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Using Procedural Blanks to Generate Analyte-Specific Limits of Detection for Persistent Organic Pollutants Based on GC-MS Analysis

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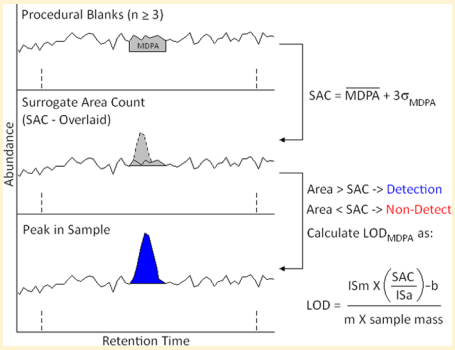
Supporting Information

ABSTRACT: Several methods are used to generate a limit of detection for organic pollutants measured by gas chromatography−mass spectroscopy (GC-MS); all have theoretical and practical drawbacks. The current project investigated two common existing methods (statistical and empirical) for applicability to chromatographic properties from real samples, comparing these with a new proposed method using procedural blanks to estimate a minimum detectable peak area. Weaknesses of all three methods are discussed. The proposed method was superior to other examined methods in that it provided analyte-specific limits of detection linked to the recovery of mass-labeled internal standards for every analyte within every sample. Other identified quality assurance benefits included the following: enhanced protection against false positives; providing a sensitivity performance metric across batch, analyst, and instrument; enabling chemists with discretionary decisions specific to every analyte regarding detectability and interferences; and some strengths of both statistical and empirical techniques without major drawbacks of either. In marine sediment samples, the proposed method of calculating the limit of detection increased reporting of trace level (low- to subppb) GC-MS data for polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) by up to 400% compared with the statistical method.



Conceptually, the term "limit of detection" (LOD) is straightforward in organic metrology, but implementation of the concept has often been confusing in the literature and has not always evolved side-by-side with technology. Paradigms regarding this concept have been maintained even while methodology has changed radically (e.g., external vs internal standard, extraction methods, electronics advances, etc.). Terms, both linguistic and mathematical, are often interchanged, poorly defined, or even unrecognizable from paper to paper.[1−6] In past work done by the NOAA Chemical Contaminants Research lab (CCR) based at Hollings Marine Laboratory in Charleston, SC, many organic contaminants at the trace level for which chemists were confident of both identification and quantitation were being censored by LODs using regression statistics. The current project investigated methods of calculating LODs for organic contaminants in environmental samples routinely measured by gas chromatography−mass spectrometry (GC-MS). Analytes of interest included polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs). The goal was to remove some of the difficulties inherent in applying statistically estimated LODs (the previous practice) and replace them with sample-specific LODs based on real observations.

Regardless of the method used to estimate the LOD, three important concepts are associated with the point at which instrumental signal can be distinguished from "nothing": the instrumental detection limit (IDL), the method detection limit (MDL), and interferences that increase these limits. For the purposes of the present study, the IDL is defined as the minimum analyte mass at which a chromatographic peak is distinguishable from background noise given no processing- or sample-derived interferences, and it is the most basic factor affecting the LOD; it is derived using calibration points where the mass fraction of analyte to internal standard (IS) is iteratively reduced until the signal cannot be distinguished from background. The MDL is defined as the mass fraction at which a chromatographic peak can be distinguished from background noise imparted by the entire analytical method; this translates the IDL to the extraction method, including aspects such as process effects and sample

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mass extracted. Finally, the LOD was defined as the sample-specific mass fraction which can be distinguished from background noise, accounting both for sample mass and recovery of IS. The statistical confidence necessary to distinguish a peak from background is typically set by EPA guidance at 99%.[7] With current generation IS methods in which IS are added at the beginning of sample processing, accuracy is assured with high confidence to very low recovery levels, but the signal generated by any given mass fraction drops commensurately with recovery; this effect was ignored by virtually all LOD calculations reviewed for the current project.

Likely the most influential paper on the limit of analytical detection was written by Lloyd Currie.[8] Based on theoretical Gaussian distributions about zero and a minimally discernible instrument signal, that paper defined statistical levels (the critical level (Lc) and detection level (Ld)) at which there was confidence that data were neither false negatives nor false positives. Hubaux and Vos[9] extended Currie's concepts to the confidence intervals about an external standard calibration curve; at the time, this was the state of the science. This scheme, or an evolution thereof, became commonly used because of its relative simplicity. Several theoretical and practical problems exist with application of the Hubaux and Vos (HV) scheme to current generation methods, but researchers struggled to overcome the inertia behind theoretically and practically troublesome calibration-based detection limits.[3,10−14] Calculation of a HV-type LOD traditionally employs the entire calibration curve; mathematically, this becomes weighted toward greater response, somewhat mitigated by $1/x$ curve weighting or curve "clipping" where only a portion of the curve applicable to response observed in samples is used. Hubaux and Vos[9] stated directly that the highest calibration point used should be between 10 and 20 times the lowest calibration point. This is rarely the case in environmental analyses when expected concentration ranges are unknown and a large calibration range is necessary. Voigtman[13] demonstrated that even when the entire calibration curve falls within an order of magnitude of the LOD, the false-negative rate of HV LODs is much greater than $\beta = 0.05$ (the EPA recommended limit for false negatives)[7] as a result of the confidence interval about the linear regression. This in large part is due to violating the assumption of homoscedasticity in instrument response between the low and high portions of the curve; Currie[3] explained how instrument response is a heteroscedastic function of mass, invalidating the use of linear regression at low levels. Needleman and Romberg[15] criticized statistical methods as providing only the "ability to measure nothing" rather than very low analyte mass. Overprotection against false-positive results in a false-negative rate of closer to 50% for samples near the LOD in schemes not representing both a Currie style Lc and Ld.[10,14] Inaccurate estimation of the LOD decreases reliability for the sensitivity and selectivity of data points. This decreased reliability extends then through statistical assessments of environmental data sets when nondetects are present, whether those assessments include substituting values (e.g., 1/2 DL) in classical tests, or in alternative tests such as those recommended by Helsel.[16]

Several authors have therefore cited the need to validate the accuracy and precision of statistically generated LODs using spiked samples.[7,17−21] If a statistical LOD must be experimentally verified, why not use real injections of a known, low mass to generate the LOD in the first place? Efforts have been made along these lines to standardize LODs as the return of a very low-mass calibration or spike point.[7,18] This has the advantage of providing a "ground truth" to what mass can be measured reliably but becomes time-consuming as method sensitivity is pushed closer to the IDL. Many laboratories follow the procedure outlined in USEPA Title 40CFR136 Appendix B.[7] This method allows high-throughput laboratories to standardize the MDL of any given method, and though robust, the method suffers from theoretical and practical burdens. Theoretically, this process does not account for variability between extraction batches and assumes homoscedasticity at various spiking levels.[22] Practically, this method requires several iterative low-level spikes after estimation of the MDL range, setting the MDL at the time the method is established. Any change thereafter to the method, or in the presence of matrix effects, requires reestablishment and/or verification of the MDL; indeed any significant batch-to-batch variability in observed matrix interferences would seem to require reverification of the MDL unless false negatives were ignored entirely in favor of extremely conservative false-positive rates. This represents a meaningful investment both in time and material. This investment may be cost-effective for high-throughput laboratories able to default to a regulatory limit higher than what could be measured (in which case it is not then by definition a detection limit but a reporting limit). It places added burden, however, on laboratories conducting smaller projects or pushing method sensitivity to the limits allowed by instrumentation. A report by the Federal Advisory Committee[23] recommended that the EPA modify the Appendix B method by incorporating batch-specific verification as one option. That report listed 15 objectives associated with detection and quantification limits and increased the number of samples required to meet regulatory approval. The following are the major measurement quality objectives which were recommended: 20% relative standard deviation, Type I error tolerance set at or below 1%, and 50−150% mean recovery. Recovery of IS (and related analytes) from the extraction process and its impact on detectability, however, is ignored.

Taking these considerations into account, the current project sought a method of calculating LODs for environmental samples that (1) reflected instrument sensitivity and performance over time; (2) reflected extraction performance over time, method, matrix, and analyst; (3) accounted for observed recoveries; (4) leveraged existing quality assurance practices; and (5) met statistical rigor.

Process blanks (i.e., reagent blanks carried through the entire extraction procedure) were identified as the most likely candidate to meet these criteria; matrix spikes were identified for verification of blank-based detection limit calculations. True matrix blanks would give ideal results as they incorporate matrix effects, but identifying sources for all possible matrices verifiably lacking all potential target analytes is troublesome to say the least. Generation of LODs from process blanks is supported in the literature,[3,11,13,14,24,25] often as a method of accounting for interferences with the mass fraction in process blanks being compared with the low calibration point and the greater of the two being used. Estimates of the LOD from blanks can then be verified by low level spikes. Some processes begin with the signal in blanks and add to it the standard deviation from replicates of a verification sample at a known low concentration,[11] but this makes assumptions regarding homoscedasticity of response between the blank, verification sample, and real samples. It additionally requires identification of the IDL and several replicate measurements.

Rather than focusing on iterative reduction of spike mass or the mass estimated in process blanks, signal noise in blanks subjected to the extraction method can provide an estimate of the

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area count which would result from a measurable peak. This is similar in concept to the classic signal/noise (S/N) ratio approach. Many aspects of the S/N approach were unsatisfying when examined; failure to account for the risk of false negatives[3] and nullification of analyst discretion given confirmation ions and IS retention offsets[19] made it difficult to consider in this context. Additionally, signal from very low mass on GC/MS platforms does not result in a Gaussian distribution as it is necessarily constrained by positive baseline signal at "zero," weakening the value of statistical LOD estimation. An area count estimated from observed noise then, combined with the recovery of IS in each sample, could also correct for sample-to-sample variation in the true detection limit while simultaneously adjusting the reported LOD to include batch-to-batch variability. Enhanced protection against false positives is also provided as chromatographic noise imparted by the sample preparation process and elution program is captured by process blanks. It is this estimation, of an area count that would result from a quantifiable peak given the baseline response characteristics of batch-specific process blanks, which became the focus of the current project. Theoretically, this approach is more similar to Currie's Lc than Ld. The proposed method somewhat blends the two concepts in allowing for chemist discretion in interpretation of baseline characteristics as guiding the target of peak area which the chemist would not reject as noise. As instrumentation varies from application to application, this property is worth investigating for each application. A robust approach such as that presented here may potentially be applied with minimal adjustment as fundamentals of the concept are preserved.

EXPERIMENTAL SECTION

Extraction and Cleanup. Purified analytical standards were purchased from AccuStandard (New Haven, CT, U.S.A.), Cambridge Isotope Laboratories (Tewksbury, MA, U.S.A.), and O2Si (Charleston, SC, U.S.A.) and volumetrically diluted to target stock concentrations. Calibration points (n = 13) including isotope-labeled internal standards (listed below) were created in 0.5 mL n-hexane targeting analyte mass from 0.01 ng to 5000 ng (point series were specific to target analyte class) in a geometric fashion. Calibration curves were built using mass- and area-ratios of target analyte to internal standard. The IDL was defined as the lowest of any three consecutive calibration points meeting sufficient linearity (r^2 > 0.99) and slope ($m \gg 0.00$). Sediment sample aliquots (~10 g) from a variety of past projects targeting PCBs, PBDEs, PAHs, and OCPs were homogenized in ~27 g sodium sulfate using ground glass mortars and pestles; all aliquots were weighed to the nearest 0.01 mg. A second aliquot (~5 g) from each sample was dried overnight at 90 °C to determine dry mass fraction. Samples were amended prior to extraction with a total of 150 uL of ^{13}C- or ^{2}H-labeled IS stock solutions in hexane (Cambridge Isotope Laboratories, Andover, MA, U.S.A. and Wellington Laboratories, Ontario, Canada; listed in Table SI1).

Samples (n = 34) were extracted by pressurized solvent extraction on an ASE 200 (Dionex Corporation, Sunnyvale CA, U.S.A.), using 50:50 v/v dichloromethane/acetone at 100 °C and 2000 psi (supplied by purified nitrogen gas at 130 psi) with three 5 min heating/5 min static cycles using 50% flush volume and a purge time of 60 s. Residual water was removed from extracts by filtration through a sodium sulfate bed. Gel permeation chromatography removed the majority of interferences with a 78.5 min elution through a 600 mm × 37.5 mm i.d. SX-3 Biobead column (BioRad, Hercules, CA, U.S.A.) using 100% dichloromethane at 5 mL/min and collection from 34.8 to 56.7 min. Extracts were concentrated to 1 mL with purified nitrogen gas using a TurboVap II Concentration Workstation (Zymark, Hopkinton MA, U.S.A.) and quantitatively transferred to automated sampler vials (ASVs) to be cleaned with 1.8 g of alumina (5% deactivation by water, fired overnight at 550 °C and gradually cooled in a desiccator) on a RapidTrace SPE Workstation (Zymark); cartridges were conditioned with 11 mL of hexane and 3 mL of dichloromethane. ASVs were rinsed with 1 mL of hexane and eluted with 9 mL of 65:35 v/v hexane/dichloromethane. Extracts were concentrated to 0.5 mL, solvent exchanged three times to hexane, concentrated to 0.25 mL, and quantitatively transferred into ASVs with a final target volume of 0.5 mL. Final extracts were amended with 20 uL each of two recovery standard solutions in hexane yielding 50 ng of d14−1,4-diphenylbenzene and 20 ng of delta-benzenehexachloride.

Process blanks of sodium sulfate were amended likewise and extracted for each batch exactly as samples. Three accuracy control samples were processed exactly as samples in each batch: 0.5 g of NIST (Gaithersburg, MD, U.S.A.) Standard Reference Material (SRM) 1944 "New York/New Jersey Waterway Sediment" and two additional aliquots of a randomly assigned sample were used as a matrix spike and matrix spike duplicate. Two spiking solutions containing all target analytes in hexane (total of 315 uL) were added to each spike, targeting 8 ng of PBDEs, 10 ng of PCBs and OCPs, and 200 ng of PAHs. All glassware was rinsed prior to use three times each using acetone, dichloromethane, and hexane. Sodium sulfate was baked overnight at 550 °C prior to use. All solvents were high-purity GC-grade, purchased from Burdick and Jackson or J.T. Baker.

Instrumentation. Extracts and calibrants were vortexed for ≥5 s and injected onto two Agilent 6890N gas chromatographs (Agilent Technologies, Santa Clara, CA, U.S.A.) equipped with Gerstel MPS2 autosampler units (Mülheim, Germany) connected to Agilent 5873 mass spectrometers and a Varian 4000 gas chromatograph equipped with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) connected to a Varian 4000 ion trap mass spectrometer. A total of 147 analytes were investigated for which detection methods were previously optimized (Table S1). A 25 uL injection with programmable temperature vaporization (PTV) inlet onto an Agilent DB5-MS 30 m × 0.25 mm × 0.25 μm column was used with an electron impact (EI) source to separate and detect 84 PCBs, 13 PBDEs, and 6 DDTs. A 25 uL injection with PTV inlet onto a Restek Rtx-5MS 30 m × 0.25 mm × 0.25 μm column was used with a negative chemical ionization (NCI) source to separate and detect 19 OCPs. A 2 uL injection onto a Varian VG-Xms 30 m × 0.25 mm × 0.25 μm column was used to separate 25 PAHs with an EI source. Carrier gas was helium at 1 mL/min (8.8 psi) for all Agilent instruments and 2.2 mL/min (18.0 psi) for the Varian. Software for instrument control and data collection/processing were MSD ChemStation E.02.00.493 and Varian Workstation 6.9.2. Analytes were quantified by area- and mass-ratio calibration curves with appropriate internal standards using selected ion monitoring with positive identification by retention time (RT) and ion ratios established from calibration standards.

Three approaches were used to calculate LODs for comparison: an HV-style regression modified for application to the area- and mass-ratio IS approach, the IDL, and the proposed minimum detectable peak area (MDPA) method. All were adjusted postcalculation for sample mass. All LODs from this point will be referred to by their method of calculation: LOD_{HV}

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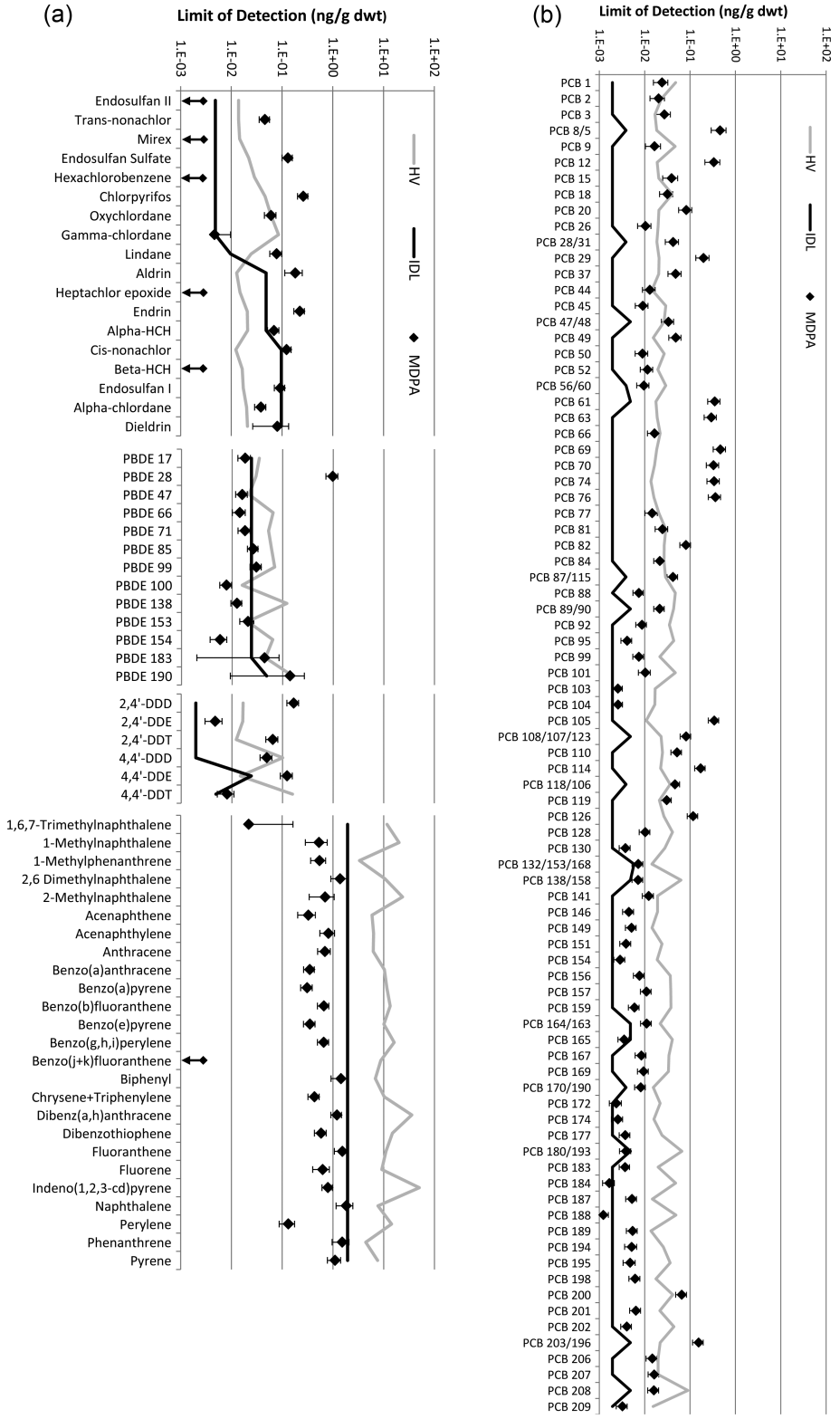

Figure 1. Limits of detection calculated by modified Hubaux–Vos regression (HV), instrumental detection limits (IDL), and minimum detectable peak area (MDPA) for (a) organochlorine pesticides, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and (b) polychlorinated biphenyls that are part of the standard suite of analytes measured by CCR.

was derived from regression, LOD_{IDL} from the IDL, and LOD_{MDPA} from the blank-based approach under investigation.

Integration of Blanks. A total of seven process blanks were used; blanks chosen represented four extraction batches processed by three different chemists. Blanks were first assessed for acceptable batch quality (e.g., contamination, baseline stability, acceptable noise, etc.). To generate an area count corresponding to the theoretical MDPA, data files for blanks were duplicated prior to integration and chromatographic properties (e.g., noise, process interferents, etc.) guided manual

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integration of an area below the response baseline giving rise to an area count corresponding to a S/N ratio of approximately three. Integrations were centered on the expected RT and covered a width approximately equal to the width of corresponding peaks from well-defined low calibration points. With very noisy quantitation baselines, confirmation traces (if clean) were used to approximate an area that would be integrated by ion-ratio matching. Examples have been provided as Supporting Information (Figure SI1). Area counts were then saved to the data file for each analyte as if that area were a peak. Any ion-ratio and retention time verified peaks present were integrated normally. Every blank was assessed in this manner by three separate chemists to assess potential variability in MDPA from human bias.

Data Post-Processing. A custom macro was developed for MS Excel 2010 using Visual Basic for Applications (VBA) which processed raw instrument output and generated LOD_{MDPA}. Specifically, a form running VBA code was used to prompt to the user to select software reports (exported as either .csv or .txt files) for blanks only, the entire batch, and the calibration parameters. This could be replicated with code modifications to accept any report with a known format; any report containing the MDPA values and the analytes to which they apply will suffice. A surrogate area count for each analyte, equal to the mean MDPA in blanks plus three standard deviations, was calculated in VBA. This surrogate area count was then programmatically applied with sample- and analyte-specific IS peak area counts to calculate the lowest detectable mass for each analyte in each sample, automatically adjusting for recovery effects. The surrogate area count for a given analyte was thereby treated as if it were the peak area for that analyte in all samples, and the mass corresponding to the LOD_{MDPA} calculated by the IS area/mass ratio approach (eq 1); the macro was used only to simplify and automate the process, but this could be accomplished in a spreadsheet as well. The LOD_{MDPA} was compared with the lowest detectable mass from the calibration curve. Using the greater of the two provided a "reality check" that the final LOD fell within the calibration curve. Finally, this was divided by the extracted mass of the sample during reporting to generate an analyte- and sample-specific LOD. (A graphical representation of the approach and examples of the calculations are provided in Supporting Information [Figure SI2].)

$$\mathrm{LOD}_{\mathrm{MDPA}_{ji}} = \frac{\mathrm{ISm}_{ji} \times \left(\frac{\mathrm{SAC}_i}{\mathrm{ISa}_{ji}} - b\right)}{m} \quad (1)$$

Where:

j = sample

i = analyte

ISa = corresponding internal standard area

ISm = corresponding internal standard mass

$\mathrm{SAC}_i = \overline{\mathrm{MDPA}_i} + 3\sigma_{\mathrm{MDPA}_i}$

m and b = calibration slope and intercept, respectively

LOD as a Function of Recovery. To demonstrate the response of the proposed LOD scheme and investigate accuracy across decreasing recoveries, the above-mentioned matrix-spike and IS amounts were added to six ASVs containing analytical-grade *n*-hexane to a final volume of 0.5 mL. Three of these were iteratively diluted by half with 0.5 mL *n*-hexane resulting in a total of seven points in triplicate targeting simulated recoveries from 100% to approximately 1%. After dilution, the same recovery standard masses as above were added to each ASV.

RESULTS AND DISCUSSION

Limits of Detection. The LOD_{IDL} fell well below LOD_{HV} for the vast majority (134 of 147) of analytes with LOD_{IDL} being on average 19 ± 25% of LOD_{HV} excluding OCPs which were highly analyte-specific (Figure 1; Table SI2). For 10 of the 19 OCPs measured by NCI, LOD_{IDL} was on average 25 ± 14% of LOD_{HV}. The remaining nine compounds were highly variable at 452 ± 182% of LOD_{HV}, which is due largely to calibration curves truncated at the low end when response ratios became negligible or nonlinear. This demonstrated that regression can be inapplicable to certain situations; regressions with very tight confidence intervals may predict a LOD below the mass at which any signal is verifiably present. This information was still somewhat valuable in that it indicated calibration curves could be adjusted if an increase in sensitivity was desirable. Often this is overlooked if calibration sensitivity meets project objectives. Refinement still relies on iterative measurements near the predicted LOD. Regardless, as neither approach considers chromatographic characteristics, the near order of magnitude difference between LOD_{HV} and LOD_{IDL} indicated an overly conservative estimation of instrument sensitivity for most analytes when using a regression-derived LOD. A large drawback of directly using LOD_{IDL} is that it does not account for process interferences; extracting the calibration curve alongside each batch of samples would address this but effectively increases the number of samples taken through all extraction and instrument steps by the number of calibration points.

Theoretically, if IDLs are established as sensitively as instrumentation allows, LOD_{MDPA} should be consistently greater than LOD_{IDL} due to less-than-ideal recovery and processing influences. As LOD_{IDL} is based on the mass fraction in the lowest calibration point for all analytes, LOD_{MDPA} should be more variable, reflecting changes in chromatographic properties through the elution; analytes toward the beginning and end of a chromatographic run, or in regions where several analytes elute in close proximity, typically have poorer chromatographic properties than those between. This was generally the case for all analyte classes except PAHs and, as would be expected, was analyte dependent (Figure 1, Table S1). For PAHs, LOD_{MDPA} was 37 ± 27% of LOD_{IDL} for 25 of 25 analytes, indicating the calibration curve should be extended to lower levels.

Perhaps the best example of this approach's utility is the case of PCBs (Figure 1), which has areas of known interference with this analytical method where quantification at the trace level can become troublesome. For PCBs, LOD_{IDL} was 10 ± 6% of LOD_{HV} for all 84 congeners measured, indicating consistent performance of both LOD methods without consideration of process interferences. In chromatographic regions known to suffer from increased noise (e.g., PCBs 61−76) or analytes with known interference (e.g., PCBs 8/5, 12, 105), LOD_{MDPA} was far greater than LOD_{IDL} (~20× on average), reflecting the impact of noise on detectability in these regions. The opposite case was true for regions with very low noise from the same chromatograms.

In some cases, predominantly that of OCPs, the LOD_{MDPA} estimation resulted in a negative mass estimation. This was due to positive interference at the low end of the calibration curve. Although this would appear to be a drawback to the method, it provided meaningful information about the performance of the method at the trace level; specifically, this indicates that low level calibration points are being enhanced in some manner (e.g., potentially from incompletely labeled IS). If curves are forced

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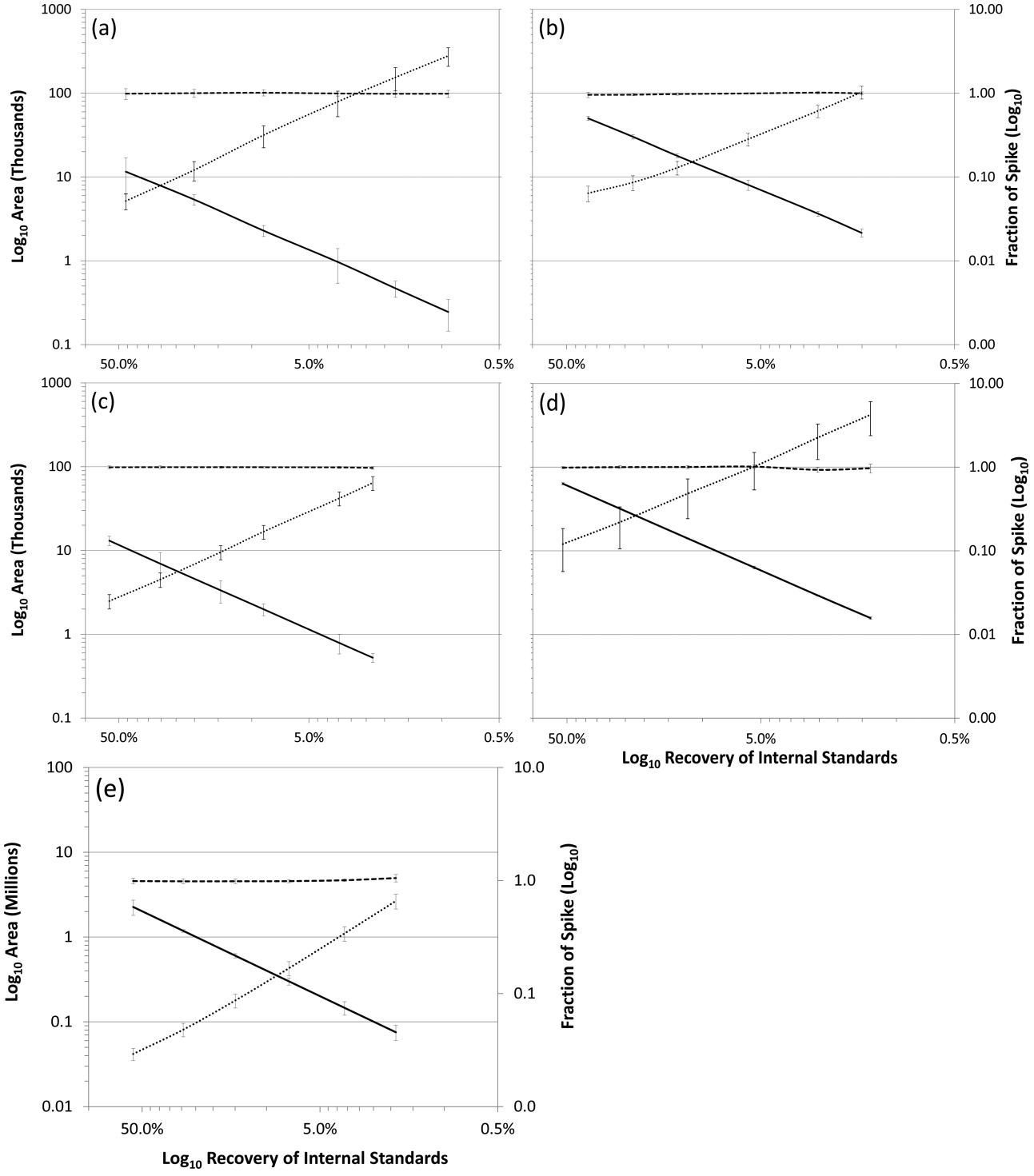

Figure 2. Mean area response of internal standards (left axis, solid line —), mean spike accuracy (right axis, dashed line --), and the limit of detection (by minimum detectable peak area, right axis, dotted line ····) across decreasing recovery for the following compounds: (a) polybrominated diphenyl ethers; (b) organochlorine pesticides; (c) polychlorinated biphenyls; (d) dichlorodiphenyltrichloroethane and byproducts; and (e) polycyclic aromatic hydrocarbons. Error bars for internal standard areas and mass accuracy represent one standard deviation of the mean; error bars for the LOD represent the standard error of the mean.

through the origin, this issue disappears, but the information about positive interference is lost. Cases where LOD_{MDPA} was less than LOD_{IDL} were flagged for investigation and possible calibration curve modification. Because LOD_{IDL} represented an actual measurement and LOD_{MDPA} represented an estimate of what should be measurable given chromatographic and calibration curve properties, the greater of the two is recommended as the final LOD, but the process yielded meaningful information about areas where lower calibration points are possible.

Effects of Recovery. Recovery of internal standards from the extraction process was roughly consistent (43 ± 22%) throughout all samples across all analytes. Mean area counts of IS peaks decreased with recovery as expected and accuracy of

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spike measurements was verified to approximately 1% recovery (Figure 2) for the majority of analytes. The possibility of recovery-dependent effects on accuracy from matrix interferences was not investigated here. As expected, final LODs calculated by the LOD_{MDPA} approach were highly analyte dependent. Because the LOD_{MDPA} method is based directly on the IS area, LODs were inversely proportional to IS area counts and in cases (e.g., PBDEs 183 and 190) coincided with complete loss of analyte quantification at low recoveries as IS signal approached the IDL for that IS. In some cases below 5% recovery, LOD_{MDPA} was greater than the spike level. Although accuracy was demonstrably maintained below this level for known analyte amounts, precision was decreased. Variance of calculated masses increased with decreasing recovery in an inverse power relationship (PBDE examples in Figure 3, compare with Figure 2a). Variance increased for all analyte classes below the 5% recovery level (*F*-test, α = 0.05). For example, quantification of PBDE 183 was completely lost below 3.5% recovery, and variance increased by >10× at the 3.5% recovery point. Most analytes showed a similar pattern when quantification was lost, exhibiting an increase in variance at the adjacent greater recovery point. Some increase in variance could theoretically be attributed to data processing or injection differences (each ASV was measured only once). The majority is more likely a numerical function of low area count; as recovery decreases, small changes in peak area represent larger proportions of the area count for both IS and analytes with an increasing impact on mass calculation. In this exercise, the LOD_{MDPA} method somewhat protected against this loss of precision by using the same MDPA, derived from related blanks, with all samples; this limited variability to the IS area. Spike levels here were chosen specifically to assess accuracy over a wide recovery range; at lower spike levels, this effect would likely become more pronounced. Potentially, this impact could provide a measurement of the recovery threshold below which mass calculations lose satisfactory precision (e.g., the inflection point of the power curve) on an analyte-, method- and platform-specific basis.

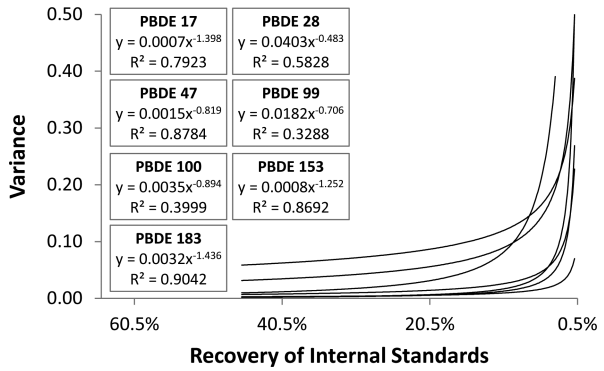

Figure 3. Model trend lines for a selection of polybrominated diphenyl ethers (n = 3, nominal spike mass of 5 ng) showing variance of calculated mass as an inverse power function of recovery.

Advantages of the Proposed MDPA Approach. Several distinct advantages were identified for the MDPA approach over other approaches considered here. Most beneficial was the inclusion of recovery and chromatographic properties specific to each analyte in the LOD. As LODs are calculated specifically for every sample in the MDPA approach, this provided an easy cross-batch metric for instrument and method performance over time. Analytes with known interferences in these analytical methods (e.g., 4,4′-DDE, aldrin, biphenyl, fluoranthene, PCBs 8/5, 82, 110, 126) showed commensurately greater LODs than other analytes of the same class. The proposed method provided excellent protection against false positives (e.g., matrix/process enhancement); as with any approach, process blanks should be evaluated for meaningful process contamination and interpreted appropriately as part of robust quality assurance. Results for analytes with much greater-than-average LODs due to process interferences should be interpreted carefully as would be the case from any other quality metric. Unlike other LOD methods, the MDPA approach readily flagged such troublesome compounds for investigation as the analyte-specific LOD is much greater than others when signal enhancement is present, rather than masking or ignoring the interference. Furthermore, calibration curves were able to be evaluated for sensitivity in a more robust manner; this benefit would extend to any given project and can greatly assist in calibration curve refinement for the needs of any given project. If an increase in sensitivity is desired, extension or refinement of calibration curves can be guided using LODs generated in this manner; rather than time-consuming iterative reduction of calibration points, the low end of the curve can be targeted directly using observations from previous batches. It is recommended that the greater of the LOD_{MDPA} or lowest point of the calibration curve be used as the final LOD to ensure reported values actually fall on the calibration curve, but if the IDL is suitably identified, this becomes a nonissue.

This process, compared with other processes discussed (e.g., additional verification or calibration points, extraction of the calibration curve, etc.) adds little cost in time (provided a minimum of three process blanks are part of current QA procedures) and does not require extraction of the calibration curve. Implementation is extremely straightforward for laboratories performing calculations external to instrument software; simply calculate the mean MDPA plus three standard deviations and use it as a surrogate area count as if it were a measured peak. Any positive interference (e.g., blank contamination) is automatically included in the LOD. Manual integration of blanks added only a negligible cost in time during data processing. Additionally, it enables chemists to use their discretion to make fully informed decisions regarding the LOD using observed signals. Since the LOD will be by definition greater than a minimum detectable peak area determined from blank chromatogram characteristics, Type II error risk is limited by choosing the MDPA as it relates to observed noise; false negatives due to censoring from overly conservative detection limits are eliminated. Other choices of target S/N or Type I risk level (i.e., increasing the number of standard deviations in the MDPA calculation, decreasing α from 0.05 to 0.01 or lower) allow for easy refinement of this approach to meet quality criteria as project needs or regulations dictate. By not artificially censoring data (i.e., inflating Type II error risk) with an overly conservative LOD, the reportability of data at the trace level was greatly enhanced in the current project, especially for PCBs. In a search comparison of historical data produced by CCR, nearly 80% of PCB congener data at the low- to subppb level censored by the statistical approach were not censored by the MDPA approach. This makes possible pattern analysis studies previously precluded by a high rate of censoring.

Caveats of the Proposed MDPA Approach. Proper sample preparation and chromatography are key to the approach presented here, as is the case with all trace level analyses. Responses in process blanks must reflect responses observed in

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samples. This assumption, if violated, could result in unacceptably high false-positive rates. To offset this somewhat, there is no limit to the number of blanks that may be included in the calculation, and no discrete upper limit on chosen S/N or σ multiplier. Additionally, inconsistent noise in the blanks will by definition result in commensurately greater LODs using this method as it relies on those characteristics. Extraction and cleanup steps must remove the majority of matrix interferences in samples as this approach assumes similar chromatographic characteristics between blanks and samples. Internal standards must accurately reflect losses for associated analytes as the LOD is scaled to the response of the internal standard. Analysts must be familiar with the characteristics of chromatograms produced by their process and highly trained to allow estimation of an area that would result from a defensible peak. This caveat is tied in with one of the major advantages of the MDPA approach; it empowers chemists to leverage their discretion regarding detectability of analytes within each batch based on observed properties. Currently, instrument software does not include this approach and MDPA-based LODs must be calculated externally; using an automated approach similar to the VBA solution used here is straightforward but requires additional expertise for implementation. Contact the primary author to request an example of the VBA code solution.

The MDPA method is similar in practice to the Estimated Detection Limit (EDL);[26] this method was not included for comparison. One main difference lies in that where EDL by definition can only be applied when a peak is absent, the proposed approach generates LODs for all analytes in all samples. This adds the capability of comparing concentrations with the LOD during review but sacrifices characterizing the noise in real samples (e.g., matrix effects). The EDL method is also more complex, requiring relative responses, a fixed multiplication factor of the noise from the expected peak region (requiring duplication of all data files and a second pass integration for all nondetect analytes), and a known dilution factor. The proposed method requires only slightly more information collected during processing data from a typical analytical run. To cover matrix effects, S/N adequate to discriminate a signal from observed noise in samples (e.g., from matrix effects) can be used as the target during MDPA integration. If additional protection against false positives is desired, a greater number of standard deviations can also be used; in samples examined here, S/N $\approx$ 3 and 3σ was adequate for all samples. Between these two very simple modifications to the proposed method, most project QA criteria can be easily satisfied. This method has not been investigated outside the present context: GC-MS analysis using IS added at the beginning of the analytical process and a positive detector signal for zero mass. Applications outside of this framework merit further investigation; this approach should be tractable with other detectors returning positive response for zero mass but may be less useful for others (e.g., detectors with true zero response at zero mass or Gaussian signal distribution about zero mass), though the theoretical framework is believed to be fairly extensible.

CONCLUSIONS

This is to our knowledge the first proposal of a method for sample- and analyte-specific LODs by formally tying the limit of detection to batch-specific chromatographic properties and recovery of internal standards within each sample. While the statistically based LOD calculation generally provided excellent protection against false positives, it resulted in an extremely high rate of false negatives at the trace level; the current project demonstrably supported previous criticisms of such approaches for the vast majority of compounds. Using batch-specific information from the blanks to generate a limit of detection greatly enhanced the reportability of data at the trace level (especially for PCBs, where as much as 80% of trace level data points were previously censored). Generation of LODs by MDPA additionally provided a metric for extraction and instrument performance over time, as well as the ability to assess performance of calibration curves at the trace level. Allowing for the discretion of highly trained chemists rather than relying on problematic statistical estimation or tiresome iterative calibration/spike reduction greatly enhanced confidence in, and the speed of deriving, the limit of detection. This approach is believed to be superior to others examined here as it relies on direct observation of chromatographic properties, provides a performance metric across batches, and accounts for recovery of analytes through the entire analytical process. While the approach has theoretical (e.g., assumptions regarding sample-to-blank interference variability) and practical (e.g., implementation) drawbacks—as do all LOD approaches considered here—these were more than offset by the wealth of information added.

ASSOCIATED CONTENT

Supporting Information

List of ^{13}C- or ^{2}H-labeled internal standards (in hexane) added to each sample prior to extraction; limits of detection (mean $\pm$ 1 SD) calculated by modified Hubaux–Vos regression (HV), instrumental detection limit (IDL), and minimum detectable peak area (MDPA) for four classes of persistent organic pollutants; conceptual description and example of the semi-automated method behind the minimum detectable peak area (MDPA) approach; example chromatograms showing integration of blanks to support the MDPA method. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

▲The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. J.M.R. and D.L. contributed equally.

Notes

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The authors declare no competing financial interest.

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