VASE - Vacuum Assisted Sorbent Extraction
Odor, Pesticide, & PAH Analysis for Dairy Products

Extending Quantitative Headspace to Include Less-Volatile Compounds in Difficult Matrices

Application Note Brief:

V-3741-03

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Overview

The headspace of common dairy products, including chocolate chip cookies, cheese, and milk were analyzed utilizing a new solvent-free technique called Vacuum Assisted Sorbent Extraction (VASE).

Dairy products are typically challenging to analyze using headspace techniques due to the low volatility of many compounds of interest, and the high fat content, which creates a high affinity for most organic compounds to the sample matrix. Most headspace techniques do not yield much information from fatty matrices, leaving solvent extraction as the only effective technique which recovers low level flavor and odor compounds, as well as contaminants such as polycyclic aromatic hydrocarbons (PAHs).

VASE enables reproducible headspace extractions of VOCs to SVOCs, including low volatility compounds with minimal matrix effects, therefore increasing the number of applications compatible with headspace analysis. Heavy volatile compounds with low vapor pressures having little to no response by SPME are extracted 10-50x more efficiently with reproducible recoveries.

The top of the Sorbent Pen outside of the vial contains a seal which allows the vial to be evacuated through the adsorbent immediately after insertion. Once under vacuum, the VOCs and SVOCs can diffuse into the headspace and onto the adsorbent faster than when extraction is performed at atmospheric pressure. VASE allows the sample and headspace to come to an equilibrium in a closed system, causing analytes to diffuse onto and collect at the front of the adsorbent bed. Therefore, VASE achieves a much better recovery of heavier compounds while eliminating the common carryover issues.

Running multiple extractions offline in parallel allows for high sample throughput despite the longer extraction times. Data includes chromatograms featuring the full range of VOC to SVOC aroma profiles of dairy products, calibration curves of organochlorine pesticides and PAHs in water, and PAH recoveries from milk samples.

Headspace Analysis

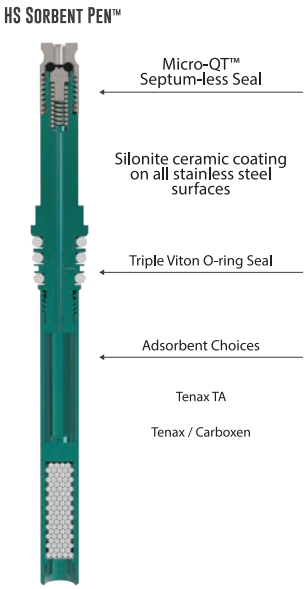


Figure 1 - The adsorbent inside the Headspace Sorbent Pen is positioned starting 3mm recessed from the bottom to provide protection from aerosols generated during rapid agitation. A hole between the bottom two O-rings allows the desorb gas to back-desorb the extracted sample into the GC when an upstream valve is turned on.

Introduction

VASE Advantages Include:

- Operates at near equilibrium to improve sensitivity and quantitative accuracy for extractions from liquid, solid, and gas samples
- Performs exhaustive vacuum extraction of full range VOCs to SVOCs
- Solvent free extractions with and without heating
- Amount of phase is increased 150 times relative to SPME
- Sample extracts much faster under vacuum than while at atmospheric pressure, enhancing the recovery of low volatility compounds
- Static headspace technique allows analytes to diffuse onto and collect at front of adsorbent bed allowing recovery of heavier compounds while eliminating carryover and channeling
- Not in contact with matrix, fewer artifacts created and longer life time
- Cost effective, simple to use and maintain by lab chemist
- Manual or automated

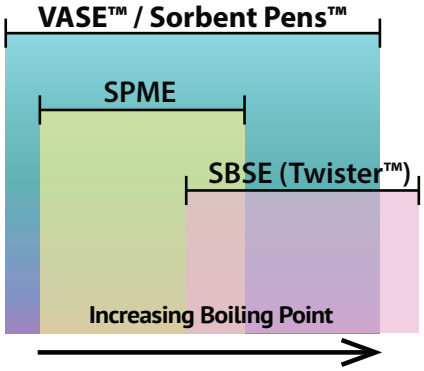


Figure 2 - Using Sorbent Pens, the VASE technique recovers compounds starting even lighter than SPME and out to compounds nearly as heavy as those recovered by SBSE. Most applications achieved by SPME or SBSE can be more easily performed with a wider compound range, higher sensitivity, and better reproducibility using VASE.

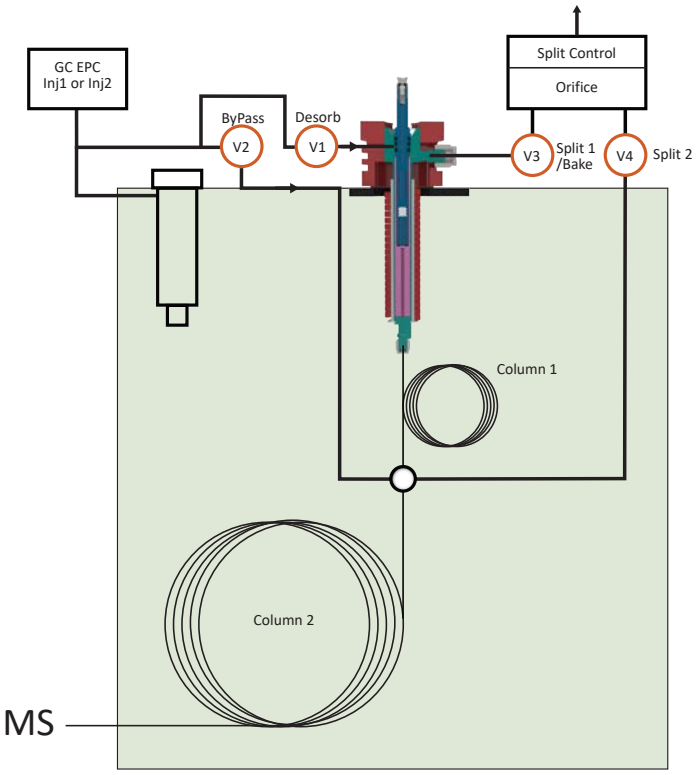
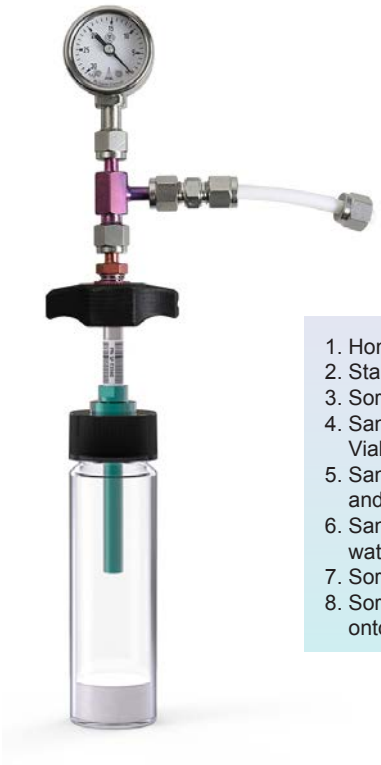


Figure 3 - Using a series of two columns in the flow path after thermal desorption, with forward and backward flushing capabilities for the first column, a wide range of applications can be performed to analyze compounds ranging in boiling points from -100°C to 500°C. Water management is performed through condensation, splitting, and back flushing. A series of 4 valves controls each method process and the location of each valve is shown: Desorb, Valve 1 (V1); Bypass, Valve 2 (V2), Split 1/Bake, Valve 3 (V3), and Split 2, Valve 4 (V4).

Figure 4 - A 5800 SPDU and CTC PAL Autosampler installed onto an Agilent Technologies GCMS. After sample extraction, remove the Sorbent Pen from the isolation sleeve and simply insert it into the 5800 SPDU, then press START on the 5800 Controller manually or upgrade with an autosampler. VASE is CTC PAL compatible. Up to 90 pre-extracted samples can be placed into sample trays for continuous analysis on most GC and GCMS systems.

Methods

Chocolate chip cookies, milk, and cheese samples were chosen to study how VASE would perform in dairy matrices. All analyses were performed by thermally desorbing Tenax Sorbent Pens using a 5800 Sorbent Pen Desorption Unit (SPDU) on a 7890B/5977 GCMS (Agilent, Palo Alto, CA).



VASE Sample Preparation

1. Homogenized sample is placed into vial
2. Standards/surrogate standards are spiked into sample (if applicable)
3. Sorbent Pen is inserted into vial creating seal with lid liner
4. Sample vial is evacuated down to <0.01 atm through Sorbent Pen with Vial Evacuation Tool (**Figure 5**)
5. Sample vial is placed into 5600 Sorbent Pen Extraction System (SPES) and equilibrates at a specific temperature, duration, and agitation speed
6. Sample vial is removed from 5600 SPES and placed onto cold tray for water management
7. Sorbent Pen is removed from vial and placed into isolation sleeve
8. Sorbent Pen is transferred from sleeve to 5800 SPDU for injection onto GC column

Figure 5 - The vacuum tight seal between the lid liner and the Sorbent Pen allows samples to remain under vacuum after a 30 second evacuation, allowing elevated rates of static diffusion to collect significantly more headspace compounds on the adsorbent than can be collected at atmospheric pressure. Samples were evacuated through the Micro QT valve at the top of the Sorbent Pen using an oil free, dual stage diaphragm pump capable of achieving a vacuum of <0.01 atmospheres was used with the Vial Evacuation Tool to create a vacuum through a micro seal at the top of the Sorbent Pen. A restrictor on the Vial Evacuation Tool ensures the vial is evacuated slowly enough so samples do not significantly foam up, therefore liquid never contacts the adsorbent. Sorbent Pens are all labeled with a barcode sticker. This allows the operator to simply scan each Sorbent Pen into the sequence table to record its identity and track each Sorbent Pen through its lifetime of hundreds to thousands of extractions and desorptions.

Results

Full volatility range experiments were performed on chocolate chip cookies, cheese, and milk samples. **Figure 9** shows the reproducibility for duplicate cheddar and brie cheese samples, and clean blanks between runs. The trace analysis experiments performed show calibration curves for 8 organochlorine pesticides (Table 1) and for 14 PAHs, ranging from 1-6 ring compounds (Table 2) from water. Analysis of spiked and non-spiked milk samples were performed to verify the ability to perform measurements pf PAHs in matrices with varied complexity and affinity for the target analytes (Table 3).

Analysis of Chocolate Chip Cookie

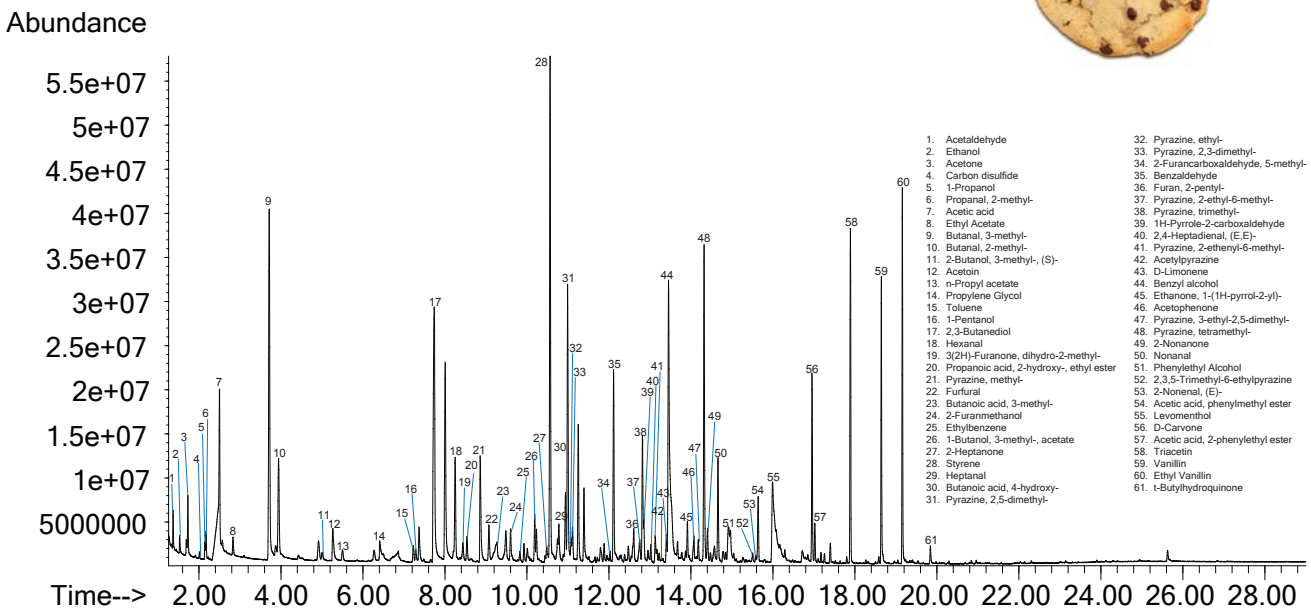


Figure 6- A wide range of compounds were extracted, desorbed, and identified using the VASE technique on 0.5g of chocolate chip cookie. Extraction was performed at 25°C for 15 hours with split injection.

Lactones and Maltol Identified in Chocolate Chip Cookie

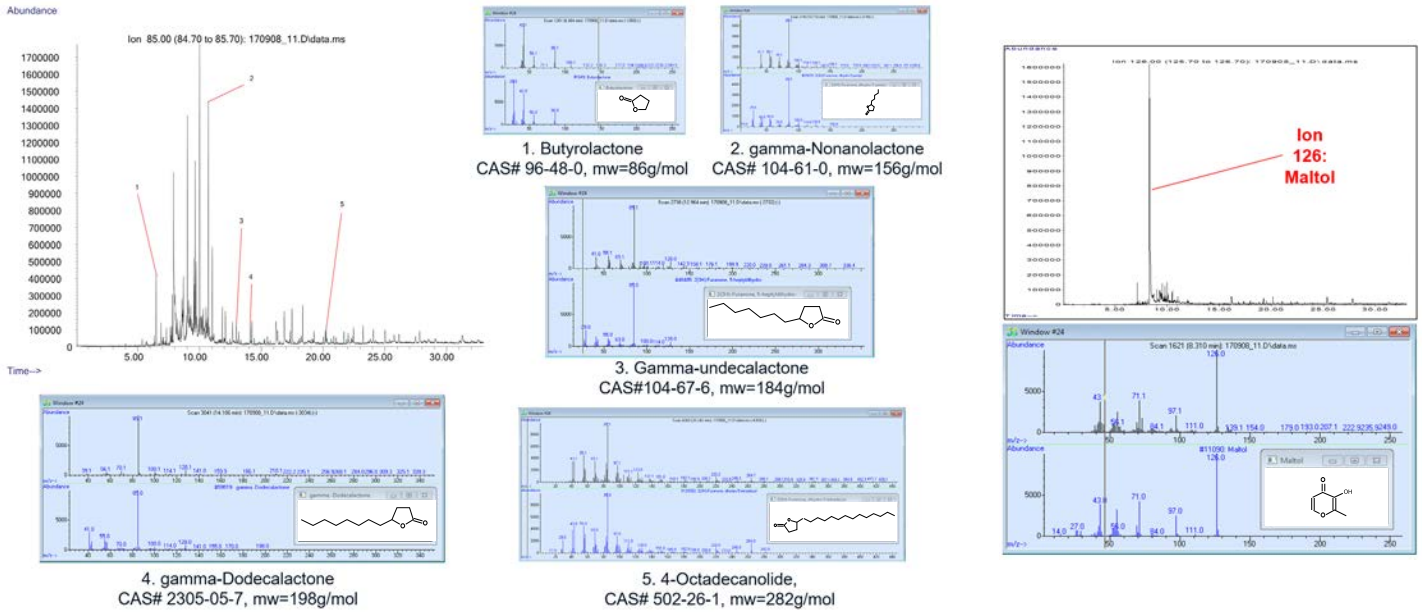


Figure 7 - Maltol and lactones ranging from light to heavy were extracted using VASE at 25°C for 15 hours. Ion chromatograms and matches to the NIST library are shown.

Analysis of Cheddar Cheese

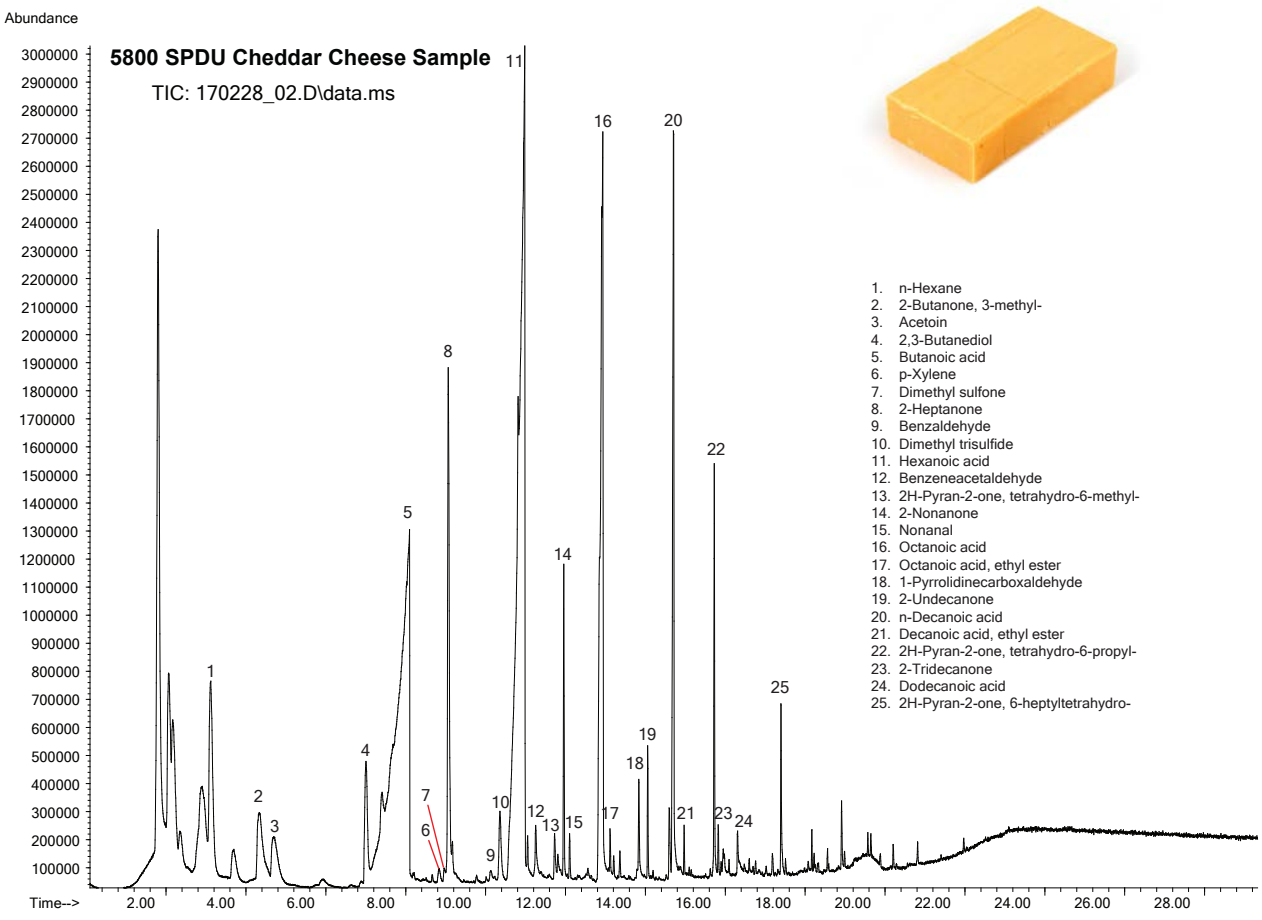


Figure 8 - Analysis of cheddar cheese. Light acids including butanoic acid do not chromatograph well on the non-polar column used, and would be better handled by a polar or intermediate polarity column. Analysis was performed by split injection.

Cheese Duplicates with No Carry Over

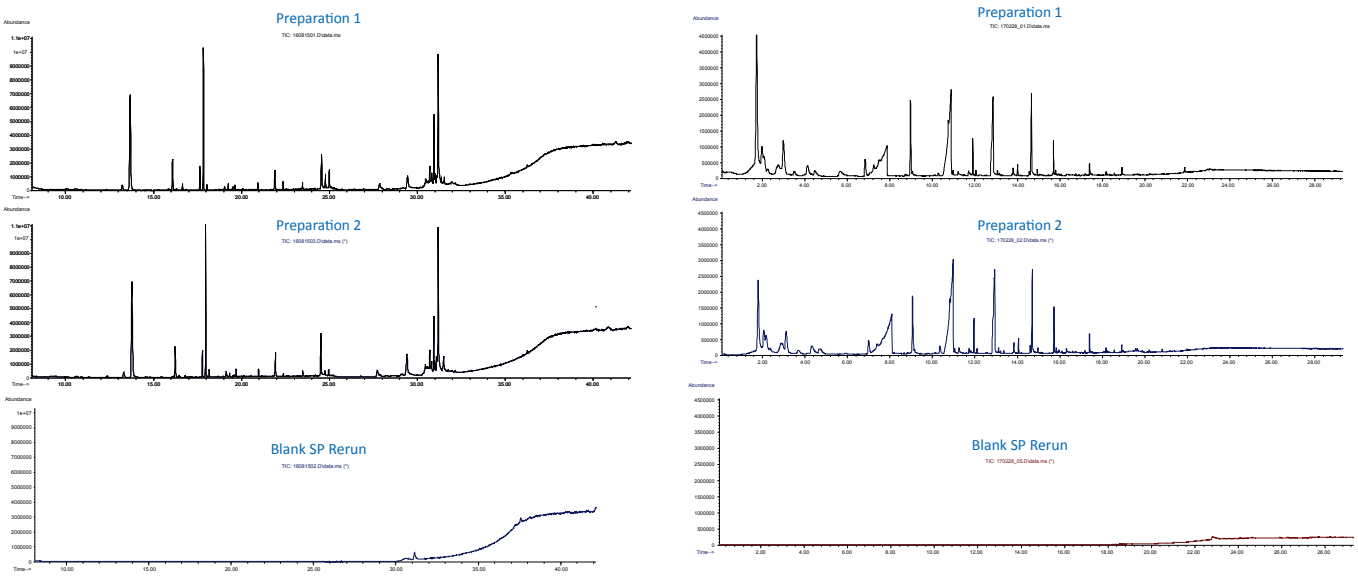


Figure 9 - VASE duplicate analysis of cheddar cheese and brie cheese. The analysis is very reproducible, shown by scaling the Y Axis the same in both respective analyses. Other than a slight difference in the size of the injected air peak at the beginning of cheddar chromatogram, the intensities and recoveries are very similar. The slight difference in free fatty acids of the brie cheese is believed to be real, based on the difficulty in preparing duplicate cheese samples with identical levels of triglyceride oxidation. To obtain these analyses, the cheese was sliced to the same thickness, and then 3/4" diameter round samples were made using a 316 stainless tube turned into a "cookie cutter" sample preparation tool to keep surface areas the same.

Table 1: Pesticides External Calibration Curve 0.5-10ppb

Analyte	0.5ppb	1ppb	2ppb	5ppb	10ppb	Average	%RSD
b-BHC	5.79	3.85	5.30	5.11	4.97	5.01	**14.36**
a-BHC	3.22	3.58	3.26	3.46	3.27	3.36	**4.62**
Heptachlor	2.42	2.16	1.52	2.45	2.48	2.21	**18.29**
Aldrin	5.98	4.63	5.23	5.19	4.85	5.18	**9.91**
Heptachlor Epoxide	1.26	1.01	1.29	1.22	1.19	1.19	**9.06**
4,4DDE	1.54	0.98	1.31	1.20	1.12	1.23	**17.27**
Dieldrin	1.30	1.07	1.26	1.30	1.29	1.24	**7.76**
4,4DDD	1.30	0.90	1.17	1.26	1.11	1.15	**13.62**

Table 1- External calibration curve generated with unique Sorbent Pens for each level using a splitless injection in full scan mode with a single quad GCMS. Standards were spiked into vials containing a final volume of 5mL water samples. Standards prepared in MeOH were added in equal amounts for each level. Values in red highlight the outliers of the curve. The 1ppb calibration point seems to be the cause of higher %RSDs for b-BHC, 4,4 DDE, and 4,4 DDD. Future work will include using surrogate internal standards to minimize MS fluctuations, extraction from only 1mL sample to further investigate sensitivity, and pesticide extraction from dairy products and produce.

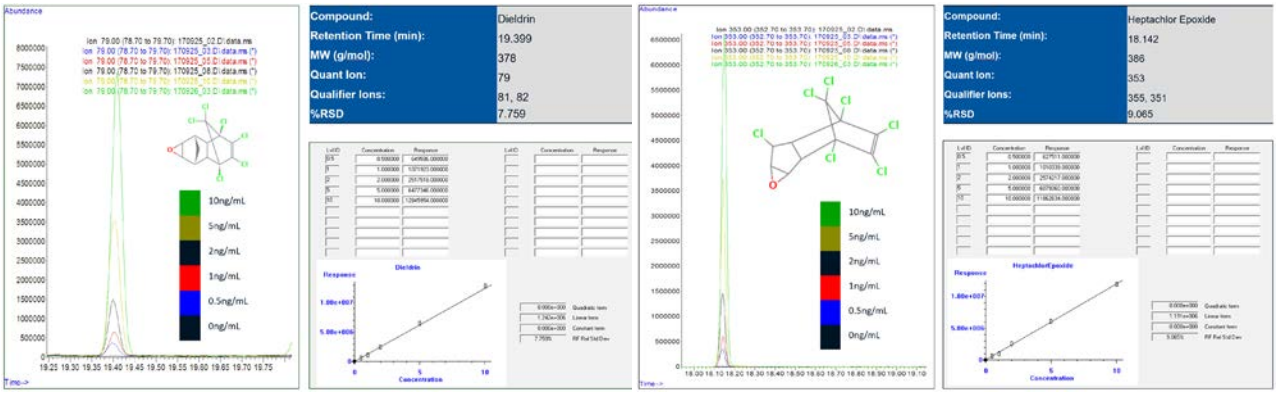


Figure 10 - Ion chromatograms of two banned organchlorine pesticides, Dieldrin and Heptachlor Epoxide, from 0.5-10ng/mL (ppb). The Heptaclor Epoxide maximum contamination level set by the EPA is 0.2ppb. Both chromatograms show excellent signal to noise ratios for the lowest point shown here, 0.5ppb. Therefore, using SIM, even greater sensitivity should be achieved, making it possible to regulate Heptachlor Epoxide and other harmful pesticides in water using VASE.

Table 2: PAHs Calibration Curve 0.5-20ppb

Analyte	0.5ppb	1ppb	5ppb	20ppb	Average	%RSD
Napthalene-d8 Group 1						
Napthalene	0.08	0.07	0.05	0.05	0.06	**28.78**
Acenaphthylene	0.13	0.13	0.16	0.15	0.14	**10.09**
Acenaphthene-d10 Group 2						
Acenaphthene	0.16	0.17	0.17	0.17	0.17	**2.87**
Fluorene	0.12	0.13	0.13	0.12	0.13	**4.09**
Phenanthrene-d10 Group 3						
Phenathrene	0.65	0.54	0.47	0.42	0.52	**19.64**
Anthracene	0.18	0.14	0.18	0.14	0.16	**14.38**
Fluranthene	0.51	0.55	0.57	0.41	0.51	**13.65**
Pyrene	0.53	0.61	0.63	0.47	0.56	**13.75**
Chrysene-d12 Group 4						
Benza(a)Anthracene	1.14	1.01	1.14	NA	1.10	**6.62**
Chrysene	1.41	1.27	1.44	NA	1.37	**6.69**
Perylene-d12 Group 5						
Benzo(k)fluroanthene	2.61	1.98	2.23	NA	2.27	**13.93**
Benzo(a)Pyrene	1.67	1.27	1.29	NA	1.41	**15.85**
Indeno(1,2,3-cd)Pyrene	1.96	1.61	1.32	NA	1.63	**19.62**
Benzo(g,h,i)Perylene	2.40	2.01	1.39	NA	1.93	**26.13**

Table 2 - Naphthene, a 1-ring aromatic, has the highest %RSD, possibly due to breakthrough of the 2m precolumn at higher concentration levels. A longer precolumn should prevent breakthrough of Naphthene, as the surrogate response at 2ppb was stable for each level. Group 4 and Group 5 recoveries for 20ppb point started to drop off, and were removed from this Table. Future work will include addition of salt to increase the ionic strength of the matrix, which should increase recovery of larger compounds.

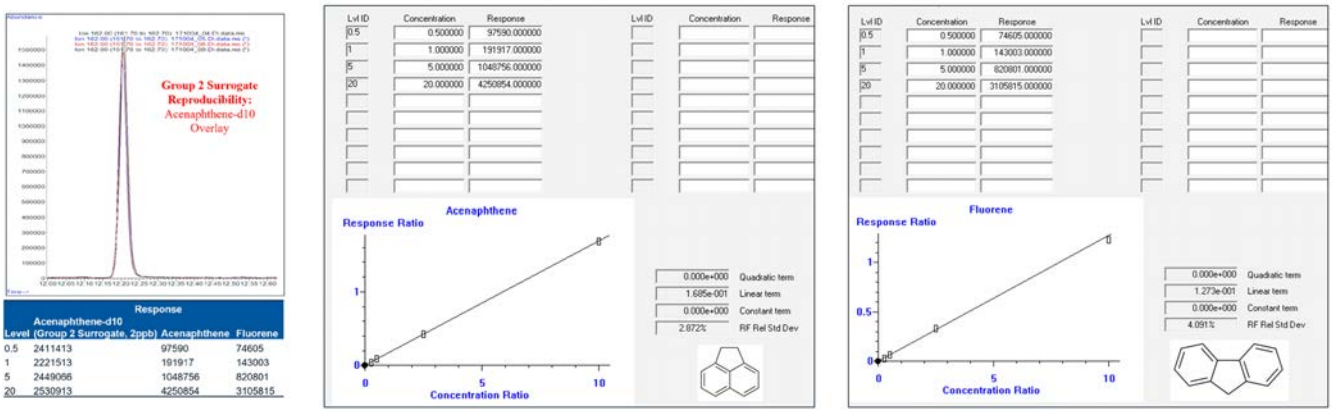


Figure 11 - Calibration curves generated with unique Sorbent Pens for each level using a splitless injection in full scan mode with a single quad GCMS. Standards and surrogate internal standards were spiked into vials containing a final volume of 1mL water samples. Standards prepared in MeOH were added in equal amounts for each level. An Ion Chromatogram of the Group 2 Surrogate Internal Standard, Acenaphthene-d10 and response table below it demonstrates its extraction reproducibility.

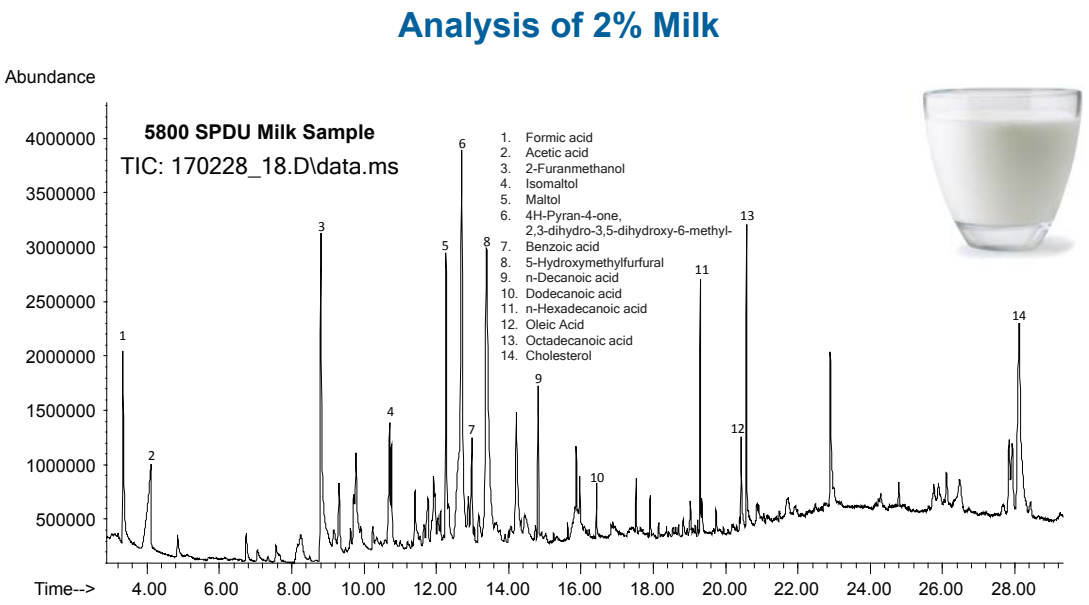


Figure 12 - A 15 hour VASE extraction at 25°C with a 6:1 split injection was performed. This VASE analysis of milk shows recovery over a range of volatile and low volatility compounds from formic acid to cholesterol.

Table 3: Recovery of 2ppb PAHs Spiked into 2% Milk Sample

Internal Standards	Retention Time	Qion	Spiked Sample Concentration (ppb)	Spiked Sample Concentration (ppb)
Naphthalene-d8	9.09	136	2.00	2.00
Acenaphthene-d10	12.20	162	2.00	2.00
Phenanthrene-d10	15.47	188	2.00	2.00
Chrysene-d12	21.80	240	2.00	2.00
Perylene-d12	25.31	264	2.00	2.00
Target Compounds	**Retention Time**	**Qion**	**Spiked Sample Concentration (ppb)**	**Concentration (ppb)**
Naphthalene	9.12	128	6.80	<0.5
Acenaphthylene	11.92	152	2.73	<0.5
Acenaphthene	12.27	152	2.23	<0.5
Fluorene	13.36	166	1.57	<0.5
Phenathrene	15.53	178	2.69	<0.5
Anthracene	15.64	178	2.57	<0.5
Fluranthene	18.34	202	2.98	<0.5
Pyrene	18.87	101	1.45	<0.5
Benza(a)Anthracene	21.78	228	1.73	<0.5
Chrysene	21.87	228	2.91	<0.5
Benzo(k)fluroanthene	24.45	252	<0.5	<0.5
Benzo(a)Pyrene	25.32	252	<0.5	<0.5
Indeno(1,2,3-cd)Pyrene	29.49	276	<0.5	<0.5
Benzo(g,h,i)Perylene	30.69	276	<0.5	<0.5

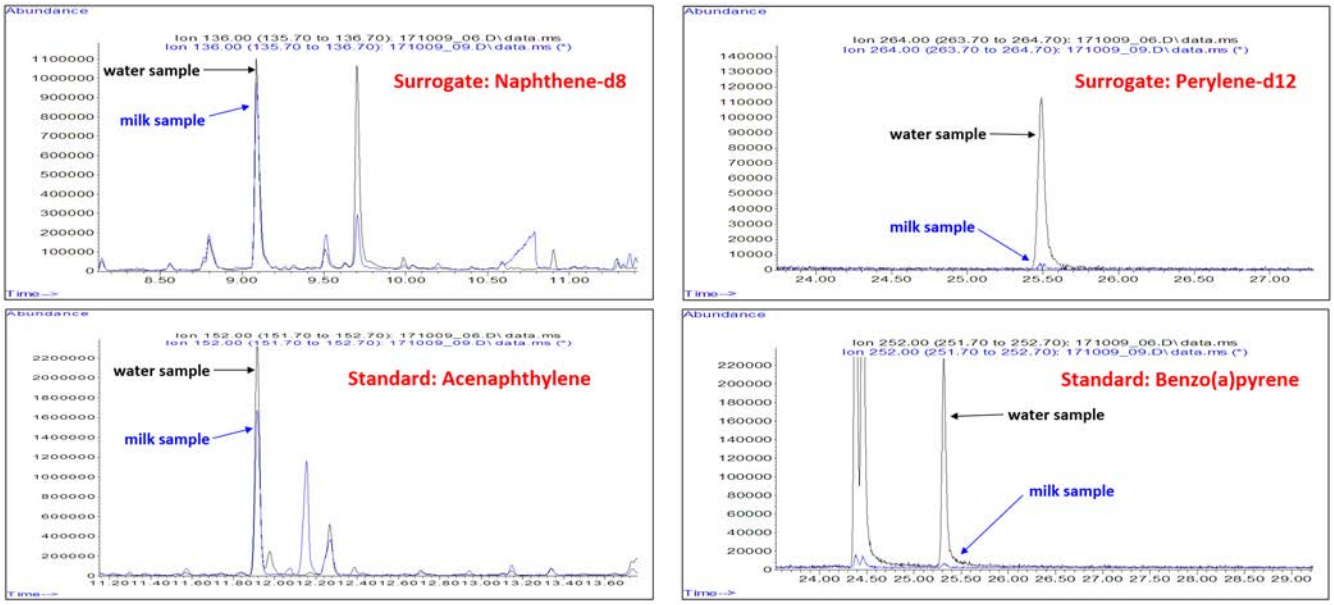


Figure 13 - Table 3 shows recoveries for PAHs spiked at 2ppb into a 1mL 2% milk undiluted sample. High recovery for Napthlene in spiked sample maybe due to breakthrough of the precolumn. Table 3 values in red represent the recovery drop off point, which is illustrated above. Group 1 surrogate Napthene-d8 and target compound Acenaphthylene show similar recoveries for the water and milk samples, however, the Group 5 surrogate, Perylene-d12 and target compound both show very low recoveries for the milk sample in comparison to the water sample. Future work will include addition of salt to increase the ionic strength of the matrix to further encourage volatility of the larger target analytes.

Conclusions

- ☑ A wide range of VOCs to SVOCs were extracted reproducibly from dairy products without carryover using VASE technique
- ☑ VASE was shown to be very promising in preliminary trace analyses experiments extracting organochlorine pesticides and 1-6 ring PAHs from water and milk samples at ppb levels
- ☑ Recovery of low volatility compounds using headpsace extraction is improved without having to perform isotope dilution for each target compound

The potential for VASE, or Vacuum Assisted Sorbent Extraction, is substantial, as it eradicates most of the problems associated with other extraction techniques, and offers an opportunity to eliminate solvents even when analyzing heavy analytes in difficult matrices. The combined approach of using larger amounts of phase, extraction to equilibrium, vacuum to speed up the extraction process, and a unique GC injector/desorber to introduce the extracted sample quantitatively makes the VASE technique distinctively capable of recovering both VOCs and SVOCs from liquid or solid samples with boiling points from -50°C to over 500°C.

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