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Application Note 00946

Fast LC/MS/MS Quantitation of *N*-Methyl Carbamate Pesticides in Food Using Bond Elut™ Plexa™ and Pursuit™ XRs C18

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Abstract
A fast quantitation method for the analysis of *N*-methyl carbamate pesticide residues in vegetables and grains has been developed with Bond Elut Plexa clean-up, Pursuit XRs C18 columns, and 320-MS LC/MS/MS. High recoveries were obtained for most carbamates screened in all matrices tested at 1 ppb levels. Good linearity of the calibration curves was observed for all analytes, over the range from 1 ppb to 200 ppb levels, with r^2 greater than 0.99.

Introduction
N-methyl carbamate pesticides are widely used as insecticides or fungicides. Even when applied in accordance with Good Agricultural Practices (GAP), they can leave residues, which can be detrimental to food safety [1]. There is a growing pressure from governmental agencies and private companies to improve the analytical performance in pesticide residue analysis, making it necessary to increase the efficiency and lower the cost and time of these analyses [2].

Carbamates are currently quantitated using HPLC with fluorescence detection. Since HPLC-fluorescence detection does not provide the required specificity for determining pesticide residues, tandem LC/MS methods have recently been adopted. The main advantage offered by tandem LC/MS is that the chromatography data acquisition is much faster than that obtained from HPLC-fluorescence.

We have adapted to an efficient, and cost-effective acetonitrile-based liquid-liquid extraction method and Bond Elut Plexa clean-up, which yields good recoveries for a number of food matrices tested.

Instrumentation
- Varian 320-MS LC/MS/MS

Materials and Reagents
- Bond Elut Plexa 200 mg, 6 ml tubes (Varian Part No. 12109206)
- 0.45 µm PTFE syringe filter (Fisher-Gelman Acrodisc Part No. 09730152)
- Carbamate pesticide stock solutions and standard mixtures were ordered from AccuStandard

Experimental
Matrices tested: Leafy vegetables like lettuce, and grains, like rice and wheat flour, were selected for screening carbamate pesticides.

Carbamate pesticides screened: aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbaryl, carbofuran, 3-hydroxycarbofuran, methomyl, oxamyl, methiocarb, propoxur, and BDMC (4-bromo-3,5-dimethylphenyl-N-methylcarbamate) as an internal standard (IS).

Sample Preparation
The sample preparation was based on a modified CDFA (California Department of Food and Agriculture) method with liquid-liquid extraction, followed by SPE clean-up and solvent exchange.

1. Weigh 50 g of matrix sample, add 100 ml of acetonitrile, spike matrix with 1, 20 or 100 ppb each of 10 analytes.
2. Blend for 2 min.
3. Filter, then add 10 g of sodium chloride, stir the mixture in a round-bottomed flask hooked to a stirrer for 5 min, let water and organic layers clearly separate (not for grain samples*).
4. Take 10 ml aliquots of organic layer, add 2.5 ml water. Load onto Bond Elut Plexa SPE cartridge preconditioned with 2 x 5 ml 80:20 acetonitrile:water. Collect the eluent, then rinse with 5 ml 80:20 acetonitrile:water, add this to 12.5 ml eluent collected earlier.
5. Collect total 17.5 ml elute, evaporate to dryness or near dry in a test tube at 40 °C, reconstitute in 10:90 acetonitrile:water with 0.1% formic acid up to 4.0 ml, bring final volume to 5.0 ml with water with 0.1% formic acid, pass through 0.45 µm PTFE syringe filter before chromatography.

   * For grains, exclude step 3 because the dry grains do not contain aqueous phase.

Standard stock solutions of each analyte and standard mixture of all 10 analytes, with 0.1 mg/ml concentration in acetonitrile were used. The standard mixture was diluted to 1, 2, 5, 10, 20, 50, 100 and 200 ppb. Before LC/MS injection, 1 µl of 100 ppm BDMC as internal standard was spiked into 1 ml of each standard solution.

LC/MS/MS Conditions

The chromatography method was based on EPA Methods 531.1 and 531.2, which are used for drinking water or wastewater analysis [3]. The original methods employed HPLC with fluorescence detection and had a 40 min run time. The first step involved getting a separation with a 40 min run using a Pursuit XRs column and Varian 320-MS Tandem MS (Method 1). Each analyte had baseline separation on the column. The second step used a shorter column to get faster elution; it was developed using a 100 mm column length with an injection-to-injection time of 10 min (Method 2).

HPLC Conditions

Method 1 (40 min method, in addition to 10 min re-equilibration):

Column: Pursuit™ XRs C18 3 µm, 150 x 4 mm ID (Varian Part No. A6001150X040)

Mobile Phase A: H_2O + 0.1% HCOOH

Mobile Phase B: CH_3OH + 0.1% HCOOH

Gradient:	Time (min:sec)	%A	%B	Flow (ml/min)
	0:00	95.0	5.0	0.6
	4:00	95.0	5.0	0.6
	30:00	40.0	60.0	0.6
	40:00	10.0	90.0	0.6
	40:06	95.0	5.0	0.6

Method 2 (10 min method):

Column: Pursuit™ XRs C18 3 µm, 100 x 3 mm ID (Varian Part No. A6001100X030)

Mobile Phase A: H_2O + 0.1% HCOOH

Mobile Phase B: CH_3CN + 0.1% HCOOH

Gradient:	Time (min:sec)	%A	%B	Flow (ml/min)
	0:00	80.0	20.0	0.6
	1:30	80.0	20.0	0.6
	5:00	40.0	60.0	0.6
	7:00	20.0	80.0	0.6
	8:00	20.0	80.0	0.6
	8:06	80.0	20.0	0.6
	10:00	80.0	20.0	0.6

API Conditions For Both Methods:

Ionization Mode: ESI Positive

Collision Gas: Argon

A total of 11 carbamates were screened in all the matrices tested. Their identity, molecular weights, parent and two daughter ions monitored, and log P values are listed in Table 1.

Results & Discussion

Chromatography

Figure 1 shows an overlay of normalized chromatograms on Pursuit XRs C18 3 µm 150 x 4.0 mm with a 40 min run. Baseline separation is achieved for all analytes in the mix. Figure 2 shows chromatograms overlaid on Pursuit XRs C18 3 µm 100 x 3.0 mm with a 10 min run. With tandem LC/MS, the analytes with different parent ions or daughter ions can be separated easily with MRM scans, which speeds up the HPLC run time from 40 min to 10 min. Figure 3 displays individual chromatograms of each analyte in Figure 2. Decent chromatographic retention is observed for extremely hydrophilic species like aldicarb sulfoxide (log P: -0.78), oxamyl (log P: -0.47), and aldicarb sulfone (log P: -0.57), while reasonably hydrophobic pesticides like methiocarb (log P: 2.92) elute within 7 mins. The separation power of Pursuit XRs comes from a combination of advanced bonding technologies developed on a high surface area, 100Å, ultra-pure silica support.

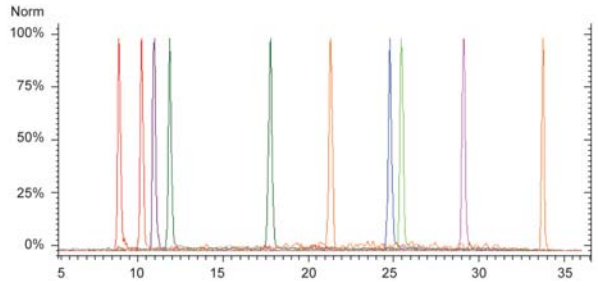

Figure 1 LC/MS/MS analysis of 10 carbamates (normalized chromatograms) on Pursuit XRs C18 with a 40 min run. From left to right: aldicarb sulfoxide, oxamyl, aldicarb sulfone, methomyl, 3-hydroxycarbofuran, aldicarb, propoxur, carbofuran, carbaryl, and methiocarb.

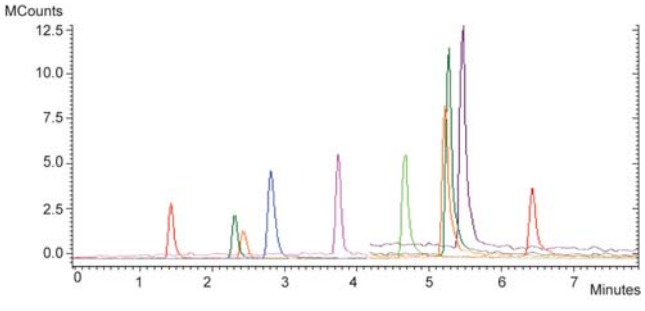

Figure 2 LC/MS/MS analysis of 10 carbamate standards (200 ppb each) on Pursuit XRs C18 with a 10 min run (overlaid chromatograms).

Compound and M.W.	MS → MS1 Ions (Precursor → Product 1)	MS → MS1 Ions (Precursor → Product 2)	ESI Mode of Analysis	Log P
Aldicarb 190.27	208.1 → 89.0	208.1 → 116.0	(+)	1.13
Aldicarb sulfone 222.26	240.1 → 86.0	240.1 → 223.1	(+)	-0.57
Aldicarb sulfoxide 206.27	207.1 → 89.0	207.1 → 132.0	(+)	-0.78
Carbaryl 201.23	202.1 → 145.0	219.1 → 145.1	(+)	2.36
Carbofuran 221.26	222.1 → 123.0	222.1 → 165.0	(+)	2.32
3-Hydroxycarbofuran 237.26	255.1 → 163.0	255.1 → 220.0	(+)	0.76
Methomyl 162.21	163.1 → 88.0	163.1 → 106.0	(+)	0.6
Oxamyl 219.26	237.1 → 72.0	237.1 → 220.0	(+)	-0.47
Methiocarb 225.31	226.2 → 121.0	226.2 → 169.0	(+)	2.92
Propoxur 209.25	209.9 → 111.0	209.9 → 168.0	(+)	1.52
BDMC (internal standard) 258.11	258.1 → 122.0	258.1 → 200.9	(+)	-

Table 1 Identity, molecular weights, parent and two transitions, and log P of carbamates screened in lettuce, rice, and wheat flour.

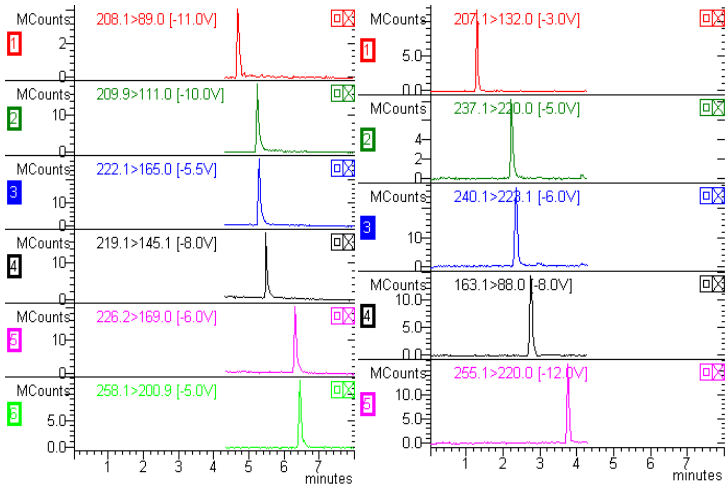

Figure 3 LC/MS/MS analysis of 10 carbamate standards (200 ppb each) with internal standard on Pursuit XRs C18 with a 10 min run (stacked chromatograms).

Confirmation and Quantitation

For each analyte, two fragment ions were monitored for confirmation purposes. The area of the primary ion is used for quantitation while the peak area ratio of primary and secondary ions is used for confirmation. Figure 4 is an example. At 1 ppb level, two fragment ions of methomyl show a peak area ratio of 0.57, which is used as confirmation of methomyl. The signal-to-noise ratios were 140 and 108 for the two fragments ions. All other analytes were identified the same way.

Carbamates in Standard and Spiked Matrices

A comparison of chromatograms of 1 ppb carbamates in standard and spiked matrices is displayed in Figure 5. Most of the analytes are detectable at this concentration in all three matrices tested, which include lettuce, rice, and wheat flour. Good linearity of the calibration curves was observed for all analytes, over the range from 1 to 200 ppb levels, with r^2 greater than 0.99.

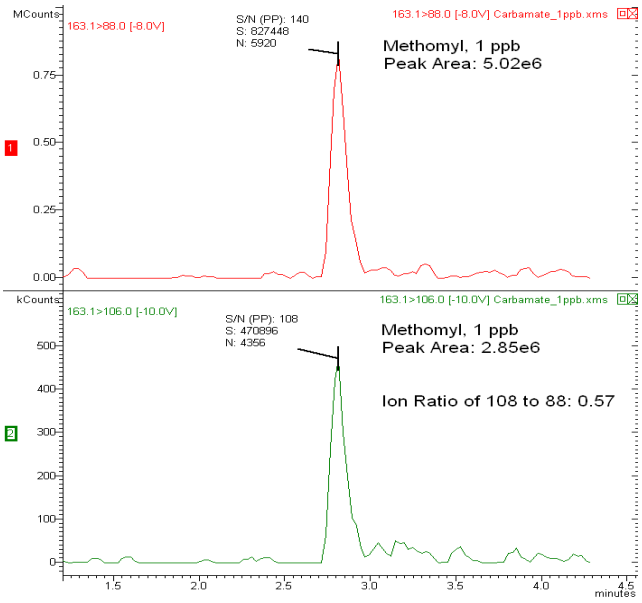

Figure 4 Two transition confirmation and quantitation of methomyl.

Recovery

The recovery of all analytes was above 60% except aldicarb sulfoxide in lettuce at 20 ppb level (Figure 6). Aldicarb sulfoxide most likely undergoes degradation reflecting in decreased recoveries. For most analytes, the recovery was above 80%. These recoveries are within the EU (60-140%) and CDFA requirements [4].

Conclusion

A fast, efficient, and cost-effective acetonitrile-based liquid-liquid extraction followed by a Bond Elut Plexa solid phase sorbent clean-up method for monitoring N-methyl Carbamate pesticides in various food matrices has been developed. The LC/MS/MS quantitation method developed offers tremendous time-savings and does not require any post-column derivatization typically used in traditional EPA methods for the analysis of these compounds. High recoveries were obtained for most carbamates screened in all matrices tested at 1 ppb levels using a Varian 320 tandem mass spectrometric detector. Pursuit XRs C18 columns employed for the determination of these residues offer enhanced separation power and can readily be incorporated to yield a high-resolution separation in an existing EPA method for carbamate analysis, or can be used for fast quantitative analysis. Complex mixtures of environmental herbicides in food matrices can be easily separated by a combination of Bond Elut Plexa sorbent, Pursuit XRs C18 high-resolution columns, and tandem MS/MS high sensitivity detectors.

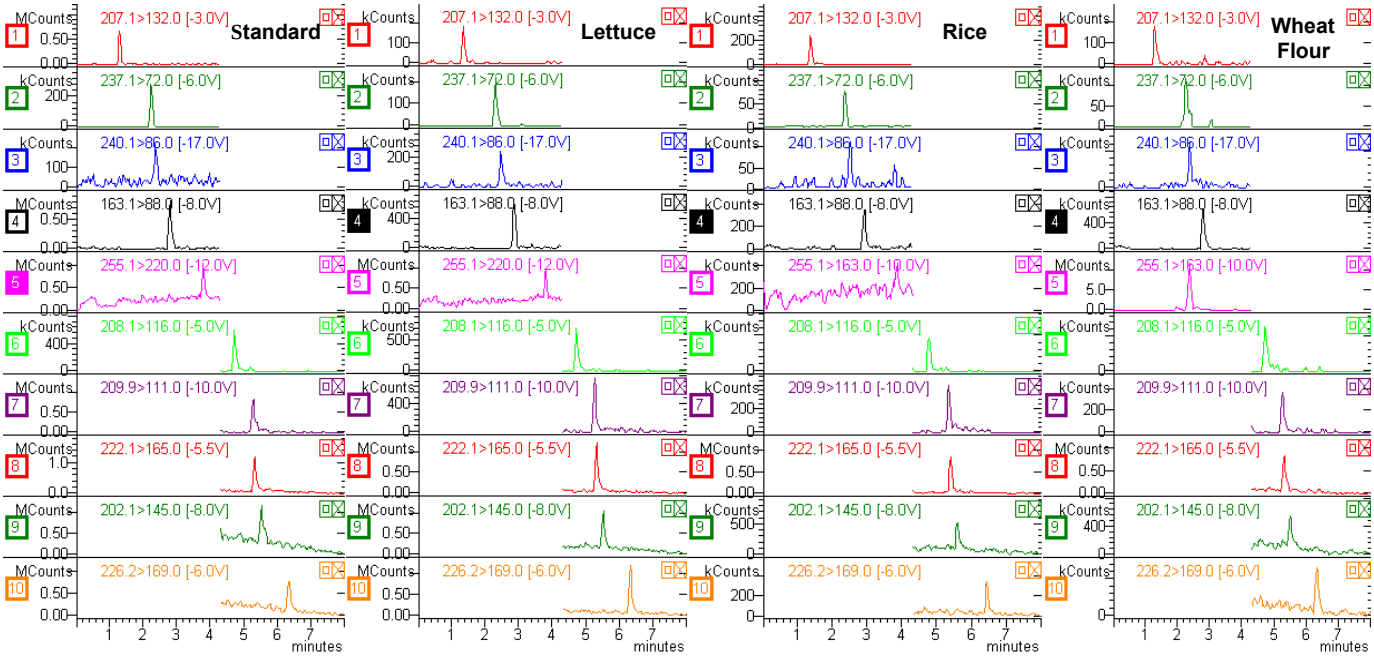

Figure 5 From left to right: Chromatogram of 1 ppb carbamates in standard, lettuce, rice, and wheat flour.

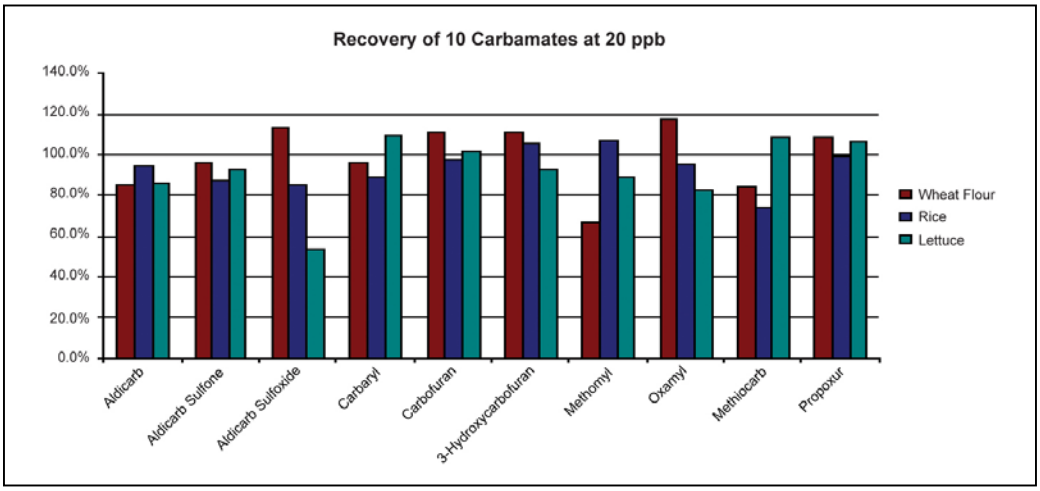

Figure 6 Recovery of 10 carbamate pesticides in lettuce, rice, and wheat flour.

References

1. R. Stepan, J. Ticha, J. Hajslova, T. Kovalczuk, V. Kocourek, *Food Addit. Contamin*. 22 (2005) 1231.
2. A.R. Fernandez-Alba (Ed.), *Chromatographic-Mass Spectrometry Food Analysis for Trace Determination of Pesticide Residues*, Vol. XLIII, Elsevier, Amsterdam, The Netherlands, 2005, 113.
3. EPA Method 531.2, EPA # 815-B-01-002, http://www.epa.gov/safewater/methods/sourcalt.html
4. "Quality Control Procedures for Pesticide Residues Analysis", Document N° SANCO/10232/2006 24/March/2006 http://ec.europa.eu/food/plant/resources/qualcontrol_en.pdf

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