Waters Lab Highlights

An Internal Communication of Applications and Techniques

LAH 0435 6/90
AN/ES,FA,HO/
MD/PS/CU

Waters™ Carbamate Analysis System II. Sensitivity – a Key Benefit of Ternary Gradient

The primary objective in designing Waters Carbamate Analysis System [CAS] was to maximize analyte sensitivity and method reliability. The benefits from reaching this goal would be the lowest possible limits of detection and the highest possible confidence in the precision and accuracy of the analytical results.

A key feature of Waters Carbamate Analysis Method which enables the CAS to meet the design objective is the use of Waters Carbamate Analysis Column with a multi-segment ternary gradient. Each solvent – methanol, acetonitrile, and water – is pumped from a separate reservoir; mixing occurs in the pumping system, thereby eliminating a common, though often overlooked, interlaboratory source of variance in LC methods: manual preparation of mobile phase component mixtures.

In an earlier Lab Highlight[1], the favorable chromatographic selectivity of Waters Carbamate Analysis Column for the analytes specified in EPA Method 531.1[2] was demonstrated (Figure 1a). As shown in Figure 1b, however, an optimized ternary gradient can shorten analysis time for increased sample throughput and improve the resolution of critical pairs such as baygon [propoxur] (7) and carbofuran (8) for better precision in quantitation.

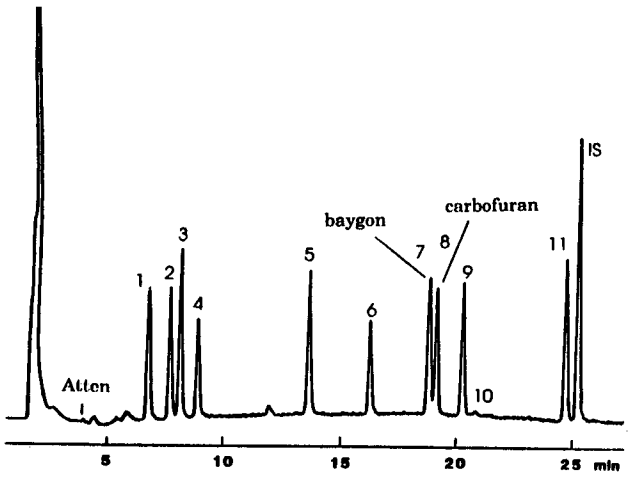

Figure 1a. EPA Method 531.1, Rev. 3.0
Waters Nova-Pak® C_{18} Column
EPA linear binary gradient: MeOH/water

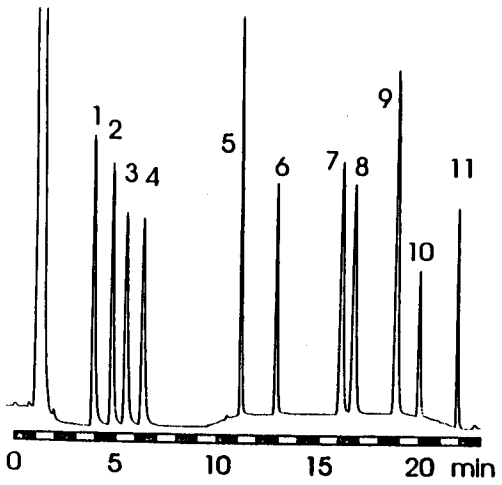

Figure 1b. Waters CAS
Waters Carbamate Analysis Column &
Carbamate Analysis Method: nonlinear ternary gradient: MeOH/ACN/water

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In Figure 1b, peaks 1-4 elute in MeOH/water only. Then, a baseline "plateau" rises at about 10 minutes and falls at about 22 minutes, indicating the portion of the gradient where acetonitrile (ACN) is present. Quantitation accuracy of peaks 5-11 is not affected by the plateau since the gradient has been optimized for maximum resolution, and the critical pair (7/8) elutes on the flat crest of the plateau.

Experiments to determine the cause of this ACN-shift implicate some trace primary amine impurity in the ACN (but not in the MeOH) which reacts with the OPA/ME post-column reagent. This impurity is common to ACN obtained from four different vendors and is ***bottle***, not ***lot***, specific. It may be trace ammonia resulting from ACN hydrolysis; hydrolysis may be accelerated by poor storage conditions. We have alerted various manufacturers of HPLC-grade ACN to this situation; they are now exploring ways to improve the quality of ACN[3].

Though the ACN-plateau does not affect the analysis, even at sub-ppb levels, why not eliminate it for aesthetic/economic reasons and use a MeOH/water binary gradient? The answer to this question is found in Figure 2 below. Two consecutive runs were performed on the CAS; on the left, the standard CAS method was run using a particularly poor lot of ACN giving a pronounced plateau. On the right, the same sample was run immediately afterwards under identical conditions except that an ***optimized*** MeOH/water *binary* gradient was substituted for the normal *ternary* gradient. The analysis time is the same (22 min), and the selectivity is similar, though the spacing of peaks 9-11 is not quite as good. There is still a slight plateau in the chromatogram on the right caused by larger pressure changes during the gradient, due to the increased viscosity of intermediate MeOH/water mixtures. *Notice however the ~2x reduction in peak heights of the last seven analytes.* By countering the fluorescence quenching due to MeOH alone, the presence of ACN in the ternary gradient effectively ***doubles the sensitivity*** for these important analytes.

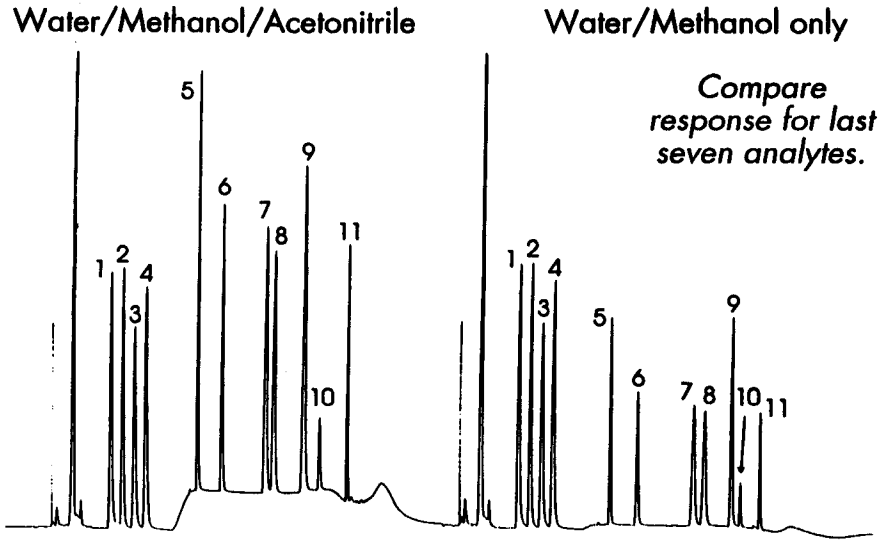

Figure 2. Comparison of ternary (left) and binary (right) gradients on Waters CAS/470.
10 ng of each analyte on column, 100µL injection volume of standards in water.
Fluorescence detection: 339 nm ex λ/445 nm em λ.

References:

1. LAH 430 3/90.
2. R.L. Graves, *Method 531.1, Rev. 3.0,* USEPA (1989).
3. Due to a plant explosion, ACN is currently in short supply; see *C&E News* (3/26/90) p. 15.

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