Waters Lab Highlights

An Internal Communication of Applications and Techniques

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Waters Carbamate Analysis System III. Troubleshooting Tips: Solvent Grade & Sparge Gas Specifications Are Important.

Recently a Carbamate Analysis System [CAS] user called Waters with a problem. He had been running his CAS very successfully to analyze water samples for carbamates. Then he converted his system, column and post-column chemistries, to do some glyphosate analyses. And finally, when he converted back to the carbamate application, he got chromatograms such as the one shown in Figure 1a below.

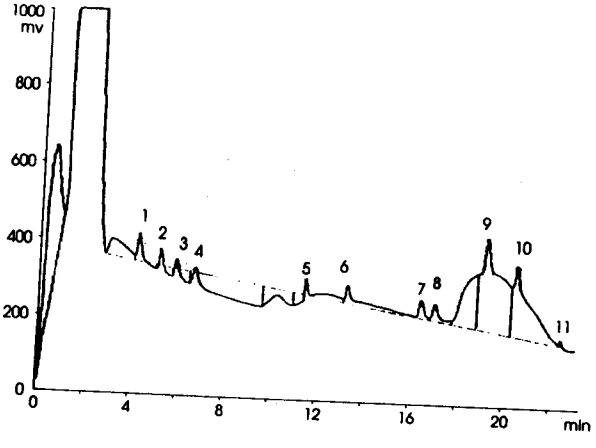

Figure 1a. Customer Chromatogram – 25 ppb Carbamate Standards in Water on Waters™ CAS/470. Note the random, elevated baseline which swamps the analyte peaks.

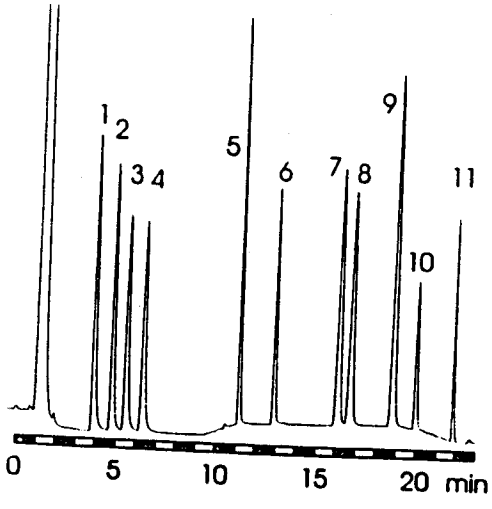

Figure 1b. What Figure 1a should look like under normal conditions. Note that chromatograms in **1a** & **1b** are *not* shown on the same scale; **1b** is taken from Waters CAS Method Manual.[1]

The chromatogram in Figure 1a exhibits a very high and randomly changing background. The analytes in this standard sample are eluting at the correct retention times, but something else in the sample, mobile phase, or post-column reagent solutions is clearly reacting with the *o*-phthalaldehyde/mercaptoethanol [OPA/ME]. Further evidence for this conclusion was given by the fact that when the OPA/ME pump was turned off, the baseline was flat and normal in appearance.

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By running each of the mobile phase solvents separately through the system, with the column bypassed but the post-column reaction chemistry on stream, it was determined that the methanol seemed to be causing the problem. Since the lab's supply of *HPLC grade* methanol[1] had run out, a high quality "*Purge & Trap*" grade from a reputable supplier had been substituted. Upon our advice, a new supply of HPLC grade methanol was immediately ordered. When the switch back to HPLC grade methanol was made, the problem baseline went away!

What caused the problem? A check of the manufacturer's literature showed that "Purge & Trap" grade, a premium quality specified for GC assays in a number of Series 500 EPA procedures for drinking water analysis, is made by taking high purity solvent and bubbling nitrogen or other inert gas through it for an extended period of time to remove all traces of volatile organic compounds. It is highly likely that if traces of ammonia or other primary amines are present in the purge gas, the amine could concentrate in the methanol. Then, when the methanol is used on the CAS, the dissolved amine would react with the OPA/ME post-column reagent, thus causing the observed baseline problem.

However, the customer's difficulties did not end there. Once he got the methanol situation under control, he began to lose prime on his 600E pump. Upon further inquiry, it was learned that he had run out of *helium* sparge gas and had substituted another inert gas, *nitrogen*, in its place. Unfortunately, this is not the way to remove dissolved air (nearly 80% of which is nitrogen) from mobile phase solvents! Now that helium is once again on hand, everything is working well.

The moral of this story is three-fold:

First, the Carbamate Analysis Method specified in detail in Waters CAS Method Manual should be followed carefully, especially with regard to the kind of solvents, reagents, and standards used. Even seemingly harmless substitutions using high quality materials on hand may lead to significant problems, as shown above.

Second, even experienced, highly capable chemists may run into problems when dealing with new technologies and new applications, as when moving from the world of GC to HPLC. This is why the CAS is designed as an integrated system, complete with methodology and separation chemistry.[2] When the CAS method is followed strictly, performance is guaranteed, making it easy for chemists new to HPLC to be successful in carbamate analysis.

Third, if problems should arise, comprehensive customer support is only a phone call away. It is much easier to find a solution to a problem or an answer to a ***system*** question from a single source than to go back and forth between separate chemistry and instrument vendors.

References:

1. Refer to Waters *Carbamate Analysis System Method Manual*, p. 3-22 for standard chromatogram, p. 3-5 for solvent specifications and p. 2-10 for sparge gas specifications.
2. P.D. McDonald, W.P. Leveille, A.E. Sims, W.J. Wildman, V.R. Zener, A.D. Scarchilli, Water Quality Technology Conference, 12-16 November 1989, Philadelphia, PA, *Proceedings Volume*, American Water Works Association, 1990, pp 631-649.

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