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Article

Utilization of Internal Standard Response Factors to Estimate the Concentration of Organic Compounds Leached from Pharmaceutical Packaging Systems and Application of Such Estimated Concentrations to Safety Assessment

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Substances from packaging systems that are leached into packaged medical products may have a safety impact on patients to whom such medical products are administered. The potential safety impact depends on the identity and concentration of the leached substances. The concentration above which a leachable must be identified in order to assess its safety impact is frequently estimated using an internal standard to "calibrate" the analytical response of a chromatographic system. Such an estimate is accurate to the extent that the responses of the internal standard and leachables are similar. To establish the accuracy of the internal standard approach, a database of gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS) responses was generated for thirty-eight leachables and eight internal standard candidates. Although the FID and MS responses of many of the leachables and internal standards fell within a narrow band, acidic and basic compounds produced responses that were discernibly different from those of neutral analytes. While most of the internal standards were suited for concentration estimation, three of the candidates, dimethylphthalate, triphenylphosphate and 4,4-dibromobiphenyl, produced the smallest mean error in estimated concentration for the analytes examined. As the FID and MS responses were linear, internal standards could be used to estimate leachables concentrations even when the difference in leachable versus internal standard concentrations was as great as a factor of 25. A multiplier may be appropriate to adjust an estimated concentration to its greatest possible value, and it is this value that is used to convert an estimated Analytical Evaluation Threshold (AET) into a working or final AET.

Introduction

Plastic materials are widely used in medical items, such as product packaging, transfusion sets, transfer tubing, devices and manufacturing systems. The physiochemical nature of these materials provides medical products with their necessary, desirable performance characteristics. While an important performance characteristic of plastics used in medical/pharmaceutical applications is chemical inertness, interactions between a plastic material and pharmaceutical products it contacts are well documented. One such interaction is leaching; the release of plastic material components to the product, where both the identities of the leached substances and their accumulation levels may affect the material's ultimate compatibility with the product. The extent of leaching, specifically what compounds are leached and at what levels, may impact the safe utilization of the medical product, as the leached substances are co-administered with the medical product. The process of establishing the safety impact of contact between a product and a plastic material is termed safety assessment.

One aspect of safety assessment that is of particular interest can informally be stated as "how low do you go in trying to quantify and identify leached substances?" In a more rigorous sense, this question asks "What is the largest amount of a substance, regardless of its identity, that can be leached from a plastic and incorporated into a medical product without adversely impacting the health of the patient receiving the medical product?" This amount, which has been termed the Safety Concern Threshold (SCT), represents a threshold below which a leached substance, regardless of its identity, would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects (1). Because the SCT is a dose, it is, in and of itself, not of direct use in analytical efforts to identify and quantify leached substances. Rather, the SCT must be "converted" to an Analytical Evaluation Threshold (AET) considering such factors as product dosing and the conditions of contact. Once calculated, the AET becomes "the threshold above which a chemist should begin to identify the extracted substance and report it for potential toxicological assessment" (2–4). Leached substances whose concentrations are above the AET must be identified and considered for toxicological safety assessment while substances whose concentrations are below the AET do not need to be either identified or assessed because they are generally recognized as safe (GRAS).

The determination of whether a leached substance's concentration is above or below the AET may thus be performed before it has been definitely identified. In such a situation the substance's concentration cannot be established by use of an external standard, since by definition the external standard is derived from and contains the substance itself. Rather, it is common for the substance's concentration to be determined by use of an internal, or surrogate, standard. That is, an internal standard is added to a sample at a known concentration (C_i) and the leached substance's concentration (C_a) is estimated from the analytical responses (A) as follows:

$$C_a = C_i \times (A_a/A_i)$$

The accuracy of such a concentration estimate depends on the similarity of the method's mass response for the analyte (M_a) and internal standard (M_i). The more the ratio

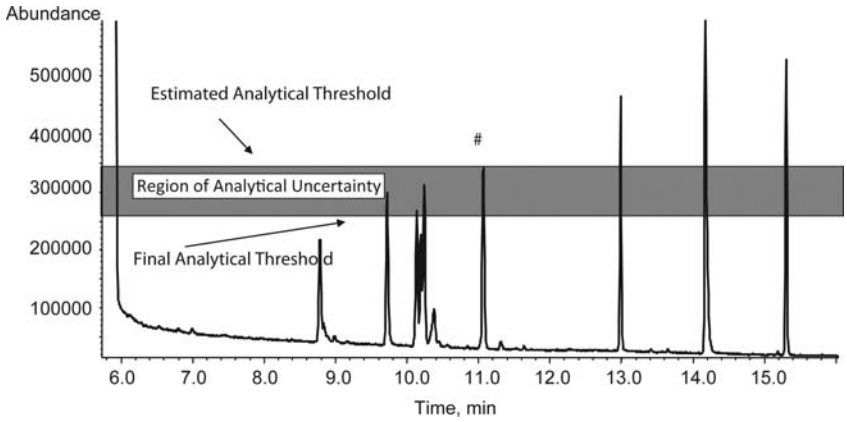

Figure 1. The Practical Application of the Analytical EvaluationThreshold (AET). The peak denoted by the # is the internal standard, which was added to the tested sample at a level equal to the Estimated AET. The use of the internal standard to represent the Estimated AET is based on an average response factor and is more or less a reflection of the average response factor for a set of compounds which make up a response factor database. The proper use of the internal standard to reflect the AET requires that that the AET be corrected to account for the variation in responses factors of all the compounds in the database. This corrected AET is termed the Final AET, which is the lowest value the AET can possibly have after its "correction" for the inherent analytical uncertainty.

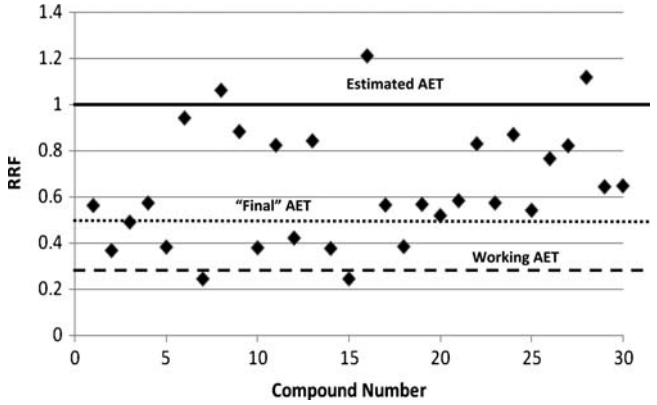

Figure 2. Response Factor Diagram Illustrating the Various Practical Aspects of the Analytical Evaluation Threshold (AET). The data illustrated is the relative GC/MS response factors (RRF) reported for 30 model compounds versus 2-Fluorobiphenyl as the internal standard (data obtained from reference 5). If the internal standard is prepared at a concentration equal to the AET, then the Estimated AET corresponds to a RRF value of 1. Per the PQRI Recommendations for OINDP, the Estimated AET is "adjusted" by a factor of 2 to produce the "Final" AET, which accounts for the variation in response, analytes versus the internal standard. All compounds with an RRF of 0.5 or greater fall above the "Final" AET and thus are not of toxicological concern. However, it is clear that a number of the analytes fall below this RRF and thus may be concluded to be of toxic concern. An "adjustment" of nearly a factor of four is required so that 90% of the compounds are "covered" by the Final AET.

M_a/M_i deviates from 1, the less accurate the concentration estimate is.

It is clear from this discussion that an AET calculated from the SCT (which has been termed the estimated AET) must be "adjusted" for the uncertainty inherent in analyte quantitation by internal standard response factors (see Figure 1). Such an adjustment results in the calculation of a Final AET. The practical "issues" associated with the determination of the Final AET and its utilization to quantify compounds at trace levels were considered by Mullis et al. (5). In order to ascertain the uncertainty in the internal standard approach, these authors studied the gas chromatography with mass spectrometric detection (GC–MS) responses of thirty-two compounds (thirty "known" extractables/leachables and two internal standards). For this database of 30 compounds, the authors report the following mean response factors: 0.642 ± 0.254 for one internal standard (2-fluorobiphenyl) and 0.487 ± 0.184 for the second internal standard (p-terphenyl-d_{14}). These authors conclude that these response factors support recommendations that in general a factor of two is an appropriate multiplier between the Estimated AET and Final AET[1], as this factor corresponds to roughly one standard deviation in the response factors. Given this situation, one expects that an examination of the response factor data (see Figure 2) would indicate that a number of the target compounds fall below the RRF value that corresponds to the "factor of 2" Final AET (RRF 0.5). The significance of this observation is as follows. The RRF value for compound 18 is approximately 0.38, meaning that the actual concentration of this compound in an extract would be 1/0.38 or 2.6 times the estimated concentration calculated using the internal standard. Adjusting the estimated concentration by a factor of 2 would underestimate somewhat the actual concentration of this compound. Use of such an underestimated concentration in a safety assessment could result in an underestimated safety impact (i.e., the potential conclusion that a compound is safe when in fact it is not). An alternate adjustment to produce an Estimated AET that encompasses 90% of the data population would require a factor of approximately 4 to establish the Final AET.

The use of response factors for concentration estimation is well established and the issues associated with the assumption of a universal response for a large population of compounds has been extensively considered (6–13). It goes without saying that the validity of any conclusions drawn about the utility of a response factor database is increased as the content of the database increases. Thus the purpose of the study discussed in this manuscript was to obtain response factors for additional extracted substances and potential internal standards. Additionally, this manuscript considers the use of response factors obtained with both flame ionization (FID) and MS detection and examines the impact of internal standard concentration on the response factor calculation.

Table I
List of Compounds Used in the Study.

Compound	CAS RN	Formula	MW
Model Compounds			
1. Dibutylamine	111-92-2	C8H19N	129.24
2. 2-Ethylhexanol	104-76-7	C8H18O	130.23
3. Caprolactam	105-60-2	C6H11NO	113.16
4. 2,4-Diaminotoluene	95-80-7	C8H10N2	122.16
5. 2(3)-Tert-butyl-4-methoxy phenol (BHA)	25013-16-5	C11H16O2	180.25
6. 2,4-Dimethylphenol	105-67-9	C8H10O	122.17
7. o-Toluenesulfonamide	88-19-7	C7H9NO2S	171.21
8. 1,4-Dioxacyclotetradecane-5,14-dione	5578-82-5	C12H20O4	228.28
9. 3,3-Dimethyl-1,5-dioxacyclopentadecane-6,15-dione	94113-50-5	C15H26O4	270.35
10. 7,9-Ditert-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione (Irganox degradate #1)	82304-66-3	C17H24O3	276.37
11. Palmitic acid	57-10-3	C16H32O2	256.42
12. 3-(3′,5′-di-t-t-butyl-1′hydroxy-4′-oxacyclohexa-2′,5′-dienyl) propanoic acid (Irganox degradate #2)	20170-32-5	C17H26O3	278.39
13. Stearic acid	57-11-4	C18H36O2	284.48
14. Mono- (2-ethylhexyl) phthalate	4376-20-9	C16H22O4	278.34
15. Erucamide	112-84-5	C22H43NO	337.58
16. Bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate (Tinuvin 770)	52829-07-9	C28H52N2O4	480.72
17. 2,4-bis(1,1-dimethylethyl)phenol, 1,1′,1″-phosphate (Irgafos 168 phosphate)	95906-11-9	C24H63O4P	662.92
18. 2,6-Di-tert-butyl-4-methyl phenol (BHT)	128-37-0	C15H24O	220.35
19. p-Toluenesulfonamide	70-55-3	C7H9NO2S	171.21
20. N-Ethyl-4-benzenesulfonamide	80-39-7	C9H13NO2S	199.27
21. Dibutylphthalate	84-74-2	C16H22O4	278.34
22. 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A)	80-05-7	C15H16O2	228.24
23. Oleamide	301-02-0	C18H35NO	281.48
24. Di-(2-ethylhexyl) phthalate (DEHP)	117-81-7	C24H38O4	390.56
25. Irganox 1076	2082-79-3	C35H62O3	530.86
26. Tris(2,4-di-t-butylphenyl)phosphate (Irgafos 168)	31570-04-4	C42H63O3P	646.93
27. Aniline	62-53-3	C6H7N	93.13
28. 2-Ethyl hexanol	104-76-7	C8H18O	130.23
29. Acetophenone	98-86-2	C8H8O	120.15
30. 2-Phenyl-2-propanol	617-94-7	C9H12O	136.19
31. Octanoic acid	124-07-2	C8H16O2	144.21
32. 2,4-Di-t-butyl phenol	96-76-4	C14H22O	206.33
33. Dodecyl acrylate	2156-97-0	C15H28O2	240.38
34. Docosane	629-97-0	C22H46	310.60
35. Hexacosane	630-01-3	C26H54	366.71
36. 30-Crown-6	64001-05-4	C24H48O6	432.63
37. Diethylene glycol	111-46-6	C4H10O3	106.12
38. 2-Ethylhexanoic acid	149-57-5	C8H16O2	144.21
Internal Standard Candidates			
39. 2-Fluorobiphenyl	321-60-8	C12H9F	172.20
40. Dimethyl phthalate	131-11-3	C10H10O4	194.19
41. Anthracene-d10	1719-06-8	C10D14	134.16
42. Triphenyl phosphate	115-86-6	C18H15O4P	326.29
43. Pentadecane	629-62-9	C15H32	212.41
44. 4-bromophenyl phenyl ether	101-55-3	C12H9BrO	249.11
45. 4,4′-Dibromobiphenyl	92-86-4	C12H8Br2	312.00
46. p-Terphenyl (1,4-diphenylbenzene)	92-94-4	C18H14	230.31

Experimental

Chemicals and reagents

This investigation included the thirty-eight model compounds and eight candidate internal standards listed in Table I. Reference materials for these compounds were either obtained commercially in the highest appropriate purity or synthesized and qualified internally. Stock solutions containing groups of these compounds were prepared at a concentration of approximately 500 mg/L (ppm) by dissolving an appropriate amount of the compounds in methylene chloride. Working standards containing approximately 5, 10, 25, 50 and 100 mg/L of the internal standard candidates were prepared by appropriate dilution of the stock with methylene chloride. Working samples containing the model compounds and appropriate internal standard candidates were prepared at a concentration of 40 mg/L via a similar dilution.

Table II
Operating Parameters, GC FID/MS.

Operating Parameter	Operating Value
Column	Phenomenex Zebra ZB-5HT Inferno, 30m x 0.25mm, 0.25 μm film thickness
Oven Program	Start at 40°C, hold for 1 min; ramp at 10°C/min to 360°C, hold for 6 min
Carrier Gas	He at 2 mL/min
Injection	Splitless; 1 μL.
Injector Temperature	280°C
FID Detector Temperature:	300°C
MS Transfer Line Temp.	280°C
MS Detection Details	70 eV (+), mass range of 30 −700 amu (4.5 min solvent delay)

GC system

The chromatographic system was an Agilent (Santa Clara, CA) 6890 series GC system, which included a Flame Ionization Detector (FID) and a 7683 series Injector. The effluent from the chromatographic column was split via an Agilent Model G3180-61500 Compact Splitter, with a portion directed to both the FID and an Agilent 5973N Mass Selective Detector (MSD). The chromatographic column was from Phenomenex (Milford, MA), specifically a Zebra ZB-5HT Inferno capillary column, 30 m x 0.25 mm i.d. x 0.25um film thickness, 7HG-G015-11.

Chromatographic conditions and analysis

The chromatographic conditions used are summarized in Table II. Typical chromatographic performance under these operating conditions is illustrated in Figure 3. Four or more injections of each working sample and standard were made and the resulting peaks areas were processed. The retention times of the individual compounds were confirmed via examination of their mass spectra.

Results and Discussion

Typical chromatograms obtained for the analytes of interest are shown in Figure 3. In general the peaks obtained for the analytes were well-shaped and well-resolved. However, the analytes had to be placed in multiple groups in order to produce unobstructed peaks for all the targeted compounds.

The FID and MS responses obtained for the target leachables and internal standard candidates are illustrated in Figures 4 and 5. In general, the MS and FID responses of all the analytes were similar and fell within a relatively narrow band. The variation in the response factors for the entire dataset is reflected in the %RSD of the responses obtained, which was 44.0% for the FID response and 50.9% for the MS response, similar to what was reported by Mullis et al. These data suggest that concentration estimation using an internal standard would be more or less equally accurate if it is based on either the MS or the FID response. As in indicated in these Figures, noteable "outliers" included the acidic and basic leachables. For example, the most prominent "outliers" in both Figures 4 and 5 are peak 4 diaminotoluene, peak 11 palmitic acid, peak 13 stearic acid, peak 14 mono-(2-ethylhexyl) phthalate, peak 12 an acidic Irganox degradate, and peak 27 aniline. Similarly, the original set of

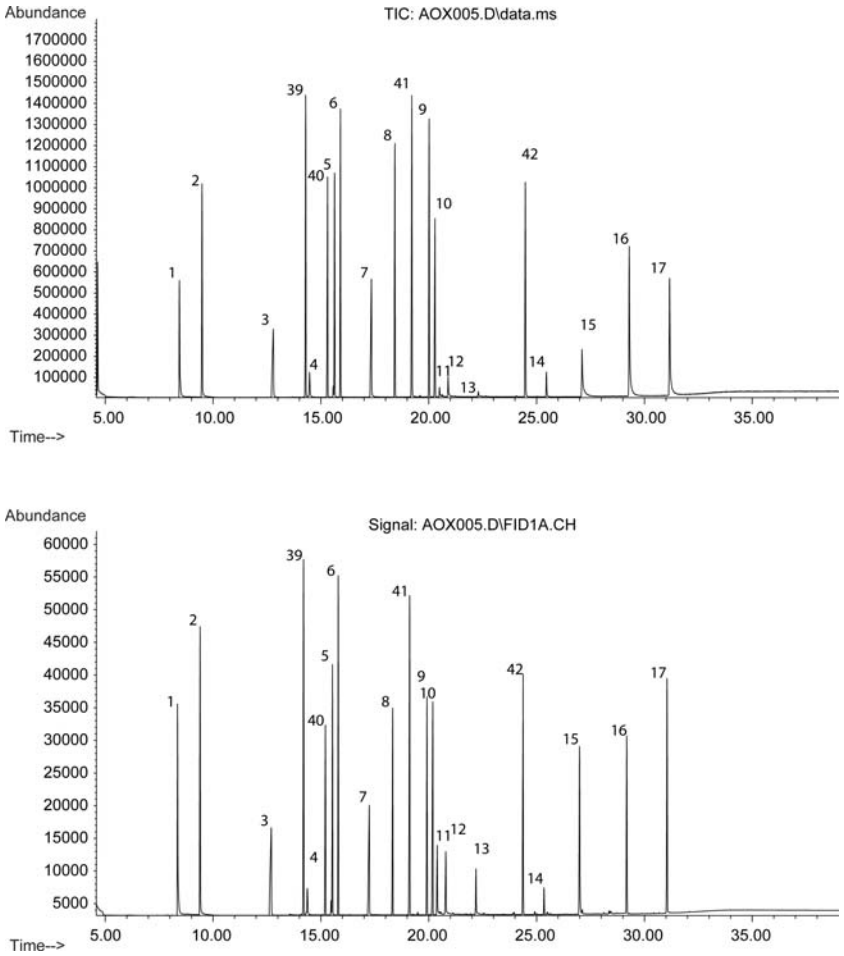

Figure 3. Typical Chromatograms Illustrating the Performance of the Analytical Method used in this Study. Compound numbers correspond to those listed in Table I.

leachables included two other basic amines, dibutylamine and triisopropanolamine. However, these analytes were not included in the final dataset as their FID and MS responses were even smaller than those reported for the other amines (e.g. aniline).

The behavior of the responses shown in Figures 4 and 5 suggest that the MS and FID responses for this dataset of compounds are roughly proportional. As noted in Figure 6 this is the case and the ratio of MS to FID response generally falls within a narrow band for a majority of the studied compounds. In fact, the variation in the MS/FID response ratio (31.7% RSD) is less than the variation in either the FID or MS responses alone. However, there are certain analytes where the MS response is significantly suppressed versus the FID response; most notable are the three organic acids (palmitic acid, stearic acid and Irganox Degradate #1) and Irganox 1076.

The MS/FID ratios illustrated in Figure 6 may provide insight into the reasons that certain analytes produced reduced FID and MS responses, as shown in Figures 4 and 5. Because amine-type analytes have constant MS/FID ratios, their responses in both MS and FID were proprtionally reduced versus the other analytes. This behavior suggests that the root of the poorer MS and FID responses for these analytes is due to a chromatographic issue as oppossed to detector issues. Alternatively, the acid-type analytes have both reduced absolute responses for MS and FID and reduced relative responses, MS versus FID. This suggests that the root of the poorer responses for these analytes is due to both chromatographic and detector issues.

When considering the proper internal standard to use, it is noted that the internal standards which most effectively reflect the response characteristics of all the targeted leachables are those internal standards whose responses fall near the average lines drawn in Figures 4 and 5. Although almost all of the internal standards would be suitable for estimating the concentration of the targeted leachables, dimethylphthalate (peak 40), triphenylphosphate (peak 42) and 4,4-dibromobiphenyl (peak 45) all have responses closest to the center line and thus produce the smallest mean error in estimated concentration for the analytes examined.

If a sample to be analyzed contains multiple leachables at varying and largely unknown concentrations, then it not practically possible to match the concentration of the analytes with that of an internal standard added to the sample for analyte quantitation. It is pertinent to consider whether a concentration mismatch between the internal standard and the analyte

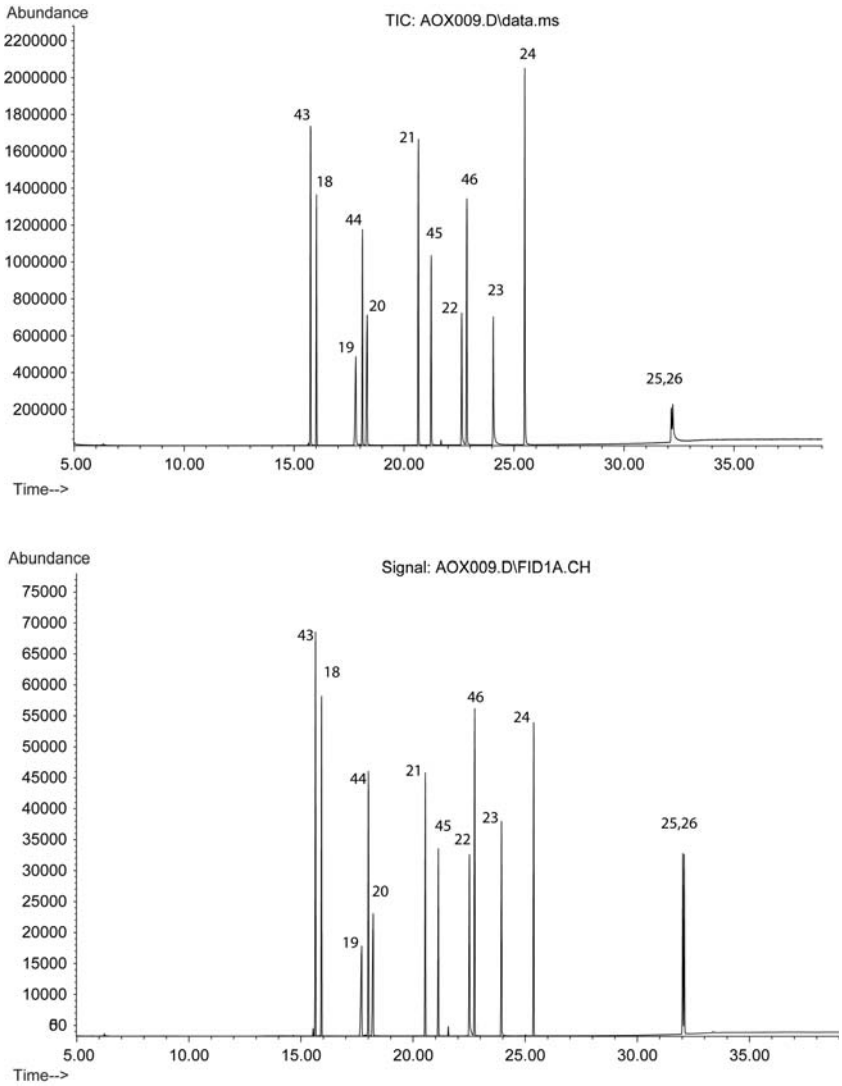

Figure 3 (Continued)

that is being quantitated adversely affects the accuracy of the concentration estimate. If the response versus concentration functions for the analyte and the internal standard are similar (co-linear and share a similar intercept), then the impact of a concentration mismatch on the accuracy of analyte quantitation would be small. However, if the response functions of the analyte and internal standard were different, then a concentration mismatch would greatly reduce the accuracy of analyte quantitation.

To examine this aspect of concentration estimation with internal standards, standards containing varying amounts of certain of the analytes and internal standards were prepared and analyzed. Response versus concentration data for these compounds is shown in Figure 7. In general there is a clear linear correlation between analyte concentration and response for all the analytes over the concentration range investigated (5 to 125 mg/L). The correlation coefficients of the best fit lines (both MS and FID responses) are poor not due to lack of linearity but rather due to the variation in response between analytes. Thus accurate analyte concentration estimates can be obtained even when the analyte and internal standard concentrations differ by as much as a factor of 25.

As was noted in the Introduction, the ultimate purpose of establishing the variation in the responses of the analytes and internal standards is to establish the magnitude of the "correction factor" that is applied to the Estimated AET to produce the Final) (or Working) AET. This objective is achieved by plotting the relative response factors of the analytes versus a chosen internal standard. Such a plot is shown in Figure 8, wherein DMP is used as the internal standard. If the internal standard is prepared at a concentration equal to the AET, then the Estimated AET corresponds to a RRF value of 1. The question that needs to be addressed by examining Figure 8 is "what is the RRF value such that a majority of the model compounds fall above the Final AET?" As shown in Figure 8, this RRF value is roughly 0.25. For this dataset, an adjustment of up to a factor of 4 to

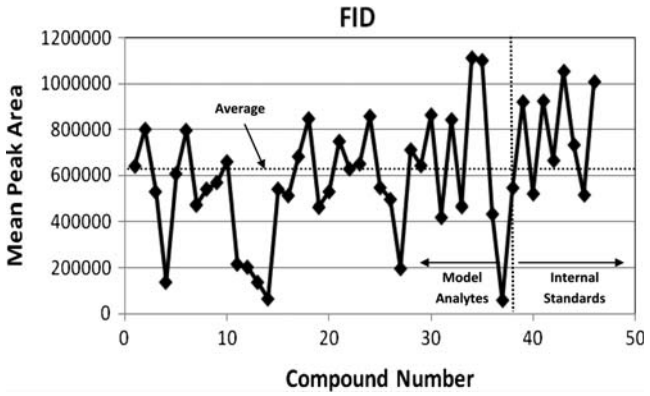

Figure 4. Mean FID Responses to a Solution containing approximately 1 mg/L of the Model Analytes and Candidate Internal Standards. Although the responses of many of the compounds examined in this study were similar and fell within a narrow band, several analytes, mainly acidic or basic compounds, produced responses that were discernibly different from those of the other analytes. Analytes with such disparate responses would be difficult to effectively quantitate via an internal standard.

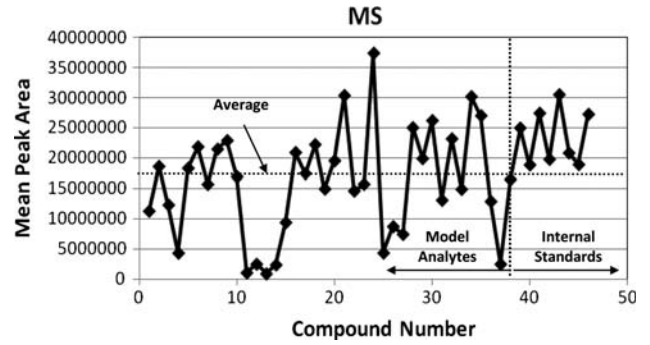

Figure 5. Mean MS Responses to a Solution containing approximately 1 mg/L of the Model Analytes and Candidate Internal Standards. Although the responses of many of the compounds examined in this study were similar and fell within a narrow band, several analytes, mainly acidic or basic compounds, produced responses that were discernibly different from those of the other analytes. Analytes with such disparate responses would be difficult to effectively quantitate via an internal standard.

produce the Final AET accounts for much of the variation in response, analytes versus the internal standard. This is roughly the same result noted in the Introduction for the Mullis data set.

Conclusion

A database of GC–FID and GC–MS responses factor has been generated for thirty-eight leachables and eight internal standard candidates. Although the FID and MS responses of many of the leachables and internal standards fell within a narrow band, acidic and basic compounds produced responses that were discernibly different from those of neutral analytes. The nature of the response ratio, MS response versus FID response, suggests that the behavior of the amine results from chromatographic effects while the behavior of the acids is due to both chromatographic and detector effects. While most of the internal standards were suited for concentration estimation, three

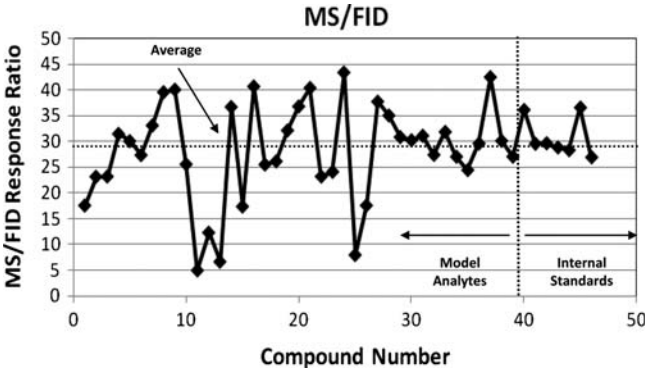

Figure 6. MS Versus FID Response. Figures 4 and 5 suggest that individually the FID and MS responses are similar for a majority of the investigated analytes. This suggests that in general the MS and FID responses should be roughly proportional. This Figure demonstrates that the MS and FID responses are roughly proportional and are not compound specific, except for isolated analytes.

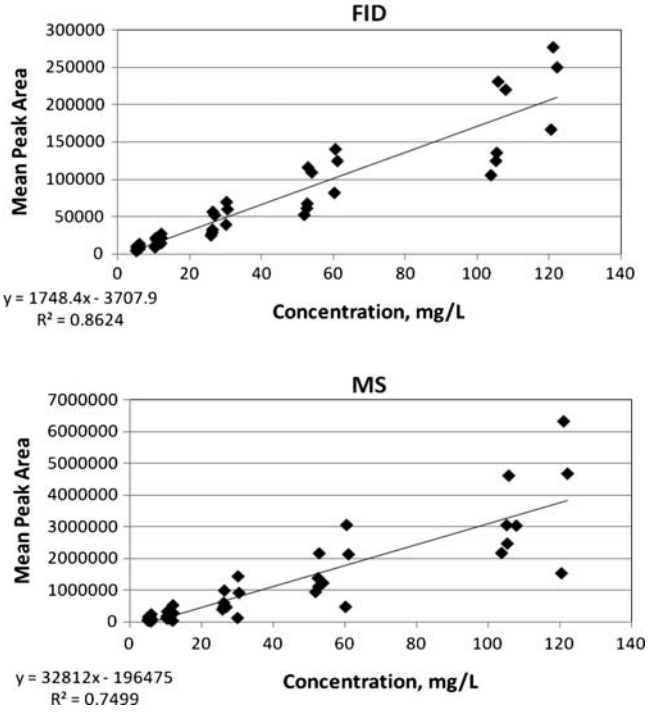

Figure 7. Linearity of Response for the Internal Standard over the Range of ≈ 5 to 125 mg/L. As the responses can roughly be linearly correlated to analyte concentration, internal standard responses factors are appropriate for concentration estimation over at least a 25-fold concentration range.

candidates, dimethylphthalate, triphenylphosphate and 4,4-dibromobiphenyl, produced the smallest mean error in estimated concentration for the analytes examined. As the FID and MS responses were linearly related to analyte concentration, internal standards could be used to estimate leachables concentrations even when the difference in leachable versus internal standard concentrations was as great as a factor of 25. The database generated in this study is consistent with previously reported information and recommendations that suggest that a factor of 2 correction in an estimated AET represents roughly one standard deviation among the response factors exhibited by

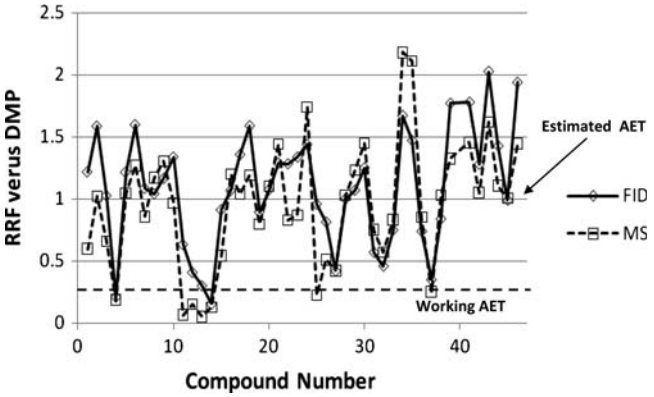

Figure 8. Response Factor Diagram. Model Analytes and Other Internal Standard Candidates versus Di-methylphthalate (DMP) as the internal standard. If the internal standard is prepared at a concentration equal to the AET, then the Estimated AET corresponds to a RRF value of 1. For this dataset, an adjustment of approximately a factor of 4 to produce the Final Working AET accounts for the variation in response, analytes versus the internal standard.

the compounds in the database. A multiplier of approximately 4 is required so that the final AET accounts for a majority of the compounds in this database.

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