GC-Tandem Quadrupole Mass Spectrometry as an Alternative to High-Resolution Mass Spectrometry for the Investigation of Polychlorinated Dioxins and Furans

ASMS 2009

Anthony Macherone, Agilent Technologies, Inc

WPY 593

Introduction

Dioxins and furans refer to a general class of chemical compounds comprised of poly-chlorinated di-benzo ring systems with a dioxin or furan core. This chemical class is considered highly toxic and ubiquitous in the environment. Common sources of dioxins and furans include paper and pulp bleaching, incineration of municipal wastes and contamination of some phenoxy herbicides. Ninety percent of the exposure to dioxins and furans occurs through the diet. *Homo sapiens* possess no pathway to metabolize these compounds although females are unfortunately able to eliminate these through gestation or lactation. Samples are most commonly analyzed by high-resolution mass spectrometry (HRMS). This study evaluates the viability of GC-tandem MS as an alternative method for the analysis of dioxins and furans.

Dioxins and Furans

Relevance

- Source
  - — Paper and pulp bleaching
  - — Incineration of municipal wastes
  - — Contaminant in some phenoxy herbicides
- 90% of the exposure to polychlorinated dioxins and furans occurs through the diet
- Considered ubiquitous in the environment
- Implicated as carcinogens

Agilent 7890 / 7000

Experimental

A mixture of seventeen dioxins and furans and ten internal standards was prepared in toluene at two levels. One microliter was injected into a GC-tandem quadrupole mass spectrometer in splitless mode with the injector temperature at 300°C. The carrier gas was He flowing at 1.0 mL/min in constant flow mode. The column was a 15 m HP-5MS UI with 0.25 mm ID and a 0.25 micron film. The system was configured with a purged Ultimate Union (Agilent part # G3186B) to allow back-flush capabilities. The oven was ramped through a step-wise thermal gradient ranging from 100 through 310°C and the run time was approximately 23 minutes. The transfer line temperature was 310°C, the source temperature was 300°C and the quadrupole temperatures (Q1 = Q2) was 150°C. See the tables and the figures below for conditions and configuration.

GC-MS/MS Conditions

Column 1	HP-5MS UI 15 m x 0.25mm x 0.25 µm
Flow	1.0 ml/min, constant flow: -1.0 ml/min in backflush
Column 2	0.65 m x 0.15 mm ID x 0 um
Flow	1 psi, constant pressure: 40 psi in backflush
Carrier	He
Inject	1 ul, splitless @ 300 deg C

Oven Table

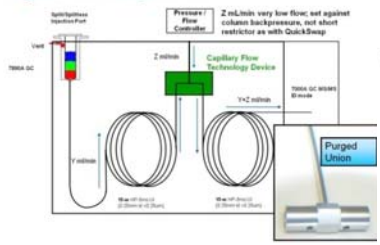
ramp (deg C / min)	Tf (deg C)	Hold t (min)
	100	0.4
100	200	6
7.5	235	4
20	310	3

Transfer line T	310 deg C
Source T	300 deg C
Quad T	150 deg C (Q1=Q2)
Quad resolution	1.2 amu (Q1=Q2)

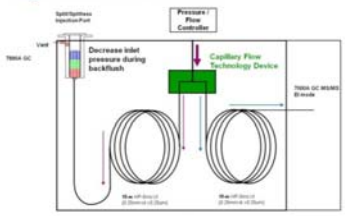
7000A MRM Transitions

Compound	Time Segment	Precursor Ion	MS1 Resolution	Product Ion 1	Product Ion 2	MS2 Resolution	Dwell	Collision Energy 1	Collision Energy 2
TCDF	1	306	1.2 amu	243	241	1.2 amu	75 ms	30	30
TCDD	1	322	1.2 amu	259	257	1.2 amu	75 ms	25	25
P_5CDF		340	1.2 amu	277	275	1.2 amu	75 ms	35	35
P_5CDD		356	1.2 amu	293	291	1.2 amu	75 ms	25	20
H_6CDF		374	1.2 amu	311	309	1.2 amu	75 ms	35	35
H_6CDD		390	1.2 amu	327		1.2 amu	75 ms	20	
H_7CDF		410	1.2 amu	347	345	1.2 amu	75 ms	35	35
H_7CDD		426	1.2 amu	363	300	1.2 amu	75 ms	25	45
OCDF		444	1.2 amu	381	279	1.2 amu	75 ms	25	25
OCDD		460	1.2 amu	397		1.2 amu	75 ms	25	

Purged Union: Analysis Mode



Purged Union: Backflush Mode



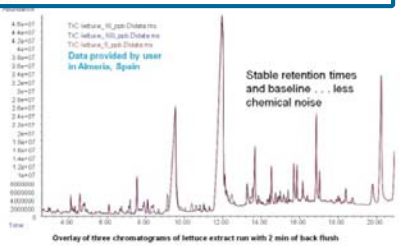
Options

Analytical Reality of GC/MS/MS Methods

GC/MS/MS needs backflush as much or more than GC/MS to avoid "invisible" problems:
- source contamination
- loss of sensitivity

And to avoid the visible problems:
- changing retention times

With Backflush: No Increased Background (Less Spectral Noise) and Consistent Retention Times



Overlay of three chromatograms of lettuce extract run with 2 min of back flush

Technical Advantage: Agilent Multimode Inlet
Split/ Splitless and LVI

Agilent Next Generation Sampler for GC

Sample Prep Programming Flexibility

Sandwich injections (up to 3 layers with air gap)

Simple liquid manipulation

- ISTD addition
- Reconstitution
- Mixing (Requires Bar Code Reader / Heater/ Mixer option)
- Dilution
- Derivatization
- In-Vial Extraction

Agilent Multimode Inlet Features

Hardware

Temperature range of -160C to 450C

Heating @ 15C/sec

Septum/Liner Easily Exchangeable

Injection Modes: Hot S/SL, Cold S/SL, all in pulsed mode, solvent vent mode, residue removal mode

Support for single stroke injections from 0.1 µL to 250 µL

EPC Compatible with Packed Liners

Compatible with 7890A, 5975C, 7683, CTC Combi PAL

Software

Ten temperature ramps

Solution for solvent vent timing

Fully integrated into ChemStation, MSD ChemStation, EZChrom, MassHunter

Results and Discussion

EPA methods 1613 and 8280 both evaluate the 17 dioxin and furan congeners albeit by differing MS methodology. EPA 1613 requires high resonance mass spectrometry (HRMS) while EPA 8280 is a low resonance (unit resolution) mass spectrometry (LRMS) method. The advantage of HRMS over LRMS is mass accuracy (specificity) and the ability to eliminate interference from the analyte matrix thus resulting in improved signal to noise (sensitivity). While tandem quadrupole (MS/MS) mass spectrometers have unit resolution, as do all quadrupole mass spectrometers, high specificity is achieved via multiple reaction monitoring (MRM) ion transitions. Specificity can be evaluated via a point system wherein 4 or better is the goal. Indeed, the European point system assigns a specificity value of 2 to HRMS and as much as 5 for MS/MS experiments with two MRM transitions per analyte (see table at left). High sensitivity is also achieved through the inherent nature of MRM to negate interfering matrix effects thus increasing overall signal to noise.

In our experiments, we evaluated specificity and sensitivity in two matrices: air particulate matter and vegetation extracted via QuEChERS. As low as 250 fg on-column was observed in the air matrix with excellent signal to noise and 400 fg on-column in the vegetative matrix. Specificity points of 5 for 15 of the 17 analytes can be assigned and 3 for the remaining 2 analytes (see chromatograms and tables section).

Specificity:
The Identification Point (IP) system

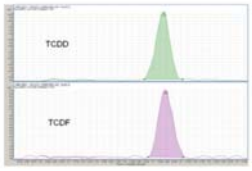
MS – Technique	IP / ion
Single Quadrupole ion (GC/MS)	1.0
GC/MS/MS precursor ion	1.0
GC/MS/MS transition products	1.5

The IP system:
Tolerance of relative intensities

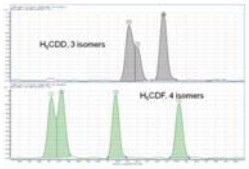
Rel. intensity (% of base peak)	GC-MS/EI	GC-MS/CI, GC-MS/MS
> 50 %	± 10 %	± 20 %
> 20 % - 50 %	± 15 %	± 25 %
> 10 % - 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

Chromatograms & Tables

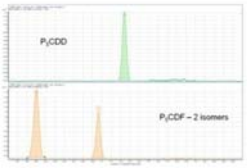
TCDD & TCDF: 250 fg/µl each



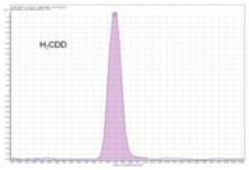
H_6CDD & H_6CDF: 500 fg/µl each



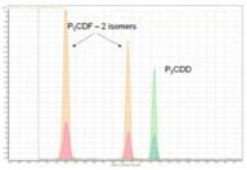
P_5CDD & P_5CDF: 500 fg/µl each



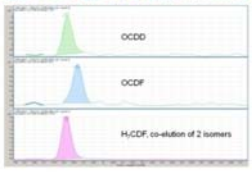
H7CDD, 500 fg/µl



Representative MRM's for 1000 fg & 250 fg on column

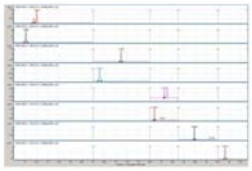


OCDD, OCDF & H_7CDF:
1000 fg/µl & 1000 fg/µl & 500 fg/µl, respectively

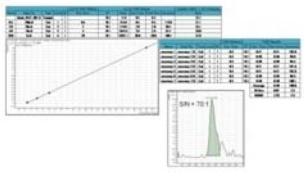


Dioxin/Furan Standards

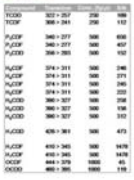
Internal Standards



400 fg TCDF in vegetative matrix on column



S/N for 17 congeners
Matrix = Air particulate



Conclusions

Currently, EPA requires HRMS for method 1613 and regulated environments must adhere to this stipulation but in non-regulated laboratories, MS/MS is a viable alternative to HRMS. GC-MS/MS provides the required specificity and sensitivity in analytical matrix and ease of operator use and maintenance. EPA method 8280 stipulates unit mass resolution and is therefore a prime candidate for transition to MS/MS to improve specificity and sensitivity.

The set of compounds in this analysis was comprised of congeners ranging from tetrachloro through octachloro species with zero to four homologues in each set. The concentration of the analytes ranged from 250 fg/µL through 1000 fg/µL. Fifteen of the seventeen analytes were identified via their unique molecular transitions (MRM) or, in the case of some homologues, via retention time. Two homologues, heptachlorodibenzofuran, co-eluted. This was also observed in the HRMS spectrum and may be resolved with more rigorous chromatographic method development. The heptachlorodibenzofuran homologues also co-eluted with octachlorodibenzodioxin but this was easily resolved in the GC-MS/MS data as a result of differing MRM transitions for the congeners. The signal to noise ratio was greater than 100:1 in all cases except that of 1000 fg/µl octachlorodibenzofuran wherein the S/N was 45:1. The S/N via GC-MS/MS suggests that a four-fold improvement in sensitivity may be attained. In general, the GC-MS/MS data suggests that the detection limits for all compounds in the set can be improved by four-fold as illustrated above through as much as 100-fold in the case of heptachlorodibenzofuran. Selectivity is also enhanced through the unique precursor-product ion transitions (MRMs) used for both quantitative and qualitative information and identification while simultaneously reducing matrix interferences.

With the added technology of capillary flow technology, designed to facilitate day-to-day prime performance, a multi-mode inlet that has been shown to improve signal intensity via cold-split/splitless or large volume injection with solvent venting and the Agilent 7693A sample prep station, the Agilent 7000 GC-MS/MS system is poised to push the envelope of sensitivity in every chemical analysis discipline.

Acknowledgements

The author would like to acknowledge Stephan Baumann, Gas Phase Applications Chemist, Agilent Technologies, for data and his collaborative effort.