	21	99.74 ± 0.54	13	12.50 (4)
	2	0.56	2	25	19	99.63 ± 0.57	17	
	3	0.53	5	20	18	99.67 ± 0.66	15	
	4	0.52	6	13	15	99.57 ± 0.87	18	
EtOH : H_2O (50 : 50, v/v)	1	0.56	2	14	16	99.66 ± 0.94	16	11.08 (1)
	2	0.5	8	1	1	98.55 ± 1.85	22	
	3	0.48	10	1	1	98.41 ± 1.96	23	
	4	0.46	12	1	1	98.61 ± 1.65	21	
EtOAc	1	0.51	7	41	22	99.8 ± 0.17	12	12.50 (5)
	2	0.46	12	9	13	99.86 ± 0.26	7	
	3	0.42	17	4	10	99.02 ± 2.21	20	
	4	0.4	19	3	9	99.97 ± 0.01	2	
EtOH	1	0.54	4	57	24	99.53 ± 0.36	19	12.83 (6)
	2	0.49	9	30	20	99.81 ± 0.25	10	
	3	0.45	14	16	17	99.87 ± 0.23	6	
	4	0.44	16	12	14	99.98 ± 0.01	1	

average peak reduction per wash was 99.6 ± 0.11% across all solvents. Only one exception was observed, where a higher number of compounds detected was obtained in a higher wash, W4 ($n = 2$ compounds), compared to W3 ($n = 1$ compound) when using acetone at 15 000 ng L^{-1}. This was attributed to benzoylecgonine (BZE), cocaine's primary metabolite, and could be due to this compound adsorbing on the plastic during the first three washes, then leaching when further solvent is used. Previous studies have shown that some CECs can accumulate in plastic due to their hydrophobic nature, with examples of microplastics adsorbing CECs such as pharmaceuticals and personal care products in aqueous media.[45] CECs have the potential to desorb or leach from plastic.[45,46] Although most research has been focused on highly hydrophobic chemicals, and that the size of the plastic and the hydrophobicity of the chemical can predict the adsorption rate, Tseng *et al.*, (2022) found that chemicals with low hydrophobicity may still be adsorbed onto a range of plastics (*i.e.*, polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC)).[46] There are several mechanisms involved, such as hydrophobic interaction, electrostatic repulsion and attraction, pore blockage (or residency in stagnant pores), and site competition.[45] However, BZE's peak area in W4 was at LOD level, and therefore, it could be that it was just simply not detected (<LOD) in W3.

CEC removal varied widely between the different solvents and washes. Examples of the different removals when using different solvents on the same compounds can be seen in Fig. 2(a) for three compounds, cilazapril, cocaine and diclofenac, with different log *P* values (1, 2.3 and 4.48, respectively (Table S1, SI 2)). Compounds such as diclofenac, a nonsteroidal anti-inflammatory (NSAID), were completely removed at all five concentrations with W1 when using solvents such as EtOH : H_2O (50 : 50, v/v) and MeCN. However, when using 1% NA (aq), only two of the lowest concentrations (*i.e.*, 500 and 1000 ng L^{-1}) were successfully removed in W1 to below LOD, while higher concentrations (*e.g.*, 10 000 and 15 000 ng L^{-1}) were not removed after W4.

3.2 Green metric assessment for washing regimes

To compare the wash effectiveness to greenness, an AGREEprep value was calculated for each of the six solvents and washes (W1–W4), where 1% NA (aq) was found to be the greenest chemical solvent (score = 0.61) for W1, followed by EtOH : H_2O (50 : 50, v/v) (score = 0.56), EtOH (score = 0.54), EtOAc (score = 0.51), MeCN (score = 0.48) and acetone (score = 0.45). All AGREEprep scores decreased with additional washes (Table S7, S1). However, further washes were considered necessary to reduce the carryover of most compounds. Therefore, all solvents and washes were ranked by AGREEprep value together with the number of remaining CECs detected in the carryover solutions and the % of peak area reduction (Table 1). Based on this ranking, averages across these parameters were calculated, and the four best chemical solvents of the six were selected to investigate the rest of the pipette tips: EtOH : H_2O (50 : 50, v/v),

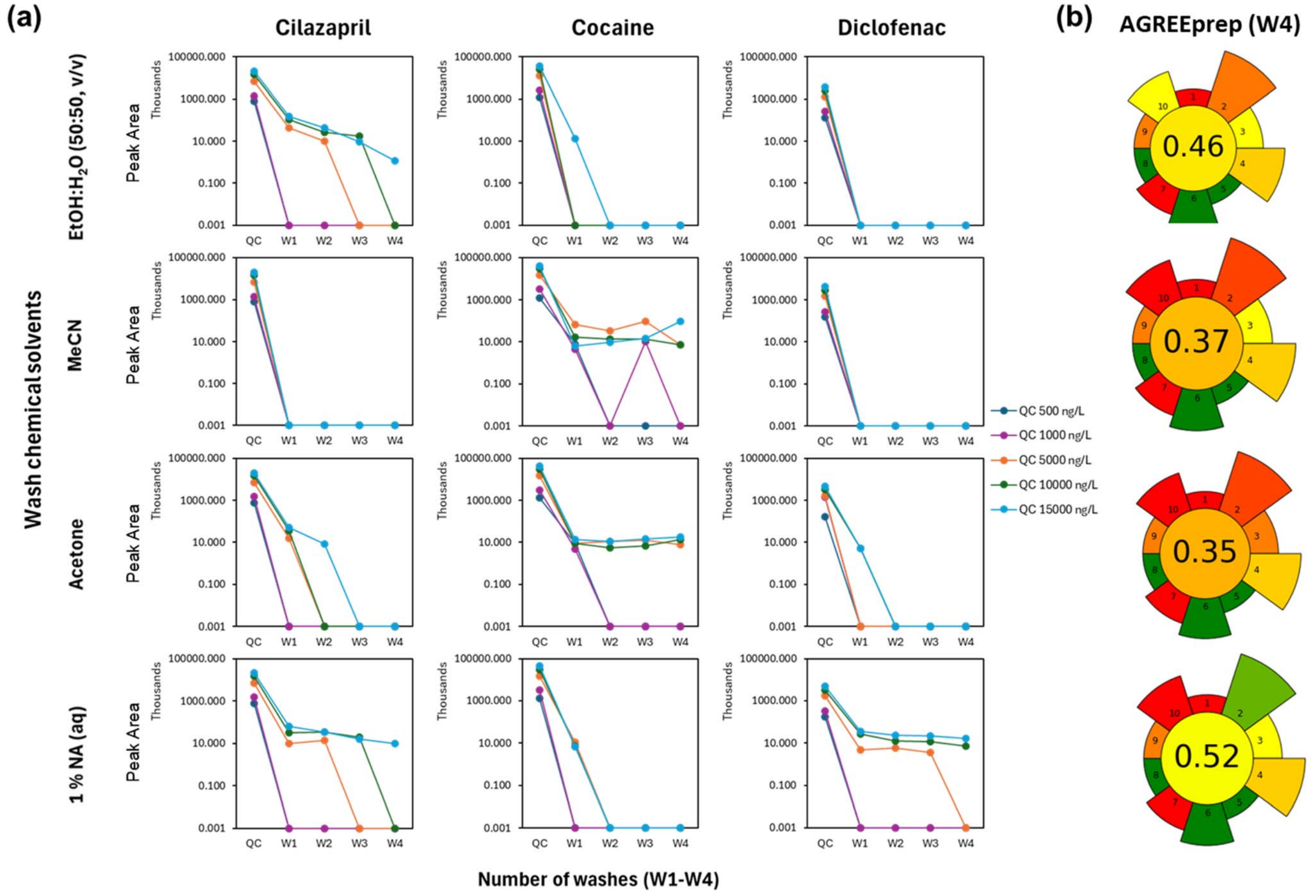


Fig. 2 (a) Graph showing the reduction of peak areas by washes (W1–W4) for three compounds in increasing order of octanol–water partition coefficient (log *P*) (cilazapril = 1, cocaine = 2.3 and diclofenac = 4.48) using the four selected chemical solvents, and washes (W1–W4) for the five different QC concentrations investigated (*i.e.*, 500, 1000, 5000, 10 000 and 15 000 ng L^{-1}). (b) AGREEprep values for each of the four chemical solvents investigated when using W4.

acetone, MeCN, and 1% NA (aq). Two solvents had the same average ranking position, 1% NA (aq) and EtOAc, however, due to the common use of NA in washing other plastic and glassware in the laboratory, such as centrifuge tubes and bottles,[47] and it having the best AGREEprep values obtained, 1% NA (aq) was considered further. Furthermore, a PCA of these four solvents was performed using the average normalised peak areas across all concentrations and washes, and no clusters were observed. The total variance explained by PC1 and PC2 was ~61% (Fig. S5, SI 1), so no specific solvent was outlined from the four selected, indicating that all four should be investigated. PCA has several limitations, including the sensitivity of results to variable scaling among others.[48] In this study, no scaling adjustments were applied, which may influence the findings.

3.3 Cross-tip type chemical residue carryover evaluation

The same carryover experiment was performed for the other pipette tips (*i.e.*, yellow, CP100 and CP1000) using the four solvents selected. The number of compounds detected across the four chemical solvents and tips varied widely across the four washes, with yellow tips ranging from 0 to 59, CP100 from 0 to 58 and CP1000 from 2 to 94 compounds in UP H_2O (Fig. S6, SI 1). Several common compounds were detected across the chemical solvents and tip types (Fig. 3(a)). An example is carbamazepine, an anti-epileptic drug, which was detected across all tips in all solvents except MeCN. Cocaine was not detected in any of the washes when using CP100 and yellow tips, despite being present in higher volume tips and no difference in washing methods. This could be due to the smaller volume aliquoted and smaller plastic surface area available to interact with the analyte, which may have fallen below the method LOD.

Peak area reduction also varied across the chemical solvents and tips (Fig. S4, SI 1). An example can be seen in Fig. 3(b) when using acetone at the highest concentration level tested, where the number of compounds and concentrations decrease overall, and a higher number of compounds is removed for CP100; the rest of the tips can be observed in Fig. S7 (SI 1). While the blue and yellow tips behaved similarly, there was a difference between CP1000 and CP100. For CP1000, even if the number of compounds was reduced by wash, the average peak area of any remaining compounds stayed steady across the multiple washes, while for CP100, most compounds were removed by W1 (95.5% compounds removed), with just one compound being detected in W4. This could be due to the difference in the

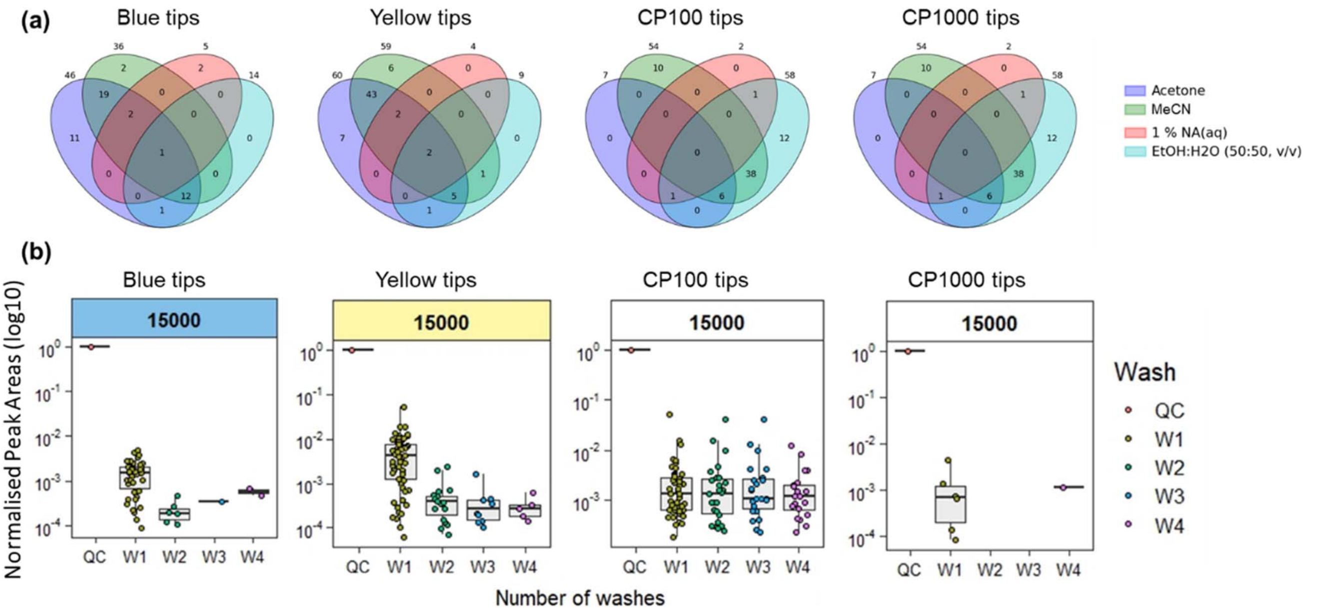


Fig. 3 (a) Venn diagrams for compounds detected across four washes (W1–W4) and the four selected chemical solvents per type of tips investigated. (b) Normalised peak area ratios of compounds detected after each wash (W1–W4) using the chemical solvent acetone at the highest concentration level (15 000 ng L^{-1}), compared to the normalised quality control (QC) peak area.

piston's plastic types impacting CEC adsorption and wash chemical interactions, as the CP100 piston was made of HDPE and the CP1000 made of POM. All individual data can be found in Table S3 of SI 2. Correlations between the compounds detected in W4 at 15 000 ng L^{-1} and their log *P* values were also investigated for the different tips (Fig. S8, SI 1). Only eight solvent and tip combinations had enough data to investigate correlations. For most CECs, no correlations were observed. However, one of the solvents showed a medium correlation, EtOH : H_2O (50 : 50, v/v) for CP1000, indicating a potential hydrophobicity dependency. As non-polar polymers, PP and POM interact with solutes primarily *via* van der Waals forces. The EtOH : H_2O (50 : 50, v/v) solvent, being moderately polar and protic, can dissolve a broad range of compounds. However, highly hydrophobic compounds (high log *P*) may still preferentially associate with the non-polar phases (or the surface of the tip), hence the medium correlation, reducing their solubility in the solvent phase and leading to lower removal. The absence of correlation in other solvent systems may be due to their poor solubilisation range or incompatibility with compound polarity. These results highlight that chemical residue removal is likely governed by a combination of solute hydrophobicity and solvent polarity using mixed-polarity solvents like EtOH : H_2O (50 : 50, v/v), rather than direct hydrogen bonding with the pipette tip surface.

AGREEprep values for the yellow, CP1000 and CP100 tips followed the same trend as the blue tips (Tables S7–S9, SI 1). W1 had an average score of 0.58 ± 0.08 across the four chemicals and tips, decreasing to an average of 0.48 ± 0.08 for W4. Overall, 1% NA (aq) averaged the greenest score (0.60 ± 0.05), with acetone as the least green solvent (0.45 ± 0.07) across the tip types. However, overall, all peak areas and number of compounds are reduced by W4 for all tip types investigated; therefore, W4 is deemed necessary to minimise any potential carryover for targeting all 133 CECs.

3.4 Matrix investigation – application to environmental samples

Carryover of the analytes might not be the only problem when reusing plastic consumables. The matrix of the sample can have a major impact, and due to its high complexity, wastewater was selected to apply the washing regime to real samples. Because the presence of organic matter may influence the adsorption of chemicals on plastics,[45] peak area reduction with all tips was tested with the four chemical solvents (Table S4, SI 2). The majority of solvents and tips showed a higher number of detected compounds in the carryover solutions when using wastewater after W4 compared to UP H_2O after the same number of washes, demonstrating less effective removal (Fig. S6, SI 1). This could be attributed to target CECs already being present in the wastewater sample, which, when spiked, would result in a higher overall concentration. This increased concentration makes it more challenging to remove compounds through the tested wash regime, as higher concentrations are generally harder to eliminate, as observed in previous results in UP H_2O. However, there were some exceptions: for blue tips, 1% NA (aq) resulted in five fewer compounds compared to UP water, for yellow tips EtOH : H_2O (50 : 50, v/v) had one fewer compound, and for the CP1000, acetone and EtOH : H_2O (50 : 50, v/v) had three and seven fewer compounds, respectively. These variations highlight how the solvent and tip combinations can influence compound retention and removal. A PCA was performed for the four tips and the four solvents investigated in wastewater, where no distinct clusters were observed, and no difference between tips was observed when using either UP H_2O or wastewater (Fig. S9 (a) and (b), SI 1). Average peak area reductions across concentrations and washes ranged from 86.7% (CP1000 using 1% NA (aq) to 99.8% (blue tips using acetone); Fig. S10, SI 1. These were slightly lower when compared to UP H_2O, which ranged from 88.2% (CP1000 using MeCN) to 99.9% (acetone using blue tips). Differences between normalised peak areas obtained from the compounds detected were investigated for the four chemical solvents and four tips, where 10/16 were significantly different between UP H_2O and wastewater (Fig. S11, SI 1).

When comparing individual solvents to average peak area reductions, the major difference obtained was for CP1000 when using 1% NA (aq), where UP reductions were 12% higher when compared to wastewater. Only in six cases, the reduction was higher when using wastewater as a matrix, mainly for the blue tips, where all but acetone showed this. However, and overall, the maximum difference of average peak area reductions between UP H_2O and wastewater was −4.35%, for CP1000 using MeCN, where 32 analytes were only identified in UP H_2O ($\log P$ ranging from 0.31 to 5.67). This could be due to the analytes binding into the organic matter (*i.e.*, proteins, carbohydrates, *etc.*) by van der Waals interactions rather than the surface and being eluted with the matrix, explaining why a higher % of reduction was found in some of the wastewater samples. Chemicals can bind, adsorb and/or form complexes in the organic matter, and this will depend on the pH of the wastewater sample. More hydrophobic analytes have a positive correlation with the binding to organic matter.[49] Some other compounds, as mentioned before, could bind into the plastic when in contact with UP H_2O as there is no competition between the analyte and the organic matter. However, compounds with higher $\log P$ would be expected to bind into the organic matter, and three compounds were identified only in wastewater: nicotine-d_4 ($\log P = 0.82$), levocabastine ($\log P = 4.48$) and atorvastatin ($\log P = 4.36$). Furthermore, non-significant correlations were found for most solvents and tip combinations between the compound's $\log P$ and the normalised peak areas detected in the carryover solutions (Fig. S12, SI 1), with 5/16 having weak correlations, and 2/16 strong correlations (CP1000 and CP100 both for EtOH : H_2O (50 : 50, v/v)). Binding interactions cannot only be based on hydrophobicity, many compounds in this study are weak acids that can interact with electron donors and functional groups of the organic matter, as well as many of them possessing aromatic rings.[49] Additionally, cation bridging, a mechanism where metal ions facilitate binding between negatively charged compounds (*e.g.*, tetracyclines) and organic matter, may play a role.[50] This is why agents like ethylenediaminetetraacetic acid (EDTA) are often added to samples to chelate metals and reduce complexation.[51–53] For instance, in a previous study, compounds with lower hydrophobicity showed a higher binding affinity to organic matter,[49] making it difficult to draw a conclusion in this study, suggesting that further research is needed. Also, a PCA was performed to compare the UP H_2O and wastewater results, where no clusters were observed (Fig. S9 (c), SI 1), resulting in no overall major difference between the matrices.

3.5 LCA

Following matrix analysis, LCA data was reviewed to further shortlist chemical solvents based on their GWP. For blue tips W4 for one reuse cycle (*i.e.*, a new tip used, washed and reused once), the GWP was found to be lowest for 1% NA (aq) at 4.1×10^{-3} $kgCO_2e$ compared to EtOH : H_2O (50 : 50, v/v) (6.2×10^{-3} $kgCO_2e$), acetone (9.4×10^{-3} $kgCO_2e$) and MeCN (1.3×10^{-2} $kgCO_2e$). Interestingly, using two new tips yielded 4.4×10^{-3} $kgCO_2e$. This GWP ranking followed the same order for all four chemical solvents across all pipette tip types (Table S2, SI 2). This GWP outcome, in combination with a low overall AGREEprep ranking compared to 1% NA (aq) and EtOH : H_2O (50 : 50, v/v), and insufficient wash effectiveness compared to MeCN, meant acetone wash investigations were not continued further.

3.6 Pipette tip degradation

To further assess physical changes, all the different types of pipette tips were submerged in a beaker containing the respective wash solvents for ~72 hours at room temperature, and the pipette tips were observed by light microscopy. Most tips were found to be intact, as can be seen in Table S10 (SI 1), where images of the surface taken by the microscope showed no impact on the tips. However, the CP100 pistons of the positive displacement tips were different from the blanks when using MeCN, showing slightly more degradation. This could be associated with the different type of plastic on the capillary pistons than the rest of the tip (*i.e.*, PP). In a solvent compatibility study, HDPE (*i.e.*, CP1000 piston) exposed to MeCN showed no/little surface deterioration at 50 °C as per our results; however, POM (*i.e.*, CP100 piston) was not recommended to use at room temperature (~21 °C) or at all when using MeCN and 1% NA (aq) solvents respectively (Table S11, SI 1). Nevertheless, the *p*-values for our gravimetric tip investigation using MeCN showed 0.29 (blue tips), 0.11 (yellow tips), 0.31 (CP1000) and 0.18 (CP100), indicating washed tips were not significantly different from blank tips of the same kind after W4. For EtOH : H_2O (50 : 50, v/v), average *p*-values for all tips were also >0.05 for W4 when compared to measurements using unwashed blanks, showing that the volume capacity of the pipette tips was not significantly different before and after cleaning. CP1000 and blue tips also gave *p*-values >0.05 for 1% NA (aq) washes compared to a blank. However, though the blanks did not show any difference with the 1% NA (aq) washed tips, *p*-values obtained for the CP100 and yellow were significantly different after W4 (0.01 and 0.03, respectively). This suggests that using this chemical wash with these tips may alter their inner volumes. Both tips were determined to be unsuitable for tip washing using this solvent. Several interaction mechanisms can contribute to these changes, including chemical attack on the polymer chain, physical alterations such as solvent absorption leading to plastic softening or swelling, solvent permeation and dissolution, and stress cracking caused by the interaction of a "stress cracking agent" with internal or external stresses.[42] Further investigation was performed under the investigation of the maximum pipette tip reuse based on these results and LCA.

3.7 Maximum reuse cycle investigation

An LCA was performed to investigate the maximum reuse cycle using 40 washing cycles (*i.e.*, W160) and compared to each of the shortlisted wash chemical solvents. For new air displacement tips, the GWP was found to be $2.2 \times 10^{-3} \pm 2.4 \times 10^{-6}$ and $7.5 \times 10^{-4} \pm 1.1 \times 10^{-6}$ $kgCO_2e$ on average for a single blue or yellow pipette tip, respectively; this equated to 0.089 $kgCO_2e$ for 41 blue tips and 0.031 $kgCO_2e$ for 41 yellow tips. This was significantly lower than the GWP of 40 washing and reuse cycles of MeCN (*i.e.*, the W4 process repeated 40 times to mimic reusing a tip), which was 478 ± 0.42% and 279 ± 0.36% of the GWP of using 41 blue and 41 yellow tips once, respectively. This imbalance increased the more wash and reuse cycles the tips underwent (Fig. 4(a) and (b)). The main reason for this is the high GWP of MeCN production, assumed to be through the Standard Oil of Ohio (SOHIO) process.[54,55] Washing and reusing positive displacement tips with MeCN was also found to be ineffective at reducing GWP compared to using new single-use CP1000 and CP100 s. Performing 40 MeCN wash cycles on one CP1000 tip produced 265 ± 0.01% of the GWP of using 41 new single-use CP1000 tips and 169 ± 0.05% for washing one CP100 tip compared to 41 new (Table S2, SI 2). Although the LCA shows that washing may not be greener than using single-use tips, this LCA is very finite in the data it is using and should be treated as preliminary. As we only account for cradle-to-gate of polymer formation here, and do not consider the GWP of tip formation, shipping, use or disposal, the GWP is likely underestimated in this study. A more holistic LCA of cradle-to-grave for both pipette tips and chemical solvents would improve the accuracy of GWP. Despite these LCA results, as MeCN showed the best wash effectiveness thus far, all tip types were investigated for maximum wash and reuse cycle carryover, and data is provided in SI 1. Overall, compounds were detected with low carryovers, where most of the peaks were close to LOD levels. *P*-values of gravimetric calibration checks after 40 wash and reuse cycles showed no significant difference when compared to the blanks for all tips, varying between 0.09 for CP1000 tips and 0.41 for CP100 tips. The interiors of the pipette tips were also analysed by SEM, and some alterations were observed between unwashed and washed tips (*e.g.*, striations, precipitate/contamination debris on the tip, some pitting) (Fig. S13, SI 1). Particularly, the capillary pistons for the CP1000 and CP100 did show some degradations regarding the surface, as seen in Fig. 5(b) when using MeCN. However, these did not impact any of the calibrations. These results suggest that carryover removal is effective for this number of cycles without altering the volumetric capacity of tips, despite some alterations to interiors; therefore, all tips were deemed still suitable for reuse at this stage.

LCA results for 1% NA (aq) showed a reduction in GWP for all tip types when undergoing 40 wash and reuse cycles compared to using 41 new tips, with the largest saving observed for CP100 (8.2×10^{-3} $kgCO_2e$ for washing, *versus* 2.3×10^{-2} $kgCO_2e$ for new tips) (Fig. 4). However, gravimetric results assessed for tips

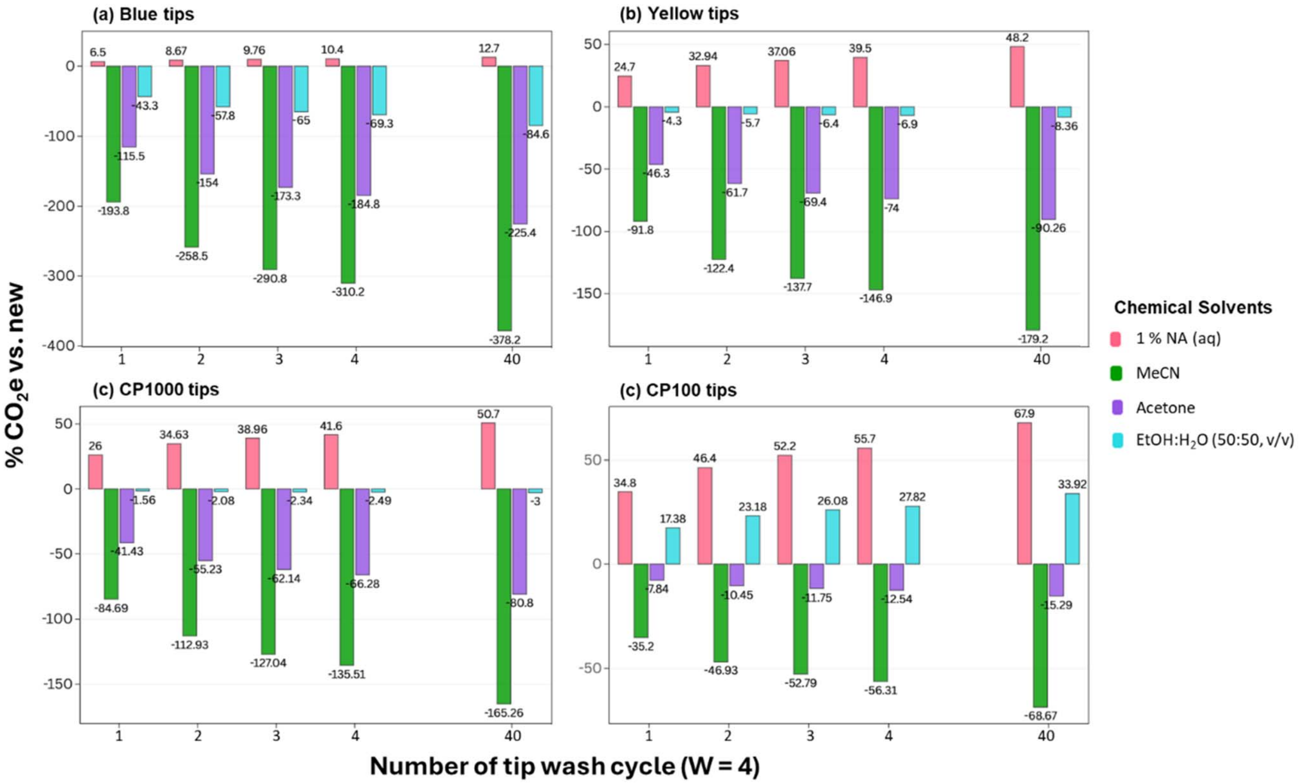


Fig. 4 Percentage of $kgCO_2e$ saved (%) compared to using new pipette tips, based on four rinses per wash and reuse cycle ($n = 40$ cycles) for (a) blue, (b) yellow, (c) CP1000 and (d) CP100 tips, for the four chemical solvents selected.

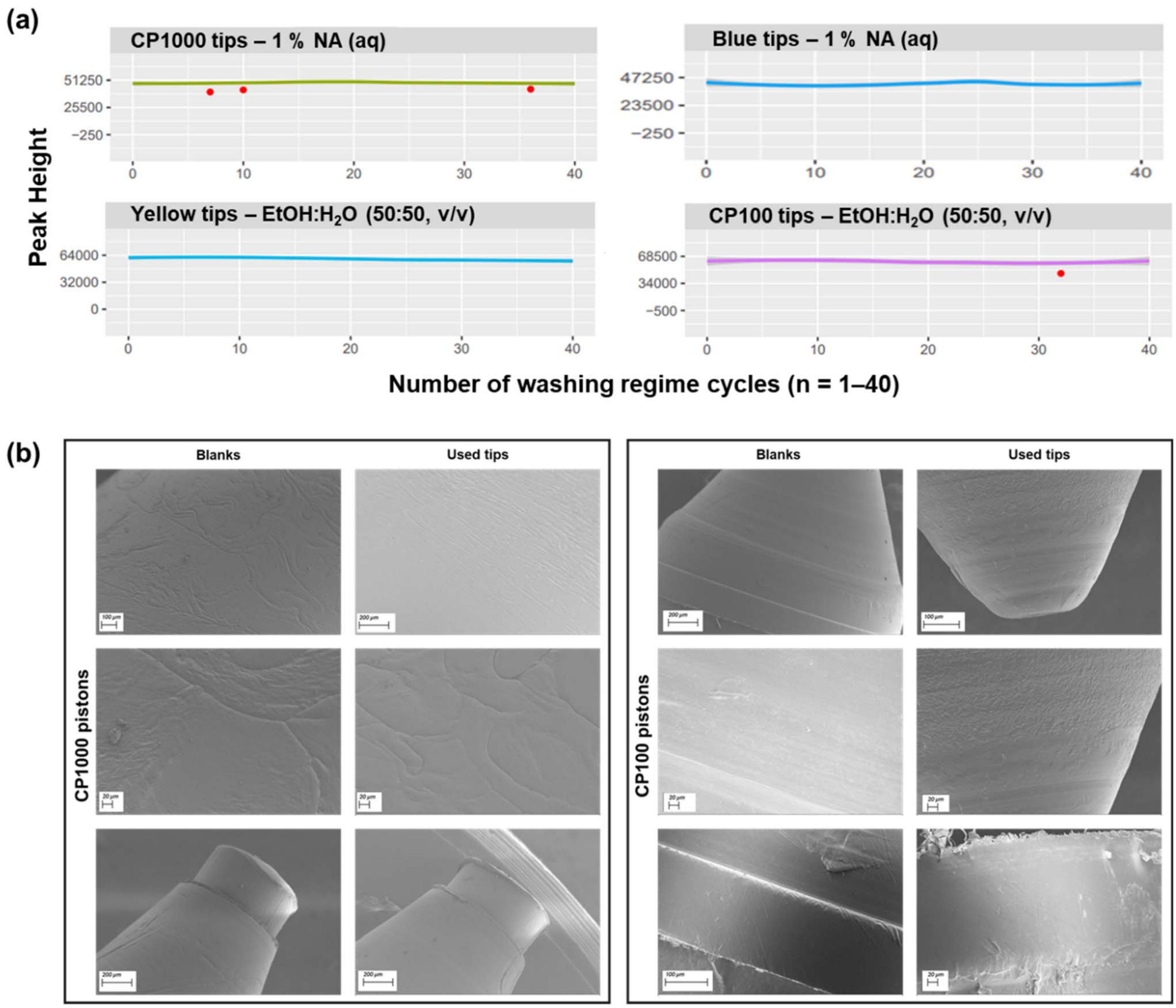


Fig. 5 (a) Benzocaine washing regime series plot of peak areas across 40 reuse cycles of a standard sample prepared at 500 ng L^{-1} in wastewater when using different pipette tips and chemical washing solvents. Confidence interval bands of 95% of the locally estimated scatterplot smoothing (LOESS) regression are shown in grey, and outliers in red. (b) Scanning electron microscopy (SEM) images of the pistons of the positive displacement pipette tips (left) CP1000 and (right) CP100, before (*i.e.*, blanks) and after (*i.e.*, used tips) reusing the tip for a total of 40 times using a wastewater sample spiked at 500 ng L^{-1} and MeCN as the chemical solvent for the washing regime ($n = 4$ washes after every sample aspiration). Magnification ranges from 20 to 200 μm.

that passed the W4 stage without showing degradation, blue and CP1000 only, yielded *p*-values of <0.05 and 0.13, respectively, after 40 wash and reuse cycles. This indicates that, despite a potential saving in CO_2e when washing and reusing tips 40 times with 1% NA (aq), reusing tips this many times may lead to plastic degradation for all but CP1000 pipette tips. Accordingly, SEM imaging was conducted on the tip following 40 cycles, during which no evidence of degradation was observed (Fig. S14, SI 1). Further investigation is warranted to determine the maximum number of wash and reuse cycles possible for the blue tips using this solvent. To maximise tip reuse whilst minimising degradation, fewer wash steps may be applied to each reuse cycle, as carryover results suggest that W4 may be unnecessary for certain analytes. This would offer an opportunity to reduce both process time and environmental impact. For example, substituting W4 for W3 over 40 wash cycles of 1% NA (aq) with CP1000 tips could reduce CO_2e by approximately 12.5%, thereby lowering the GWP of the cleaning process.

For EtOH : H_2O (50 : 50, v/v), LCA results showed a 17.4 ± 0.01% CO_2e saving for CP100 compared to single-use tips when using W4 per cycle. However, all other tip types washed with this solvent exhibited a higher GWP when using new tips (Fig. 4). This is likely due to the higher tip mass-to-volume-capacity ratio of the CP100 compared to other tip types evaluated. Gravimetric analysis indicated that average *p*-values for blue and yellow tips washed with this solvent did not significantly impact their performance ($p = 0.28$ and 0.11, respectively). In contrast, CP100 tips showed a difference ($p < 0.01$), implying degradation. Despite CP100 showing the greatest potential GWP reduction over 40 washing cycles, it was also the most affected by repeated washing. However, SEM images confirmed no degradation in any tips. Furthermore, as observed with 1% NA (aq), both degradation and GWP associated with the washing regime could be reduced by minimising the number of wash steps where appropriate, particularly when W4 may not be necessary for effective analyte removal.

No consistent accumulative trend in carryover was observed across the 40 cycles for any of the 145 compounds, which varied depending on the tip and solvent tested. EtOH : H_2O (50 : 50, v/v) generally resulted in low analyte identification across all pipette tip types. Blue tips showed four detectable compounds, yellow tips three, while CP100 tips performed best with the lowest carryover (*i.e.*, one compound). In contrast, 1% NA (aq) led to substantially higher carryover overall (*i.e.*, four compounds detected for blue tips and six for CP1000). Trace contaminants detected in the washes appeared either at relatively consistent levels or as infrequent, random occurrences. No clear evidence as to whether $\log P$ governed their carryover was obvious. These findings suggest that EtOH : H_2O (50 : 50, v/v) is more effective at minimising carryover than 1% NA (aq). ICH and FDA guidelines suggest that carryover should not exceed 20% of the LLOQ.[56,57] However, usually, for highly sensitive methods laboratories aim for <5% carryover.[58] Therefore, and considering the worst-case scenario, we do not recommend reusing yellow tips using EtOH : H_2O (50 : 50, v/v) for analysis that include nortriptyline as an analyte. When performing 40 cycles using 1% NA (aq) blue washed tips, ronidazole and cocaine are not recommended for analysis, and for CP1000 tips for ronidazole, and trimethoprim, which obtained >5% of carryover. Also, refer to the list of compounds previously detected when using higher concentrations (Table S4, SI 2).

3.8 High-resolution chemical screening for maximum reuse cycle investigation

To broaden the range of chemical compounds, the carryover solutions were also run under suspect screening analysis. For EtOH : H_2O (50 : 50, v/v), a total of 67 extra compounds were detected when using blue tips, namely ketoprofen and quetiapine, 58 when using yellow tips, for example diltiazem, gabapentine, and riboflavin, and 70 when using CP100 tips, including adenosine, indomethacin, and rivaroxaban (Fig. S5–S9, SI 2). For 1% NA (aq), a total of 68 extra compounds were detected when using blue tips, including paracetamol and codeine, and 56 when using CP1000 tips, such as aciclovir, naproxene, and propranolol (Fig. S8–S9, SI 2).

Total ion chromatograms (TICs) from the blank sample (W0) and the 40-cycle wash carryover solution (W160) were overlaid to assess potential differences. While most overlays showed no visible differences (Fig. S15, SI 1), TICs alone are limited in resolving subtle changes. Therefore, peak-level comparisons were performed using EICs following chromatographic alignment. No significant differences were observed, as the majority of features clustered around $M = 0$ in MA plots, indicating similar intensities between W0 and W160. This suggests minimal carryover or contamination from the washing procedure. A symmetrical distribution around $M = 0$ further supports the absence of systematic bias toward either sample. An example is shown in Fig. S16 (SI 1) for the blue tips (positive mode) washed with EtOH : H_2O (50 : 50, v/v). In contrast, the yellow and CP100 tips (positive mode) exhibited several features with large negative M values (down to −15), indicating that these peaks were more intense in W0 and likely removed during washing. Few features showed large positive M values, suggesting low contamination in W160. Overall, no significant differences were found across any EIC-derived peak areas (Fig. S17, SI 1), except for the CP100 tips in negative mode washed with 1% NA (aq), which showed notable deviations. These differences were also evident in the corresponding MA plot (Fig. S18, SI 1), indicating substantial carryover or contamination after 40 reuse cycles, particularly for specific features. This suggests that the washing procedure may be less effective under negative ionisation conditions for this tip type, or that certain compounds are more susceptible to leaching or retention in these conditions.

Furthermore, the wastewater samples used for the 40-wash cycle investigation were also run under suspect screening analysis. Because no standards were available for all compounds confirmed, peak areas of all samples were plotted against the 95% confidence interval of the LOESS regression to investigate any potential outliers across the 40 cycles (Fig. S1–S9, SI 2). With EtOH : H_2O (50 : 50, v/v) for the blue tips, 101/5319 of the measurements were outliers across 45 compounds, ranging from one to nine outliers in each compound. This tip had a total of 130 compounds confirmed, 59 of which were not spiked, and came from the wastewater sample. For the yellow tips, 91/4326 of the measurements were outliers across 38 compounds, ranging from one to five outliers per compound. A total of 105 compounds were confirmed, with 56 not spiked but present in the sample. Lastly, for the CP100 tips, 85/5281 of the measurements were outliers across 41 compounds, ranging from one to seven outliers per compound. A total of 133 compounds were confirmed, with 62 not spiked, but present in the sample. For tips washed with 1% NA (aq) and for the blue tips, 107/5247 of the measurements were outliers across 58 compounds, ranging from one to five outliers per compound. A total of 127 compounds were confirmed, with 58 not spiked but present in the sample. For the CP1000 tips, 81/4347 of the measurements were outliers across 37 compounds, ranging from one to eight outliers per compound. A total of 106 compounds were confirmed, with 51 not spiked but present in the sample. Overall, all tips had less than ∼1.9% of outliers of the total dataset across all compounds and tips (ranging from 1.61 to 2.10% when using EtOH : H_2O (50 : 50, v/v) and 1.86 to 2.04 with 1% NA (aq)). Also, most outliers were found at the beginning or end of the 40 cycles, with only a few compounds showing outliers throughout the middle. An example can be seen in benzocaine, a local anaesthetic drug that relieves minor pain and discomfort, where outliers varied depending on the tip and chemical washing solvent tested (Fig. 5(a)).

Outliers could also be associated with the manual sample preparation or instrument variability across the batches, indicating that if any carryover exists, they are low and not consistent, not carried over after a large number of reuse cycles. Therefore, ~98% of the data has not been affected by carryover from the reuse of the tips across the 40 cycles.

4 Conclusions

This study is the first to demonstrate the feasibility of reusing plastic pipette tips for high-throughput environmental monitoring through solvent washing, targeting >100 CECs. A systematic screening approach identified four effective solvents, 1% NA (aq), EtOH : H_2O (50 : 50, v/v), MeCN, and acetone, based on low carryover in UP H_2O from 11 solvents initially investigated. These were further evaluated across multiple pipette tip types and wash cycles. Results showed that compound removal efficiency depended on both solvent properties and analyte hydrophobicity ($\log P$), with W4 proving necessary to ensure minimal residual contamination. The application of these washing protocols to a wastewater matrix indicated additional challenges due to organic matter interactions, but similar trends were observed compared to UP H_2O.

Incorporating LCA and AGREEprep as environmental tools further refined the solvent selection. Overall, EtOH : H_2O (50 : 50, v/v) was identified as the most balanced solvent, offering low GWP, strong removal efficiency, and minimal degradation of tips across 40 reuse cycles. SEM imaging and gravimetric tests confirmed minimal tip wear, although some exceptions (*e.g.*, CP100) require further validation before routine reuse. While both acetone and MeCN were effective at removing CECs, their relatively high carbon footprints, particularly for MeCN, limited their sustainability under standard multi-wash protocols. The environmental impact of all wash solvents investigated could be reduced by optimising the number of washes based on the specific analytes of interest. This would not only lower GWP but also extend pipette tip lifespan. Furthermore, the combination of solvents (*e.g.*, 1% NA (aq) followed by EtOH : H_2O (50 : 50, v/v)) was not explored in this study but may offer improved cleaning efficiency. This approach warrants further investigation in future research.

Finally, this study provides a comprehensive profile of compound-specific removal efficiency for each solvent, enabling researchers to select cleaning strategies tailored to their target analytes. Based on the greenest findings of this study, substituting 20 000 new tips with 500 reused ones using W3 of EtOH : H_2O (50 : 50, v/v), could save up to ~5 kgCO_2e a year. However, this will need further optimisation. This approach supports greener workflows without compromising analytical integrity. To support broader adoption, a user-friendly calculator has been developed, allowing laboratories to estimate both solvent wash efficiency and associated reductions in GWP. Together, these tools and findings offer a practical, data-driven pathway toward reducing laboratory plastic waste and lowering the environmental footprint of high-throughput analytical processes.

Author contributions

Amber Vaughan: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, supervision, validation, visualisation, writing – review and editing. Yassir El Hadri: formal analysis, investigation. Juditha Gurumurthy: formal analysis, visualisation. William Francis: formal analysis, visualisation. Margarita White: formal analysis. Eric Auyang: formal analysis, investigation. Stephanie Wright: writing – review and editing. Leon Barron: conceptualisation, writing – review and editing. Helena Rapp-Wright: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, visualisation, writing – original draft, writing – review and editing.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5su00644a.

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