Preventing Wine Spoilage:

Rapid Screening & Quantitative Analysis of Off-flavor Phenolic Compounds by DART Mass Spectrometry

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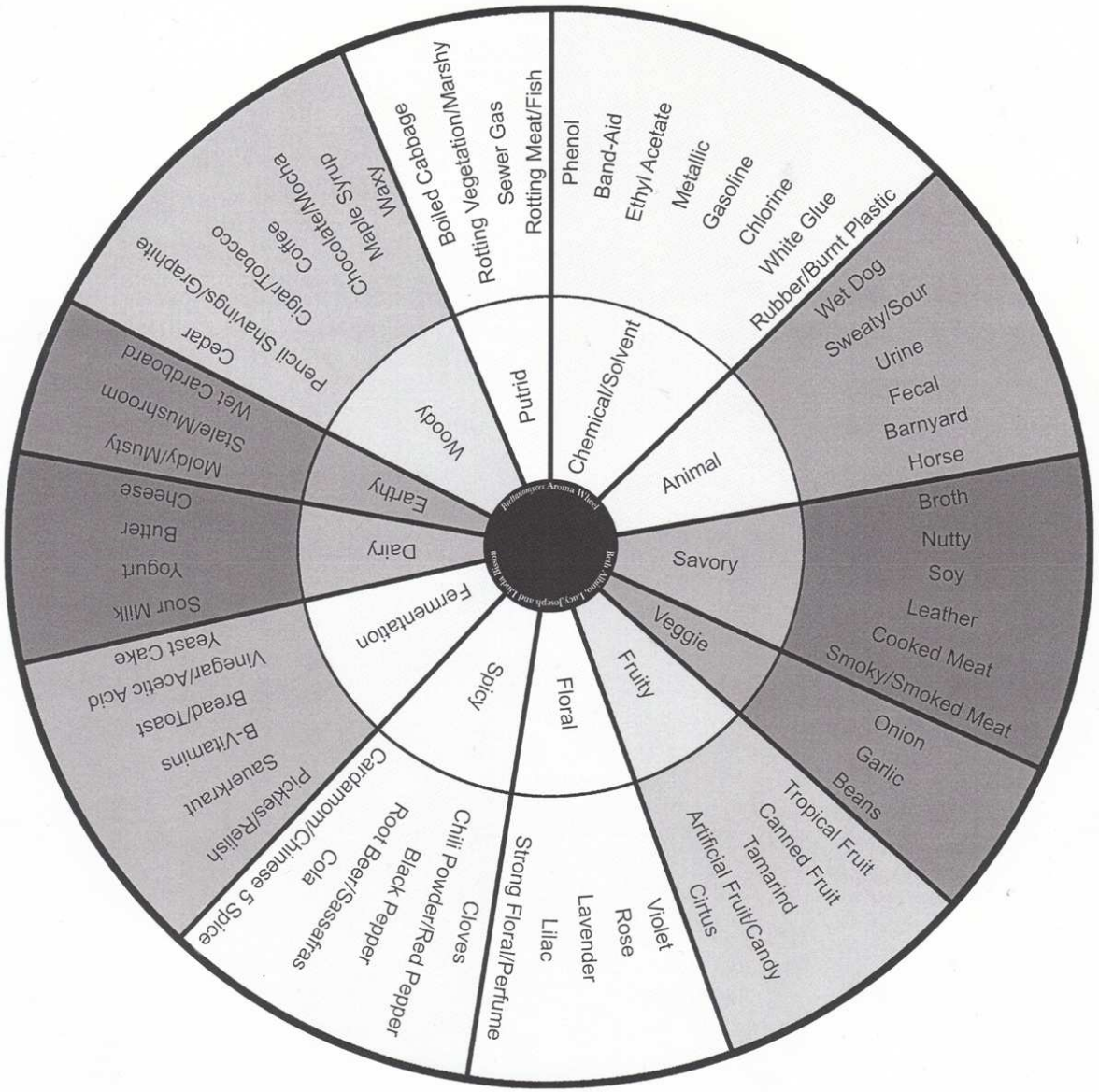
Brettanomyces

Background:

- Budding yeast found widely distributed in nature
- Discovered in beer in 1904 (Claussen), in wine (Krumbholz & Tauschanoff,1930) and again in 1940 (Custers)

- Produces a wide array of aromatic compounds
- Wine cellar contamination was widespread
- **"Brett" characters can compete with varietal characters for dominance of wine profile**



"Brett" Wheel http://heysmartbeerdude.files.wordpress.com/2013/04/brett-aroma-wheel.jpeg (Access: 10 June 2013)

When Is It Spoilage?

- High concentration, dominating wine profile
- Conflict with wine matrix characters
- Suppression of varietal character
- Enhancement of off-notes
- Lactic acid bacteria often found in wines with *Brettanomyces*

Recovery Thresholds:

- Chatonnet* has defined spoilage as:
  - >426 ppb of 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG)
  - >620 ppb of 4-EP
- 50% of tasters can detect 605 ppb in wine or 440 ppb in water of 4-EP

* Chatonnet, P.; Boidron, J. N.; Dubourdieu, D. Influence des conditions d' élevage et de sulfitage des vins rouges en barriques sur leur teneur en acide acétique et en éthyl-phenols. *J. Int. Sci. Vigne Vin.* **2003**, *27*, 277-298.

Main Aromas and Incidence of Spoilage

Associated Aromas:

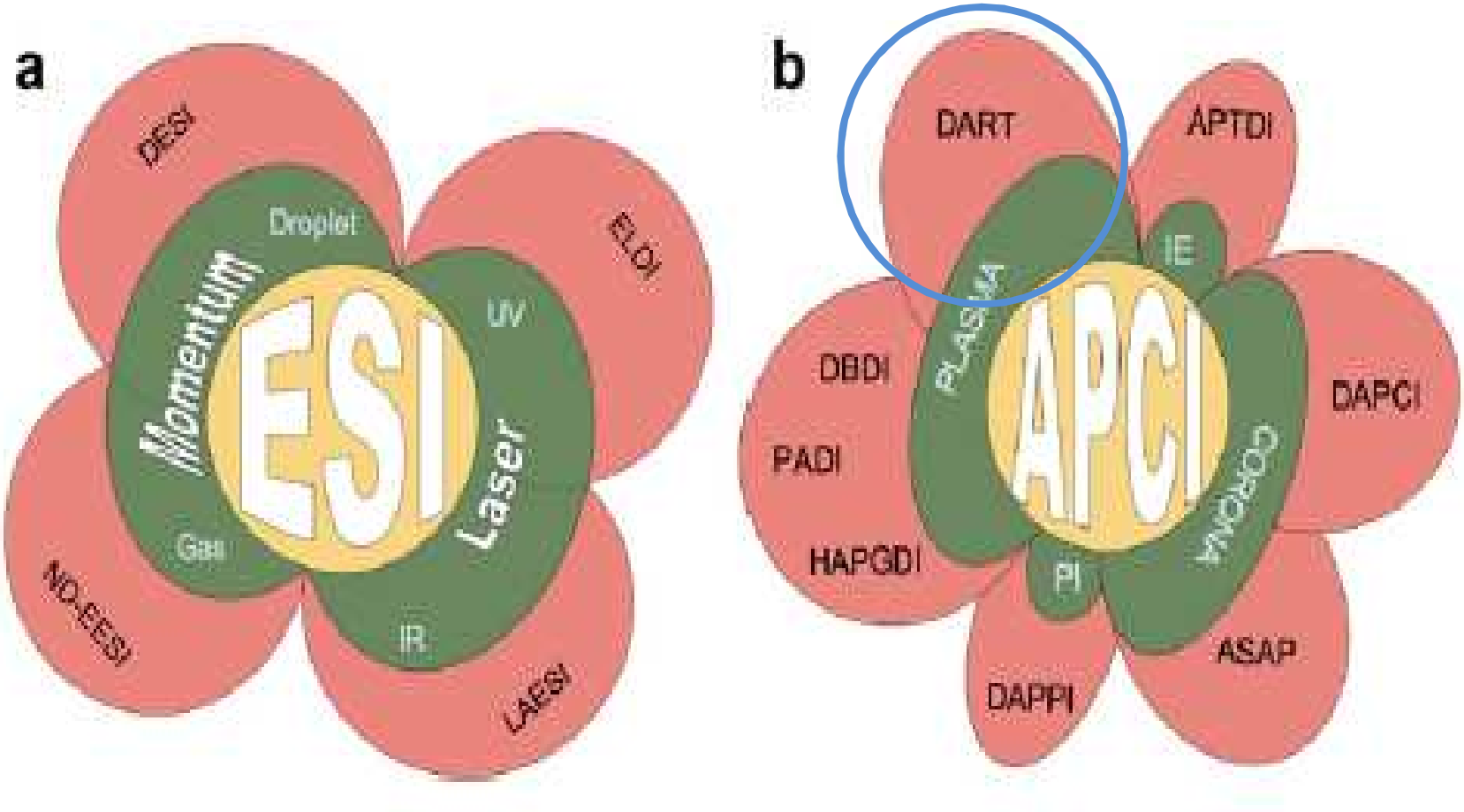
- Band-Aid (4-Ethyl Phenol)
- Earthy (Geosmin)
- Horsy
- Leather
- Putrid
- Soy
- Tobacco

Country	>426ppb	>620ppb
France	36%	28%
Italy	49%	19%
Australia	59%	46%
Portugal	42%	27%

Wines may contain up to 50 ppm (!) of 4-EP

Slide courtesy of Dr. L. Bisson, Dept. of Viticulture & Enology, Univ. of California-Davis

Evolving Open Air Ionization



Venter, A.; Nefliu, M. Cooks, R.G., "Ambient Desorption Ionization Mass Spectrometry", Trends in Anal. Chem., 27, 284-290, 2008.

Classic vs. Ambient Ionization

GC-MS Method:

Sample Preparation:

- **Total runtime = ~50 minutes**
  - **Total** sample prep time = **15 minutes** with **100% analyst involvement**
  - **GC-MS** runtime of **32 minutes**
    - Internal standards (4-EP-d4; 4-EG-d5) spiked into wines (5 mL), vortexed, solvent added (t-butyl ether), centrifugation, 5 µL injection onto GC

(Adapted form Rayne & Eggers (2008) (Am. J. Enol. Vitic. 59:92-97))

DART Ambient Ionization Method:

- **Direct sample analysis** under ambient conditions
  - **No separation** of sample components (chromatographic)
  - **Minimal** sample preparation requirements (sample concentration)
  - Analysis time of **3 minutes** per sample

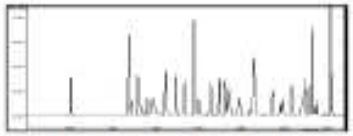
AMBIENT MASS SPECTROMETRY

CLASSIC APPROACH (GC-MS)

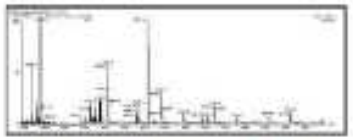
SAMPLE

SAMPLE PREPARATION

IONIZATION & DETECTION



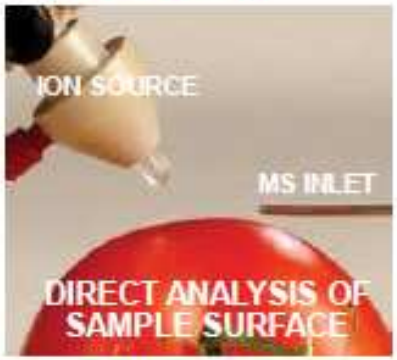
CHROMATOGRAM



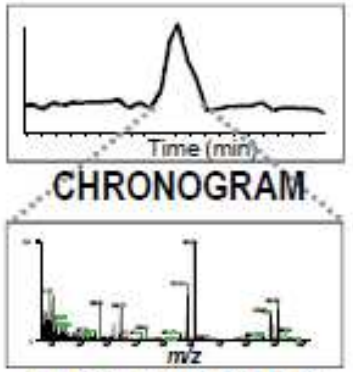
MASS SPECTRUM

AMBIENT MS APPROACH (DART–MS)

SAMPLE



IONIZATION & DETECTION



CHRONOGRAM

MASS SPECTRUM

ICT PRAGUE

Slide courtesy of Prof. Jana Hajšlová, ICT Prague, Czech Republic

Key DART Source Parameters

- **Source Parameters**:
  - Ionization gasses
    - N_2 (Standby)
    - **He (Run Mode)**
  - **Temperature** of ionization gas
  - Sample introduction **speed**

- **Ions typically observed in DART–MS mass spectra:**
  - $[M+H]^+$, $[M-H]^-$
  - $M^{+\cdot}$, $M^{-\cdot}$ (ionic compounds)
  - Use of dopants to promote ionization:
    - $[M+NH4]^+$ (ammonia)
    - $[M+Cl]^-$ (e.g. CH_2Cl_2)
    - $[M+CF_3COO]^-$ (trifluoroacetic acid)

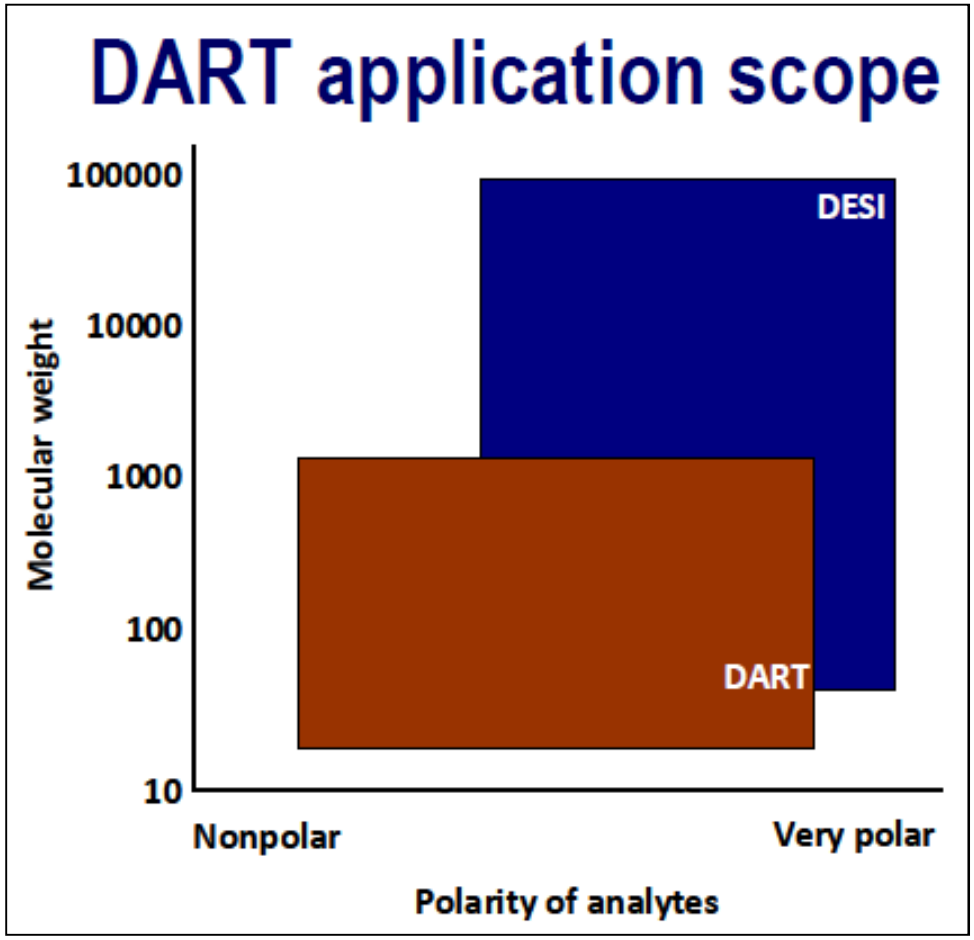


Figure courtesy of Prof. Jana Hajšlová
ICT Prague, Czech Republic

DART-SVP Coupled with Q Exactive

Targeted MS^2 Settings

- **Scan Parameters:**
  - **Negative Ion Mode**
  - **Resolution:** 35,000 FWHM at *m/z* 200
  - **NCE Fragmentation:** 45 (4-EP); 25 (4-EG)
  - **AGC Target:** $2e^5$ charges
  - **Max IT:** 250 ms
  - **Isolation Width (*m/z*):** 3.0
- **Q Exactive Inlet Parameters:**
  - **Capillary Temp:** 200° C
  - **S Lens:** 50
- **External Mass Calibration**

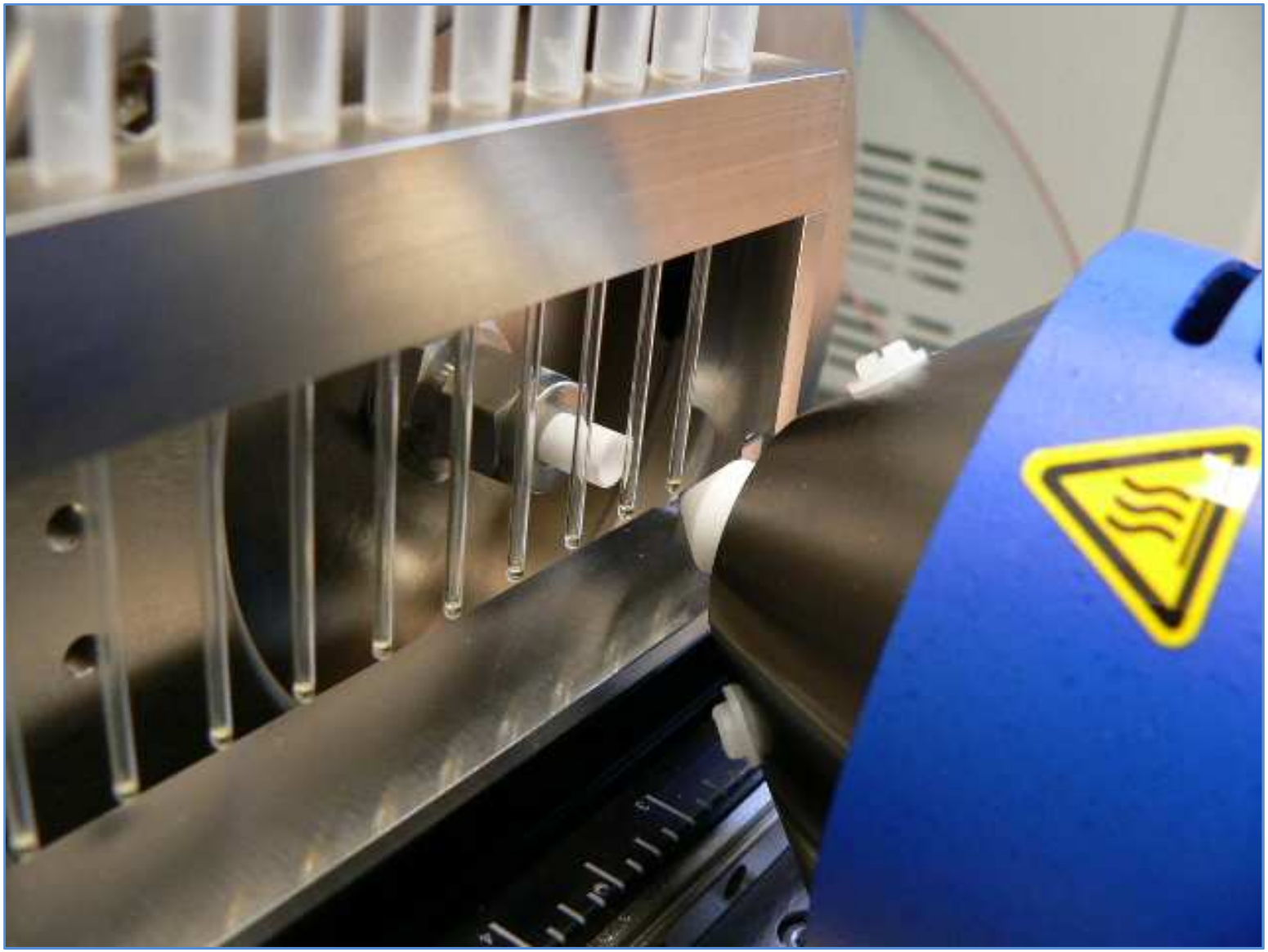
- **All of the following parameters were set to zero:**
  - Sheath Gas Flow, Aux Gas Flow, Sweep Gas Flow, Spray Voltage

DART-SVP Settings

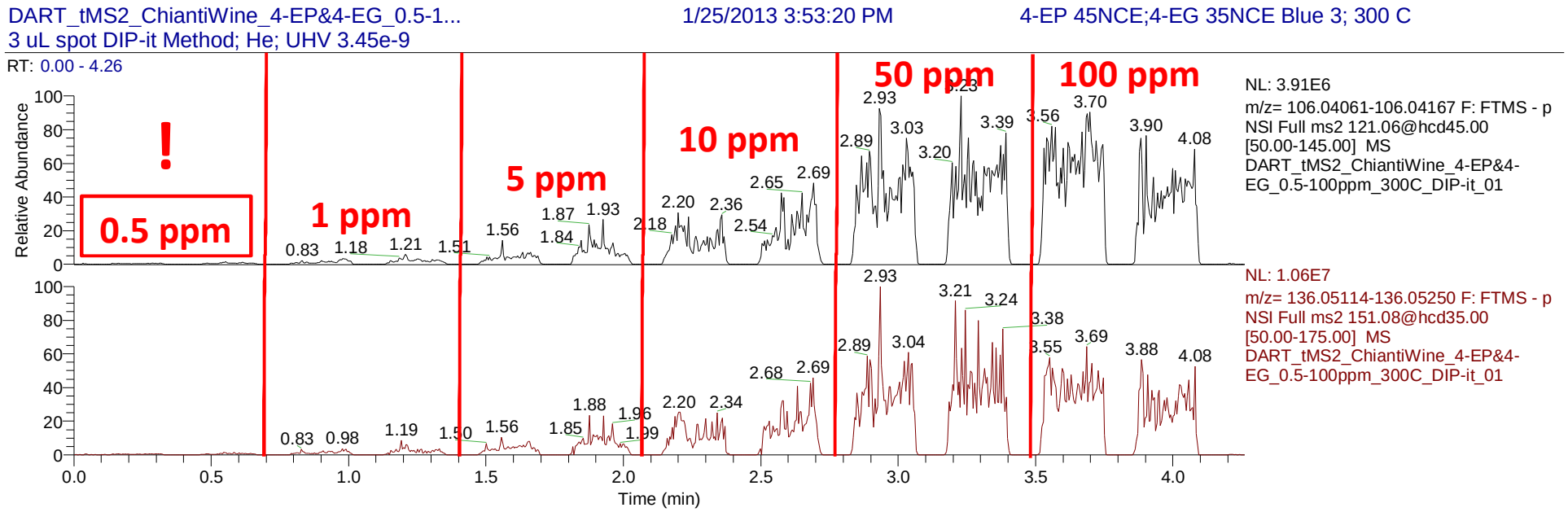
- **DART Source:**
  - **Negative Ion Mode**
  - **Heater Temperature Gradient:** **100 - 300° C**

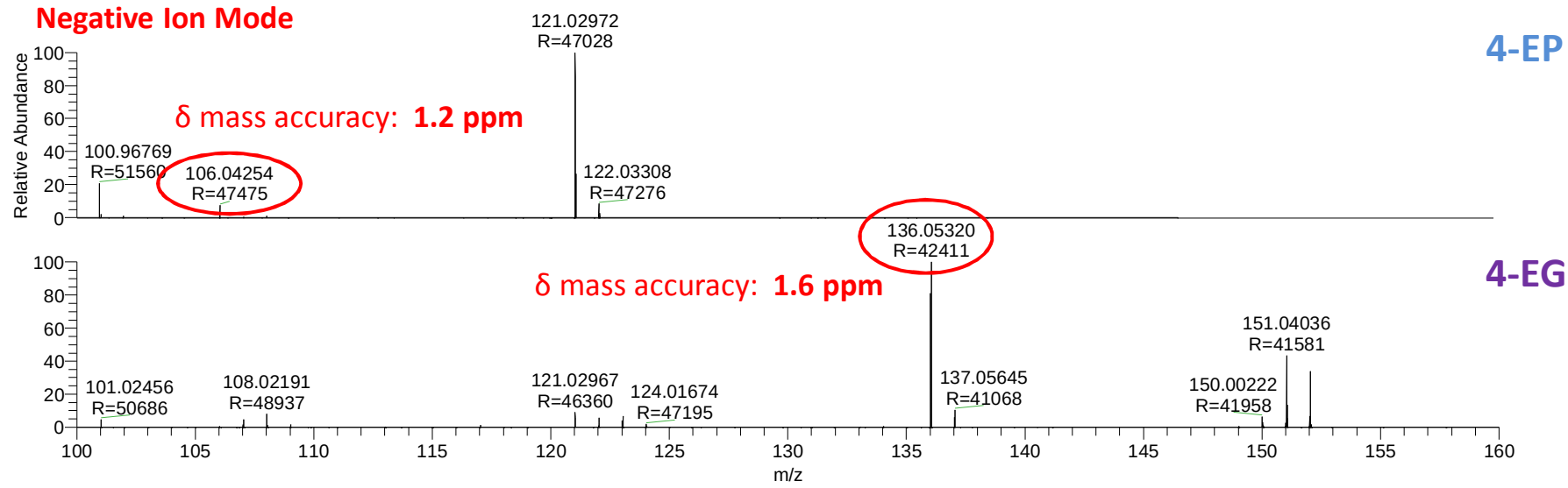
*** SVP = Standardized Voltage and Pressure**

Classic DART Sampling: Wine on Glass Tips



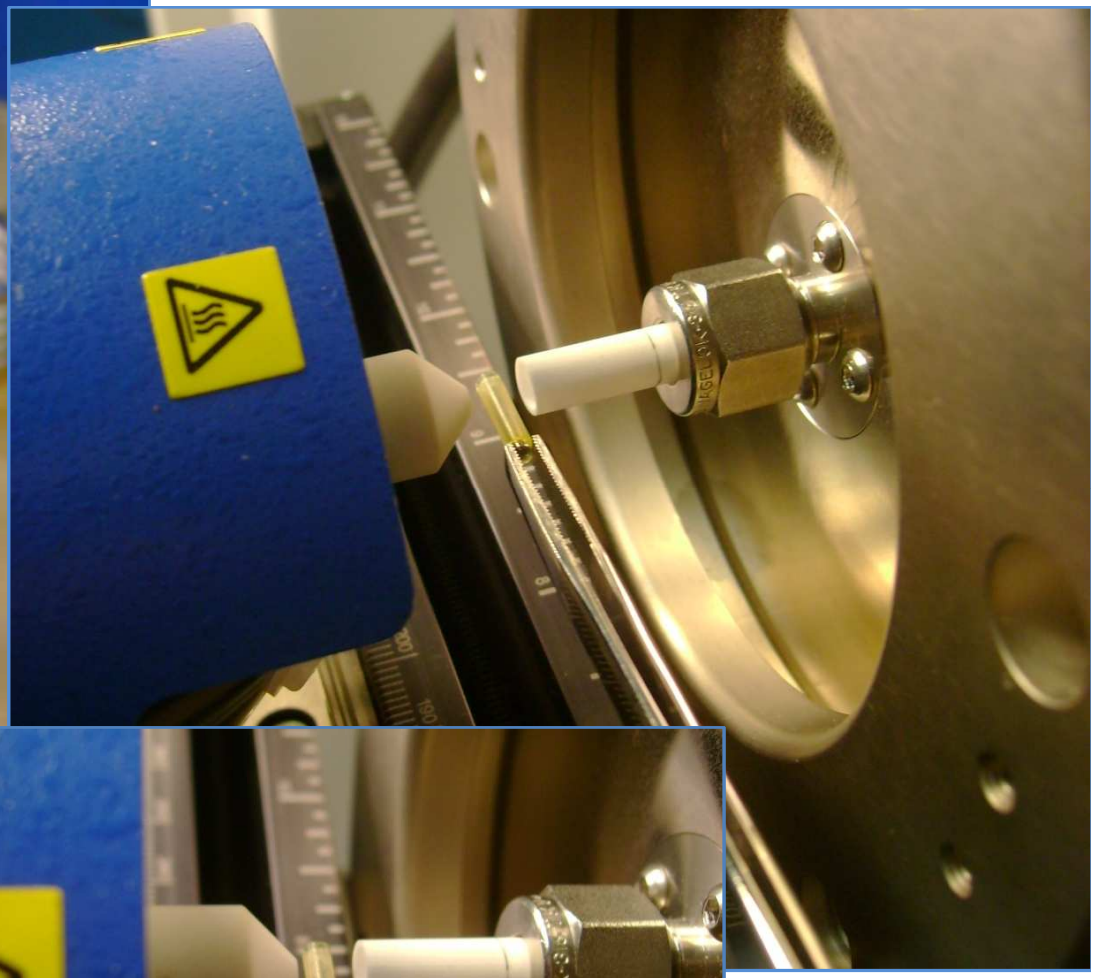
Spiked Wine Sampled Direct on Glass

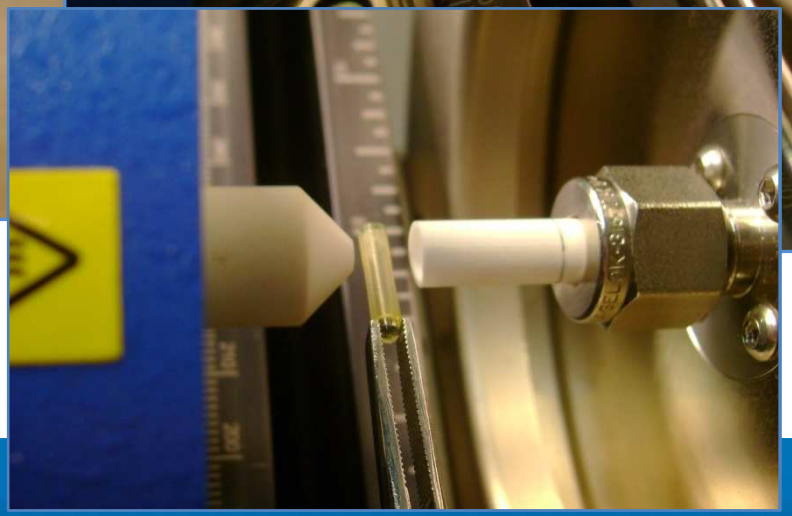




Targeted MS/MS experiment looking at major fragment ions, with direct sampling from merlot wine spiked with 4-EP and 4-EG

Gerstel Twister Stir Bar: Sample Concentration





Twister was **spun overnight** in **orange juice** with **50 ppb spiking** level of **10 pesticides**

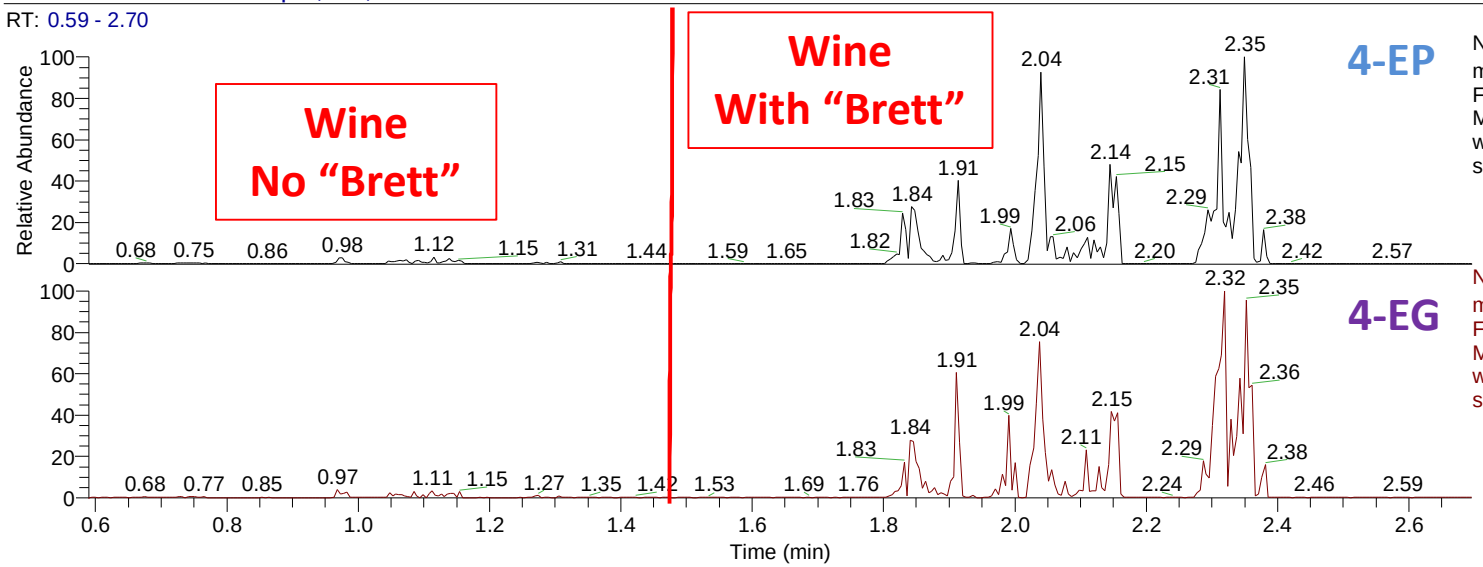
Real Wine Sample - Direct Twister

Preliminary Results - No Glass Chamber

DART_tMS2_Wine_4-EP&4-EG_Wine without...
Gerstel Twisters 24hr spin; He; UHV 3.45e-9

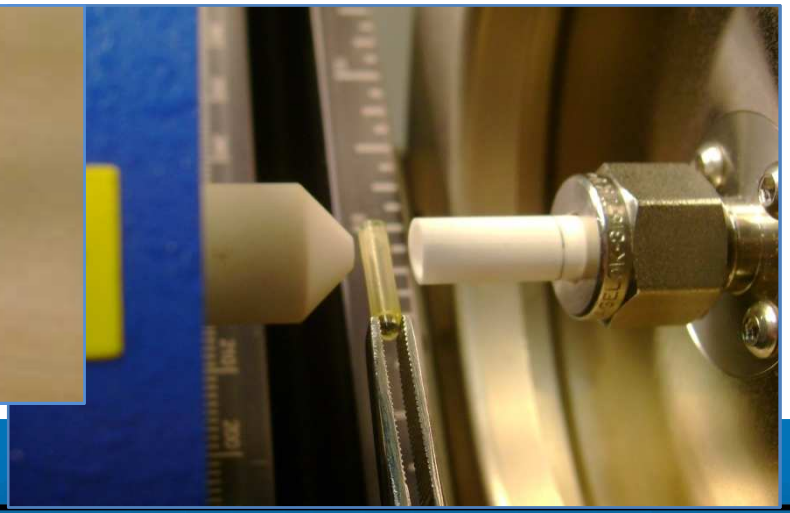
1/25/2013 4:20:00 PM

4-EP 45NCE;4-EG 35NCE Blue 3; 300 C



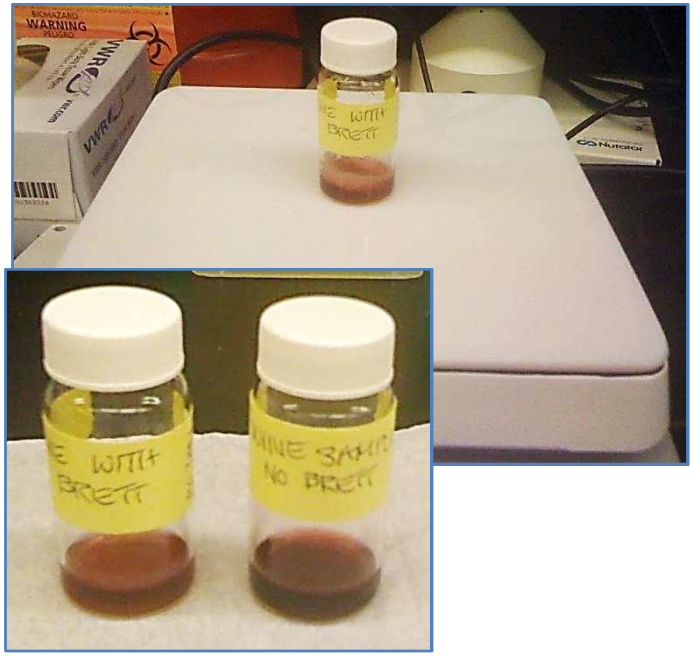
NL: 2.20E6
m/z= 106.04061-106.04167 F: FTMS - p NSI Full ms2 121.06@hcd45.00 [50.00-145.00] MS DART_tMS2_Wine_4-EP&4-EG_Wine without & with Brett_300C_Twisters_24hr spin_01

NL: 2.55E6
m/z= 136.05114-136.05250 F: FTMS - p NSI Full ms2 151.08@hcd35.00 [50.00-175.00] MS DART_tMS2_Wine_4-EP&4-EG_Wine without & with Brett_300C_Twisters_24hr spin_01



Signal from real wine sample could not be detected directly, need to concentrate analytes.

Wine Sampled with PDMS Twister

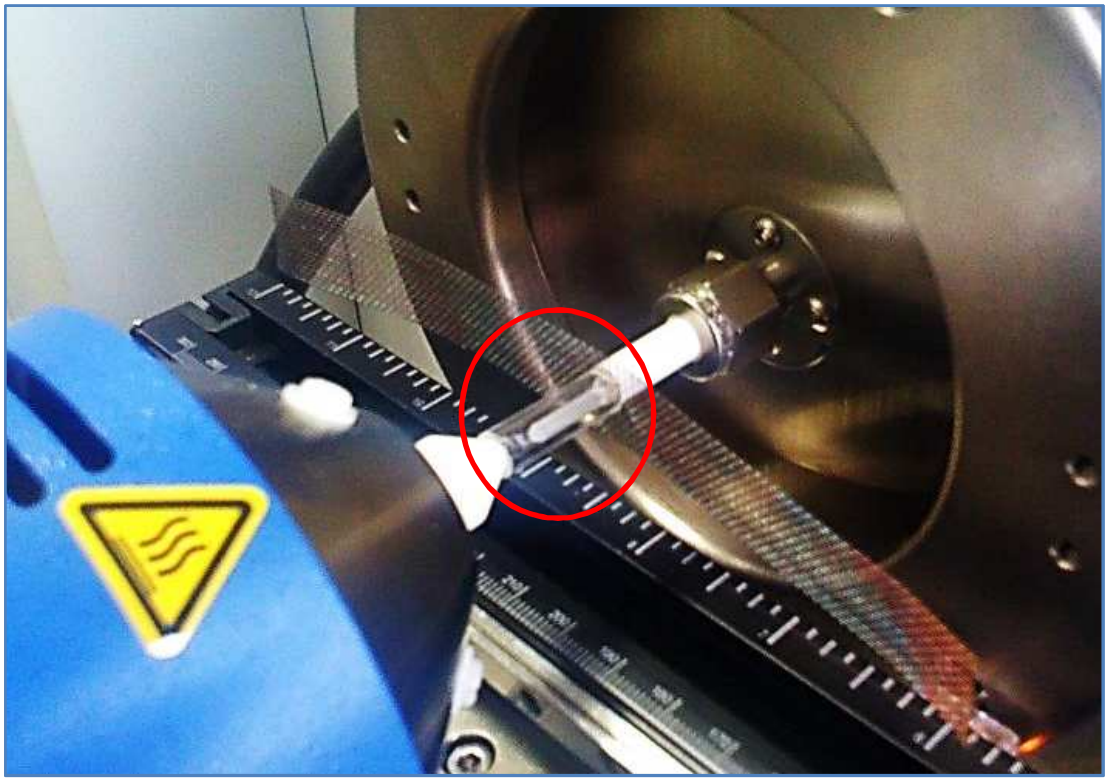


Twister stir bar
(PDMS, 0.5 mm thickness)
introduced into 2 mL wine

Minimum 30 minutes
unattended stirring

MAJOR IMPROVEMENT

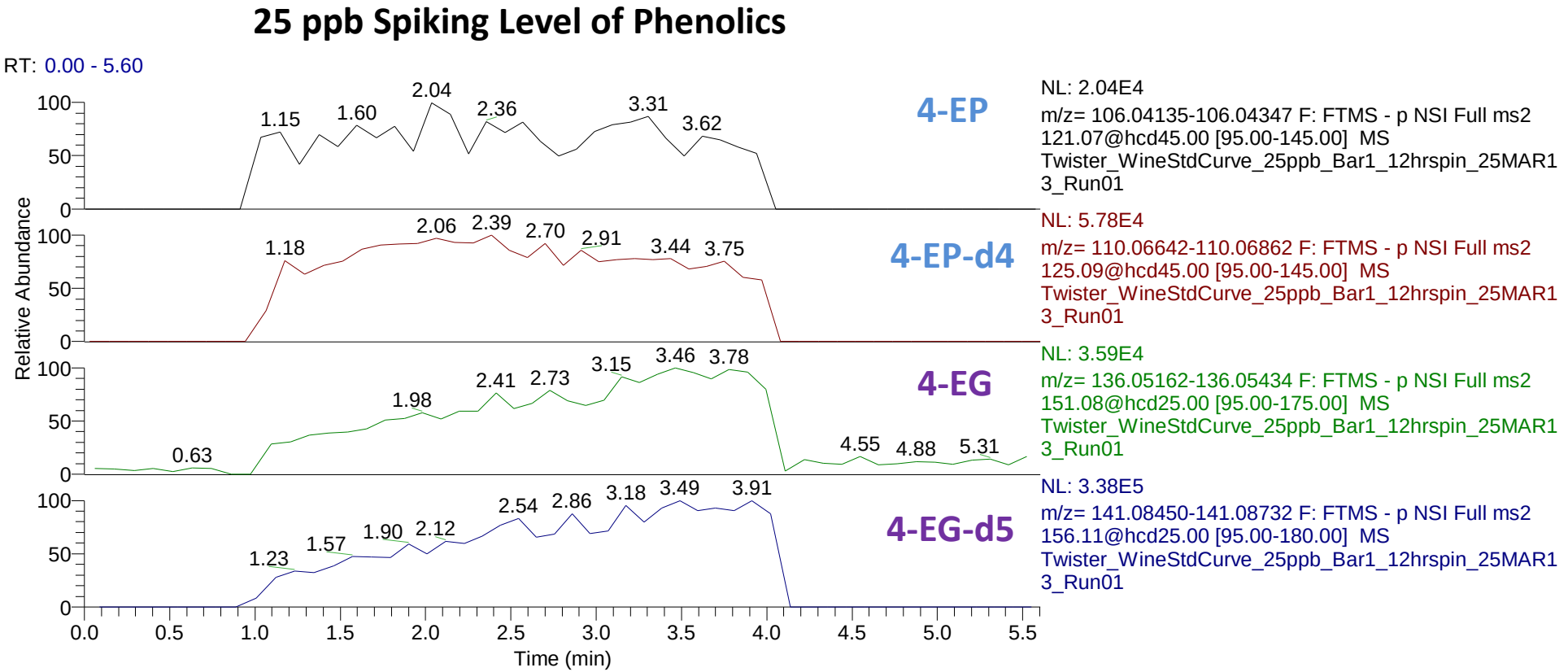
Twister placed in glass chamber = COMPLETE desorption



Typical DART-MS Chronogram from Stir Bar

Final Results - Glass Chamber Yields

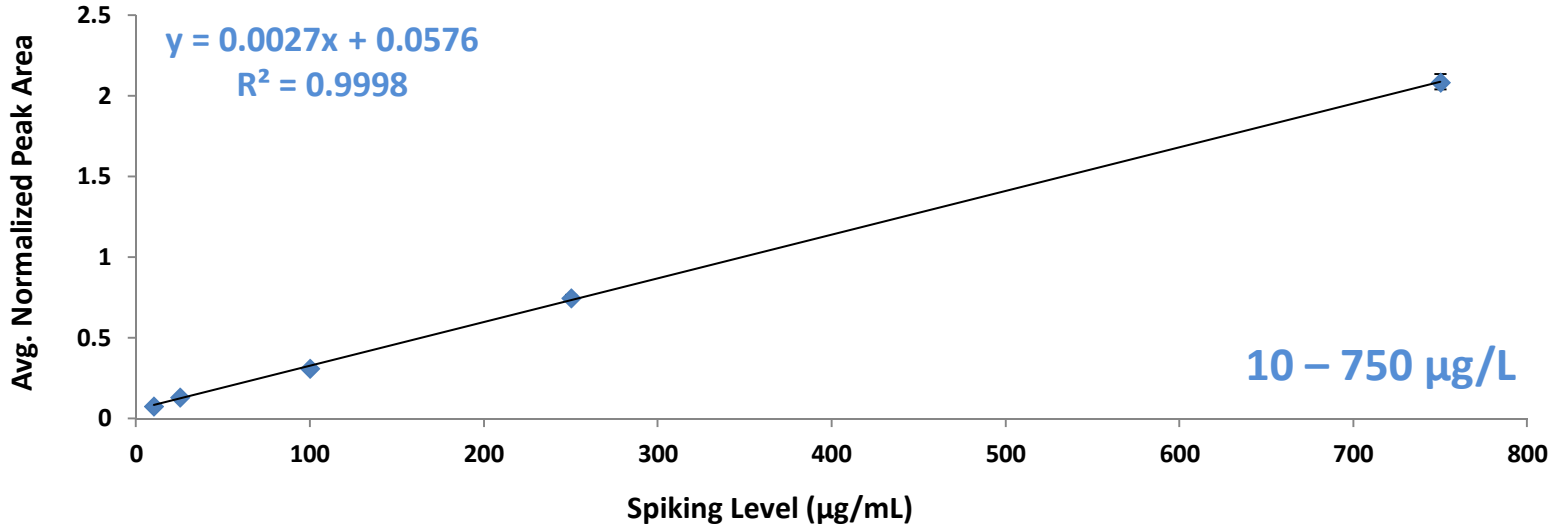
Much Improved Sampling Reproducibility



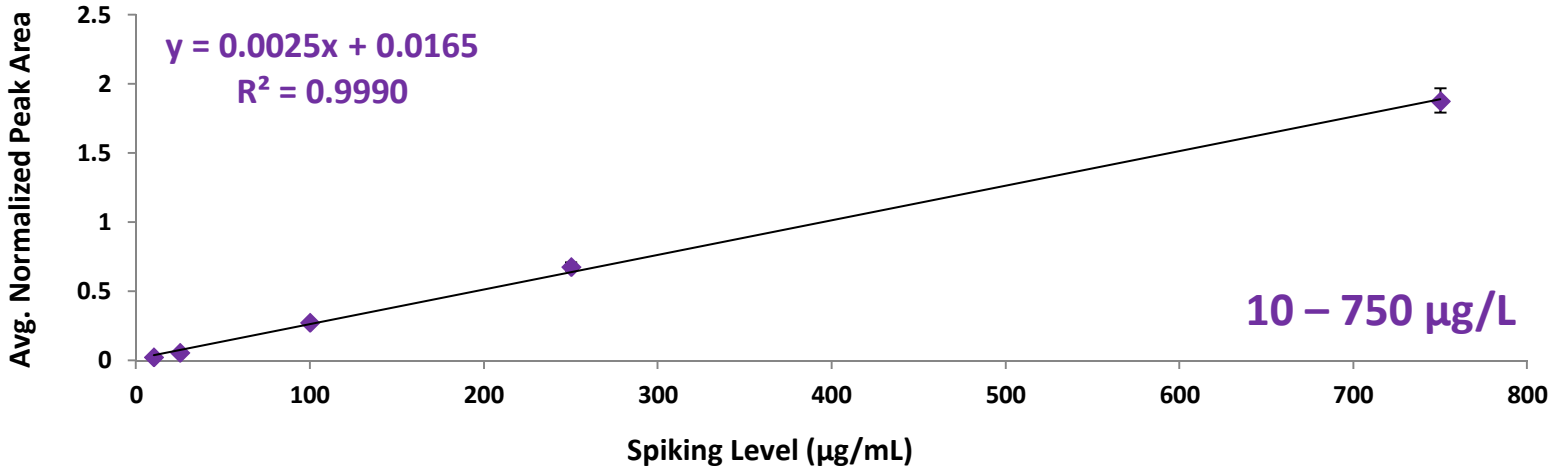
Major improvement using glass chamber for even desorption from a single Twister stir bar

Twister Stir Bar: Quantitative Results

Merlot Spiked with 4-Ethylphenol (4-EP)



Merlot Spiked with 4-Ethylguaiacol (4-EG)



DART MS/MS Method: Figures of Merit

4-EP Conc. with IS (µg/L)	1	10	25	100	250	750
1	ND	5.9	32.8	101.1	259.0	737.7
2	ND	9.8	24.4	88.4	253.4	764.9
Mean	N/A	7.8	28.6	94.8	256.2	751.3
%Bias	N/A	-21.7	14.5	-5.2	2.5	0.2
n	2	2	2	2	2	2

4-EP Conc. (µg/L)	Low	High
	50	500
1	47.7	492.2
2	58.7	509.2
3	52.1	N/A
4	41.0	N/A
Mean	49.9	500.7
%CV	14.9	2.4
%Bias	-0.3	0.1
n	4	2

Compare: Calculated Levels of 4-EP & 4-EG

Wine Sample	DART HRAM MS/MS		GC MS	
	4-EP (µg/L)	4-EG (µg/L)	4-EP (µg/L)	4-EG (µg/L)
Sample 04	854 *	197	845	203
Sample 05	518	157	563	161
Sample 06	52	ND	129	14
Sample 09	ND	ND	110	13
Sample 14	2774 *	492	2534	433

Yellow = Brettanomyces

*** Levels above selected calibration range**

Twister: Effect of Time on Concentration

Wine Sample	4-EP (µg/L)	4-EG (µg/L)
Sample 44 (30 min)	3775*	287
Sample 44 (1 hour)	3276*	295
Sample 44 (12 hour)	3587*	309
RSD %	7.0	3.6
Sample 19 (30 min)	2230*	450
Sample 19 (12 hour)	2342*	440
RSD %	3.5	1.6
Sample 36 (30 min)	1443*	289
Sample 36 (12 hour)	1277*	296
RSD %	8.6	1.7

*** Levels above selected calibration range**

Summary

- **Remarkable correlation** between classic GC-MS method and novel DART-MS/MS method
- **Effective** and **labor-free and solvent-free** analyte concentration onto the **Gerstel Twister sorptive stir bars**
  - Direct screening of volatile **phenolic compounds** at **low levels (10 - 50 ppb range)** with **3 minute sample analysis time with DART ionization**
- The stir bar **DART HRAM MS/MS** method is **quantitative** over the targeted concentration range with good precision and accuracy
  - **Limits of quantification** between **10 - 50 ppb**
- **Minimized analyst interaction** with the samples with DART method:
  - Reduces analyst error
  - Increases productivity and sample throughput
  - **Real-time monitoring = quick response to changes during wine fermentation to prevent wine spoilage (minimize revenue loss)**
- **Future work:** Apply this method for broader phenolic characterization of wines for rapid wine characteristic fingerprinting

Haunschmidt, M. et al. *Determination of organic UV filters in water by stir bar sorptive extraction and direct analysis in real-time mass spectrometry.* Anal. Bioanal. Chem., 2010, 397(1): 269-275

Questions?

Many thanks to…

Thermo Fisher Scientific

- Dr. Catharina Crone
- Dr. Markus Kellmann
- Dr. Tabiwang Arrey

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Recent Publications

- **Direct analysis in real time mass spectrometry and multivariate data analysis: A novel approach to rapid identification of analytical markers for quality control of traditional Chinese medicine preparation**. Zeng S, Wang L, Chen T, Wang Y, Mo H and Qu H. *Analytica Chimica Acta* **733**:38–47, 2012

- **Rapid detection of alkaloids in Ipecac by direct analysis in real time tandem mass spectrometry (DART-MS/MS)**. Sun L, Hu X, Liu L, Jin H and Lin R. *Zhongguo Zhong Yao Za Zhi* **37**:1426–30, 2012 (Article in Chinese)

- **Metabolomic fingerprinting employing DART-TOF MS for authentication of tomatoes and peppers from organic and conventional farming**. Novotna H, Kmiecik O, Gałązka M, Krtkova V, Hurajová A, Schulzová V, Hallmann E, Rembiałkowska E and Hajšlova J. *Food Additives & Contaminants: Part A* **29**(9):1335–1346, 2012

- **Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry**. Musah R, Domin M, Walling M and Shepard J. *Rapid Commun. Mass Spectrom.* **26**(9):1109–1114, 2012

- **Analysis of isoflavones in soybeans employing direct analysis in real-time ionization–high-resolution mass spectrometry**. Lojza J, Cajka T, Schulzova V, Riddellova K and Hajslova J. *J. Sep. Science*. **35**(3):476–481, 2012

- **Confined direct analysis in real time ion source and its applications in analysis of volatile organic compounds of Citrus limon (lemon) and Allium cepa (onion)**. Li Y. *Rapid Commun. in Mass Spectrom.* **26**(10):1194–1202, 2012

- **Chemometric Classification of Morphologically Similar Umbelliferae Medicinal Herbs by DART-TOF-MS Fingerprint.** Lee SM, Kim HJ and Jang YP. *Phytochem. Anal.* **23**(5):508-512, 2012.

http://www.ionsense.com/pdfs/DARTFoodBook18Sep12sm.pdf