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응용 자료

Analysis of Pharmaceuticals and Pesticides in Bottled, Tap, and Surface Water Using ACQUITY UPLC Systems with 2D-LC Technology

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Abstract

This application demonstrated the disruptive nature of ACQUITY UPLC Systems with 2D-LC Technology with a Xevo TQD Mass Spectrometer. The application targeted the analysis of PPCP's and pesticides in bottled, tap, and surface water. The limit of detection in this study was 1.0 ppt with a 10:1 enrichment from the extraction protocol (15 min total) and a 200:1 enrichment from the at-column dilution option, for a total of 2000:1. The recovery data for bottled, tap, and surface water samples using a micro extraction protocol shows comparable results to application with macro extraction protocols.

Benefits

Fast extraction protocol (15 min)

Introduction

LC-MS/MS and GC-MS/MS have been utilized for routine analysis since the introduction of hyphenated instrumentations in the 1970's. Those platforms play a crucial role for analyses that require trace level part-per-billion (ppb) detection limits. In environmental analysis, government agencies around the world are vigilant for both regulated and emerging contaminants in bodies of water. The list of contaminants grows every year and, as a consequence, new analytical protocols need to be developed to meet those demands.

Both gas and liquid chromatography with mass spectrometry detection are without a doubt the most popular techniques utilized for trace level analysis. By improving the level of automation, the next generation of hyphenated solutions is even better equipped to bring a measurable cost reduction to the overall analytical process (time, resources, and consumables). The typical workflow process is accomplished in two parts. First, a target analyte must be isolated from the sample matrix. This is commonly known as the "extraction process," during which a target analyte is isolated from a raw sample into an ideal format for analysis. The second phase of extraction deals with the separation and detection of a target analyte in a sample extract. The workflow for any extraction process is directly linked to the level of complexity of the sample matrix. For example, drinking water is considered to be a low-complexity matrix, meaning the level of difficulty of isolating a target analyte from that particular matrix is low. However, waste water sample is a high-complexity matrix, which means the level of interferences are at high concentration and will subsequently impact the analytical performance of the extraction protocol (recoveries, robustness, lifetime, accuracy, etc.).

Macro vs micro extraction protocol

When confronted with trace level analysis, it is often required to bring the concentration of the target analyte into the detectable range of the chosen analytical method (UV, MS, ELSD, etc.), meaning an enrichment step is required in the extraction protocol. Most applications targeting low part-per-trillion (ppt) will need to extract large sample volumes or masses. In the case of water applications, it is a common practice to extract between 500 mL to 1000 mL of sample volume. The enrichment factor is calculated from the initial volume before extraction and the final volume of the sample extract before analysis.

Most methods[1-7] will opt for a final volume between 0.5 mL and 1.0 mL, which bring an enrichment factor range from 500x up to 1000x. Figure 1 shows a macro extraction protocol using a 1000 mL water volume with a double SPE cartridge configuration. This configuration is extremely useful during method development and provides crucial information regarding the retention behavior (breakthrough, retention strength, retention mechanism, etc.) of target analytes.

Oasis MAX
6CC 150 mg

Oasis MCX
6CC 150 mg

Loading
- Condition 1: 5 mL MeOH
- Condition 2: 5 mL Water
- Load: **1000 mL @ 10 mL/min**

High grade water	**2.5 h**
Tap water	**8 h**
Surface water	**15 h**

Washing
- Disconnect stack
- Wash MAX: 5 mL 100 % H_2O + 2 % NH_4OH
- Wash MCX: 5 mL 100 % H_2O + 2 % HCOOH

Elution
- Elute 1 MAX: 5 mL 100% MeOH
- Elute 2 MAX: 5 mL 100% MeOH + 2% HCOOH
- Elute 3 MCX: 5 mL 100% MeOH + 2 % NH_4OH

Pre-Injection
- Pool all 3 elutions (15 mL total)
- Evaporate to dryness (N_2)
- Evaporation time: **1.5 hr**
- Reconstitute 1000 µL 100 % H_2O + 10 mM NH_4 Formate
- Inject volume 100 µL

Figure 1. Workflow sequence using a macro extraction protocol.

The extraction sequence starts with a sorbent conditioning step to remove potential interferences. The next step is sample loading, which extracts target analytes from the sample. Typical loading flow rate for large sample size range is between 5 mL/min and 10 mL/min. The loading flow rate is an optimized function

derived from the SPE bed mass, sample contact time, and mass transfer onto the sorbent. With a loading flow rate set at 10 mL/min, the total loading time should take 1.6 hours before proceeding with the next step of the extraction protocol. However, as seen in Figure 1, the values for high-grade water (2.5 hours), tap water (8 hours), and surface water (15 hours) samples far exceed the expected 1.6 hours. The discrepancy comes from the fact that the loading flow rate is not at a constant value for the entire sample volume. In fact, the flow rate is linked to the quality of the sample and, therefore, the extended loading time is attributed to clogging issue from particulate matter in the sample. This is necessary to reach the desired target LOD or LOQ. In some instances, it may be necessary to extract a larger sample volume to increase the enrichment factor. Once the total volume is extracted, a wash step removes weak interferences without causing breakthrough for the target analyte. The elution step breaks the retention bonds of the target analyte from the SPE sorbent. At this point in the extraction process, the target analyte sustained a solvent exchange from aqueous to an organic solvent (aqueous or non-aqueous miscible). If the final extract is dissolved in a non-aqueous miscible solvent, this indicates that the analysis will be performed with a GC-MS platform. If the analysis is performed with an LC-MS and assuming a reversed-phase separation, the final extract must undergo a second solvent exchange. This is accomplished by using nitrogen stream evaporation to evaporate the sample to dryness and reconstitute with initial mobile phase conditions.

Nitrogen evaporation is linked to the properties of the organic solvent and any remaining percentage of water collected during the elution step. In some cases, the evaporation time can be decreased by applying mild heat. It is a well-known fact that evaporative loss is always a potential cause for poor recoveries. In some instances, the evaporation rate can be at extreme low settings, which requires adding an overnight time period for completion. Finally, once the sample is reduced to dryness, yet another cause of poor performance can occur by reconstitution solvent compatibility and solubility. The overall workflow is dependent of the analytical technique used for analysis and can be extremely time-consuming and laborious.

ACQUITY UPLC Systems with 2D-LC Technology offer the same analytical performances regarding recoveries, linearity, robustness, and lifetime, but at micro-extraction level. Figure 2 shows a micro extraction protocol using a 15-mL sample volume. The smaller sample volume allows faster loading time, on average less than 10 minutes. The final elution volume was optimized at 1 mL. The enrichment ratio for a micro extraction protocol is 15:1. With the option of a wider range of injection volume and extract composition, the evaporation and reconstitution step were eliminated. With 2D at-column dilution, aqueous and organic extracts can be loaded and captured on a trap column with high efficiencies. The injection volume for this configuration is not a limitation, which gives the option to inject as much as needed to reach target detection limits. For example, if the entire final sample (1 mL) is used for the analysis, it will give an additional 100:1 enrichment factor. Therefore, the overall enrichment from hardware and extraction protocol is now calculated at 1500:1, which is higher than those seen with a macro extraction protocol. Furthermore, the entire

extraction protocol (loading, washing, and elution) was completed in less than 15 minutes for a high-grade, tap, and surface water sample.

Oasis MAX
3CC 60 mg

Oasis MCX
3CC 60 mg

Loading
- Condition 1: 2 mL MeOH
- Condition 2: 2 mL Water

- Load: **15 mL @ 10 mL/min**

High grade water	**10 min**
Tap water	**10 min**
Surface water	**10 min**

Washing
- Disconnect stack
- Wash 1 MAX: 2 mL 100% H_2O + 2% NH_4OH
- Wash 1 MCX: 2 mL 100% H_2O + 2% HCOOH
- Wash 2 MAX: 1 mL 100% MeOH
- Wash 2 MCX: 1 mL 100% MeOH

Elution
- Elute 1 MAX: 1 mL 100 % MeOH + 2% HCOOH
- Elute 1 MCX: 1 mL 100 % MeOH + 2% NH_4OH

Injection
- Inject 200 µL (elute 1 MAX & elute 1 MCX)

Figure 2. Workflow sequence using a micro extraction protocol.

Two MRM transitions (quantification and confirmation) for all pharmaceuticals and personal care products (PPCP's) and pesticides were selected and optimized. The MS conditions are listed in Table 1.

PPCP	Ion mode	Precursor ion	Cone	Product ion	CE
Enrofloxacin	ESI+	360.3	25	342.3	20
				316.3	20
Trimethoprim	ESI+	291.3	40	123.0	30
				230.2	30
Sulfamerazine	ESI+	265.1	35	92.0	25
				156.0	15
Sulfamethoxazole	ESI+	254.1	30	92.0	25
				156.0	15
Sulfadimethoxine	ESI+	311.1	40	156.0	15
				92.0	25
Salbutamol (albuterol)	ESI+	240.1	30	148.0	15
				222.1	10
Cimetidine	ESI+	253.1	30	159.1	15
				117.1	15
Tripolidine	ESI+	279.1	25	208.2	15
				193.2	35
Miconazole	ESI+	417.1	40	161.1	30
				69.0	25
Diethylcarbamazine	ESI+	200.2	25	100.1	15
				72.0	25
Levamisole (tetramisole)	ESI+	205.2	25	178.1	20
				91.1	30
Benzocaine	ESI+	166.1	25	138.1	15
				77.0	25
Procaine	ESI+	237.2	25	100.1	15
				120.0	25
Bromhexine	ESI+	377.1	30	114.1	15
				263.9	30
Buflomedil HCl	ESI+	308.3	30	140.1	15
				237.1	15
Diltiazem	ESI+	415.2	30	178.1	20
				310.1	20

Pesticides	Ion mode	Precursor ion	Cone	Product ion	CE
Methomyl	ESI+	163.0	10	88.0	10
				106.0	10
Atrazine desethyl	ESI+	188.0	35	78.0	26
				146.0	16
Simazine	ESI+	202.0	32	96.0	22
				124.0	17
Chlortoluron	ESI+	213.0	23	46.0	16
				72.0	18
Monolinuron	ESI+	215.1	20	126.0	20
				148.0	15
Atrazine	ESI+	216.1	30	96.1	23
				174.0	18
Metoxuron	ESI+	229.0	25	72.0	18
				155.9	25
Sebuthylazine	ESI+	230.0	30	96.0	26
				174.0	18
Terbuthylazine	ESI+	230.0	30	96.0	26
				174.0	18
Diuron	ESI+	233.0	30	46.3	14
				72.1	30
Dicrotophos	ESI+	238.0	17	112.0	10
				193.0	10

Loading conditions

Loading:	Water pH 7 no additives
Flow rate:	2 mL/min
At-column dilution:	5% (0.1 mL/min pump A and 2 mL/min pump B)

UPLC conditions

UPLC system:	ACQUITY UPLC 2D with at-column dilution
Runtime:	10 min
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	60 °C
Mobile phase A:	Water + 0.5 % Formic acid
Mobile phase B:	Acetonitrile + 0.5 % Formic acid
Elution:	5 minute linear gradient from 5% (B) to 95% (B)
Flow rate:	0.500 mL/min (pump C)
Injection volume:	200 µL

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI positive
Capillary voltage:	3.0 kV
Cone voltage:	30.0 V
Source temp.:	150 °C
Desolvation temp.:	550 °C
Desolvation gas:	1100 L/hr
Cone gas:	50 L/hr

Results and Discussion

Automated method development

The starting point of any analytical protocol is the selection of chromatographic parameters to achieve well-resolved peaks for qualitative and/or quantitative analysis. Method development is typically performed with a trial-and-error approach, which ultimately leads to an optimized chromatographic method in a relatively short time. Another current practice is to select the most successful conditions in a systematic screening approach with the goal of quickly reaching optimized conditions. When utilizing multidimensional chromatography, the task of selecting optimized conditions can be quite difficult. However, with automation and a selection of key parameters, a large number of methods can be screened in a short time frame. For example, Figure 3a shows a 2D configuration with at-column dilution with typical loading conditions, elution conditions, trapping chemistries, and separation chemistries. As shown, several options are listed and, with a multiplication effect, can generate a staggering number of methods (Figure 3b). This represents a collection of conditions for the analysis of a basic analyte. Each condition selected will have a key effect on the chromatographic behavior of a target analyte. In this instance, the high pH, low pH, and neutral loading conditions were evaluated to monitor the trapping efficiency versus the ionized or neutral state of the target

analyte. Figure 4 shows the retention behavior for cimetidine at low, neutral, and high pH. The low pH elution with methanol or acetonitrile were selected to monitor the polarity range of the target analyte. As seen in Figure 5, the elution profile for atrazine suggests a high affinity for methanol. The loading and eluting parameters work in tandem to ensure no breakthrough during loading and as well as minimize peak distortion during back-flush elution.

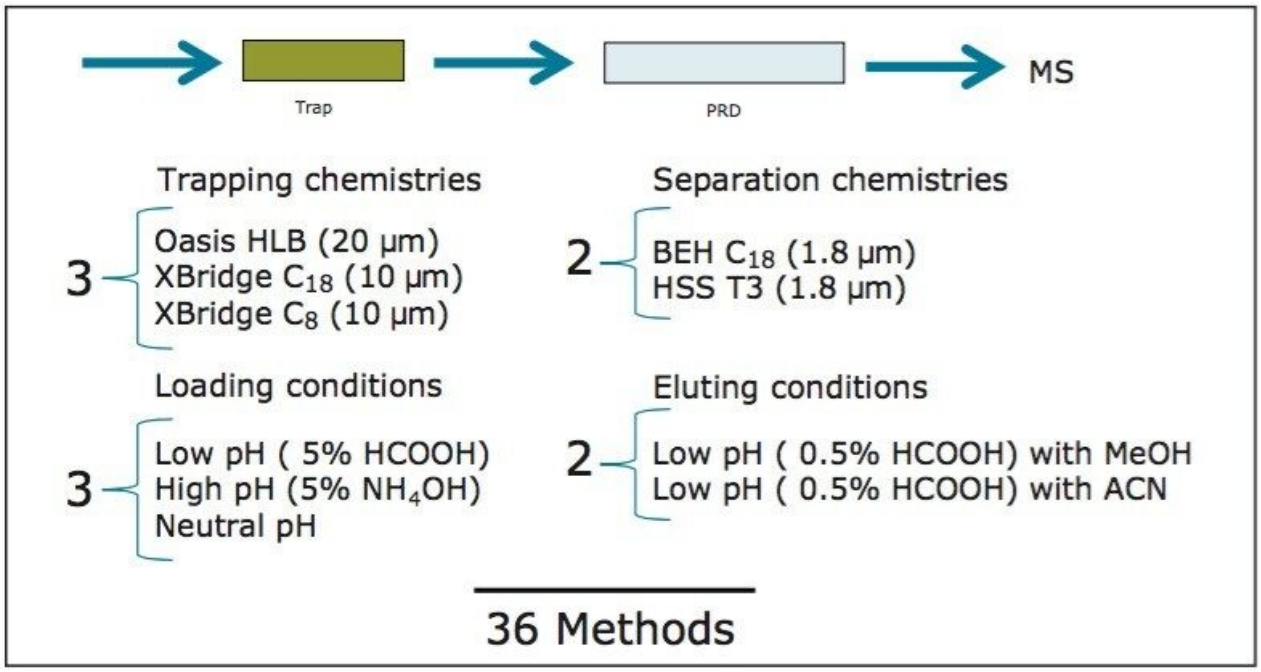


Figure 3a. 2D trap and elute configuration – loading and eluting conditions.

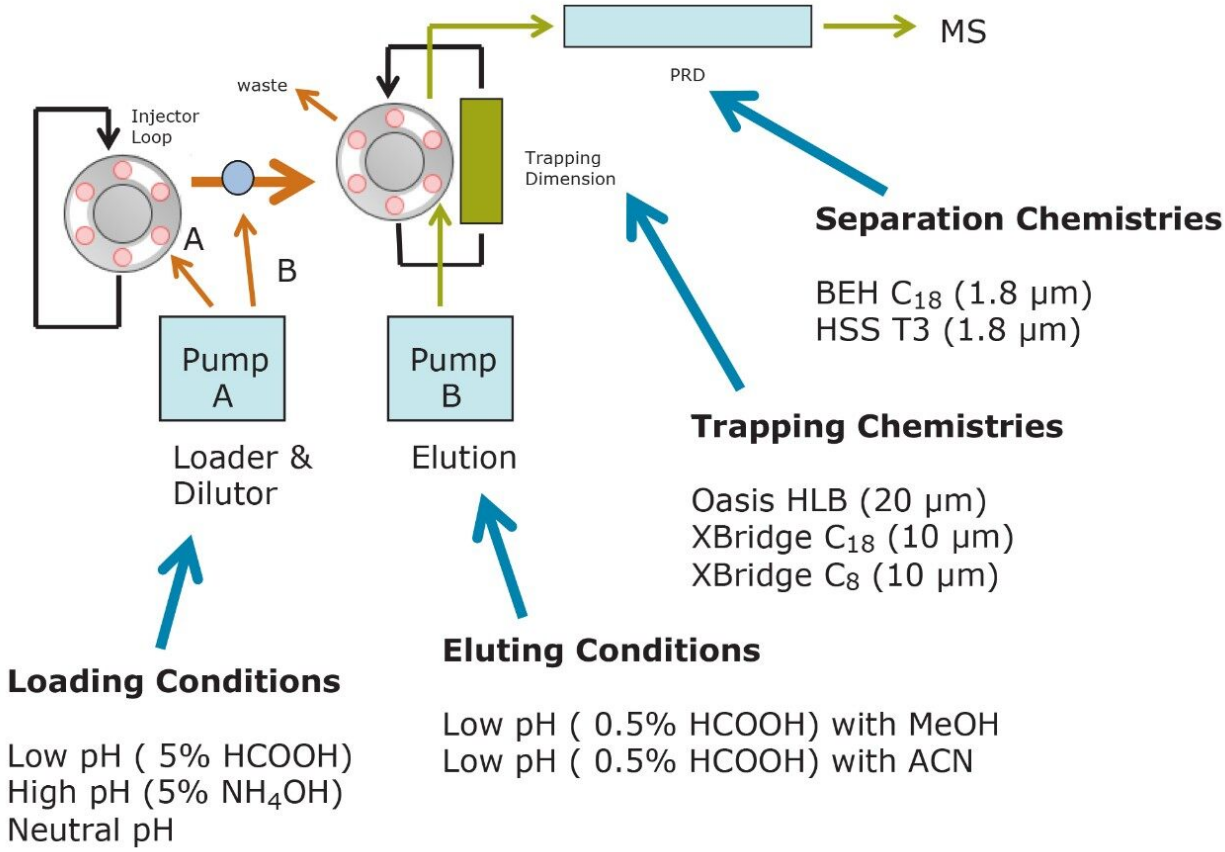


Figure 3b. 2D trap and elute configuration with at-column dilution.

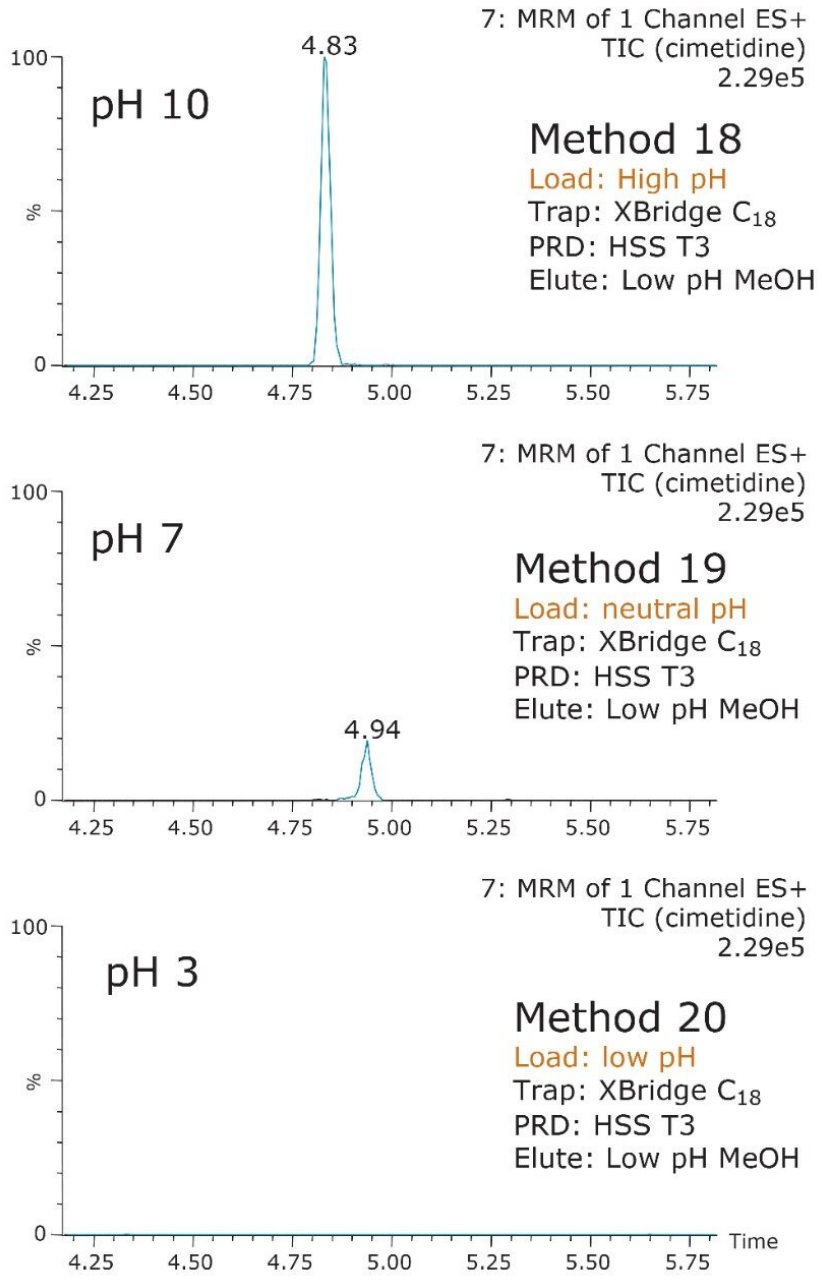


Figure 4. Trapping efficiency during loading phase.

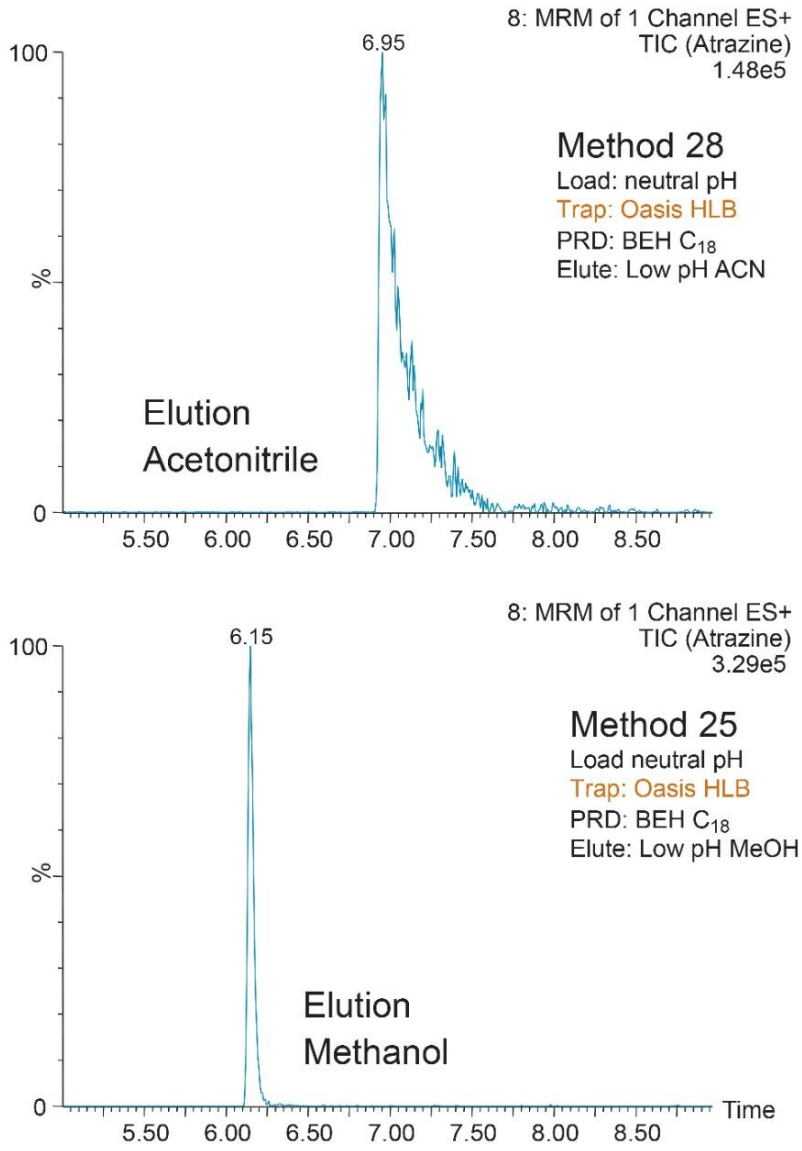


Figure 5. Elution strength during back flushing phase.

The chemistries selected for the trap also play a crucial role. The target analyte can often bind very strongly or be captured with a weak binding effect. In both cases, poor recovery can result. Figure 6 showcases the retention strength of carbamezapine with a very strong affinity for Oasis HLB.

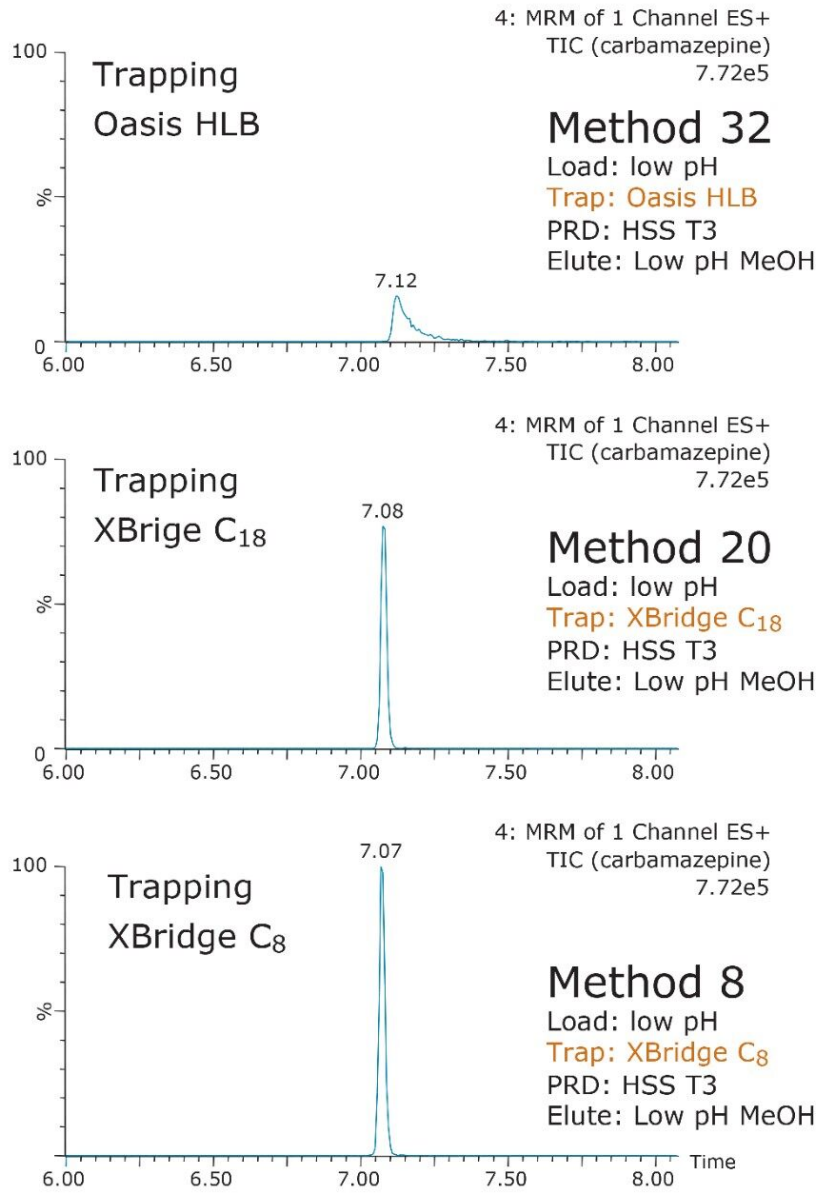


Figure 6. Retention strength during loading phase.

On the other hand, Figure 7 displays the chromatographic behavior of corticosterone versus the hydrophobic selectivity of BEH C_{18} and HSS T3. Overall, the separation chemistries complement the system performance by fine tuning the level of hydrophobicity. By multiplication, a total of 36 permutations can be setup for method development. In this application, each method uses a 3 minute loading and a 5 minute back-flush gradient for a total run time of 10 minutes. With duplicate injection per method, 3 methods per hour were recorded, thus all 36 methods tested were completed in 12 hours. With the amount of results generated in a short amount of time, a color coded chart was constructed to visualize which operating conditions gave the

best peak profile. Figure 8a shows the elution profile of trimethoprim for 3 selected methods. The chromatogram from method 1 shows no signal for the target analyte, and therefore method 1 was attributed a red tag. The chromatogram from method 25 shows an intense signal, however, and the peak shape is distorted by a peak tailing effect. Thus, method 25 was attributed a yellow tag. The chromatogram from method 28 shows a well resolved and gaussian peak shape, which received a green tag. With this screening criteria, each method was carefully identified and compiled for comparison. The comparison chart for trimethoprim (Figure 8b) shows an 83% success rate. Several pharmaceuticals and pesticides gave a 100% score, while two pesticides produce un-successful results at 0%. This is not a situation in which the hardware is at fault, but rather it points toward the expansion of operating conditions, such as flow rate, temperature, buffers, ion pairing, etc.

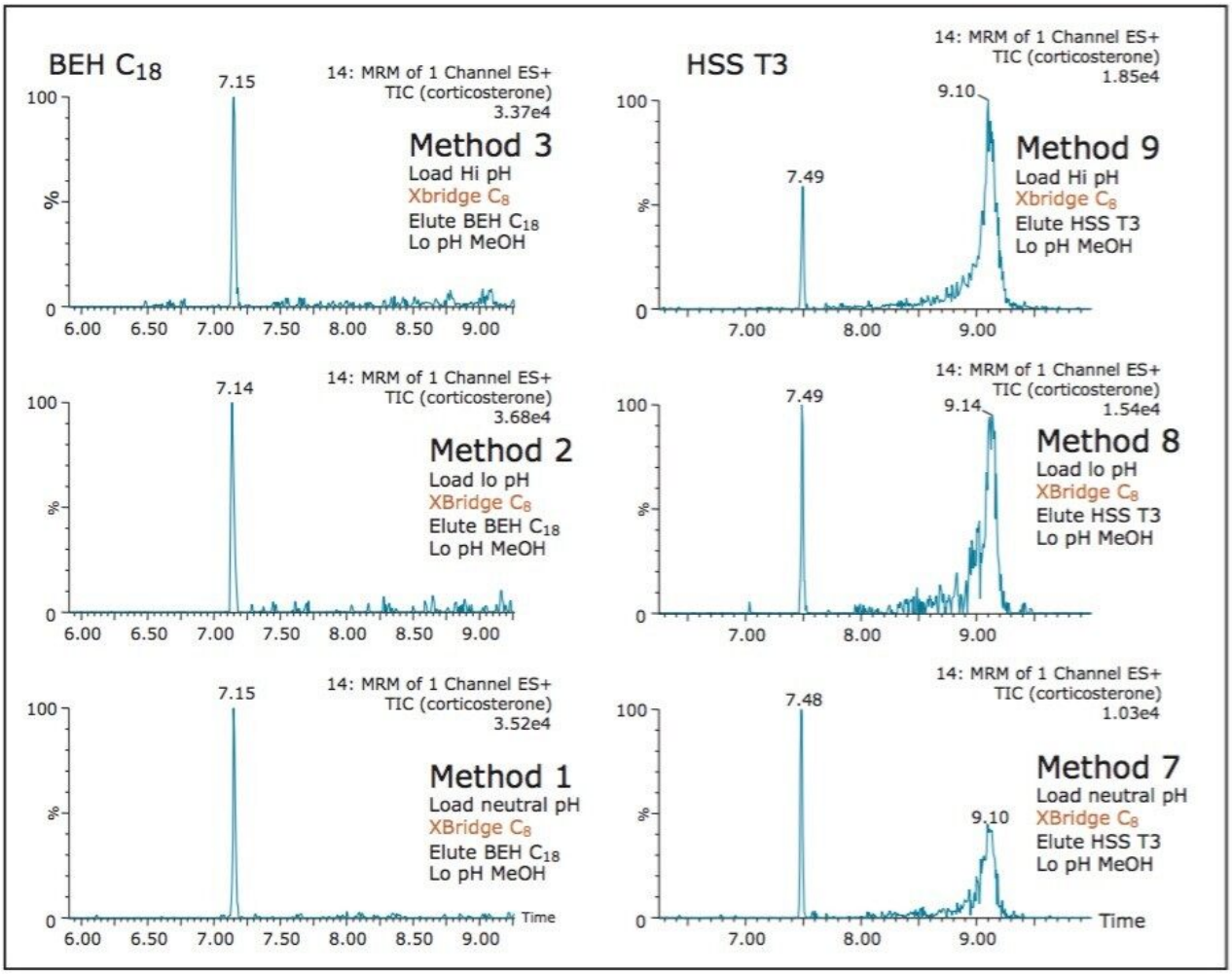


Figure 7. Retention efficiency during back flushing phase.

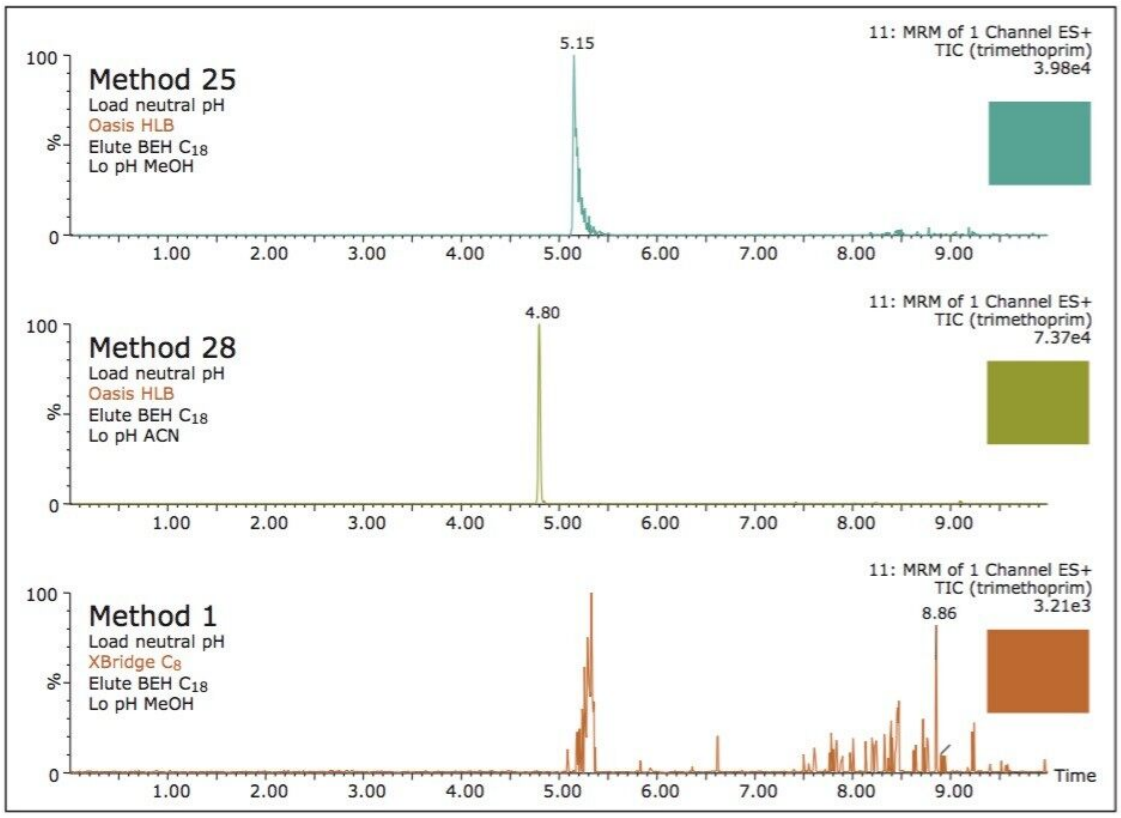


Figure 8a. Typical results during method development.

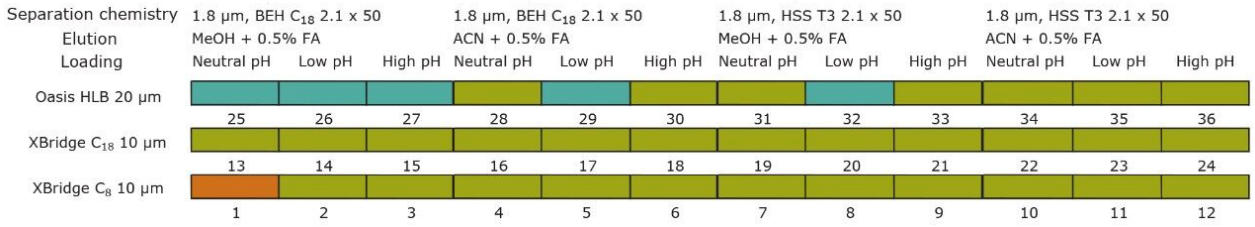


Figure 8b. Comparison chart of 36 2D methods for trimethoprim.

From the comparison chart (Figure 9a, 9b, 9c, 9d, 9e, 9f), it is apparent that a single method will not cover the entire mix of pesticides or pharmaceuticals, which brings the option to select an automated multi-method approach rather than a single multi residue protocol. For this application, method 28 was selected for pesticides and pharmaceuticals for the highest score (Figure 10).

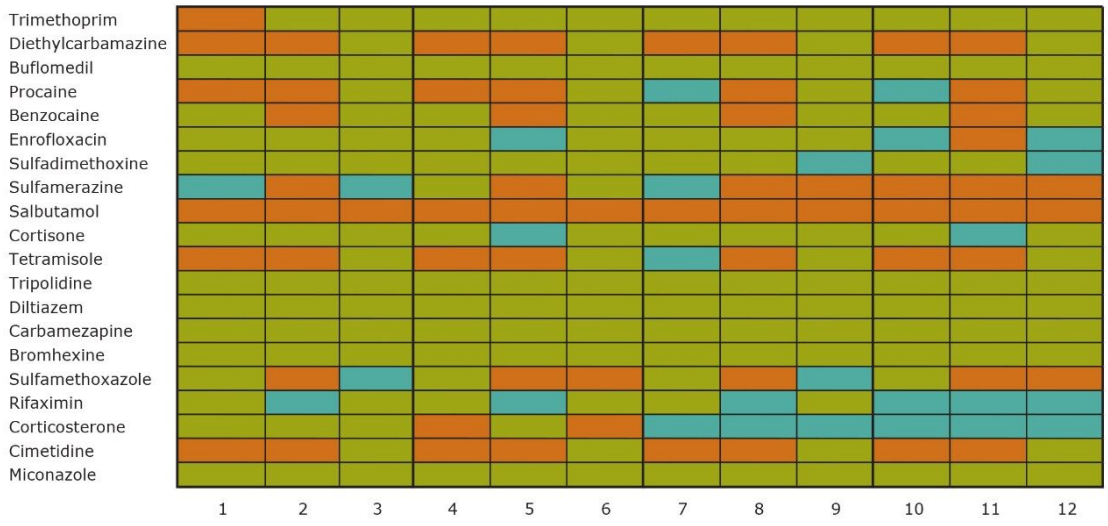


Figure 9a. Comparison chart for pharmaceutical mix using XBridge C_8 – method 1 to 12.

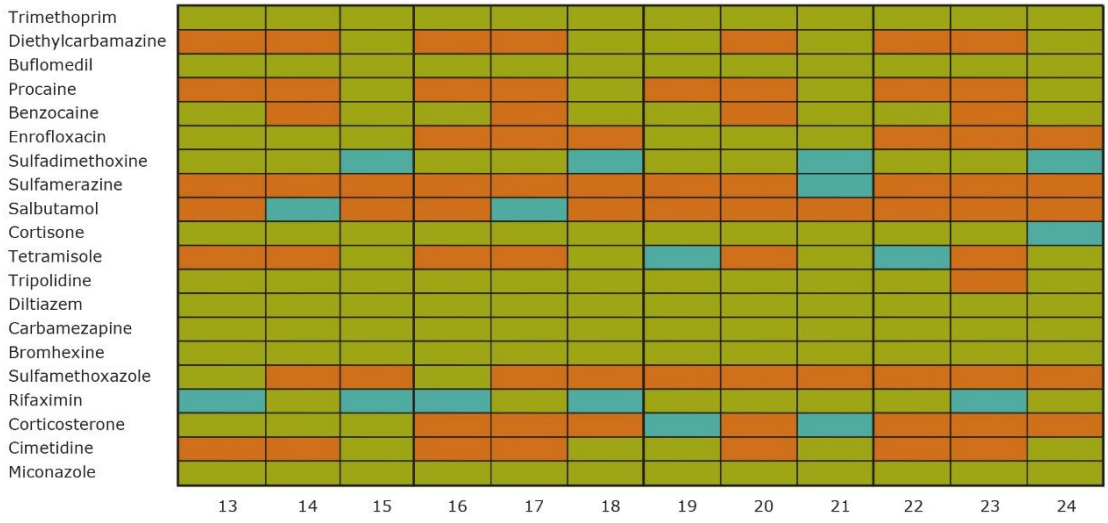


Figure 9b. Comparison chart for pharmaceutical mix using XBridge C_{18} – method 13 to 24.

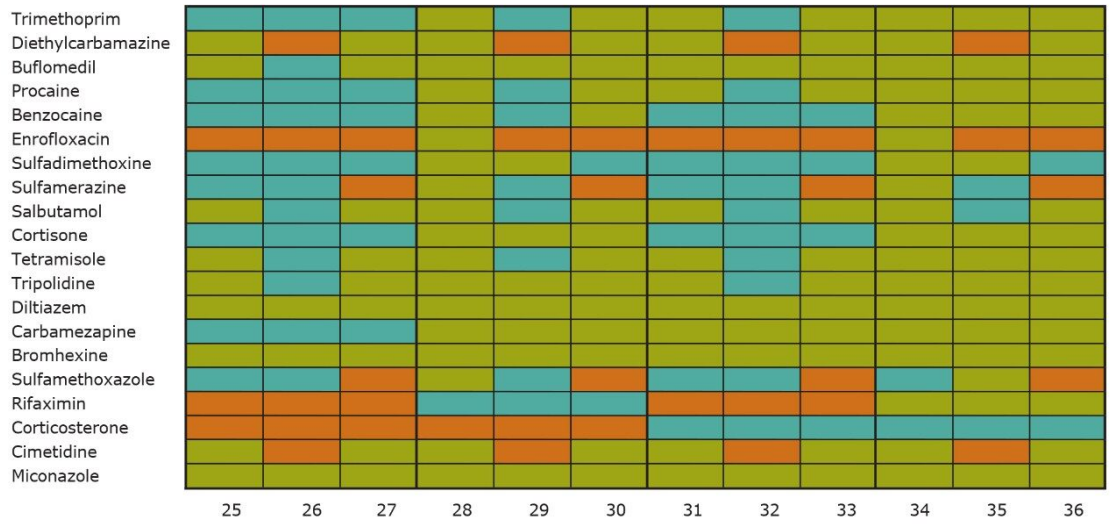


Figure 9c. Comparison chart for pharmaceutical mix using Oasis HLB – method 25 to 36.

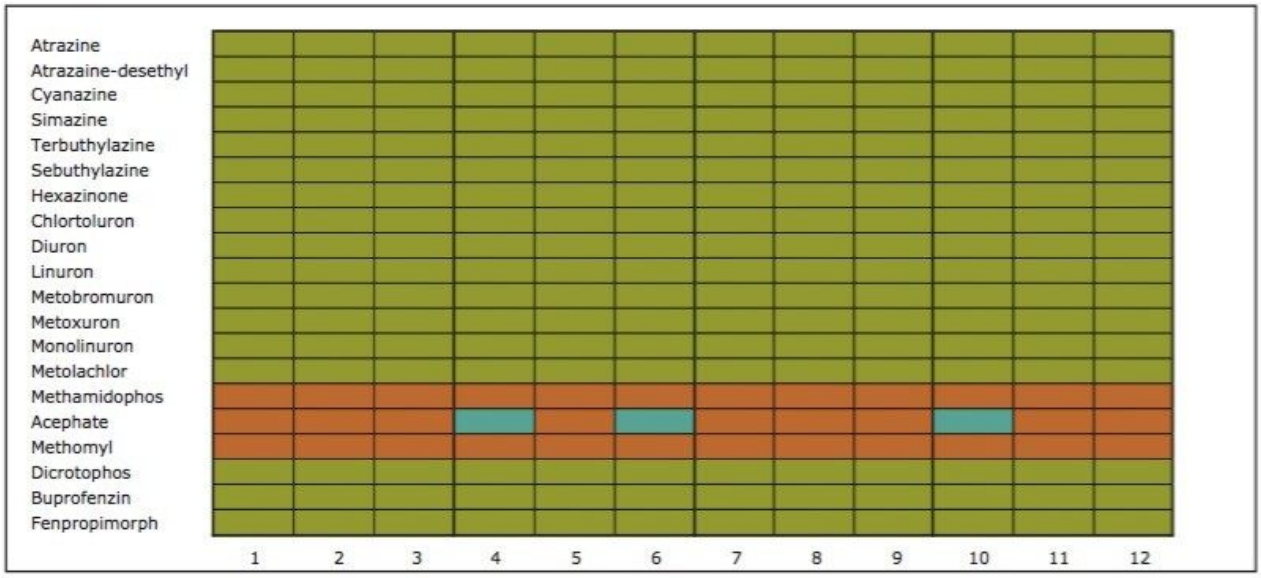


Figure 9d. Comparison chart for pesticide mix using XBridge C_8 – method 1 to 12.

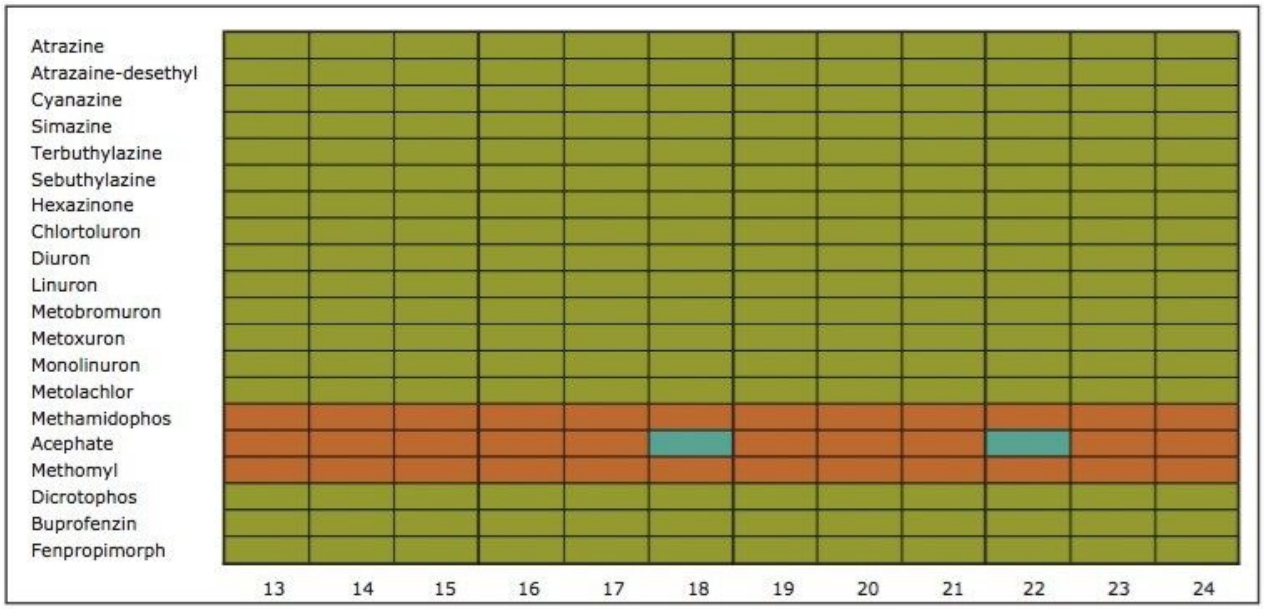


Figure 9e. Comparison chart for pesticide mix using XBridge C_{18} – method 13 to 24.

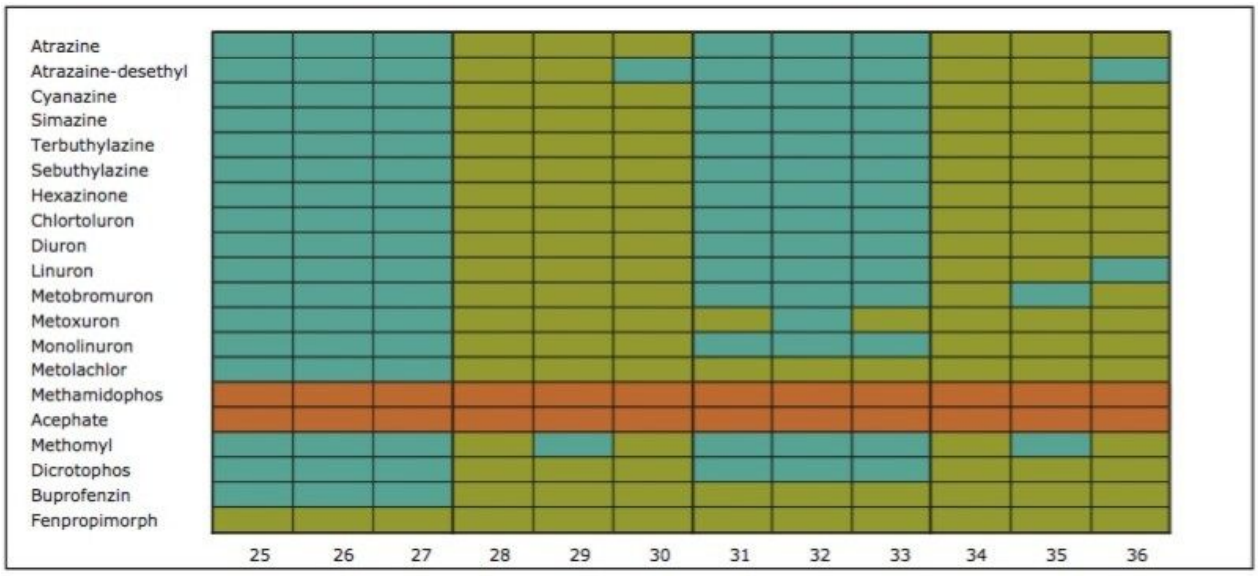
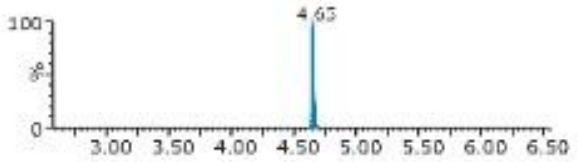


Figure 9f. Comparison chart for pesticide mix using Oasis HLB – method 25 to 36.

- *Successful*
- *Marginal*
- *-Unsuccessful*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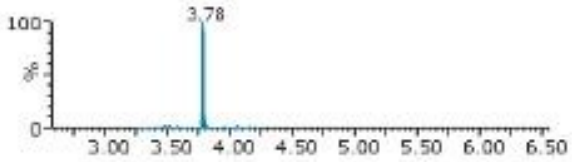
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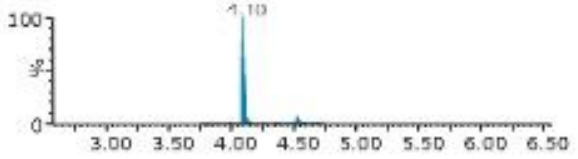
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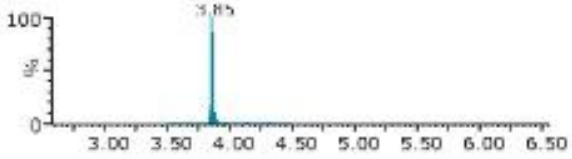
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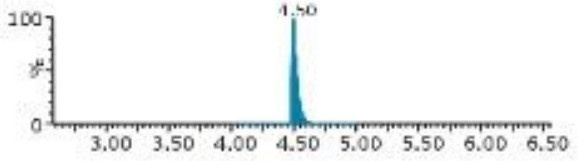
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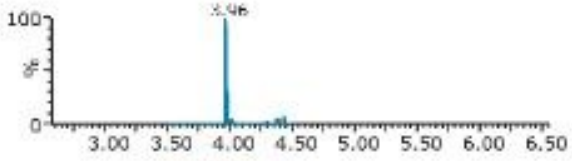
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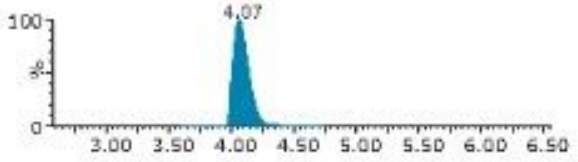
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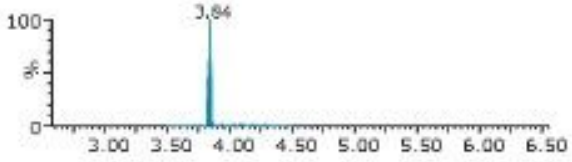
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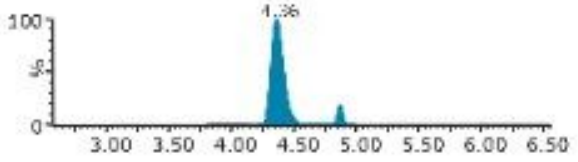
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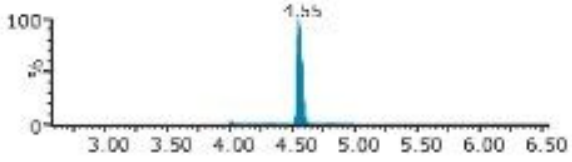
4.55
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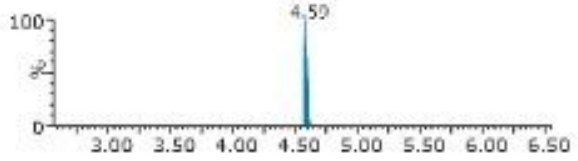
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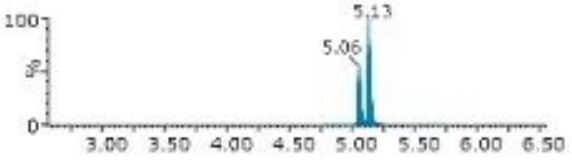
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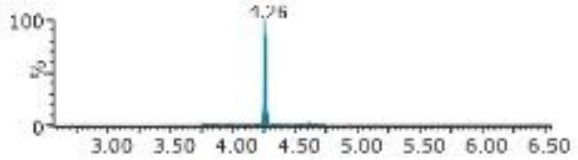
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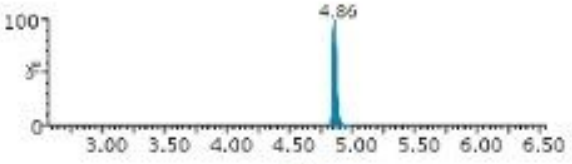
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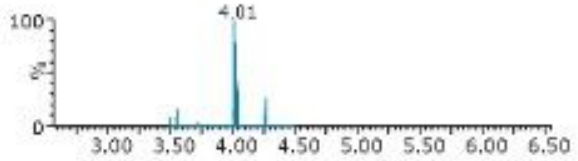
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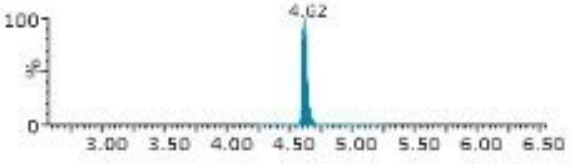
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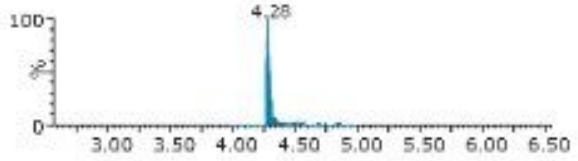
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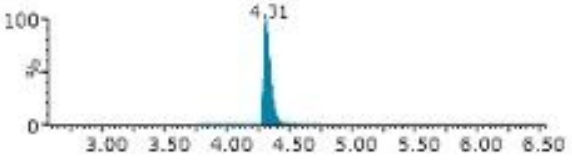
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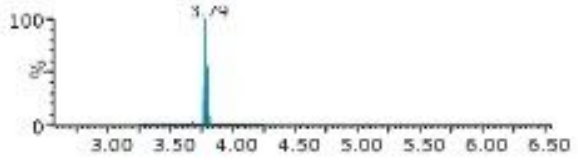
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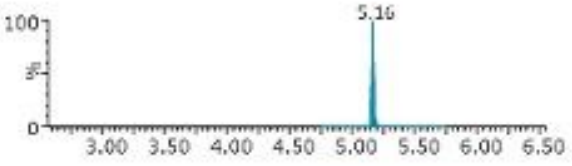
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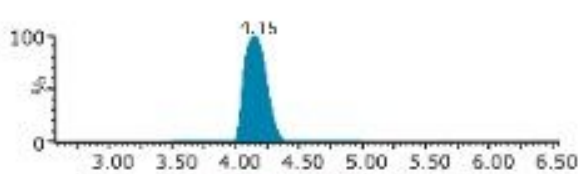
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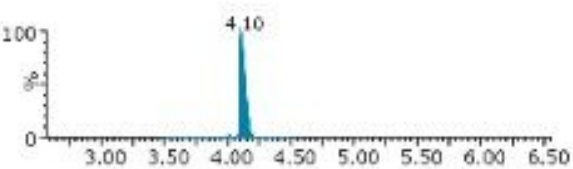
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