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Contaminant Spot Check and Removal Assay (ContamSPOT) for Mass Spectrometry Analysis

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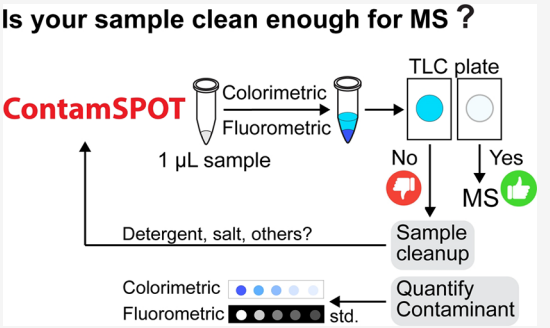
ABSTRACT: Mass spectrometry (MS) analysis is often challenged by contaminations from detergents, salts, and polymers that compromise data quality and can damage the chromatography and MS instruments. However, researchers often discover contamination issues only after they acquire the data. There is no existing contaminant assay that is sensitive enough to detect trace amounts of contaminants from a few microliters of samples prior to MS analysis. To address this crucial need in the field, we developed a sensitive, rapid, and cost-effective contaminant spot check and removal assay (ContamSPOT) to detect and quantify trace amounts of contaminants, such as detergents, salts, and other chemicals commonly used in the MS sample preparation workflow. Only 1 μL of the sample was used prior to MS injection to quantify contaminants by ContamSPOT colorimetric or fluorometric assay on a thin layer chromatography (TLC) plate. We also optimized contaminant removal methods to salvage samples with minimal loss when ContamSPOT showed a positive result. ContamSPOT was then successfully applied to evaluate commonly used bottom-up proteomic methods regarding the effectiveness of removing detergent, peptide recovery, reproducibility, and proteome coverage. We expect ContamSPOT to be widely adopted by MS laboratories as a last-step quality checkpoint prior to MS injection. We provided a practical decision tree and a step-by-step protocol with a troubleshooting guide to facilitate the use of ContamSPOT by other researchers. ContamSPOT can also provide a unique readout of sample cleanliness for developing new MS-based sample preparation methods in the future.



INTRODUCTION

Minimizing contaminations prior to mass spectrometric (MS) analysis has been a long-standing challenge for analytical chemists.[1−4] Contaminants such as detergents, polymers, solvents, and salts can significantly influence the separation and ionization efficiency of target molecules in chromatography and MS.[5] Trace amount of detergent contaminations (SDS, Triton, Tween, etc.) was notoriously known to strip packing materials from chromatography columns and further damage both the chromatography and MS instruments.[6,7] Extensive contamination can harm the ion funnel and mass analyzer beyond the ion source, leading to costly downtime for deep cleaning and repair services. Contaminants are introduced throughout the experimental workflow and may not be discovered until after data acquisition, already damaging the instrument and losing precious samples. Contamination issues can be challenging to identify when samples were collected from another laboratory for a collaborative project or received by an MS facility. Therefore, developing a fast quality checkpoint for contaminants prior to MS analysis can greatly benefit researchers and protect valuable samples and expensive instruments.

Detergents and salts are often added to buffers to facilitate the extraction of membrane proteins or lipids, which need to be removed in the downstream sample preparation steps. Various sample preparation workflows have been developed for detergent-containing samples or removing contaminants prior to MS analysis for different types of molecules.[6−12] For bottom-up proteomics, filter-based FASP[13] and S-Trap[14] methods can remove contaminants prior to protein digestion. Recently developed bead-based SP3,[15] SP2,[16] SP4[17] methods can remove contaminants at the protein level or peptide level. However, the efficiency to remove contaminants of these methods can only be assessed by examining the MS data due to the lack of a sensitive and quantitative contaminant assay. Inadequate sample cleanup compromises data quality and harms the instrument, while excessive cleanup causes sample loss and increases both the time and the cost of sample preparation. Therefore, a sensitive and quantitative contaminant assay can provide a unique readout of sample cleanliness for method development and decision-making regarding sample cleanup.

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A practical contaminant assay prior to MS analysis should be rapid and highly sensitive and require minimal sample volume. Detergents such as SDS have been traditionally quantified by spectroscopy methods in environmental analysis.[18,19] However, these methods typically require a minimal volume of 20−100 μL, and they do not provide sufficient sensitivity to quantify the trace amounts of contaminants remaining in the sample after routine cleanup steps. Ideally, the method should also be straightforward and low cost so that researchers are willing to incorporate this assay as a last-step quality checkpoint prior to MS analysis.

To address this crucial need in the field, we developed a Contaminant Spot Check and Removal Assay (ContamSPOT) that only consumes 1 μL of the sample within a few minutes prior to MS analysis. We screened various solvents and reagents to extract common MS contaminants and amplify their signals so that they could be spotted and quantified on a thin layer chromatography (TLC) plate. We developed both colorimetric and fluorometric assays to expand the applicability of the ContamSPOT assay to different types of contaminants, including ionic and nonionic detergents, salts, acids, and other chemicals. We also optimized contaminant removal methods so that contaminants can be effectively removed with minimal sample loss when ContamSPOT showed a positive result. We then applied ContamSPOT to compare several popular proteomics sample preparation methods that should remove contaminants at the protein or peptide level. We also provided a practical decision tree and a step-by-step protocol to assist users in conducting the ContamSPOT assay and making experimental decisions based on the ContamSPOT result.

EXPERIMENTAL METHODS

ContamSPOT Colorimetric and Fluorometric Assays. For the colorimetric assay, a mixture was created in a 0.2 mL tube containing 1 μL of the sample, 1 μL of ion-pairing agent (0.1% o-toluidine blue, Carolina Biological), and 3 μL of ethyl acetate (ACS grade, Fisher Scientific). The mixture was vortexed and centrifuged briefly. One to two microliters of the top layer were spotted on a TLC plate (Sorbtech). The spot dried instantly, and the blue color (indicating the presence of contaminants) can be directly visualized by the eye. An image can be taken and quantified by ImageJ software.[20] To accurately quantify the amount of contaminants from an unknown sample, calibration curve samples using contaminant standards should be spotted with the unknown sample on the same TLC plate.

For the fluorometric assay, reagents were obtained from the ProFoldin Detergent Assay Kit (DAK 1000) with a modified protocol. A mixture was created in a 0.2 mL tube containing 1 μL of sample, 2 μL of 1× ProFoldin dye A43, and 1 μL of 1× Reagent 2. The mixture was vortexed briefly and then incubated at room temperature for 5 min. Two microliters of the mixture were spotted on a TLC plate (no visible color). A Fluorescence Imager (Bio-Rad ChemiDoc MP) was used to image the plate at 632 nm followed by quantification in ImageJ. The detailed step-by-step protocol is provided in Supporting Information.

Ethyl Acetate Liquid−Liquid Extraction (LLE) To Remove Detergents. Water-saturated ethyl acetate was created by mixing pure ethyl acetate (HPLC grade, Sigma) with a small amount of HPLC grade water in a glass bottle until forming a water layer at the bottom. Water-saturated ethyl acetate was added at a 10-fold volume to the samples followed by 30 s of vortex and 30 s of centrifugation. Then, the top ethyl acetate layer was carefully removed without touching the bottom aqueous layer. This process was repeated for a total of 3−5 extraction cycles. Cleaned samples were then dried and were ready for LC-MS analysis. See Supporting Information for detailed steps.

Cell Culture and Protein Extraction. HEK293 cells were routinely cultured and harvested in the lab as described previously.[4,21] Cell pellets were lysed in either urea buffer (8 M urea, 150 mM NaCl, 50 mM ammonium bicarbonate (AmBC)) or detergent buffer (2% SDS, 1% Triton X-100, 150 mM NaCl, 50 mM AmBC) followed by sonication, clarification by centrifugation, and protein concentration measurement. Protein lysates were routinely reduced and alkylated by tris(2-carboxyethyl)phosphine (TCEP), iodoacetamide (IAA), and dithiothreitol (DTT) treatments as described previously.[4]

Protein Sample Preparation Using S-Trap, SP3, SP4, and Acetone Precipitation Methods. Reduced and alkylated protein lysates in detergent buffer were aliquoted and prepared using several commonly used methods that can remove contaminations at the protein level followed by trypsin digestion. Tryptic peptides from various methods were acidified by 1% formic acid (FA), dried, and stored at −30 °C. S-Trap sample preparation followed Protifi manufacturer protocol with minor adjustments.[14] Briefly, protein lysate was diluted with 100 mM triethylammonium bicarbonate (TEAB) in 90% methanol to 200 μL and applied to Micro S-trap columns. After three TEAB washes, trypsin (Promega, sequencing grade) was added at a 1:10 (trypsin: protein, w:w) ratio for digestion in 50 mM AmBC buffer at 47 °C for 2 h. Peptides were eluted using three washes: 50 mM AmBC, 0.1% FA in 2% acetonitrile (ACN), and finally 50% ACN, each centrifuged at 1000 *g*. SP3 sample preparation followed the published protocol with minor adjustments.[15] Briefly, protein aggregation was induced onto the Cytiva paramagnetic beads by first adding ACN to 80% of the protein lysate followed by beads addition with a bead-to-protein ratio of 1:25 (w/w). After 15 min of incubation, the beads were washed twice with 95% ACN and then twice with 70% ethanol. On-bead digestion was conducted in 50 mM AmBC by using a 1:30 trypsin to protein ratio for 16 h at 37 °C followed by peptide elution on a magnetic rack. A recently developed SP4 method was conducted using glass beads enrichment (10:1, beads: protein, w/w) at 80% ACN.[17] Sample-bead mixtures were centrifuged for 5 min at 16,000 *g*. The protein-bead pellet was then washed three times with 80% ethanol and resuspended in 50 mM AmBC. Samples were sonicated for 5 min, followed by trypsin digestion (1:30 ratio, 16 h) and peptide elution by centrifugation. For acetone precipitation, a 4-fold volume of cold acetone (−20 °C) was added to the protein lysate and placed into a −80 °C freezer overnight. Samples were then centrifuged at 15,000 *g* to precipitate proteins at 4 °C and washed with 1 mL of cold acetone three times. Samples were then air-dried and reconstituted in 50 mM AmBC and 15 mM NaCl for trypsin digestion and desalting.

Peptide Sample Preparation Using SP2, Detergent Removal Resin, and Desalting Methods. Reduced and alkylated protein lysates in urea buffer were digested overnight using 1:30 (w/w) trypsin and quenched by FA until pH < 3. Known amounts of detergents were added to the peptide samples followed by several peptide cleanup methods. For SP2 cleanup, peptide samples were dried down and reconstituted in 95% ACN with a 1:25 (w/w) beads−protein ratio (Cytiva).[16] Beads were bound to the peptides for 15 min followed by four 95% ACN washes. Peptides were then eluted twice in HPLC-

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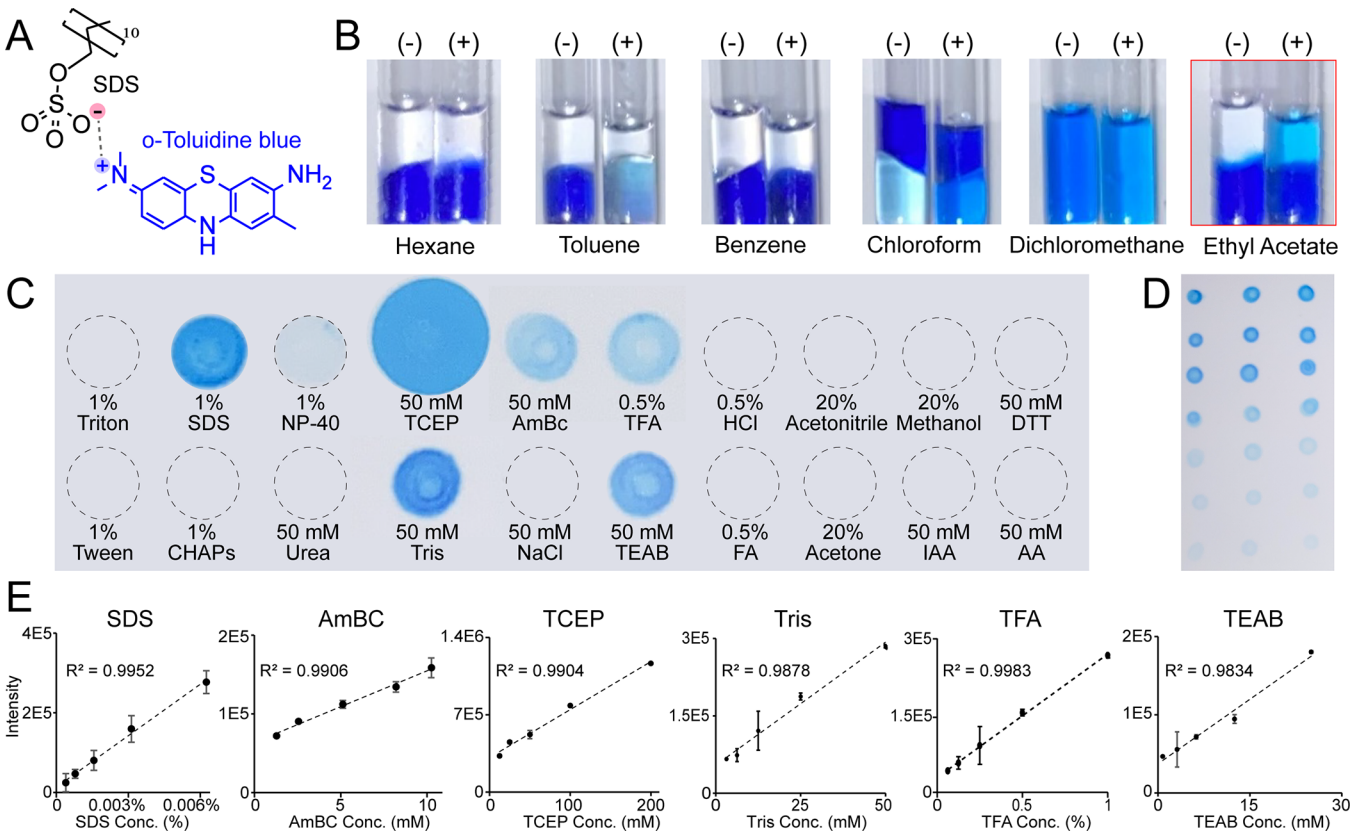

Figure 1. Development of the ContamSPOT colorimetric assay. (A) Schematic of the o-toluidine blue (OTB)-SDS ion pair. (B) Solvent screening to extract the OTB-SDS ion pair by mixing 2 μL of solvent with 1 μL of OTB dye and 1 μL of 0.1% SDS (+), compared to water control (−) in thin glass capillary tubes. (C) Screening of commonly used sample preparation chemicals. Ethyl acetate and OTB were added to each sample followed by brief vortex and centrifugation. Then, 2 μL of the ethyl acetate top layer was spotted on a TLC plate. (D) Example image of a series concentration of SDS solutions with three technical replicates. (E) Calibration curves of positively tested chemicals.

grade water. For detergent removal resin, the HiPPR detergent removal spin column kit was used here. The resin was added to the spin column and equilibrated by 50 mM AmBC three times via centrifuge at 1500 *g*. Samples were then added to the spin column, incubated for 2 min, and centrifuged for 1 min to elute the peptides prior to acidification and desalting. For reverse-phase desalting, an Oasis HLB desalting plate (Waters) was used following the manufacturer's protocol. All samples from different protein and peptide cleanup methods were dried and reconstituted in 2% ACN and 0.1% FA for ContamSPOT, a colorimetric peptide assay (Pierce), and LC-MS analysis.

LC-MS Analysis. LC-MS/MS analysis was conducted on a Dionex Ultimate 3000 nanoLC system coupled with a Thermo Scientific Q-Exactive HFX MS. A 2-h LC gradient was used with 0.1% FA in water as buffer A and 0.1% FA in ACN as buffer B. Samples were first loaded onto a PepMap 100 C18 trap column (3 μm, 100 Å, 75 μm × 2 cm) at 5 μL/min and then separated on an Easy-spray PepMap C18 column (3 μm, 100 Å, 75 μm × 15 cm) at 0.3 μL/min and 55 °C. MS1 scanned from m/z 400−1500 with a resolving power of 60,000. A top 25 data-dependent acquisition was conducted with an MS/MS resolving power of 15,000, an isolation window of 1.4 Da, a collision energy of 30%, and a dynamic exclusion time of 22.5 s. The maximum injection times were 30 and 35 ms for MS and MS/MS, respectively. Automatic gain controls were set to 10^6 and 10^4 for MS and MS/MS, respectively.

Proteomics Data Analysis. Proteomics data were analyzed with Thermo Fisher Proteome Discoverer (PD, version 2.4) software. Swiss-Prot *Homo sapiens* database (reviewed, 20,428 entries) and our custom-made cell culture-specific protein contaminant FASTA library (https://github.com/HaoGroup-ProtContLib)[4] were included for protein identification. Contaminant proteins (marked with the Cont_ prefix in our custom contaminant FASTA) were selected and removed from the data set as described in our previous publication.[4] False discovery rate cutoff was set as 1% for protein and peptide spectral matches. Maximum missed cleavages were set to 2 with trypsin as the enzyme. Precursor tolerance was set to 20 ppm. Modifications include cysteine carbamidomethylation (fixed), methionine oxidation (variable), and protein level N-terminus acetylation (variable). Further data analysis was conducted in R-studio.

RESULTS AND DISCUSSION

Developing a ContamSPOT Colorimetric Assay To Detect and Quantify Common MS Contaminants. We aim to develop a fast and sensitive contaminant detection and quantification method with minimum sample consumption for MS samples. We started with the notorious SDS detergent that was often added in protein lysis buffer but not sufficiently removed before MS. A cationic dye O-toluidine blue (OTB) has been reported to form an ion pair with SDS (Figure 1A), which can be used to quantify SDS from environmental samples (e.g., river water) using a spectrophotometer.[19] We first screened organic solvents that can extract the SDS−OTB ion pair to a separate layer, which allowed us to extract trace levels of SDS

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Table 1. Summary of Chemicals That Can Be Detected by the ContamSPOT Assay

chemical	type	ContamSPOT	R^2	CV (%)	LOD	LOQ	removal method
SDS	detergent	colorimetric	0.991	4.6	0.0004%	0.0013%	LLE[a]
TEAB	salt	colorimetric	0.98	3.1	0.9 mM	2.7 mM	desalting[b]
AmBC	salt	colorimetric	0.99	1.5	3 mM	8 mM	drying/desalting
Tris	salt	colorimetric	0.995	5.4	4 mM	12 mM	desalting
TCEP	reducing agent	colorimetric	0.99	7.5	8 mM	24 mM	desalting
NaOH	base	colorimetric	0.97	0.4	0.11 mM	0.34 mM	desalting
TFA	acid	colorimetric	0.99	6.6	0.0007	0.002	drying/LLE
Triton X-100	detergent	fluorometric	0.95	2.7	0.002%	0.005%	LLE
Tween-20	detergent	fluorometric	0.98	4.5	0.02%	0.06%	LLE
NP-40	detergent	fluorometric	0.96	2.9	0.02%	0.05%	LLE
CHAPS	detergent	fluorometric	0.99	4.5	0.02%	0.06%	cannot be removed by LLE
others[c]							

[a]LLE using ethyl acetate. [b]Desalting with reverse phase C18 or HLB cartridges/plates. [c]Tested negative in ContamSPOT assay, including NaCl, urea, iodoacetamide, acrylamide, dithiothreitol, formic acid, hydrochloric acid, EDTA, acetone, ACN, and methanol.

from a minimal sample volume. Six organic solvents were tested, as shown in Figure 1B. Ethyl acetate successfully extracted the SDS−OTB ion pair into the upper organic layer, and the control sample without SDS has no color in the upper layer. With such a low volume, drying down the organic layer and redissolving it into water for spectroscopy analysis do not have sufficient sensitivity and reproducibility. Therefore, we directly spotted 1−2 μL of the top ethyl acetate layer on a TLC plate. The spot dried instantly, and the blue color can be directly visualized by the eye, indicating the presence of SDS in the sample.

We then tested if this method could be used to detect other contaminants besides SDS. Figure 1C shows the screening results of common chemicals used in the MS-based sample preparation workflow. SDS, TCEP, Tris, AmBC, TFA, and TEAB showed positive blue color. To test the quantification performance of our ContamSPOT assay, we used standard solutions with series concentrations and quantified the blue color on the TLC image with ImageJ software. An example image is shown in Figure 1D from a series concentration of SDS solutions with three technical replicates. We then established calibration curves for all of the positive chemicals (Figure 1E). Excellent linearity and reproducibility can be obtained for all positive chemicals using ContamSPOT colorimetric assay. Although ContamSPOT is not selective to each tested chemical, their different performances can provide clues for identification. For example, the TCEP spot was significantly wider than other chemicals. Salts like AmBC, TEAB, and Tris showed pink color in the ethyl acetate layer during extraction, but the spot returned to blue after the TLC plate was air-dried. FA can be used to quench protein digestion instead of TFA in proteomics steps to avoid the interference of TFA in ContamSPOT assay. In a real experiment, researchers have prior knowledge of what chemicals were used in sample preparation steps. The sample should have been cleaned up to remove the majority of contaminants before the ContamSPOT assay. Detection limits for salts were also relatively high (mM range). Therefore, this assay is mostly useful to detect and quantify trace levels of SDS in the sample with an extremely high sensitivity (LOD of 0.0004%; Table 1).

Expanding ContamSPOT with a Fluorometric Assay for Nonionic Detergent Contaminants. ContamSPOT colorimetric assay is limited to ionic species that can form an ion pair with the OTB dye and be extracted into ethyl acetate. To expand the applicability of this assay, particularly for nonionic detergents, such as Triton, Tween, and NP-40, we expanded the ContamSPOT method with a fluorometric assay. We adopted the A43 dye from the ProFoldin Kit for nonionic detergent spectroscopy analysis. As shown in Figure 2A, the

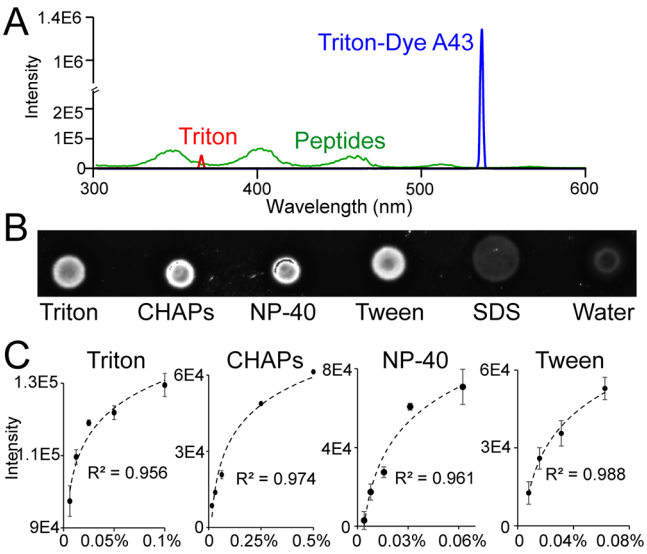

Figure 2. Development of the ContamSPOT fluorometric assay. (A) Fluorescence spectra of 0.1% Triton, 0.1% Triton-Dye A43 complex, and peptides from BSA digest. (B) Contaminant screening of common detergents used in lysis buffer; 1% concentration was used for each detergent. (C) Nonlinear calibration curves for positively tested detergents in ContamSPOT fluorometric assay.

Triton-Dye complex exhibited shifted fluorescence wavelength away from Triton standard and peptide signals (BSA digest) with a more than 10-fold increase of signal intensity compared to Triton alone. We tested various detergents by mixing 1 μL of detergent standard with ProFoldin A43 dye, spotting 2 μL of the mixture on a TLC plate, and imaging it under a fluorescence imager. As expected, nonionic detergents Triton, CHAPS, NP-40, and Tween showed positive signals, while ionic detergents SDS and water had no signal (Figure 2B). Then, we established calibration curves for all positive detergents (Figure 2C). Fluorescence signals saturated at high concentrations, leading to nonlinear calibration curves in various fluorescence emission times and concentration ranges that we tested (Figure S1). Therefore, a logarithmic function was used for the curve fitting. Excellent reproducibility and sensitivity were achieved by ContamSPOT fluorometric assay with 0.002% LOD for Triton

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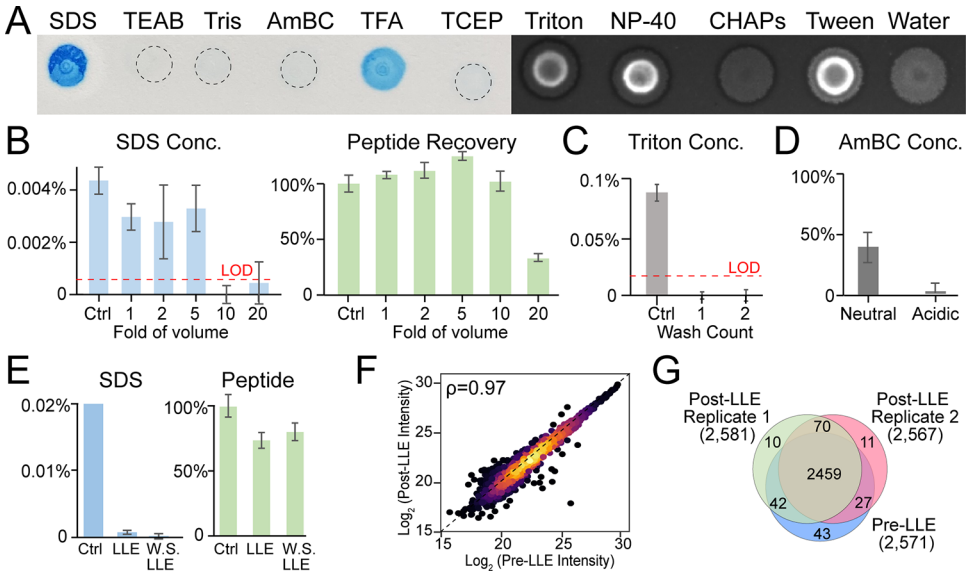

Figure 3. Optimization of the ethyl acetate LLE method to remove contaminants. (A) ContamSPOT colorimetric (left) and fluorescence (right) assay screening of the extractants from ethyl acetate LLE. (B) SDS concentrations and peptide recoveries using different amount of ethyl acetate (fold of volume to samples) with three wash cycles. (C) Triton concentration after multiple wash cycles with 10-fold ethyl acetate volume. (D) Drying down under acidic condition improved the removal of volatile salt ammonium bicarbonate. (E) SDS concentrations and peptide recoveries after pure ethyl acetate LLE and water saturated (W.S.) ethyl acetate. (F) Scatter plot showing peptide intensity correlation before and after LLE cleanup. (G) Venn diagram of number of quantified proteins before and after LLE cleanup with two technical replicates.

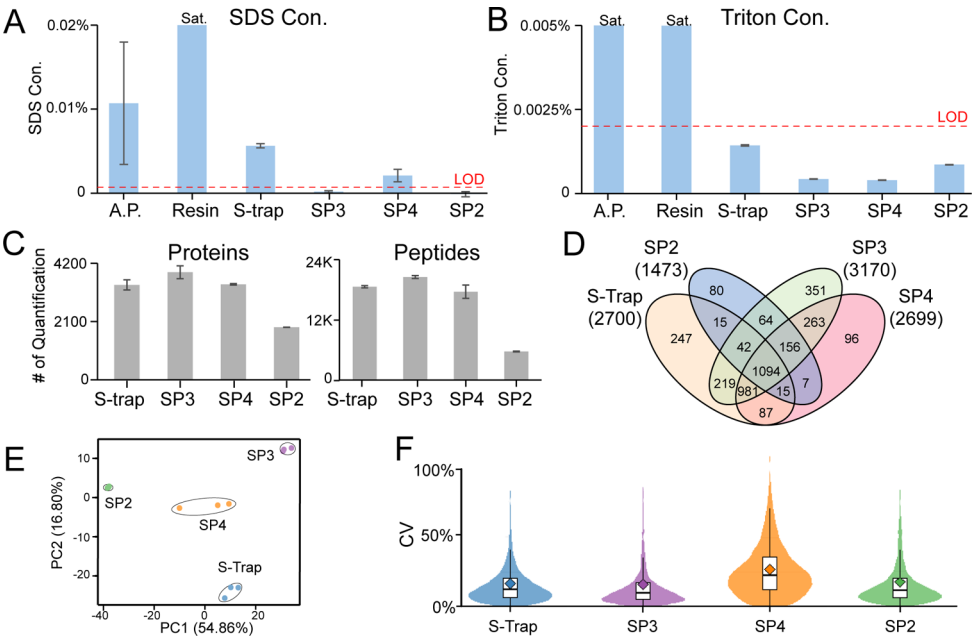

Figure 4. Applying ContamSPOT assays to compare different protein and peptide sample preparation methods. (A) ContamSPOT colorimetric measurement of SDS concentrations after various sample preparation methods. Sat. indicates saturated signal. (B) ContamSPOT fluorescence measurement of Triton concentrations. (C) Number of quantified proteins and peptides from different methods. (D) Venn diagram of reproducibly quantified proteins in different methods. (E) Principal component analysis of different methods with three replicates. (F) Violin plot showing the coefficient of variance of different methods.

and 0.02% LODs for Tween, NP-40, and CHAPS. In summary, we have tested a total of 22 commonly used chemicals in MS workflow, 11 of which showed positive results in ContamSPOT colorimetric or fluorometric assay, summarized in Table 1.

Optimizing Contaminant Removal Methods Prior to LC-MS Analysis. Our ContamSPOT colorimetric and fluorometric assays enabled fast detection and quantification of various contaminants prior to MS analysis. If ContamSPOT showed a positive result, we need to further clean up the sample to remove contaminants prior to MS injection. LLE using ethyl acetate has been successfully used to remove detergents such as SDS, NP-40, Triton, OG, and DM from proteomics samples.[22,23] Here, we further optimized the extraction experiment and screened various contaminants enabled by our ContamSPOT colorimetric and fluorometric assays. As shown in Figure 3A, SDS, Triton, NP-40, and Tween can be extracted

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by ethyl acetate from water solution. TFA can also be extracted by ethyl acetate. However, CHAPS and other salts cannot be removed by LLE. We then optimized the LLE conditions for sufficient removal of these contaminants with minimized sample loss (Figure 3B). ContamSPOT assay and Pierce Peptide Colorimetric Assay were used as readouts for contaminant and peptide concentrations. A 10-fold volume of ethyl acetate can effectively remove trace amounts of SDS after three ethyl acetate wash cycles with a 99% peptide recovery. If 5-fold volume is used, eight washes are needed to completely remove SDS with minimal sample loss (Figure S2). Nonionic detergents such as Triton showed a high affinity to ethyl acetate and were undetectable after a single wash (Figure 3C). Because ethyl acetate can dissolve up to 3% water and concentrate the peptide sample during LLE, peptide recovery showed over 100% in some samples. To minimize water/peptide loss during LLE, we can use water-saturated ethyl acetate for LLE (Figure 3E).

To further evaluate the influence of ethyl acetate LLE on LC-MS results, we analyzed cleaned HEK peptides before and after LLE with two technical replicates. Peptide abundances showed excellent linear correlation before and after LLE with minimal sample loss and excellent LLE reproducibility (Figure 3F, G). The ethyl acetate extractant showed no peptide signals in LC-MS (Figure S3). We did notice that ACS grade ethyl acetate extractant contains several singly charged contaminant peaks compared to HPLC grade ethyl acetate and therefore suggest using the HPLC grade ethyl acetate for the LLE experiment. To summarize, ethyl acetate LLE can be used as a rescue method for detergent-contaminated samples without influencing peptide recovery or LC-MS analysis. Volatile salts can be removed by drying down, and we found that drying down under acidic conditions can dramatically improve the removal of AmBC from samples (Figure 3D). Other nonvolatile salts must be removed by reverse phase C18 or HLB desalting cartridges. The methods to remove different types of contaminants are summarized in Table 1.

Evaluating Different Proteomic Sample Preparation Methods Using ContamSPOT Assay. Various proteomic methods have been developed in recent years to prepare detergent-containing samples and remove various contaminants prior to MS analysis.[13−17] However, the efficiency of removing detergents has not been comparatively evaluated due to the lack of a sensitive contaminant assay. Here, we applied our ContamSPOT assay to compare various bottom-up proteomics sample preparation methods: S-Trap,[14] SP3,[17] SP4,[17] and acetone precipitation,[24] as well as peptide cleanup methods: SP2,[16] detergent removal resin, and desalting plate. HEK protein lysate containing 2% SDS and 1% Triton was used as the starting material. ContamSPOT, the peptide colorimetric assay, and LC-MS were used as readouts. As shown in Figure 4A,B, acetone precipitation and detergent removal resin cannot sufficiently remove detergents from the sample. Beads-based sample preparation (SP3, SP4, SP2) provided more sufficient detergent removal compared to trap-resin-based spin columns (S-Trap). The detergent amount after S-Trap was below 0.01% and did not significantly influence peptide/protein identifications.[9] But we recommend using five or more washing times (the manufacturer recommends 3 times) for S-Trap to sufficiently remove detergents. SP3 provided the best overall detergent removal and peptide recovery among all methods (Figure 4C, D). SP2 cleanup sufficiently removed detergents at the peptide level but had a significant sample loss. Principal component analysis showed sample clustering based on the sample preparation methods. S-trap, SP3, and SP2 showed better reproducibility compared to the SP4 method (Figures 4E, F, and S3). However, SP4 uses glass beads and is much cheaper compared to magnetic beads or S-Trap. We also found that even a trace amount of detergent could wash away packing materials from the reverse phase (C18 or HLB) desalting column, causing serious polymer contaminations in LC-MS. Therefore, if detergents were used in the lysis buffer, ethyl acetate cleanup can be conducted as a routine step before desalting to avoid polymer contamination from desalting columns.

Practical User Guide for ContamSPOT Assay. Given the merits of our ContamSPOT assay (sensitive, rapid, simple, low sample input, and low cost), we anticipate that ContamSPOT can be a very useful method for the MS community. To assist the use of ContamSPOT in other laboratories, we provided a practical decision tree (Figure 5) and a step-by-step protocol

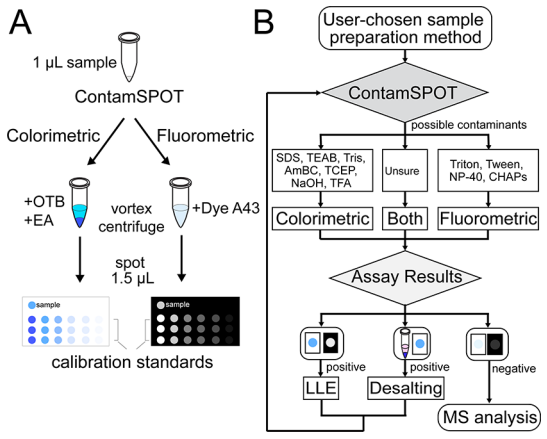

Figure 5. A practical user guide for ContamSPOT. (A) Schematic workflow for ContamSPOT colorimetric and fluorometric assay. (B) Decision tree to implement ContamSPOT to detect, quantify, and remove contaminants prior to MS analysis.

with a troubleshooting guide (Supporting Information) for users to incorporate ContamSPOT into their routine sample preparation workflow. ContamSPOT should be performed after routine protein digestion and peptide cleanup steps. Researchers would have prior knowledge of possible contaminations in the sample based on the buffers and experimental steps, which can be used to select ContamSPOT colorimetric or fluorometric assay. ContamSPOT colorimetric assay should be used if there are potential ionic contaminations, such as SDS, TEAB, Tris, AmBC, etc. ContamSPOT fluorometric assay should be used if potential nonionic contaminations exist, such as Triton, Tween, NP-40, CHAPS, etc. If both ionic and nonionic reagents were used during sample preparation, we recommend doing colorimetric assay first and then fluorometric assay, which only consumes 1−2 μL of the sample. Our ContamSPOT assay can be used for both quantitative and qualitative analyses. A quick qualitative check of sample contamination can use as little as 0.5 μL of the sample. However, accurate quantification of contaminant concentrations would require at least 1 μL of sample to reduce pipetting variability.

If ContamSPOT showed a negative result (i.e., no blue color on the TLC plate in colorimetric assay and/or no signal in fluorometric assay), the samples could safely proceed to MS injection. If ContamSPOT showed a positive result, further cleanup of the sample will be necessary. Interestingly, the

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colorimetric assay could distinguish when there are salt contaminations by showing a pink color in the ethyl acetate layer before spotting. We have demonstrated that ethyl acetate cleanup can rapidly and effectively remove detergents such as SDS, Triton, NP-40, and Tween. However, it is worth noting that ethyl acetate extraction cannot remove CHAP and salts. While volatile salts (e.g., TEAB and AmBC) could be removed by drying down, nonvolatile salts (e.g., Tris) will require additional desalting steps. For samples that use detergent in the workflow, water-saturated ethyl acetate extraction can be included as a routine cleanup step to remove possible detergent contamination and then tested by ContamSPOT. However, any additional sample preparation step can introduce possible sample loss and variation and, therefore, should be conducted with caution. Ethyl acetate cleanup is best used for hydrophilic analytes to avoid sample loss into the ethyl acetate layer. We recommend first conducting qualitative ContamSPOT colorimetric/fluorometric test (which takes 1 μL of the sample within a few minutes) to decide on further cleanup steps (Figure 5). The cleaned samples can be tested again with ContamSPOT and proceeded to MS analysis when negative results are obtained. ContamSPOT colorimetric and fluorometric assays could also be used to test detergent contamination from various LC-MS experiments for lipid, metabolite, and intact protein analyses (we did not test these experiments).

CONCLUSIONS

In this study, we developed a sensitive, rapid, and cost-effective method to quantify and remove contaminants prior to MS analysis, namely, ContamSPOT. We developed both colorimetric and fluorometric assays to detect a range of ionic and nonionic detergents, salts, and chemicals that are commonly used in MS workflow. We tested a total of 22 commonly used chemicals, 11 of which can be quantified by ContamSPOT. Particularly, trace amounts of detergents (e.g., 0.0004% SDS, 0.002% Triton) can be detected at nanogram levels to prevent damage to the chromatography and MS instruments. Although ContamSPOT is not selective to each positively tested chemical, we provided performance characteristics that can provide clues for identification. A simple Yes or No answer is sufficient to decide whether the samples are clean enough for MS analysis or should go through additional cleanup steps. ContamSPOT is also capable of high-throughput sample processing with a multichannel pipet or a liquid handler. Enabled by ContamSPOT assay as the readout, we optimized ethyl acetate LLE to rapidly remove trace amounts of detergents from peptide samples with minimal sample loss. We further applied ContamSPOT to compare various proteomic sample preparation workflows, regarding effectiveness to remove contamination, peptide recovery, and reproducibility. We provided a practical guide and a decision tree to help researchers implement ContamSPOT as a rapid quality checkpoint before MS injection. ContamSPOT assay can also be easily adopted when developing new sample preparation methods as a unique readout for sample cleanliness.

ASSOCIATED CONTENT

Data Availability Statement

All MS raw files are publicly available on MassIVE Repository with the identifier MSV000093387. Other data are available in the main text and Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c05020.

Supplemental figures: effect of emission time of the fluorescence imager on the ContamSPOT fluorometric assay; evaluation of ethyl acetate cleanup efficiency with 5-fold ethyl acetate volume; LC-MS base peak chromatograms of HEK protein digest before and after ethyl acetate cleanup; and peptide level Spearman correlation heat map across different sample preparation methods with three replicates (PDF)

Step-by-step ContamSPOT protocol: introduction of the method; ContamSpot colorimetric assay protocol; ContamSPOT fluorometric assay protocol; ImageJ analysis; ethyl acetate liquid-liquid extraction; troubleshooting guide; and list of reagents (PDF)

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Author Contributions

N.S. and L.H. designed the study. N.S., K.B., and W.M. conducted the experiments. N.S. and H.L. analyzed the data. ContamSPOT assay was tested by all Hao Lab members. N.S., H.L., W.M., and L.H. wrote the manuscript with input from all coauthors.

Notes

The authors declare no competing financial interest.

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