Automated and Simultaneous Identification and Quantification in Extractables and Leachables Analysis

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Overview

- The safety impact of leachable chemicals found in medical devices, food packaging, or pharmaceuticals is determined by accurate qualitative and quantitative chemical analysis.
- Gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) are the primary instruments used.

GC/MS or LC/MS	Polyarc/FID
Responses are compound dependent	Response per mole of carbon is universal
Compounds can give non-linear responses with concentration	This detection system is linear over seven orders of magnitude
Targeted analysis may not be possible if standards are unavailable or prohibitively expensive	Quantification can be done with a single point calibration with any internal or external standard
Surrogate compounds are used to calibrate based on functionality or retention time	No assumptions need to be made to select an appropriate standard
This results in inaccuracy of up to 71% as shown here	This results in a much more accurate quantification

Introduction

The analysis of extractables and leachables (E&L) in food and pharmaceutical packaging and medical devices requires unambiguous identification and quantification of a wide range of analytes in various matrices and concentrations.

Quantification is required when analytes are present at concerning concentrations, a paradox that often results in the need to quantify all compounds detected. Quantification is further complicated by the widely varying and unpredictable response factors of analytes in the mass spectrometer as well as sample and standard instability in certain matrices.

In this poster, we describe the use of complementary flame ionization detection in a hyphenated Cerno-calibrated GC/MS-Polyarc-FID setup to improve the accuracy, reproducibility and linearity of quantification and identification using a single calibration with an arbitrary surrogate molecule.

Methods

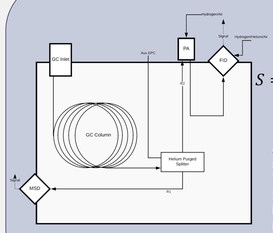
A test solution of molecules commonly found in E&L studies at unknown concentrations was prepared for evaluating the method, as well as five level calibration curves of several surrogate molecules. Calibrated mass spectra were obtained in raw mode and analyzed with Cerno MassWorks to improve spectral accuracy. Calibration curves based on the MS results were used to quantify the unknown as surrogate molecules. The Polyarc/FID results were obtained using a single internal standard concentration.

GC conditions
Front SS Inlet He
Split ratio: 10:1
Inlet temperature: 300 °C
Column flow: 2.6 sccm
Septum purge flow: 3 sccm
Inlet liner: Agilent 5190-2295
Oven: 40 °C (5 min),
15 °C/min to 275 °C (10 min)
Column: DB-5MS UI
(30 m × 0.25 mm × 0.25 µm)
Syringe: 10 µL
Injection volume: 0.5 µL

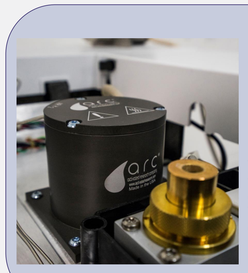
MS conditions
Energy: 70 eV
Scanning range: 33-500 m/z
Source temp: 230 °C
Quadrupole temp: 150 °C
Transfer line temp: 250 °C
Transfer line length: 0.6 m
Transfer line ID: 0.1 mm ID

FID conditions
Temperature 300 °C
H_2 Flow: 1.5 sccm
Air Flow: 350 sccm
Makeup: 5 sccm (He)
Sampling rate: 100 Hz

Polyarc reactor conditions
Temp Setpoint: 293 °C
H_2 Flow: 35 sccm
Air Flow: 2.5 sccm

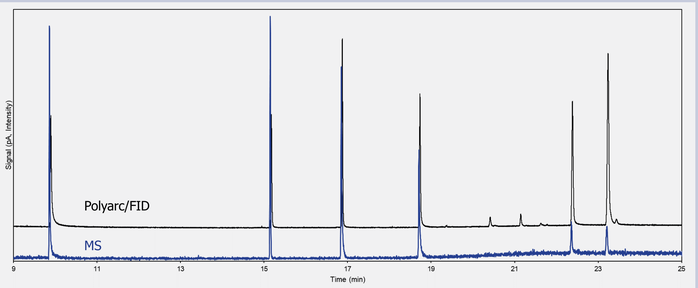


A post-column helium-purged splitter diverted column effluent from an Agilent 7890A GC in a 50/50 ratio to an Agilent 5973 MS and a Polyarc-FID. The split ratio is dictated by the above equation, with R2 representing the Polyarc transfer line and R1 representing the MS transfer line.



$$C_A = C_S\left(\frac{Area_A}{Area_S}\right)\left(\frac{MW_A}{MW_S}\right)\left(\frac{\#C_S}{\#C_A}\right)$$

The Polyarc reactor converts all organic species to methane to allow for equimolar carbon detection and accurate single point calibration. This equation was used to calculate concentration in mass terms using a single internal standard.



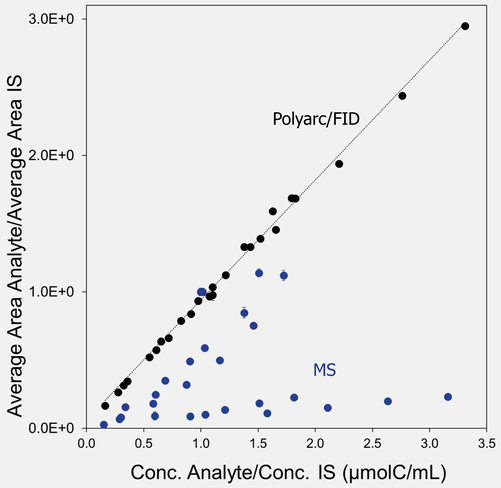
Matching retention times of the MS and Polyarc/FID signals allow for simultaneous identification and quantification. The final two peaks are Tinuvin 328 and Erucamide, and their low signals relative to the other peaks can be observed. This illustrates showing the variation in MS response by compound.

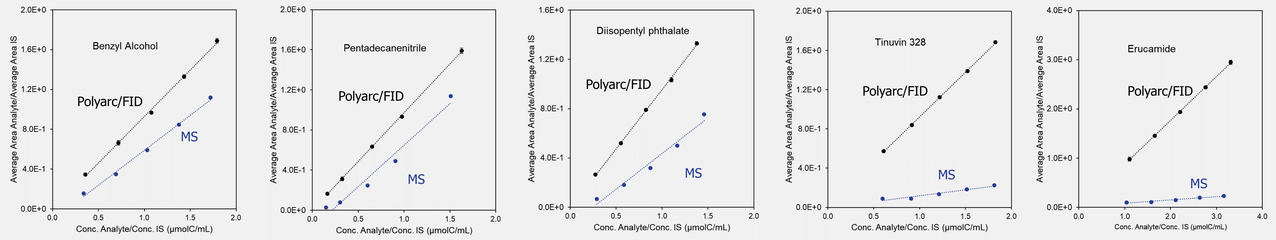
Results

Calibration Curves

The use of surrogate or reference compounds for calibration can introduce significant error into the quantification of analytes because of the variability of detector response factors and sample stability. Five level calibration curves were prepared with compounds of varying functionalities and retention times. The resulting response factors were used to calibrate for various compounds in a test mixture based on similar functionality and/or retention time.

Polyarc/FID results showed better linearity with a drastically more uniform response per mole of carbon. The plot below shows the response per mole of carbon for the Polyarc/FID in black and the MS in blue. Responses can differ by orders of magnitude in the MS, depending on the functionality, where the response is independent of molecule type with the Polyarc.

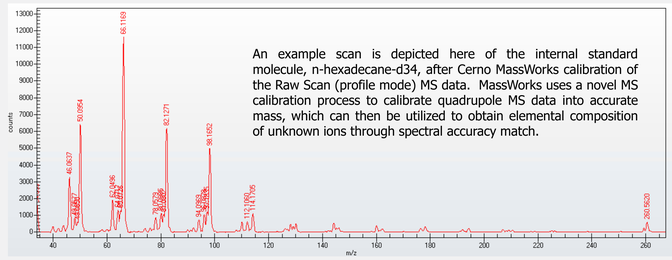




Enhanced Unknown Determination

Compound identification was improved through spectral calibration with PFTBA and subsequent software analysis with Cerno Massworks. Fragmentation patterns of several compounds did not always result in accurate identification using standard NIST library search routines. Instead, calibrated mass and spectral accuracy were used to identify several potential molecular candidates and quantification errors were compared. The results suggest that unknowns not present in library searches can be approximately identified and quantified with a single quadrupole Cerno-calibrated GC/MS-Polyarc-FID. The entire method for the identification, calibration and quantification has been automated through software to greatly increase the speed and accuracy of E&L analysis.

cerno BIOSCIENCE **MassWorks**
A software system for better MS

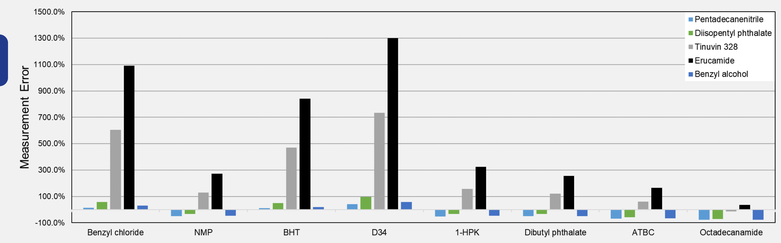


An example scan is depicted here of the internal standard molecule, n-hexadecane-d34, after Cerno MassWorks calibration of the Raw Scan (profile mode) MS data. MassWorks uses a novel MS calibration process to calibrate quadrupole MS data into accurate mass, which can then be utilized to obtain elemental composition of unknown ions through spectral accuracy match.

Accuracy

With standard GC/MS, average errors exceeded 40% and response factors did not correlate with functionality or retention time. With this novel setup, average errors were reduced, typically below 10%, and reproducibility improved to less than 2% standard deviation versus 8.7% with MS. Furthermore, all surrogate and reference molecules gave similar per carbon response factors with the Polyarc, suggesting that the choice of calibration surrogate is arbitrary. A single surrogate calibration was sufficient for full quantification. The quantification results in the table are based on surrogate molecules that matched chemical functionalities, and the chart below the table displays the variations in error that would be observed if different surrogate molecules were chosen for the MS.

Compound	MS Concentration (µg/mL)	MS Error	Polyarc Concentration (µg/mL)	Polyarc Error
Benzyl chloride	82.68	61%	53.46	4%
2-Pyrrolidinone, 1-methyl- (NMP)	21.76	-35%	34.87	4%
Butylated Hydroxytoluene (BHT)	28.44	57%	21.35	18%
n-Hexadecane-d34 (D34)	47.79	71%	28.00	0%
1-Hydroxycyclohexyl phenyl ketone (1-HPK)	30.62	-36%	52.35	10%
Dibutyl phthalate	26.13	-20%	32.75	0%
Tributyl acetylcitrate (ATBC)	35.48	-48%	68.54	0%
Octadecanamide	62.51	-2%	59.81	-6%



Conclusions

- When calculating concentrations using GC/MS with surrogate molecule assignment, errors range from 2%-71% in an unknown sample.
  - This calibration requires at least 15 different injections to create linearity plots
- Using a single internal standard, Polyarc errors are under 10% in the same unknown sample for most compounds, requiring no calibration curves.
- Choosing the incorrect surrogate compound can result in errors as high as 1300%, as shown to the right.
- Limits of detection are roughly 30x lower for Erucamide and Tinuvin 328 when using the Polyarc/FID.
- Splitting the column effluent using a helium purged splitter allows for identification and quantification in a single injection when using the Polyarc
- Cerno Massworks allows for accurate mass measurements and can identify compounds not available in the NIST database.