

How low can we go? Sensitivity and the Analytical Evaluation Threshold for Leachables

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Introduction

Over the years significant progress has been made in understanding the leachables/extractables issue and its implications for Orally Inhaled and Nasal Drug Product (OINDP) pharmaceutical development, largely due to the efforts of organizations like the International Pharmaceutical Aerosol Consortium for Regulation and Science (IPAC-RS). The culmination of this experience is found in the Product Quality Research Institute's (PQRI) recommendation document, produced by the PQRI Leachables and Extractables Working Group [1] which was submitted for comment to the USFDA in September 2006 (note that IPAC-RS is a PQRI member organization). The heart of the PQRI recommendations is the development of safety thresholds for individual organic leachables, including a Safety Qualification Threshold (QT) and a Safety Concern Threshold (SCT). Also included are "Best Practice" recommendations for leachables and extractables pharmaceutical development, in which the SCT is translated into a threshold useful in the analytical laboratory, the Analytical Evaluation Threshold (AET). The AET is the threshold at which the analytical chemist should begin to identify a potential leachable or extractable and report for potential toxicological assessment.

This poster demonstrates the capability of modern analytical instrumentation in identifying compounds at AET levels. An answer is given to the question: given both the "Estimated" and "Final AET", what are the instrument sensitivity capabilities of modern analytical instrumentation for determining the AET? How does one define sensitivity? In this case the definition is not from the conventional signal-to-noise [2] paradigm, but defined as the lowest absolute amount of an individual organic compound from which sufficient information can be acquired to elucidate the structure.

Threshold Concepts

The Analytical Evaluation Threshold (AET) is defined as "the threshold at or above which a chemist should begin to identify a particular leachable and/or extractable and report it for potential toxicological assessment" [1]. The AET is derived from the Safety Concern Threshold (SCT) which is defined as "the threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects" [1]. Along with the SCT is the Qualification Threshold (QT), defined as "the threshold below which a given leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure activity relationship (SAR) concerns" [1]. In numerical terms the SCT is defined as 0.15 µg/day total daily intake (TDI) for an individual organic leachable (the QT is defined as 5 µg/day TDI). The AET represents the translation of the SCT into terms that are useful in the analytical laboratory. In order to determine the Estimated AET for a leachable, one must take into consideration the dosing and other parameters of the particular OINDP. The following is an example of how one goes about determining the Estimated AET in both the drug product and component.

Consider an MDI with 120 labeled actuations per canister, a recommended dose of 8 actuations per day, and a critical component elastomer mass per valve of 250 mg. For an individual organic leachable derived from this elastomer, the Estimated AET would be:

$$\text{Estimated AET} = \left(\frac{0.15 \mu\text{g/day}}{8 \text{ actuations/day}} \times 120 \text{ labeled actuations/canister} \right)$$

$$\text{Estimated AET} \approx 2.25 \mu\text{g/canister}$$

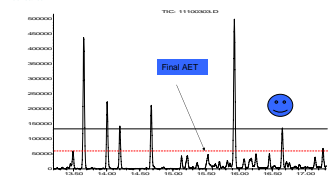
Given this amount per canister and the weight of the elastomer an Estimated AET can be determined as follows:

$$\text{Estimated AET} = \left(\frac{2.25 \mu\text{g/canister} \times 1 \text{ canister/valve}}{0.25 \text{ g of elastomer/valve}} \right)$$

$$\text{Estimated AET} \approx 9.00 \mu\text{g/g of elastomer}$$

Chromatographically this is represented in Figure 1. For a given leachables GC/MS profile a line can be drawn representing the estimated AET (black line). Peaks above this line are above the AET and must be identified. The dashed line (red) represents the analytical uncertainty in the measurement that is determined from a statistical evaluation of the data and thus represents the Final AET or the lowest level of compounds in the chromatogram that must be identified and be reported for potential toxicological assessment.

Figure 1 – Leachables GC/MS Total Ion Chromatogram



Sensitivity and AET

Since the identities of compounds above the AET is critical for toxicological assessment; how low can one go and still determine the identity? Better yet, redefine sensitivity as the lowest absolute amount of an individual organic compound from which information can be acquired sufficient to elucidate the structure.

To investigate this "sensitivity" definition a set of experiments were conducted using an Agilent 5973 GC/MSD for potential semi-volatile leachable compounds and a Thermo LTQ FT Ultra for potential non-volatile leachables. A set of serial dilutions were performed on model compounds to illustrate how low one could go and still elucidate molecular structure.

In the case of the GC/MSD, NIST library searchable EI spectra were obtained with high quality library matches as the criteria to identify the compound (Figures 2-5).

Figure 2 – GC/MS Profile of Target Leachables at the "Sensitivity" Level

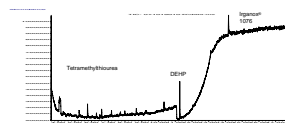


Figure 3 – EI Spectrum and library match of Tetramethylthiourea at 130 pg on-column

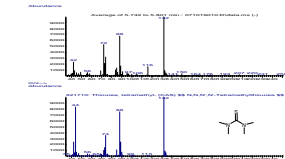


Figure 4 – EI Spectrum and library match of Bis-2-ethylhexylphthalate at 304 pg on-column

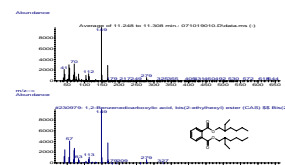
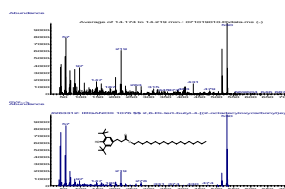


Figure 5 – EI Spectrum and library match of Irganox 1076 at 304 pg on-column



For the non-volatile leachables HPLC-LC/MSⁿ was used in conjunction with accurate mass to elucidate structure of two compounds, Irganox 1010 and Irganox 1076. The metric for "sensitivity" was how low could one go and obtain MSⁿ and accurate mass information. Figures 6-9 illustrate the ability to achieve MSⁿ and accurate mass data on single nanogram quantities.

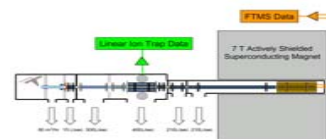


Figure 6 – Irganox 1076 LC-UV Result (Top) and APCI (Bottom), 100 to 0.22 ng on-column.

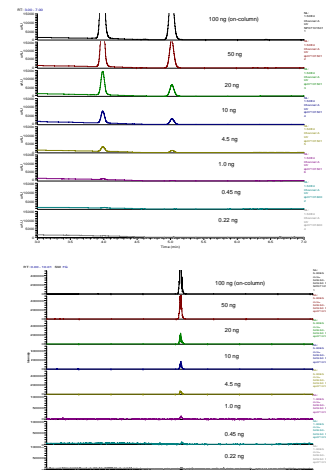


Figure 7 – Irganox 1076 APCI – Mass Spectrum on 1.0 ng on-column with 2.2 ppm mass error

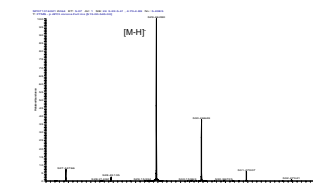
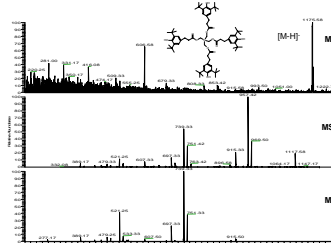


Figure 8 – Irganox 1010 APCI Negative Ion Mass Spectrum, MSⁿ on 100 ng on-column



Figure 9 – Irganox 1010 APCI Negative Ion Mass Spectrum, MSⁿ on 0.22 ng on-column



Conclusions

The sensitivity of modern analytical instrumentation for structure elucidation is within the realm of the required AET. If one back calculates from the sensitivity as shown with these model compounds, the projected AET can be determined as follows: For tetramethylthiourea, if one assumes contents of 2 MDIs into 1 mL of extract and a 1 µL of the extract is injected on-column, then the projected AET would be 65 ng/canister. Likewise, in the case of Irganox 1010, assuming 2 MDIs into 1 mL extract and a 10 µL extract injected on-column the projected AET would be 11 ng/canister. Therefore modern state-of-the-art instrumentation is not significantly challenged by the AET threshold approach.

References

1. Safety Thresholds and Best Practices for Leachables and Extractables in Orally Inhaled and Nasal Drug Products. Product Quality Research Institute. Leachables and Extractables Working Group 2006. http://www.pqri.org/pdfs/LE_recommendations_to_FDA_09-29-06.pdf. Accessed August 2007
2. Swartz, Michael E., and Krull, IRA S., Analytical Method Development and Validation, Marcel Dekker, Inc. 1997, p. 63.