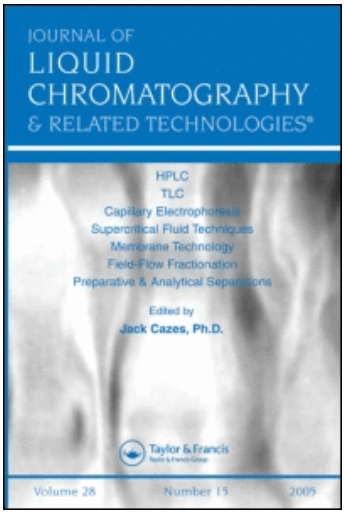

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Rasmussen, H. T. , Friedman, S. K. , Mustilli, A. J. , McDonough, R. and McPherson, B. P.(1994) 'Analysis of Methyl Neodecanamide in Lake Water by Reversed-Phase High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry', Journal of Liquid Chromatography & Related Technologies, 17: 3, 589 — 601
To link to this Article: DOI: 10.1080/10826079408013162
URL: http://dx.doi.org/10.1080/10826079408013162

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(3), 589-601 (1994)

ANALYSIS OF METHYL NEODECANAMIDE IN LAKE WATER BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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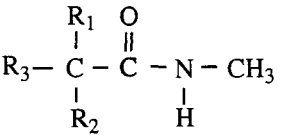
ABSTRACT

Methyl Neodecanamide (MNDA) has been quantitated in lake water at levels of 0.1 to 1000 ppm. Total recoveries from spiked placebos were 99.8 +/- 2.3% at the 1000 ppm level and 98.3 +/- 4.3% at the 0.1 ppm level (based on 54 determinations at each level). Plots of actual concentrations vs. determined concentrations were linear from 0.07 - 0.13 and 700 - 1300 ppm ($r > 0.999$). Stability of MNDA in lake water was verified by determining the composition by GC/MS immediately after dissolution and after 3 days.

INTRODUCTION

Methyl Neodecanamide (MNDA) is an isomeric distribution of secondary amides with the formula: $C_{11}H_{23}NO$ (Fig. 1). MNDA has



Isomers	R_1	R_2	R_3
	CH_3	CH_3	C_6H_{13}
	CH_3	C_2H_5	C_5H_{11}
	CH_3	C_3H_7	C_4H_9
	C_2H_5	C_2H_5	C_4H_9
	C_2H_5	C_3H_7	C_3H_7

FIGURE 1. Structure of MNDA

shown efficacy as an insect repellent (1) and is for this reason a useful ingredient in household cleaners. To assess ecological toxicity of MNDA, it has been necessary to perform teratology studies on trout, and in support of such studies to quantitate MNDA in lake water at levels of 0.1 - 1000 ppm.

Amides of similar structures to MNDA have previously been separated by GC, either directly (2), or after acylation (3) or alkylation (4). Fatty amides have been separated by normal phase HPLC (5). While each of these methods potentially allow for the analysis of MNDA, reversed-phase HPLC was chosen as the primary method of analysis since it allows for the direct analysis of aqueous samples.

Since ease of operation and the ability to transfer the method to other laboratories were additional requirements, UV detection was used.

MNDA may be monitored at 205 nm, but in order to obtain sub-ppm sensivity, large injection volumes are required, and it is useful to elute MNDA as a single peak to maximize the response. A limitation of eluting MNDA as a single peak is that it is suspected that similar compounds, such as degradation products, may coelute. To ascertain that MNDA composition remained constant, samples were analyzed by GC/MS immediately after dissolution and at the completion of the teratology study (3 days).

EXPERIMENTAL

HPLC

Samples of water used for trout teratology studies and spiked placebos for HPLC analyses were prepared by diluting the appropriate amount of MNDA (synthesized in-house) with lake water (Springborn Laboratories, Switzerland). Samples containing less than 5 ppm of MNDA were diluted to operating concentrations of 0.1 ppm with Milli-Q water (Millipore Corporation, Milford, MA); samples containing from 5 - 1000 ppm MNDA were diluted to operating concentrations of 5 ppm using 50/50 (v/v) Milli-Q water / HPLC Grade methanol (J. T. Baker Inc., Phillipsburg, NJ). Standards were prepared from serial dilution of 100 mg MNDA/100 mL HPLC Grade methanol stock solutions. (Initial solutions were prepared in methanol to expedite dissolution). Stock standards were diluted to an operating concentration of 5 ppm using 50/50 (v/v) Milli-Q

water / HPLC Grade methanol and then in Milli-Q water to an operating concentration of 0.1 ppm.

All HPLC analyses were performed using: a 15 cm x 4.6 mm Dupont Zorbax Rx C8 column (MAC-MOD Analytical, Chadds Ford, PA); a mobile phase consisting of 40/25/35 (v/v/v) acetonitrile (J. T. Baker Inc.)/methanol/water with 10 mM sodium perchlorate (Mallinckrodt Inc., Paris, KY); a Shimadzu Model LC-600 pump (Shimadzu Corporation, Kyoto, Japan) operated at 1 mL/min; and a Shimadzu Model SPD-6A variable wavelength UV detector operated at 205 nm. Samples with operating concentrations of 0.1 ppm were introduced using a Rheodyne Model 7125 injection valve (Rheodyne, Cotati, CA) with a 2 mL loop. Samples with operating concentrations of 5 ppm were introduced using a Waters 712 WISP Autosampler (Waters, Milford, MA) set to inject 200 uL. Chromatograms were monitored using a Kipp and Zonen Model BD 40 strip chart recorder (Baxter, McGaw Park, IL). Quantitation was achieved by manual measurement of the MNDA peak heights.

GC/MS

A 100 mL sample of placebo lake water was extracted with 20 mL of GC/GC/MS Capillary Grade hexane (Baxter, Edison, NJ) to determine if it contained any possible organic contaminants which could coelute with MNDA. The hexane extract was injected into the GC/MS for analysis. A sample of the 1000 ppm MNDA lake water was extracted and analyzed in the same manner. This procedure was run on the first day and third day of the teratology study.

GC/MS was performed using a Finnigan MAT 4605 Gas Chromatograph / Mass Spectrometer (Finnigan, Livingston, NJ). The GC column used for this application was a DB5 FSWCOT, 30 m x 0.25 mm I.D. capillary with a 0.25 um film thickness (J & W Scientific, Folsom, CA). Sample was introduced in the split mode using an injector temperature of 250 °C. Separations were conducted using helium as the carrier gas at a linear velocity of 357 mm/sec. The oven was temperature programmed as follows: Initial temperature = 115°C (hold time = 4 min.); 1st ramp rate = 4.0°C/min. to 170°C; 2nd ramp rate = 40.0°C/min. to 290°C; 3rd ramp rate = 20.0°C/min. to 295°C. Electron Impact (EI) mass spectra (Electron Energy = 70ev) were collected from 28 to 500 AMU using an Ionizer temperature of 130°C and an Electron Multiplier voltage of -1750 V. The Mass Spectrometer was calibrated daily with FC43 ($C_{12}F_{17}N$; MW=671g/mole).

RESULTS AND DISCUSSION

Several constraints were placed on the choice of HPLC operating conditions for the analysis of MNDA at the 0.1 ppm level. To obtain adequate sensitivity a detection wavelength of 205 nm was required along with the use of large (2 mL) injection volumes. Additionally, it was desirable to elute the MNDA isomers as a single peak, resolved from the matrix, to maximize sensitivity.

When large aqueous samples are loaded onto a reversed-phase column, less-polar organics concentrate at the column inlet during the

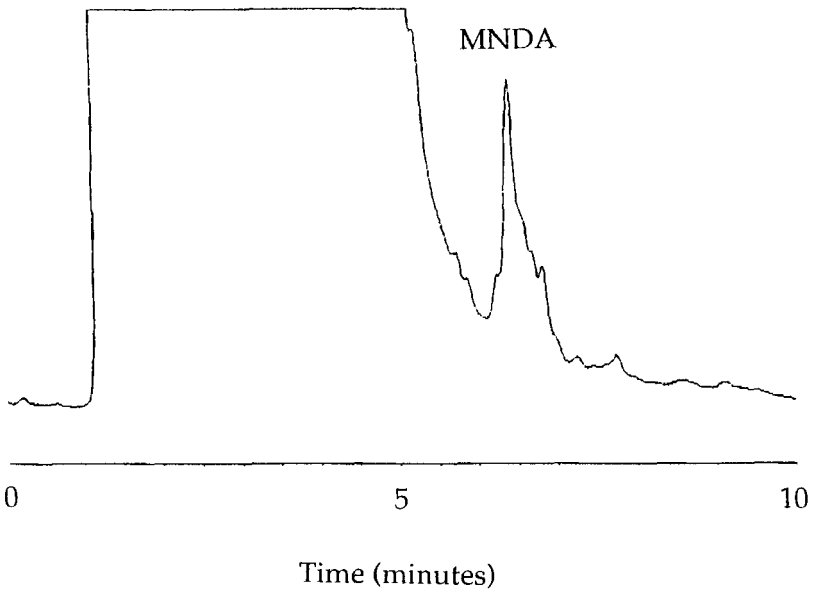


FIGURE 2. Chromatogram of a 0.1 ppm MNDA in Lake water sample. Conditions - Mobile phase: 40/25/35 (v/v/v) acetonitrile / methanol/water with 10 mM sodium perchlorate. Column: 15 cm x 4.6 mm Dupont Zorbax Rx C8 column. Flow rate: 1 mL /min. Detection: UV at 205 nm. Injection volume: 2 mL.

injection and no deleterious volume induced band-broadening is incurred (6). Previous studies have shown that base deactivated column packings such as Zorbax Rx and the addition of perchlorate minimize peak tailing of basic compounds (7-8). Consequently, it was decided to use a Zorbax Rx C8 column and a mobile phase containing 10 mM sodium perchlorate. The use of 205 nm as a detection wavelength limited the choice of solvents to methanol, propanol, acetonitrile and water. A mobile phase consisting of 40/25/35 (v/v/v) acetonitrile / methanol / water with 10 mM sodium perchlorate minimized the separation of MNDA isomers, but provided

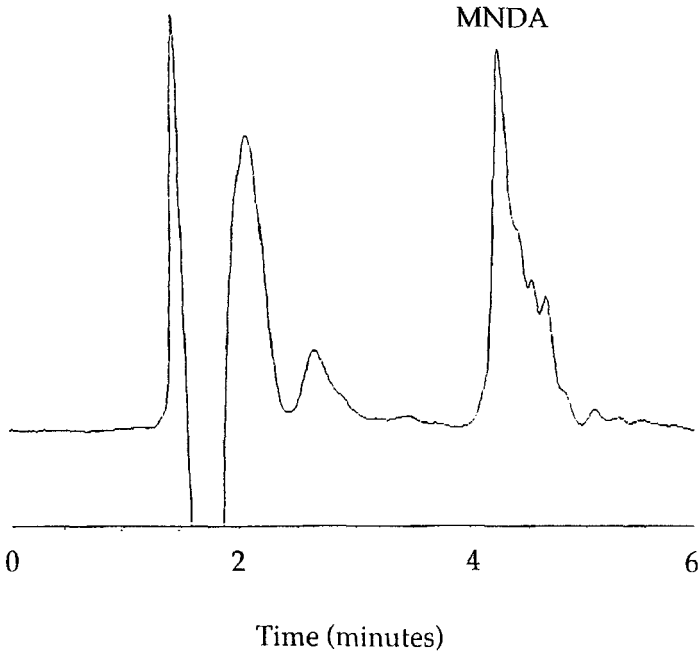


FIGURE 3. Separation of a 1000 ppm MNDA in Lake water sample (Sample was diluted to 5 ppm for analysis). Conditions are as for Fig. 2, except that the injection volume was reduced to 200 uL.

separation from the matrix. A chromatogram of a 0.1 ppm sample is shown in Figure 2.

The method as outlined was used for samples containing up to 5 ppm of MNDA, by diluting the samples to working concentrations of 0.1 ppm. To make the method applicable to the analysis of MNDA at concentrations of up to 1000 ppm, without the need of excessive sample dilution, the method was modified slightly. Samples were diluted to 5 ppm and injected using a 200 uL loop. A chromatogram of a 1000 ppm sample analyzed using the modified method is shown in Figure 3.

To validate the HPLC methods spiked placebos were prepared fresh daily, in triplicate, at concentrations of 0.07, 0.10, 0.13, 700, 1000 and 1300 ppm and analyzed. This procedure was performed by two operators on six non-consecutive days (3 operator days per analyst) for a total of 18 independent analyses at each of the 6 concentrations. To determine recoveries at the 0.1 ppm level the data obtained for 0.07, 0.1 and 0.13 ppm was pooled such that the 0.1 ppm level recovery is based on 54 determinations. (The 1000 ppm data was treated in the same way). The use of 18 determinations at each of the 70, 100, and 130% target concentration has the advantage over analyzing 54 samples at the 100% level of allowing for the determination of linearity.

The results obtained for the validation (Tables I and II) show overall recoveries at the 0.1 and 1000 ppm levels of 98.3 +/- 4.3% and 99.8 +/- 2.3%, respectively. Estimates of variance stemming from analyst-to-analyst , day-to-day and error contibutions were, as shown in the Tables, acceptable at both concentrations. Furthermore, plots of the actual concentration vs. the measured concentration were linear for both levels ($r > 0.999$).

Recoveries for other MNDA concentrations were not determined explicitly. However, it likely that these would not be worse than those reported since the possible sources of variance are maximized for the above levels. For samples containing 0.1 - 5 ppm MNDA, the largest source of variance stems from interference from the lake water matrix; this variance is maximized when the samples are undiluted and the MNDA to matrix concentration is smallest. For samples containing from 5 - 1000

TABLE I

Recoveries for 0.1 ppm MNDA samples.

Operator	Day	Level (% target)	% recovery		
			1	2	3
1	1	70 %	98.9	97.9	99.0
1	1	100 %	101.0	95.1	94.7
1	1	130 %	99.0	100.7	97.5
2	1	70 %	95.5	96.9	102.2
2	1	100 %	90.9	97.8	99.5
2	1	130 %	95.0	93.5	100.5
1	2	70 %	101.0	107.8	106.6
1	2	100 %	99.5	107.1	98.8
1	2	130 %	102.7	100.8	100.2
2	2	70 %	90.2	105.7	105.4
2	2	100 %	93.1	98.3	96.1
2	2	130 %	93.1	102.8	92.1
1	3	70 %	100.3	101.7	105.9
1	3	100 %	92.8	98.8	95.5
1	3	130 %	95.5	96.7	100.2
2	3	70 %	97.3	98.7	100.9
2	3	100 %	98.3	93.6	94.4
2	3	130 %	93.9	93.2	91.5

Overall mean +/- std. dev. = 98.3 +/- 4.3 %
Analyst-to-analyst variance = 1.96 %
Day-to-day variance = 1.46 %
Variance due to error = 3.52 %

TABLE II

Recoveries for 1000 ppm MNDA samples (diluted to 5 ppm).

Operator	Day	Level (% target)	% recovery		
			1	2	3
1	1	70 %	102.0	100.4	100.0
1	1	100 %	100.0	99.2	99.5
1	1	130 %	98.0	97.7	100.6
2	1	70 %	102.8	102.3	99.1
2	1	100 %	101.3	101.0	101.7
2	1	130 %	99.7	100.8	100.7
1	2	70 %	101.8	100.1	101.1
1	2	100 %	101.1	101.8	101.6
1	2	130 %	101.0	99.8	99.7
2	2	70 %	101.6	102.7	101.8
2	2	100 %	99.6	99.8	100.2
2	2	130 %	99.2	99.3	99.1
1	3	70 %	100.0	100.9	97.1
1	3	100 %	99.2	98.3	101.1
1	3	130 %	98.8	98.1	98.6
2	3	70 %	102.1	99.2	100.8
2	3	100 %	88.1	97.2	98.4
2	3	130 %	93.8	97.2	101.8

Overall mean +/- std. dev. = 99.8 +/-2.3 %
Analyst-to-analyst variance = 0 %
Day-to-day variance = 1.27 %
Variance due to error = 1.99 %

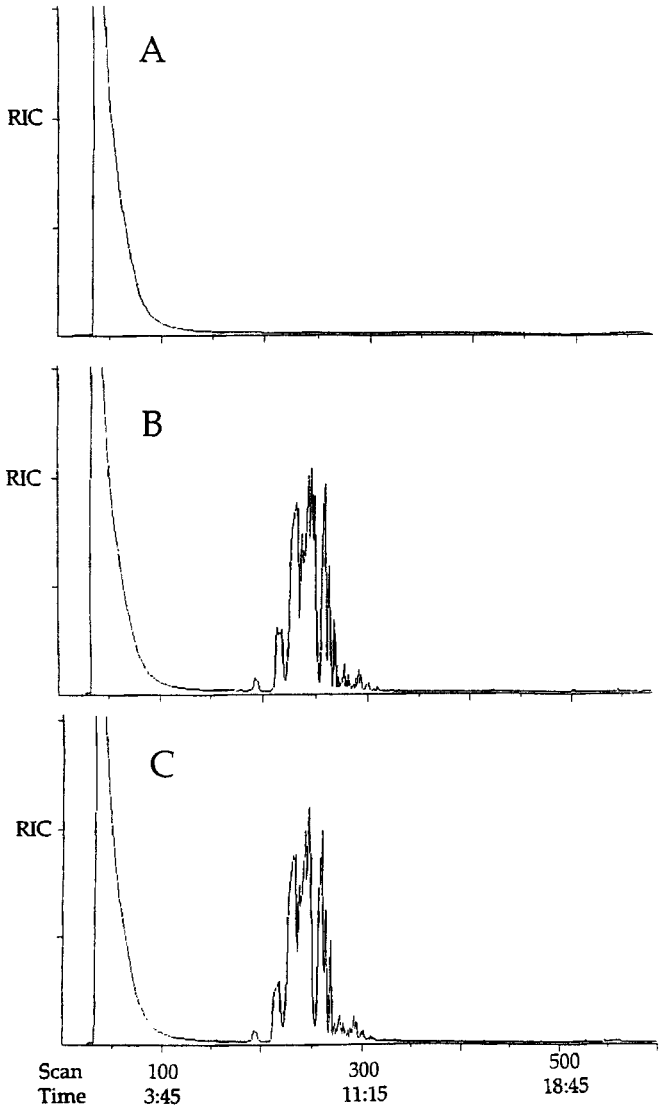


FIGURE 4. RIC Scans of (A) Lake water, (B) Lake water with 1000 ppm MNDA immediately after dissolution, (C) Lake water with 1000 ppm MNDA after 3 days.

ppm MNDA the influence of the matrix is minimal. Consequently, the largest variance is predicted to stem from the dilutions required to reduce the MNDA to the operating concentration of 5 ppm.

On the basis of the reproducibility observed for the HPLC analyses, the method was considered suitable for supporting teratology, providing that any MNDA degradates formed during the study did not coelute with MNDA and thereby provide a higher than actual assay. To verify MNDA stability, GC/MS analyses were performed. The GC/MS data showed no interfering peaks or organic contaminants present in the blank lake water (Figure 4a). The RIC Scans obtained immediately after dissolution and after 3 days for the 1000 ppm MNDA lake water samples are shown in Figures 4b and 4c. The comparison of the RIC Scans and Mass Spectral Data for the 1000 ppm MNDA lake water samples are identical and match exactly what is obtained when MNDA is prepared directly in hexane and analyzed.

CONCLUSIONS

A HPLC method has been developed for the analysis of MNDA in lake water at levels of 0.1 to 1000 ppm. The method has been validated by recoveries from spiked placebo and by GC/MS data which confirms the stability of MNDA in Lake water. The latter data is important since it dismisses the possibility of contributions to the apparent MNDA level by MNDA degradates.

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Received: May 12, 1993
Accepted: July 8, 1993