Reversed Phase HPLC of Polar Compounds

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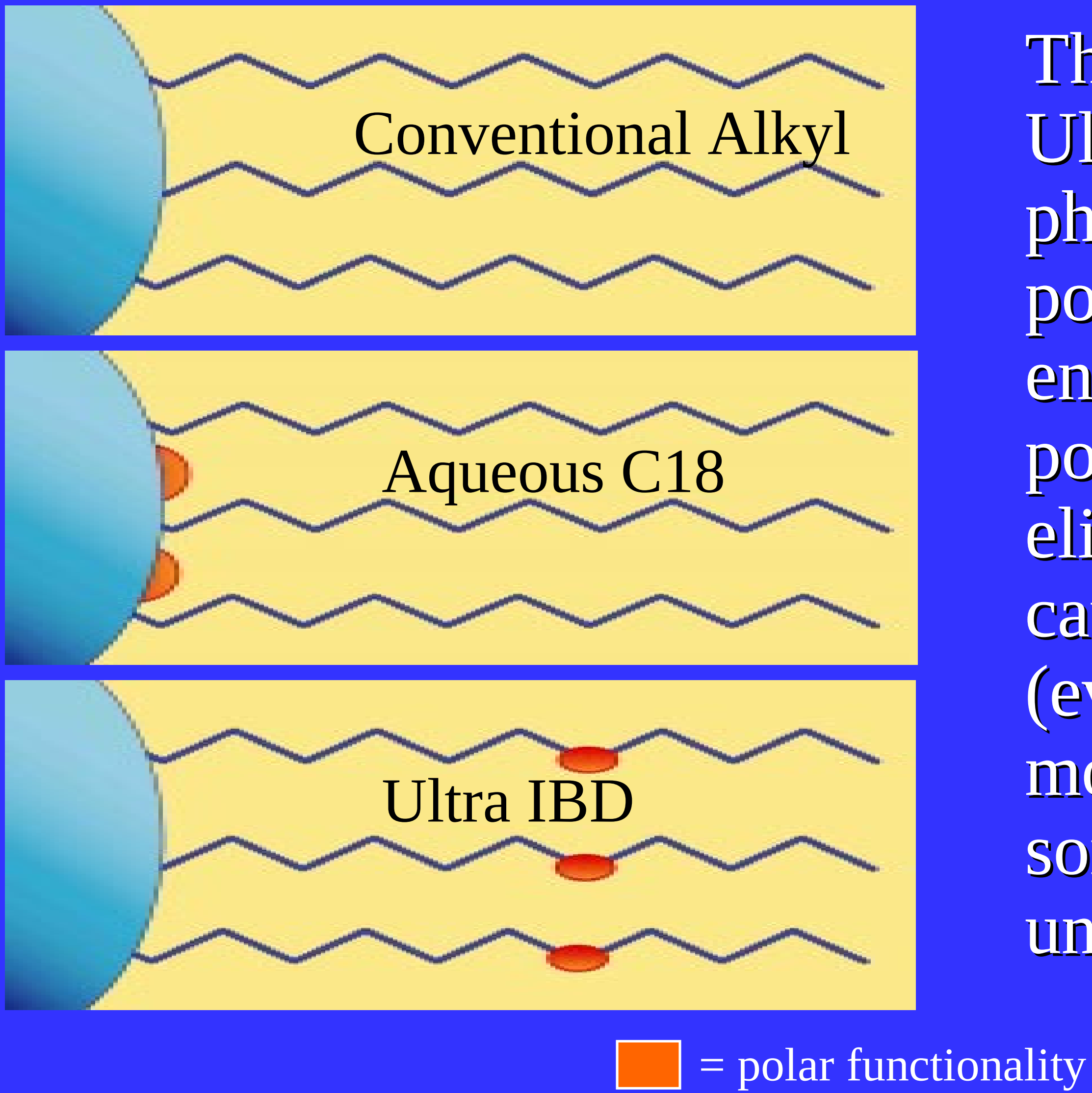
Abstract

Many polar compounds are difficult to retain using conventional reversed phase columns, even with little or no organic solvent in the mobile phase. The Ultra Aqueous C18 and Ultra IBD columns were designed to provide enhanced retention and selectivity for highly polar compounds in the reversed phase mode, as well as compatibility with highly aqueous mobile phases. The unique features of these columns are demonstrated in the analyses of nutraceuticals, pesticides, and carboxylic acids.

Figure 1. Stationary Phases



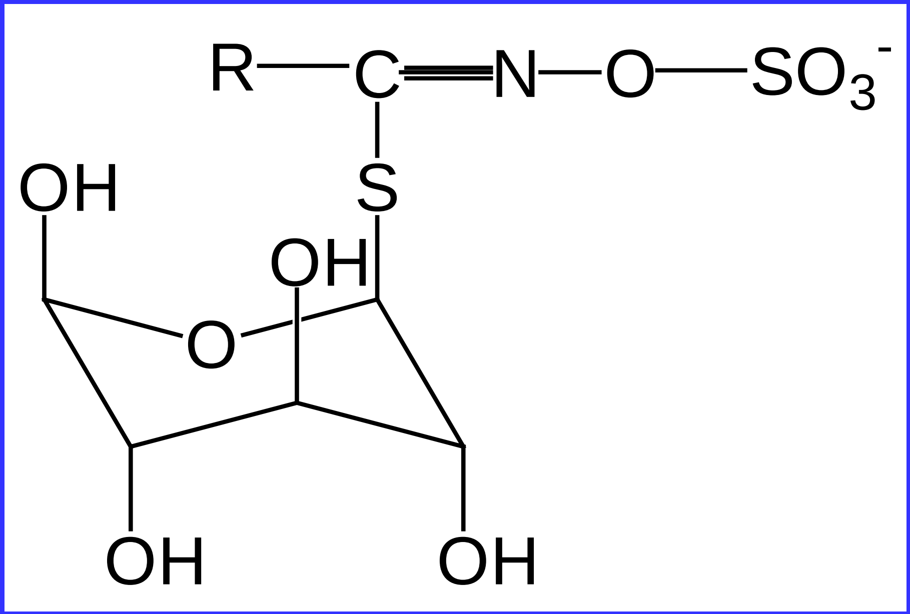
The Aqueous C18 and Ultra IBD stationary phases have secondary polar functionalities that enhance the retention of polar compounds, eliminate retention loss caused by chain folding (even in 100% aqueous mobile phase), and, in some cases, provide unique selectivity.

Nutraceuticals

Glucosinolates are contained in a number of edible plants. The consumption of these compounds is associated with a significantly reduced risk for a variety of malignant cancers. Glucosinolates are precursors of isothiocyanates, including sulforaphane (4-methylsulfinylbutyl isothiocyanate), which regulate mammalian enzymes of xenobiotic metabolism.

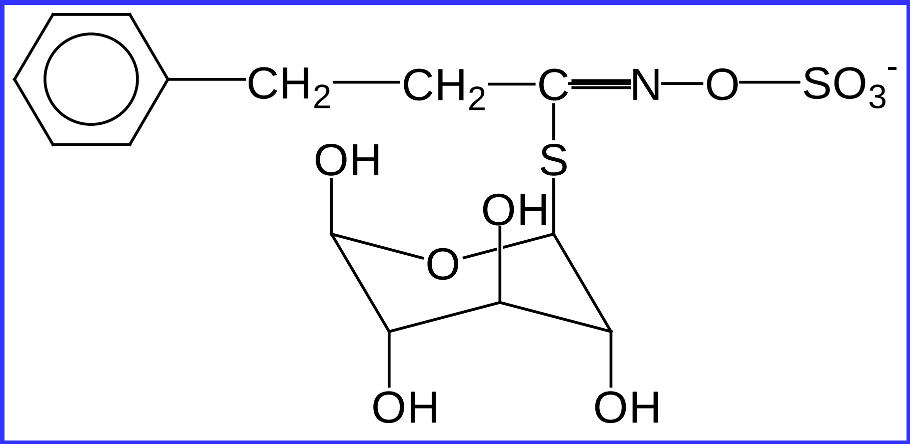
Fahey, J.W, et al, Proc. Nat. Acad. Sci. USA, Vol. 94, pp. 10367-10372, Sept. 1997.

Figure 2. Glucosinolates



Glucosinolates are β-thioglucoside N-hydroxysulfates having this general structure.

Figure 3. Phenethyl Glucosinolate



Phenethyl glucosinolate (gluconasturtiin) is one of the numerous glucosinolates widely distributed in cruciferous vegetables, and was used as a marker for glucosinolates in this study.

Glucosinolates

Figure 4 shows the analysis of the phenethyl glucosinolate standard using the Aqueous C18 column. Extracts of cabbage and watercress were analyzed using the same conditions, as shown in Figures 5 and 6. Gradient elution from 0 to 75% acetonitrile was used to retain and elute analytes with a wide range of polarities.

Figure 4. Phenethyl Glucosinolate Standard

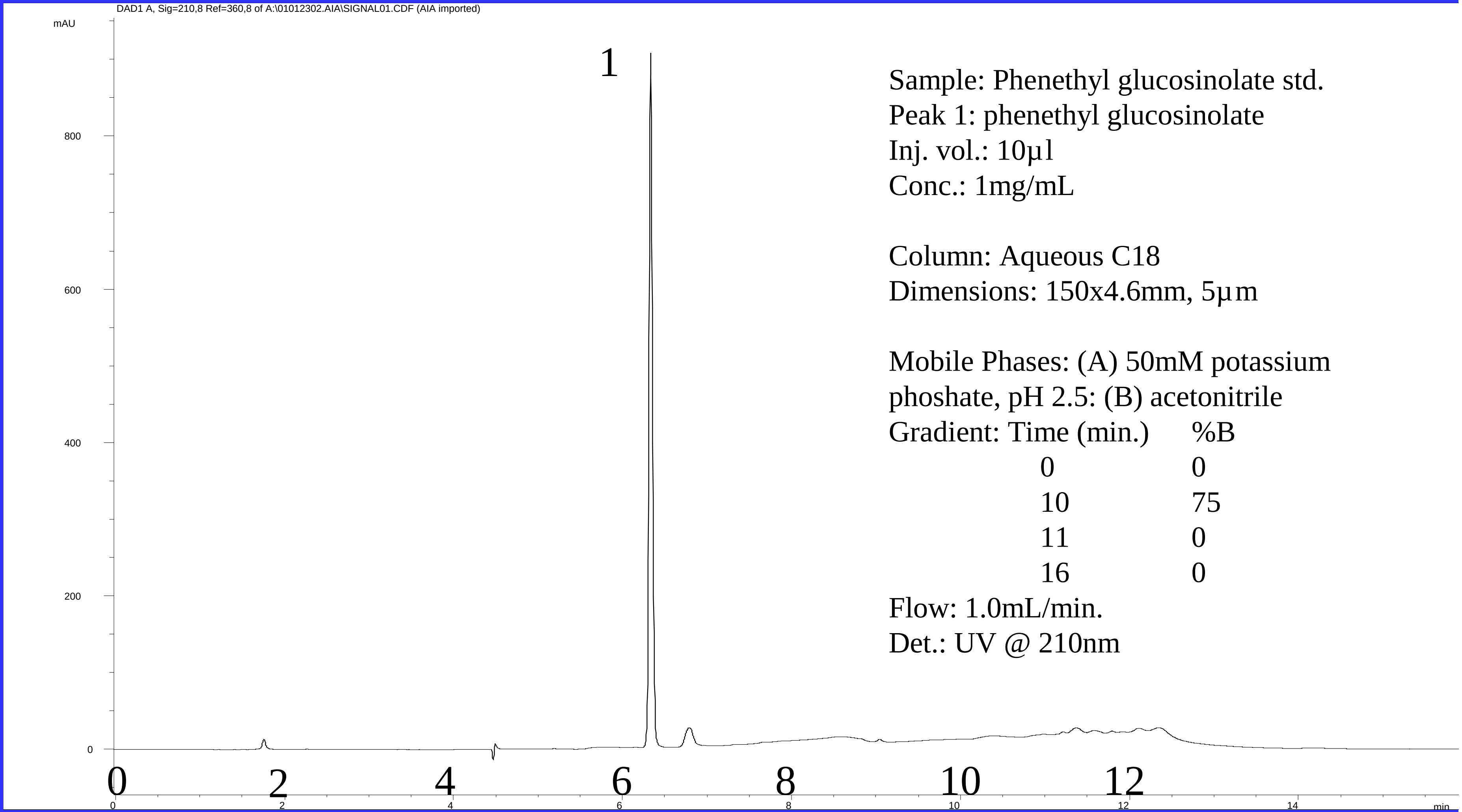
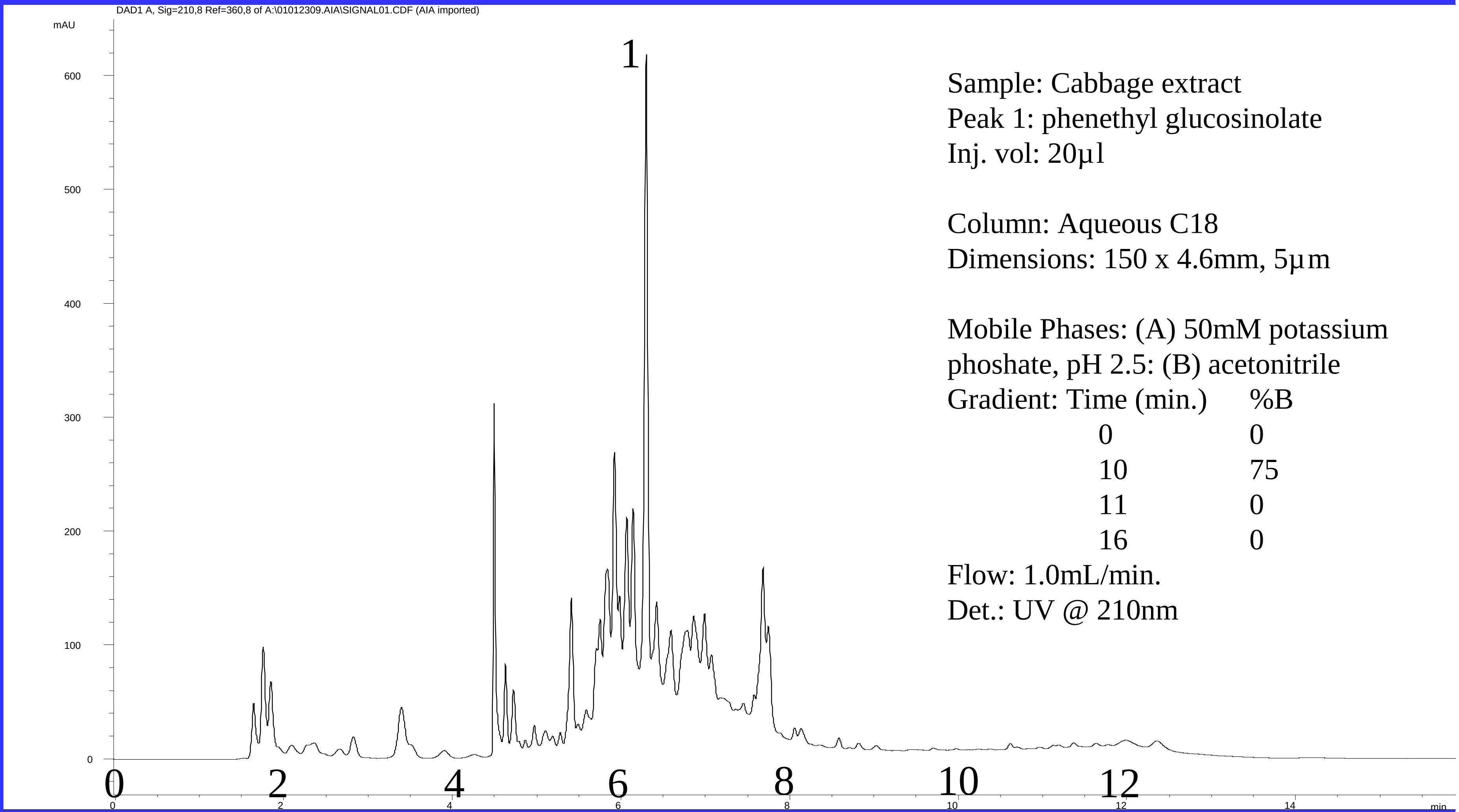


Figure 5. Cabbage Extract



Sample: Cabbage extract
Peak 1: phenethyl glucosinolate
Inj. vol: 20µl

Column: Aqueous C18
Dimensions: 150 x 4.6mm, 5µm

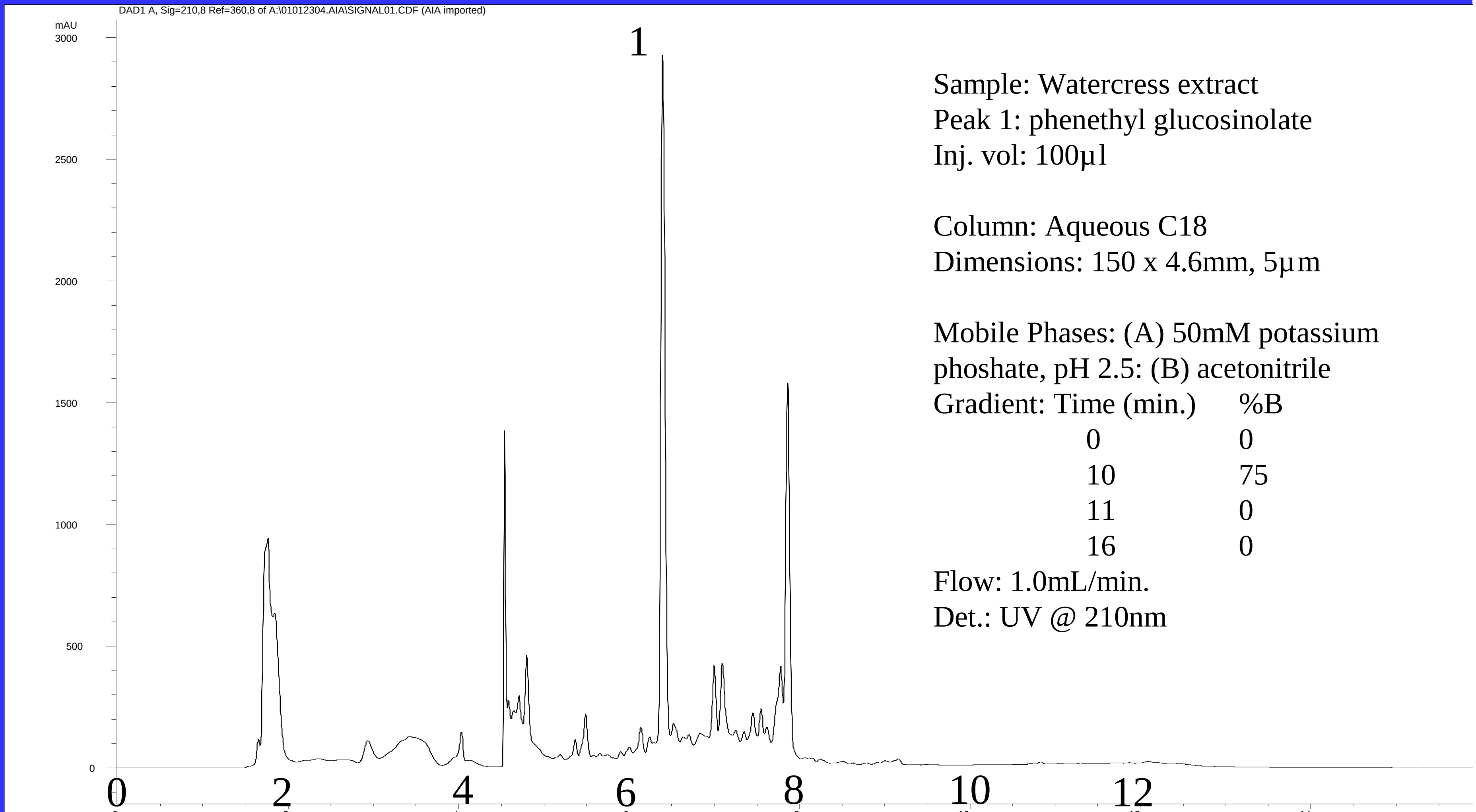
Mobile Phases: (A) 50mM potassium phoshate, pH 2.5: (B) acetonitrile

Gradient:

Time (min.)	%B
0	0
10	75
11	0
16	0

Flow: 1.0mL/min.
Det.: UV @ 210nm

Figure 6. Watercress Extract



Sample: Watercress extract
Peak 1: phenethyl glucosinolate
Inj. vol: 100µl

Column: Aqueous C18
Dimensions: 150 x 4.6mm, 5µm

Mobile Phases: (A) 50mM potassium phoshate, pH 2.5: (B) acetonitrile

Gradient:

Time (min.)	%B
0	0
10	75
11	0
16	0

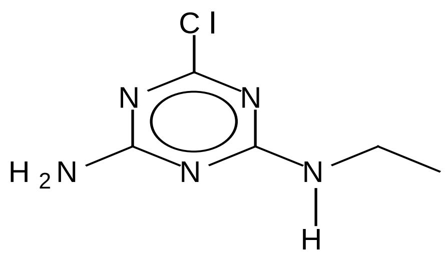
Flow: 1.0mL/min.
Det.: UV @ 210nm

Pesticides

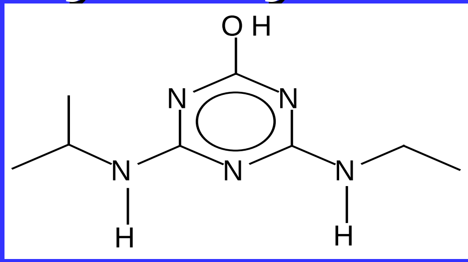
The unique selectivity of the Ultra IBD column was used to develop a two-column method for analyzing a mixture of the 17 triazine, phenylurea, and carbamate pesticides and metabolites shown in Figure 7. Figure 8 shows the separation on the Ultra C18 column in which 15 of the 17 compounds are resolved. Figure 9 shows that an Ultra IBD column with the same conditions also resolves 15 out of 17 compounds, but the pesticides that coelute on the C18 are well resolved, as indicated by the arrows.

Figure 7A. Pesticides: 17-Component Mixture

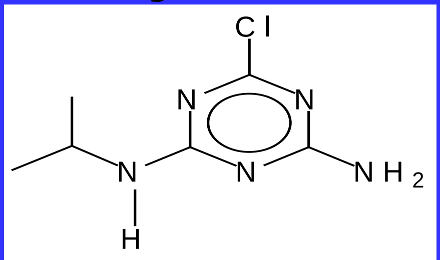
1. Desisopropylatrazine



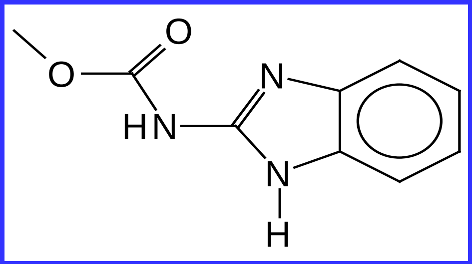
2. Hydroxyatrazine



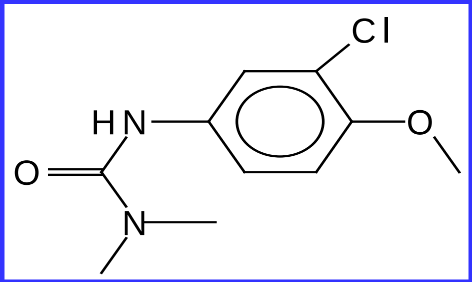
3. Desethylatrazine



4. Carbendazim



5. Metoxuron



6. Simazine

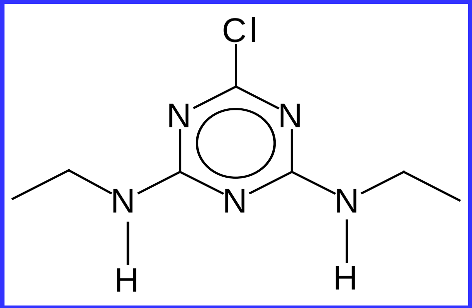
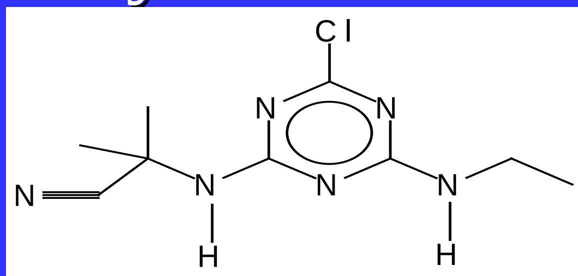
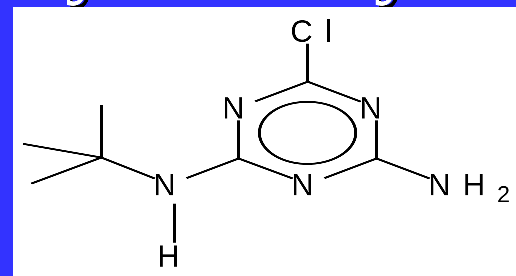


Figure 7B. Pesticides: 17-Component Mixture

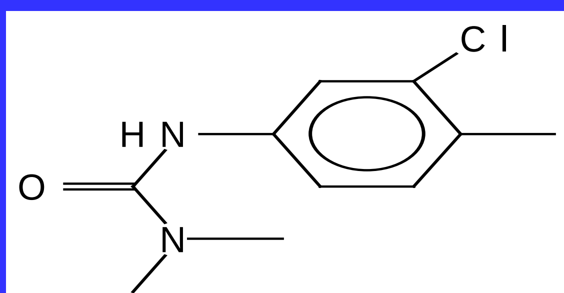
7. Cyanazine



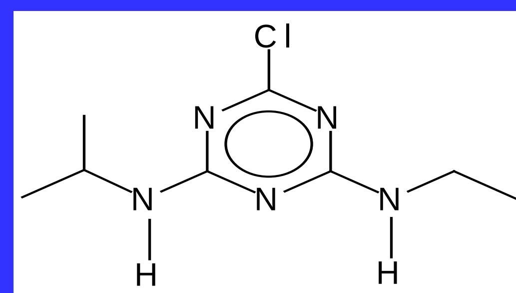
8. Desethylterbuthylazine



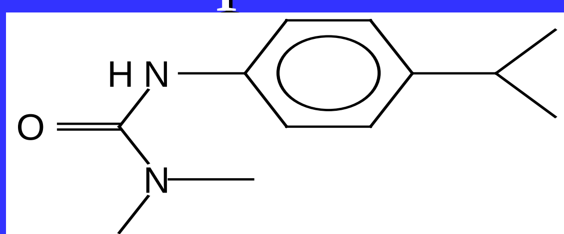
9. Chlortoluron



10. Atrazine



11. Isoproturon



12. Diuron

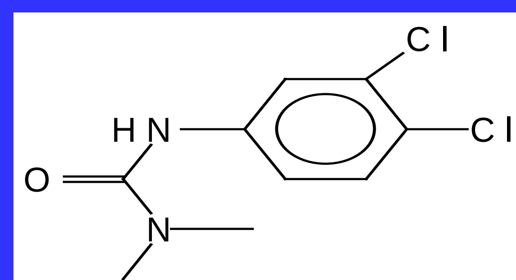
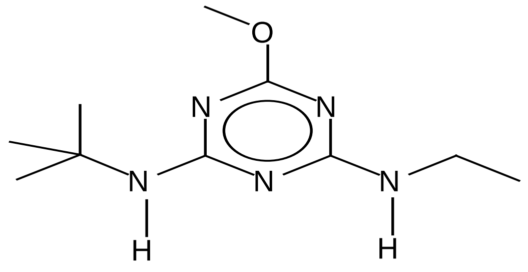
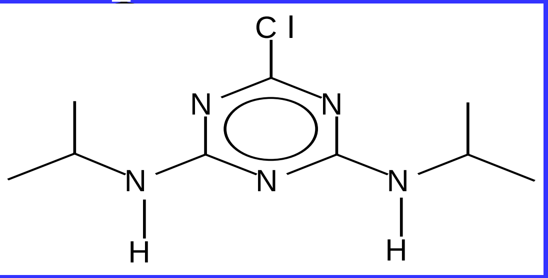


Figure 7C. Pesticides: 17-Component Mixture

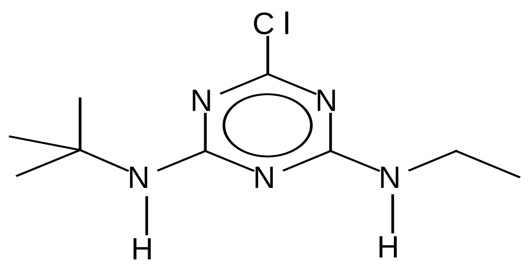
13. Terbumeton



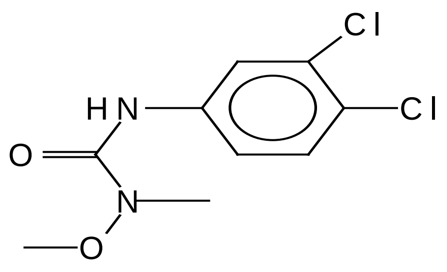
14. Propazine



15. Terbuthylazine



16. Linuron



17. Terbutryn

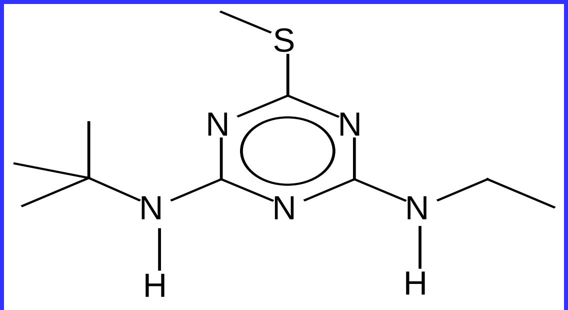


Figure 8. Pesticides on Ultra C18 Column

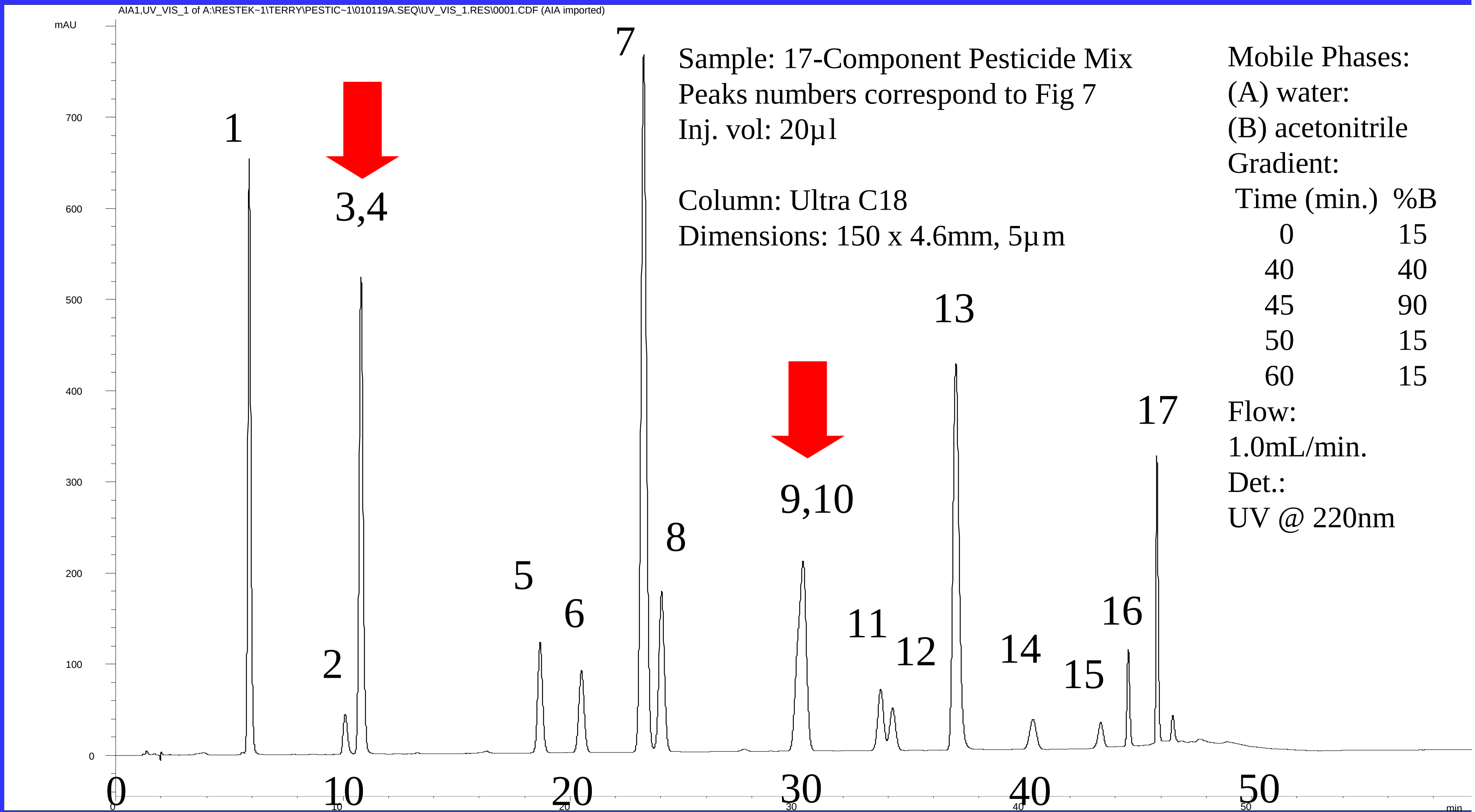
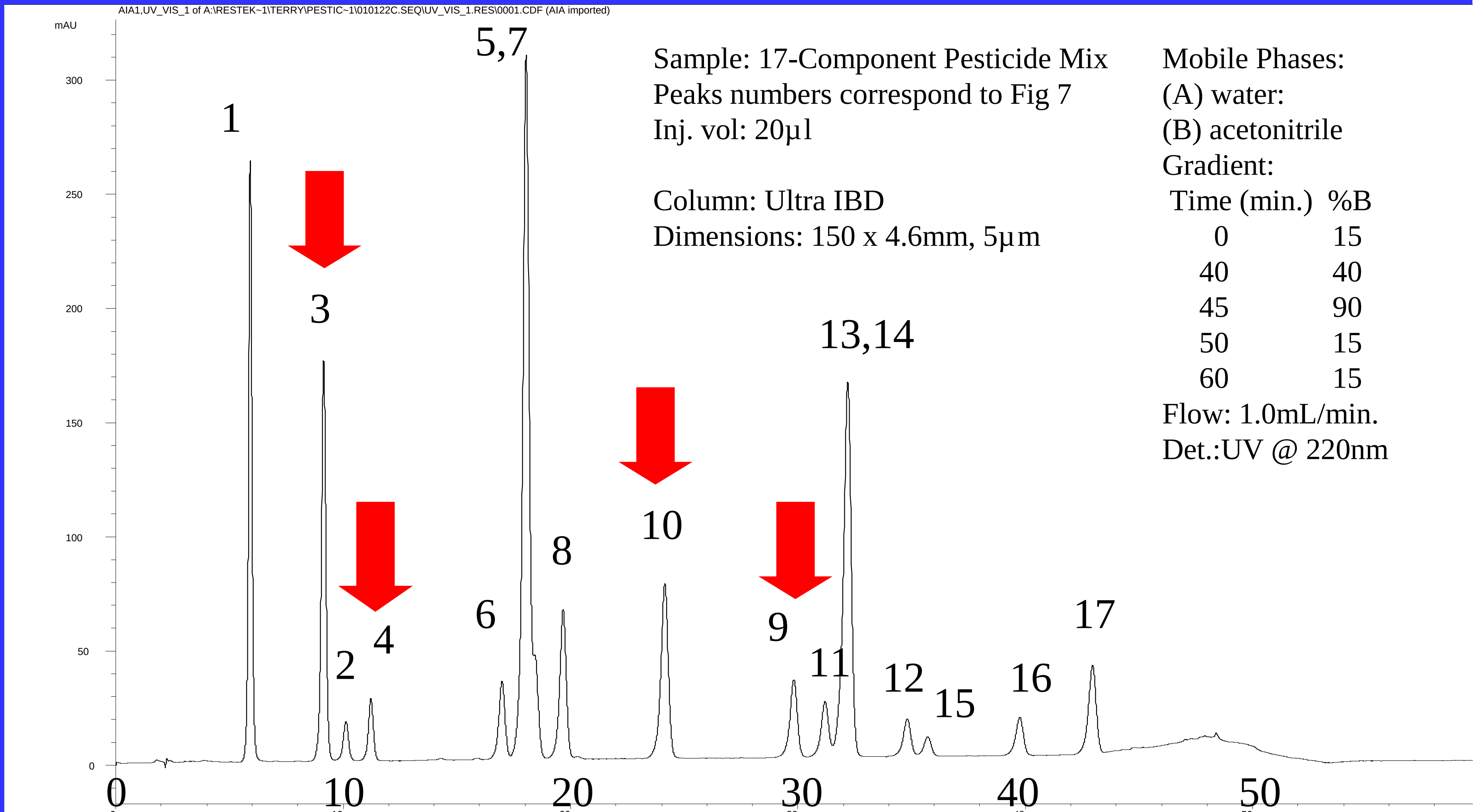


Figure 9. Pesticides on Ultra IBD Column

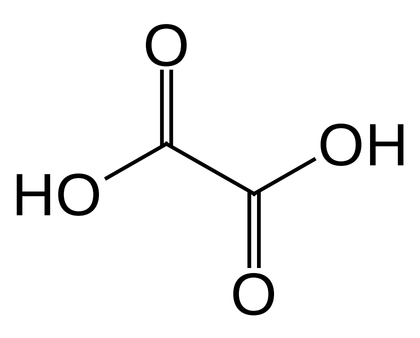


Carboxylic Acids

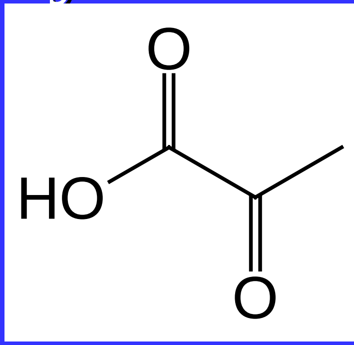
Small carboxylic acids can be difficult to retain by reversed phase HPLC, often requiring a highly aqueous mobile phase. Highly aqueous mobile phases are problematic for many C18 columns, leading to retention loss that is attributed to chain folding. The Aqueous C18 column was designed to enhance retention of polar compounds and to provide stable retention, even with completely aqueous mobile phases.

Figure 10. Carboxylic Acids

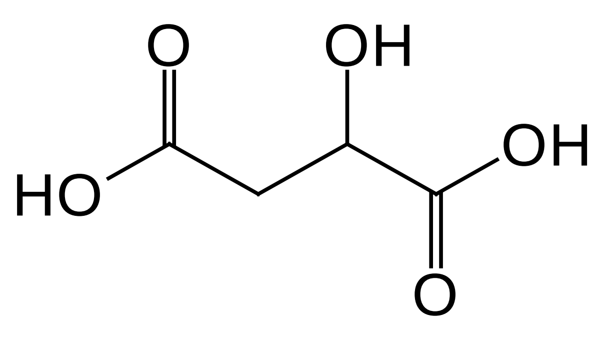
1. Oxalic acid



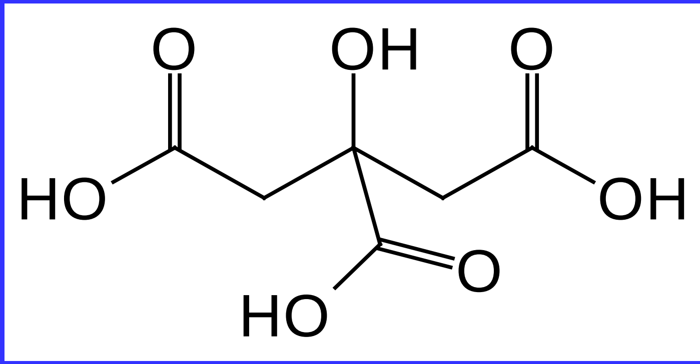
2. Pyruvic acid



3. Malic acid



4. Citric acid



Carboxylic Acids (Cont.)

Figure 10 shows four small carboxylic acids that were analyzed using the Aqueous C18 column and a completely aqueous mobile phase. Figure 11 shows the resolution of a mixture of standards and Figure 12 shows the analysis of a tobacco extract. The stability of the Aqueous C18 column is demonstrated in Figure 13, which shows the reproducible separation of the same sample as in Figure 12 after 168 injections.

Figure 11. Carboxylic Acids on Aqueous C18 Column

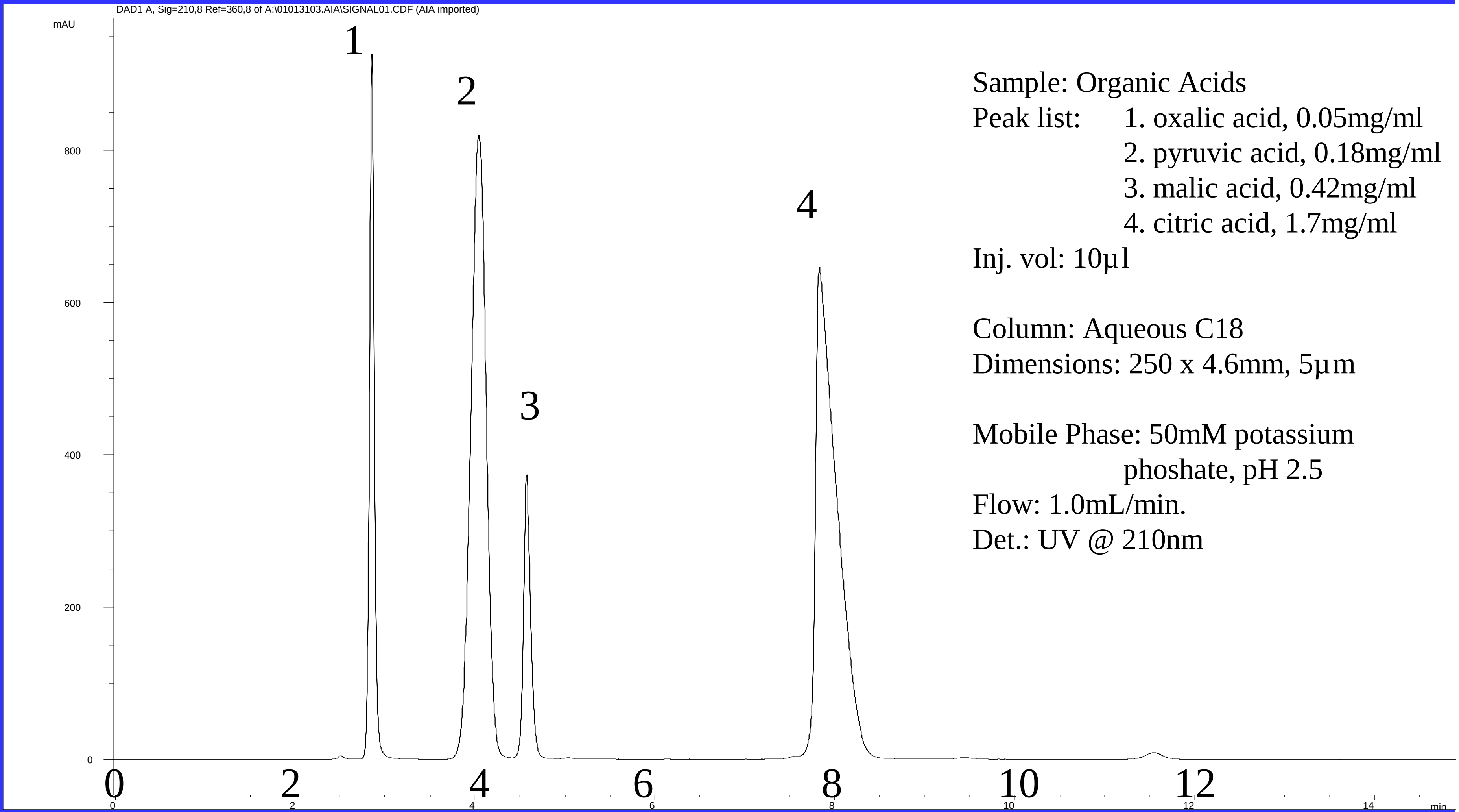
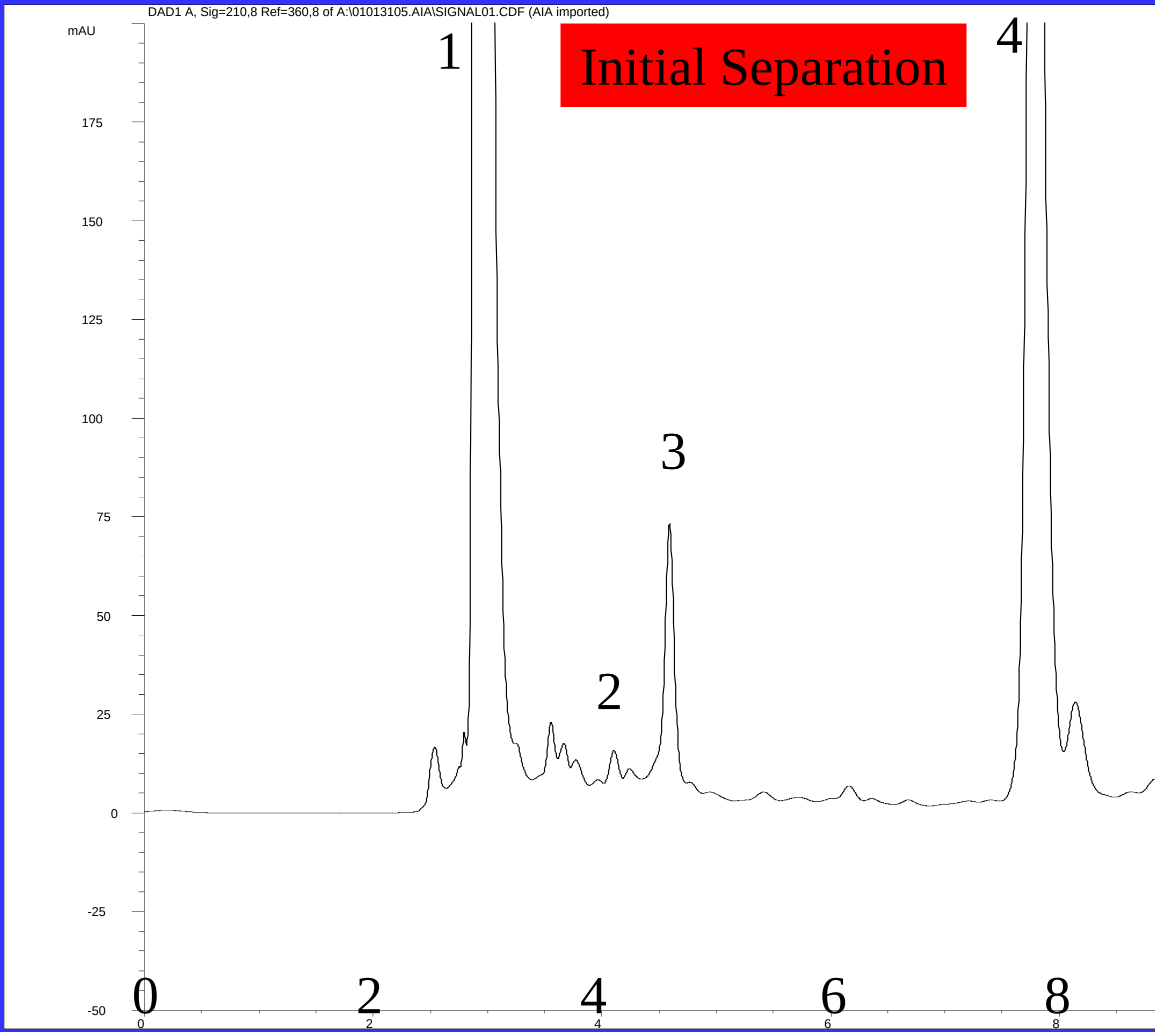


Figure 12. Tobacco Extract on Aqueous C18 Column



Sample: Tobacco Extract

Peak list: 1. oxalic acid
2. pyruvic acid
3. malic acid
4. citric acid

Inj. vol: 20µl

Column: Aqueous C18

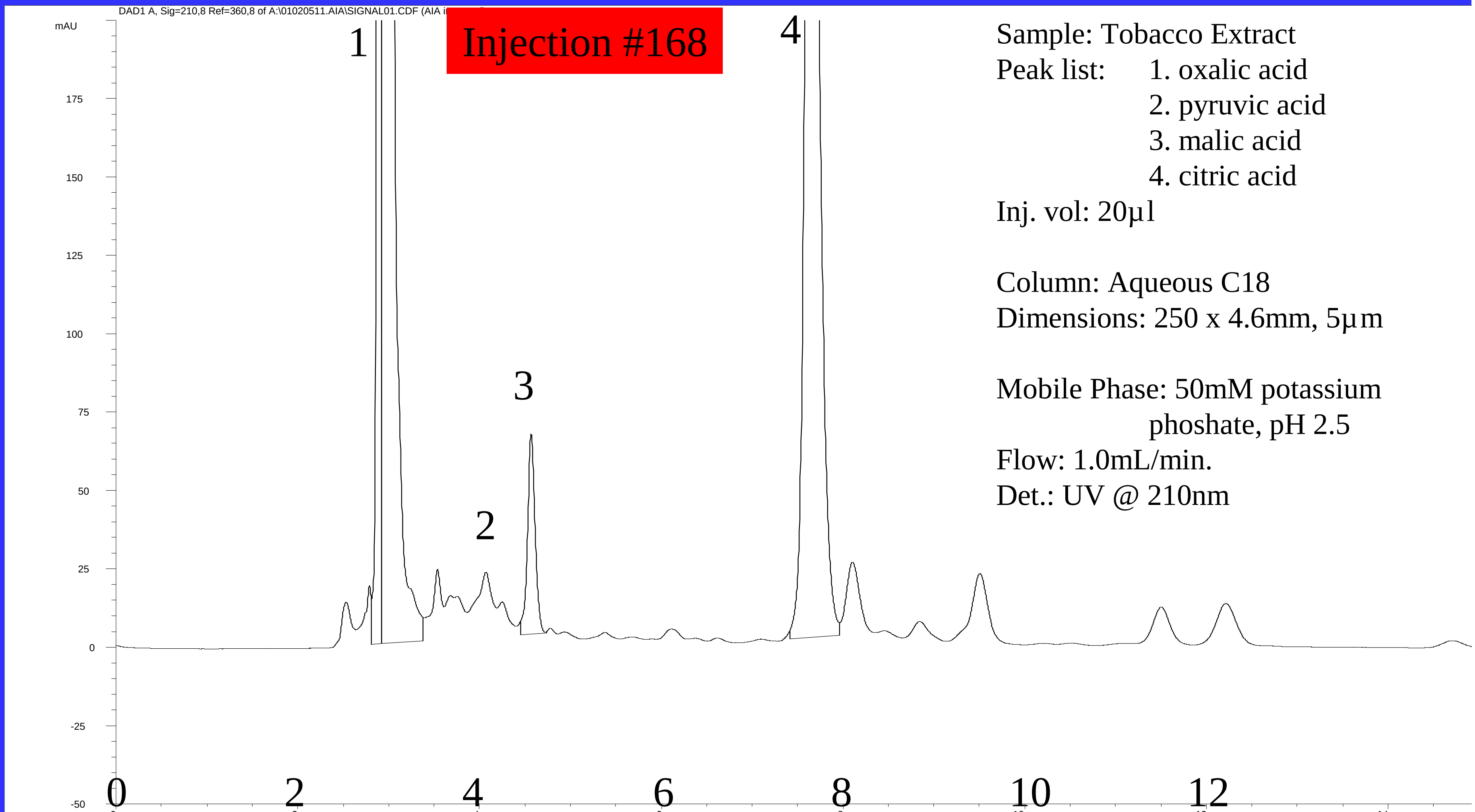
Dimensions: 250 x 4.6mm, 5µm

Mobile Phase: 50mM potassium phoshate, pH 2.5

Flow: 1.0mL/min.

Det.: UV @ 210nm

Figure 13. Stability of Aqueous C18 Column



Chain Folding

Figure 14 depicts how highly aqueous mobile phases can cause chain folding with conventional C18 columns. Chain folding can result in a total loss of retention as shown in Figure 15. Exposure to completely aqueous mobile phases at ambient pressure (no flow) accelerates the chain folding process. Figure 16 demonstrates that the Aqueous C18 column provides reproducible retention, even when stored in completely aqueous mobile phase at ambient pressure for extended periods.

Figure 14. Chain Folding (Conventional C18)

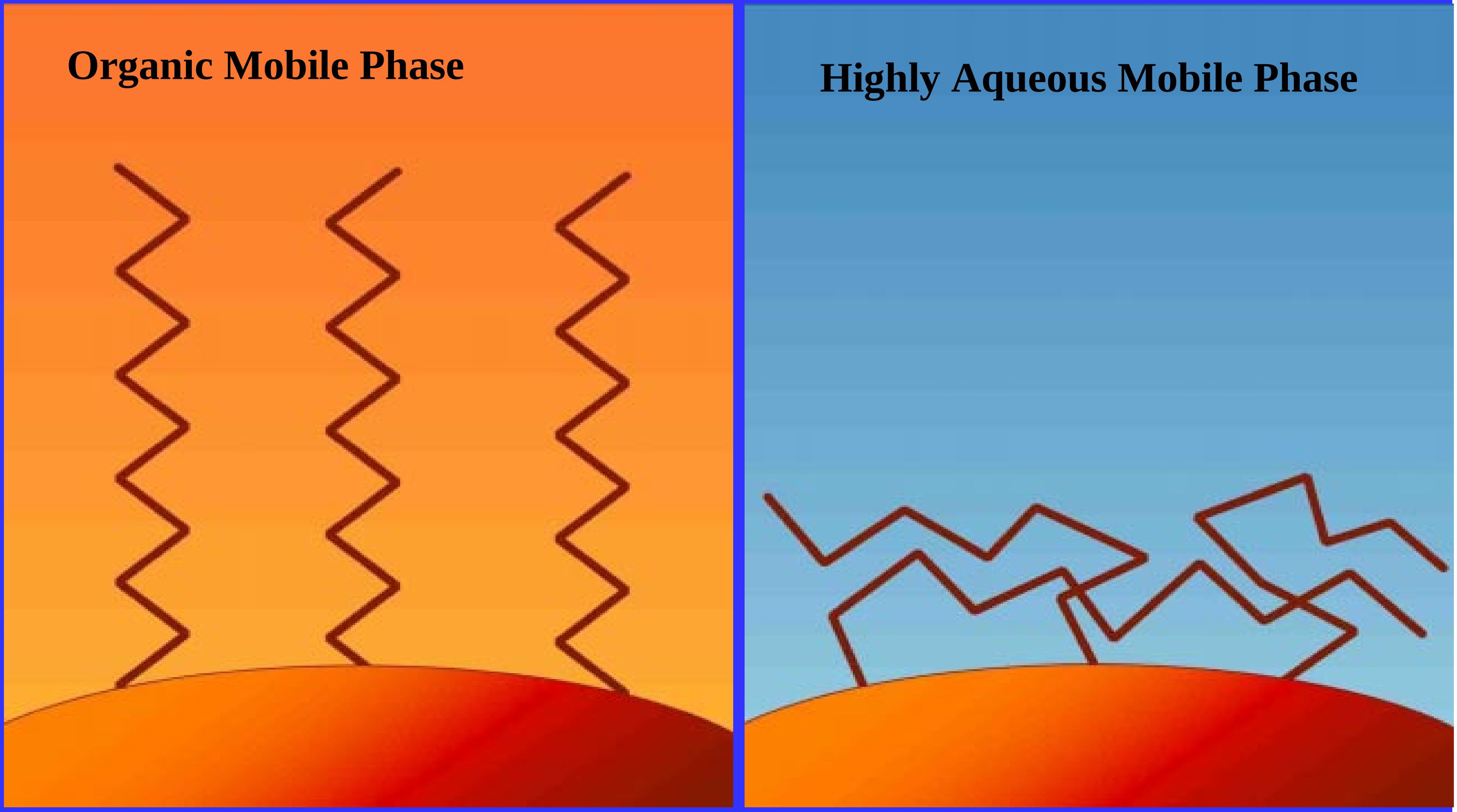


Figure 15. Chain Folding with Conventional C18

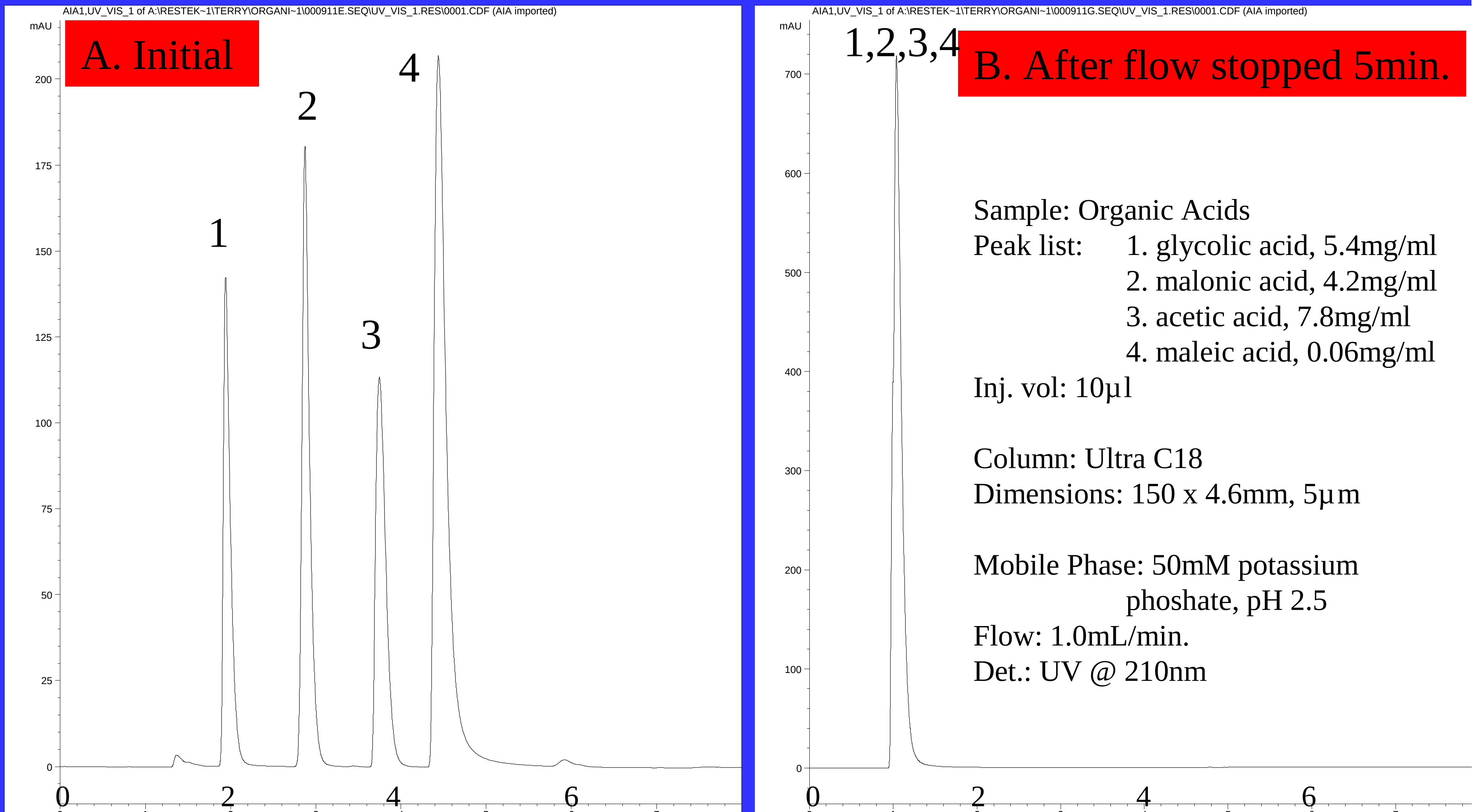
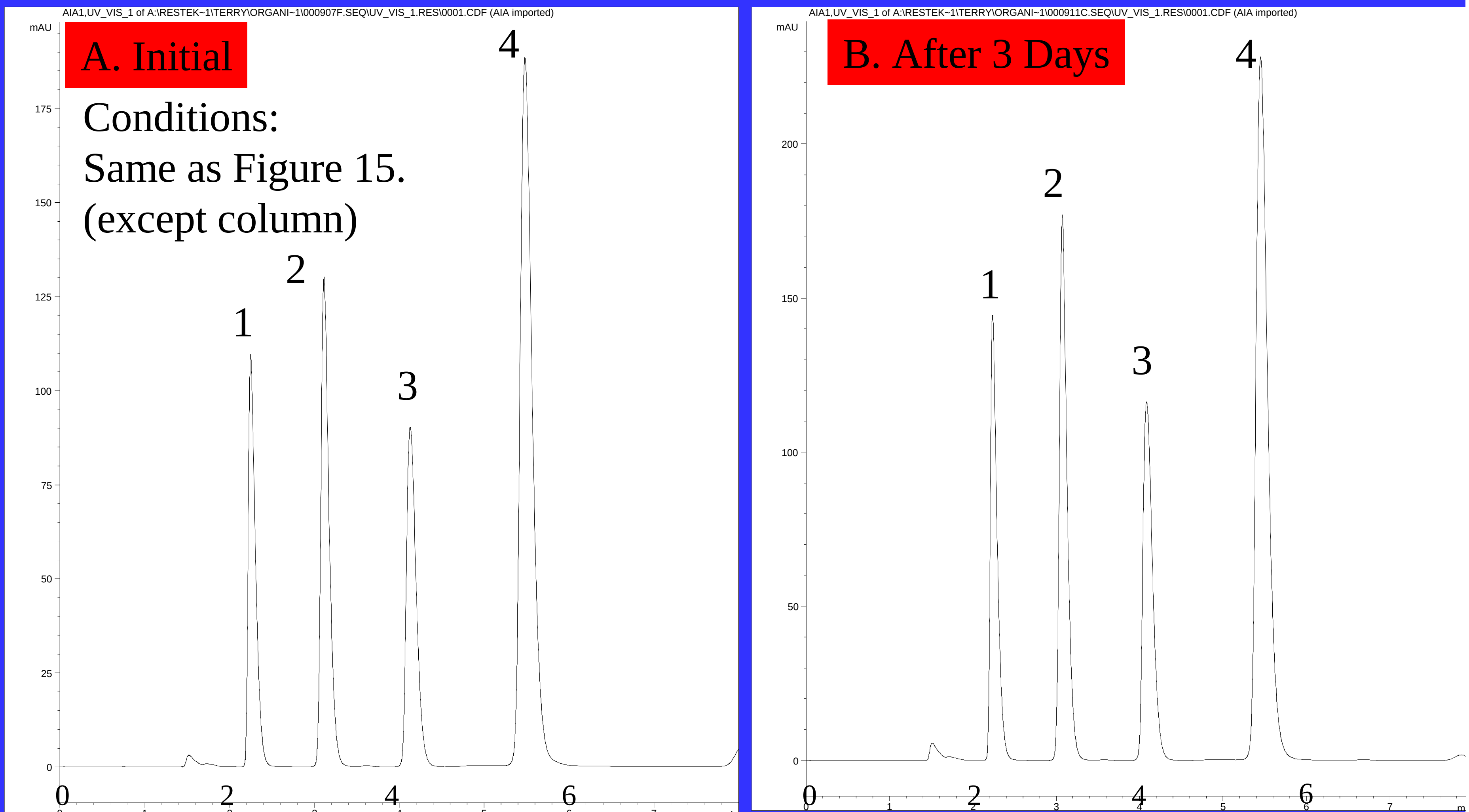


Figure 16. Stability of Aqueous C18 Column



Conclusions

The secondary polar character of the Aqueous C18 and Ultra IBD columns provide enhanced retention of polar compounds, stable retention in totally aqueous mobile phases, and unique selectivity.

The characteristics of the Aqueous C18 and Ultra IBD columns can be advantageous for analyzing a wide range of polar compounds, including nutraceuticals, pesticides, and carboxylic acids.

Acknowledgement

The phenethyl glucosinolate standard and extracts of cabbage and watercress were generously provided by Dr. Gerard Engelen-Eigles, University of Minnesota, Horticulture Department.