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Application Note

Comparing the Performance and Reliability of Waters Alliance HPLC Systems for Carbamate Analysis

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Abstract

With the release of the 2013 Alliance HPLC System, this application note shows equivalency of the Waters Alliance System for Carbamate Analysis and 2013 Alliance HPLC System relative to linearity, precision, reproducibility, and limit of detection (LOD). The data shows that similar results are obtained on the legacy and 2013 Alliance HPLC systems for Carbamate Analysis.

Benefits

Waters Alliance System for Carbamate Analysis provides a complete system solution for the analysis of N-methylcarbamate and N-methylcarbamoyloxime pesticides in drinking water and a variety of environmental and food matrices.

- Carbamate analysis at sub-ppb levels
- Compatible with US EPA Method 531.2
- Baseline separation of analytes

Introduction

The Waters Alliance System for Carbamate Analysis has been offered as a system solution since 1998. With the release of the 2013 Alliance HPLC System, we wish to show equivalency of the two platforms relative to linearity, precision, reproducibility, and limit of detection (LOD).

Figure 1. 2013 Alliance HPLC System.

Experimental

LC conditions

System:	Alliance HPLC for Carbamate Analysis (both legacy and 2013 systems)
Run time:	25.0 min
Column:	Waters Carbamate 3.9 x 150 mm, 4.0 µm at 30 °C
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile
Flow rate:	1.5 mL/min
Injection volume:	400 µL (1000 µL for 0.2- and 0.1-ppb levels)
Detection:	Fluorescence (Ex-339 nm, Em-445 nm)
Data management:	Empower 2 Software

Gradient

Time (min)	Flow rate (mL/min)	%A	%B	%C	Curve
Initial	1.5	88	12	0	*
5.3	1.5	88	12	0	1
5.4	1.5	68	16	16	5
14.0	1.5	68	16	16	3
16.1	1.5	50	25	25	7
20.0	1.5	50	25	25	6
22.0	1.5	88	12	0	5

The basic system, components, and experimental procedure are described in the Waters Alliance System for Carbamate Analysis Method Manual.[1]

Standard and sample preparation

Two reference materials, M531M and M531-IS, were purchased from AccuStandard. These were diluted with preservation solution[2] to prepare the following levels: 100-, 75-, 50-, 25-, 10-, 5-, and 1-ppb. 0.2- and 0.1-ppb mixes were also prepared. These levels were also run as unknowns for the tests described in this study.

Linearity

The seven levels described above were injected in triplicate to construct a linear calibration curve.

Precision and reproducibility

The 75-, 25-, and 10-ppb levels were injected seven times as unknowns to determine precision and

reproducibility for amount. The 21 injections (seven of each of the three levels) were used to determine precision and reproducibility for retention time.

Limit of detection

A 0.2-ppb carbamate mix was run three times as a standard, then seven times as an unknown to determine a limit of detection per 40 CFR pt 136 App, B[3]. A 0.1-ppb mix was also run for comparative purposes.

Performance evaluation

A Performance Evaluation Carbamate mix was purchased from ERA. The mix was prepared as directed, and quantified using both systems.

Results and Discussion

Figures 2 and 3 show overlaid chromatograms of the standards on the legacy and 2013 Alliance platforms, respectively. The linearity on both platforms is compared in Table 1. R^2 is greater than 0.999 for all compounds regardless of which platform was used. The reproducibility for both retention time and concentration was investigated for both platforms using seven injections of three different standard concentrations (10-, 25-, and 75-ppb) with the results shown in Tables 2 through 5. The relative standard deviation for the retention time for the 21 injections was less than 0.25% on both platforms, as shown in Table 2. Tables 3 through 5 show comparisons for the amount for seven injections each of the 75-, 25-, and 10-ppb carbamate mixes run as unknowns. The %RSD was less than 0.8% for the 75-ppb and 0.9% for the 25-ppb mixes using both the legacy and 2013 Alliance platforms. One exception to this is 1-Naphthol, which is a hydrolysis product of carbaryl[4] and is, therefore, expected to show more variability. The %RSD was shown to be less than 1.5% for the 10-ppb mix on both platforms.

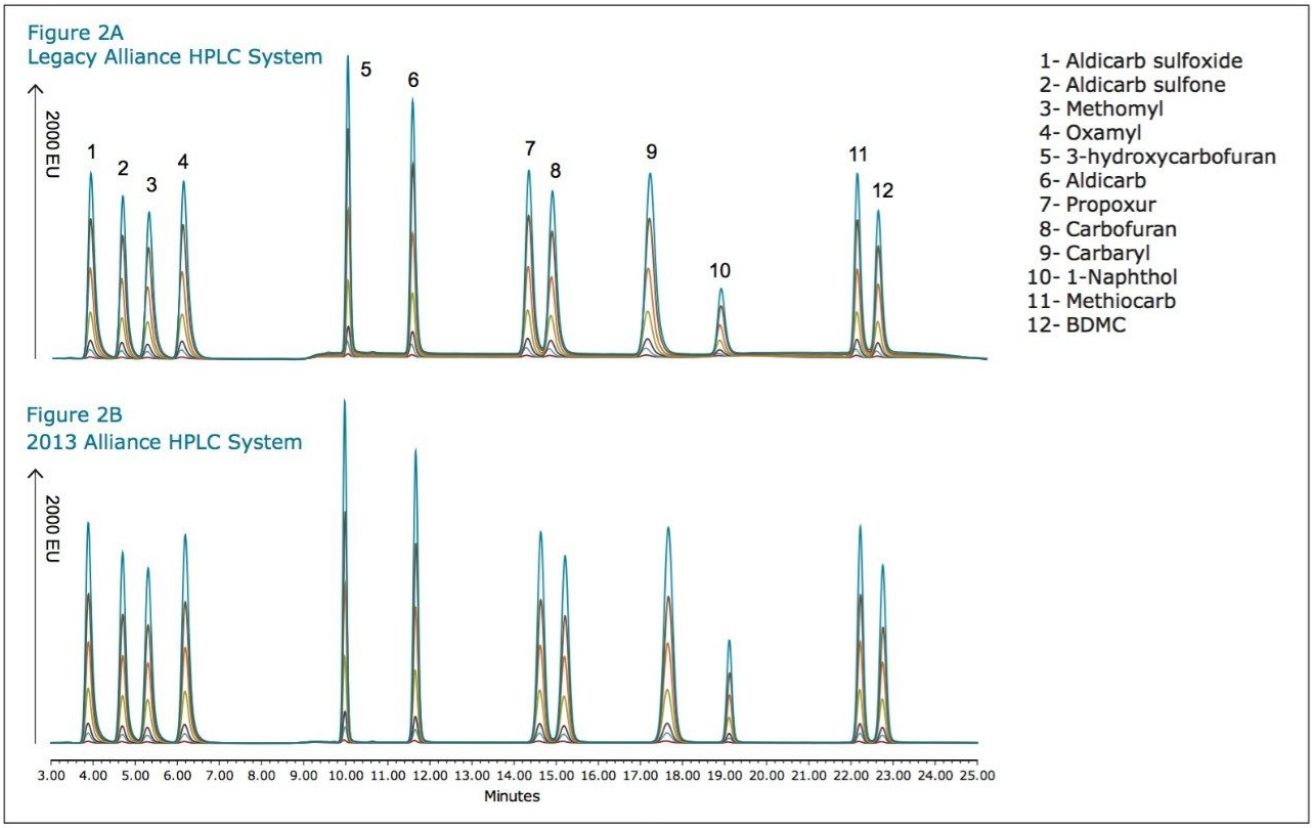

Figure 2. Chromatograms of 100-, 75-, 50-, 25-, 5-, and 1-ppb carbamate mixes using the legacy and 2013 Alliance HPLC systems.

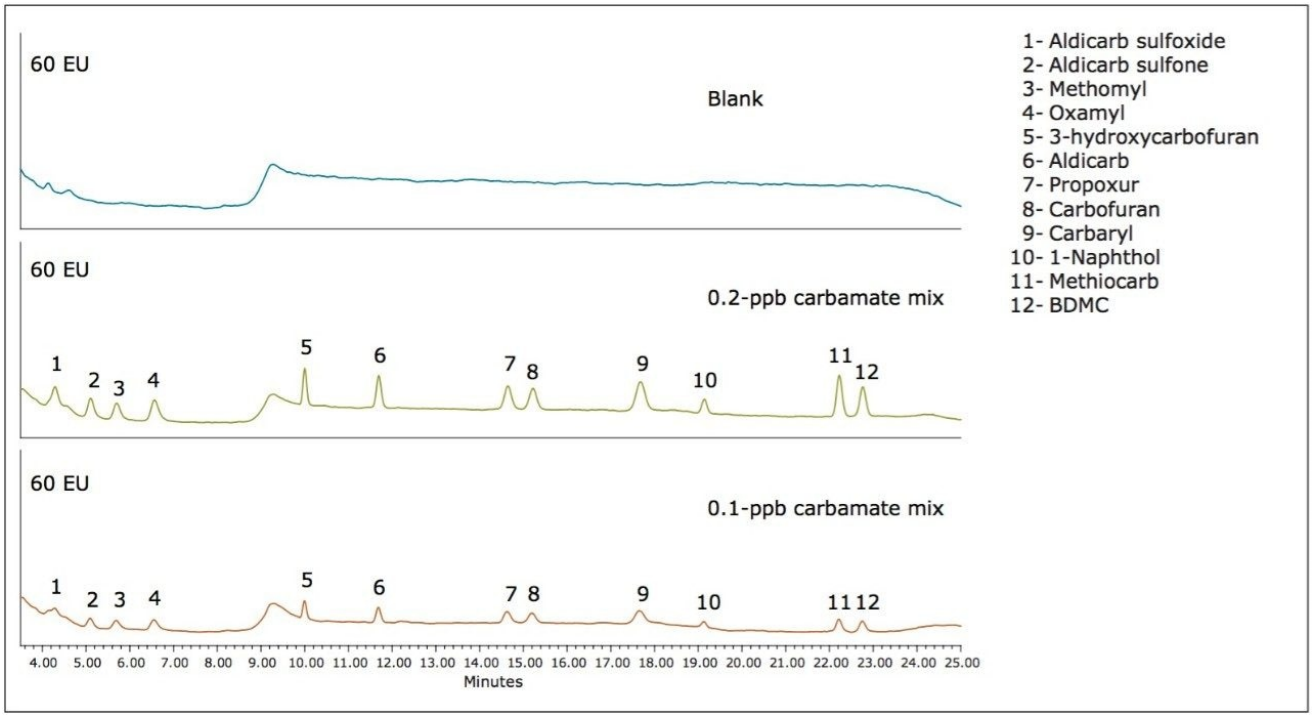

Figure 3. Chromatograms of low-level carbamate mixes on the legacy Alliance HPLC System, 1000-µL injection.

Linearity (R^2)	Legacy Alliance HPLC	2013 Alliance HPLC
Aldicarb sulfoxide	0.9998	0.9998
Aldicarb sulfone	0.9998	0.9997
Oxamyl	0.9997	0.9998
Methomyl	0.9997	0.9997
3-Hydroxycarbofuran	0.9998	0.9997
Aldicarb	0.9998	0.9998
Propoxur	0.9997	0.9998
Carbofuran	0.9997	0.9996
Carbaryl	0.9998	0.9996
1-Naphthol	0.9998	0.9995
Methiocarb	0.9998	0.9996
BDMC	0.9999	0.9996

Table 1. Linearity for carbamate analytes.

Reproducibility (RT) 21 injections	Legacy Alliance HPLC			2013 Alliance HPLC		
Analyte	Mean RT	Std Dev	%RSD	Mean RT	Std Dev	%RSD
Aldicarb sulfoxide	3.86	0.003	0.086	3.94	0.005	0.132
Aldicarb sulfone	4.59	0.008	0.174	4.65	0.008	0.170
Oxamyl	5.18	0.010	0.193	5.27	0.011	0.209
Methomyl	5.99	0.012	0.197	6.08	0.011	0.186
3-Hydroxycarbofuran	9.94	0.009	0.089	9.93	0.007	0.072
Aldicarb	11.44	0.012	0.104	11.44	0.011	0.100
Propoxur	14.12	0.017	0.119	14.13	0.020	0.144
Carbofuran	14.65	0.018	0.125	14.67	0.021	0.146
Carbaryl	16.89	0.025	0.146	16.93	0.027	0.158
1-Naphthol	18.63	0.021	0.114	18.62	0.018	0.097
Methiocarb	21.87	0.013	0.059	21.85	0.013	0.062
BDMC	22.36	0.013	0.059	22.33	0.015	0.065

Table 2. Reproducibility data for retention time for 21 injections, seven injections each, of 75-, 25-, and 10-ppb carbamate mixes using the legacy and 2013 Alliance HPLC systems.

Amount 7 injections (75-ppb)	Legacy Alliance HPLC			2013 Alliance HPLC		
Analyte	Mean RT	Std Dev	%RSD	Mean RT	Std Dev	%RSD
Aldicarb sulfoxide	75.0	0.41	0.552	74.9	0.419	0.559
Aldicarb sulfone	75.3	0.46	0.610	75.2	0.367	0.488
Oxamyl	75.3	0.59	0.779	75.5	0.246	0.327
Methomyl	74.3	0.69	0.415	77.1	0.254	0.330
3-Hydroxycarbofuran	75.9	0.32	0.415	74.7	0.318	0.427
Aldicarb	74.0	0.46	0.621	77.2	0.331	0.429
Propoxur	75.4	0.37	0.493	73.5	0.309	0.421
Carbofuran	77.0	0.25	0.329	76.3	0.303	0.398
Carbaryl	75.2	0.31	0.416	77.3	0.251	0.324
1-Naphthol	70.0	0.92	1.321	88.3	0.289	0.327
Methiocarb	76.0	0.36	0.479	74.9	0.314	0.419
BDMC	76.6	0.44	0.572	76.2	0.353	0.464

Table 3. Reproducibility data for the amount of seven injections of the 75-ppb carbamate mix using the legacy and 2013 Alliance HPLC systems.

Amount 7 injections (25-ppb)	Legacy Alliance HPLC			2013 Alliance HPLC		
Analyte	Mean RT	Std Dev	%RSD	Mean RT	Std Dev	%RSD
Aldicarb sulfoxide	25.4	0.099	0.391	24.8	0.133	0.539
Aldicarb sulfone	25.4	0.083	0.328	24.9	0.148	0.594
Oxamyl	25.6	0.108	0.422	25.1	0.076	0.303
Methomyl	25.3	0.106	0.420	25.6	0.122	0.477
3-Hydroxycarbofuran	25.7	0.095	0.369	24.8	0.115	0.465
Aldicarb	24.9	0.124	0.499	25.6	0.158	0.619
Propoxur	25.2	0.130	0.515	24.4	0.104	0.425
Carbofuran	25.8	0.108	0.418	25.4	0.154	0.607
Carbaryl	25.1	0.100	0.399	25.7	0.108	0.420
1-Naphthol	24.3	0.319	1.313	29.4	0.226	0.771
Methiocarb	25.4	0.113	0.445	24.7	0.220	0.889
BDMC	25.1	0.154	0.614	25.6	0.216	0.843

Table 4. Reproducibility data for amount for seven injections of the 25-ppb carbamate mix using the legacy and 2013 Alliance HPLC systems.

Amount 7 injections (10-ppb)	Legacy Alliance HPLC			2013 Alliance HPLC		
Analyte	Mean RT	Std Dev	%RSD	Mean RT	Std Dev	%RSD
Aldicarb sulfoxide	10.1	0.034	0.338	9.7	0.062	0.638
Aldicarb sulfone	10.0	0.041	0.415	9.7	0.070	0.724
Oxamyl	10.2	0.051	0.501	9.8	0.067	0.678
Methomyl	10.0	0.049	0.490	9.9	0.094	0.939
3-Hydroxycarbofuran	10.2	0.041	0.405	9.7	0.077	0.794
Aldicarb	9.8	0.059	0.602	10.0	0.106	1.055
Propoxur	10.0	0.057	0.578	9.6	0.076	0.793
Carbofuran	10.2	0.067	0.655	10.0	0.100	1.005
Carbaryl	10.0	0.042	0.419	10.0	0.041	0.410
1-Naphthol	10.2	0.099	0.967	11.6	0.146	1.262
Methiocarb	10.1	0.071	0.698	9.5	0.088	0.924
BDMC	10.3	0.142	1.368	9.5	0.098	1.032

Table 5. Reproducibility data for amount for seven injections of the 10-ppb carbamate mix on the legacy and 2013 Alliance HPLC systems.

To investigate the detection of low levels of the pesticides, a blank, 0.2-ppb, and 0.1-ppb carbamate mix was injected with the resulting chromatograms shown in Figures 3 and 4. The calculated limits of detection, shown in Table 6, were 0.1-ppb or less for both systems with the exception of 1-Naphthol which, as previously mentioned, is a degradation product.

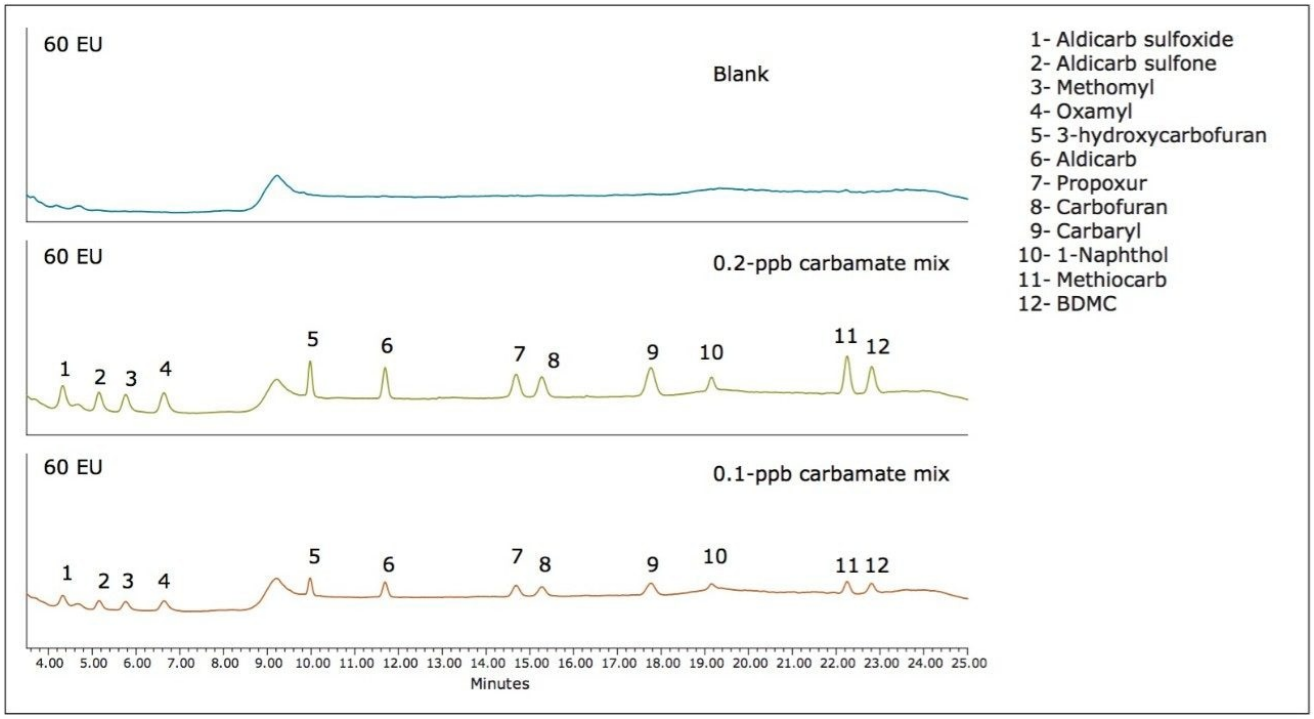

Figure 4. Chromatograms of low-level carbamate mixes on the 2013 Alliance HPLC System, 1000-µL injection.

Limit of detection	Legacy Alliance HPLC	2013 Alliance HPLC
Aldicarb sulfoxide	0.05	0.03
Aldicarb sulfone	0.06	0.03
Oxamyl	0.10	0.04
Methomyl	0.06	0.07
3-Hydroxycarbofuran	0.09	0.02
Aldicarb	0.02	0.03
Propoxur	0.04	0.03
Carbofuran	0.07	0.10
Carbaryl	0.09	0.05
1-Naphthol	0.19	0.36
Methiocarb	0.04	0.04
BDMC	0.07	0.10

Table 6. MDL data for seven injections of a 0.2-ppb mix calculated per 40 CFR pt 136 App B (ppb).

To test the two systems using a blind sample, the ERA performance evaluation sample was used. For both the previous and current systems, the calculated amounts were found to be within the acceptable QC performance limits and within 1-ppb of each other, as shown in Table 7.

ERA Certified Mix Lot #5180-707	Cert value	QC acceptable range	Legacy Alliance HPLC	2013 Alliance HPLC
Aldicarb sulfoxide	16.2	11.9-21.6	13.7	13.3
Aldicarb sulfone	19.0	14.2-23.4	17.9	18.0
Oxamyl (Vydate)	70.9	52.5-85.8	59.8	58.9
Methomyl	53.7	41.1-67.1	46.9	46.2
3-Hydroxycarbofuran	45.6	34.8-57.0	39.6	38.9
Aldicarb	30.1	21.9-37.9	26.4	26.0
Propoxur (Baygon)	42.1	32.5-51.8	36.7	36.2
Carbofuran	39.5	27.2-50.2	35.1	34.3
Carbaryl	24.4	16.9-29.8	20.3	20.0
Methiocarb	82.8	61.4-97.7	69.8	69.1

Table 7. Analysis of ERA QC carbamate mix units are ppb.

Conclusion

The data show that similar results are obtained on the legacy and 2013 Alliance HPLC systems for Carbamate Analysis, yielding the following benefits:

- Conformance to EPA Method 531.2
- Baseline separation of analytes
- Run time of 25 minutes
- Provides a complete Waters solution for carbamate analysis

References

1. Waters Alliance System for Carbamate Analysis Method Guide, p/n 71500017101 Rev D.

2. *Ibid*. 3-11.

3. US EPA 40 CFR pt 136 App, B. 565-566.

4. de Bertrand, et al. Photodegradation of the Carbamate Pesticides Aldicarb, Carbaryl and Carbofuran in Water. *Analytica Chimica Acta.* 1991; 254:235-244.

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Alliance HPLC System <https://www.waters.com/534293>

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Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

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