ELSEVIER

Journal of Chromatography A, 849 (1999) 381–388

JOURNAL OF CHROMATOGRAPHY A

Improvement of EPA method 8330: complete separation using a two-phase approach

Michael J. Lang, Susan E. Burns*

Chemistry Department, Western Michigan University, Kalamazoo, MI 49008, USA

Received 19 October 1998; received in revised form 13 April 1999; accepted 28 April 1999

Abstract

Complete separation of the 14 nitroaromatic and nitramine analytes targeted in EPA Method 8330 was achieved using a 30×4.6 mm Bondesil CN guard column in series with a 250×4.6 mm Bondesil C18 column (5 μm particles). Consistent with Method 8330 specifications, the mobile phase in the separation was 1:1 methanol:water and the flow-rate was 1.5 ml min^{-1}. The success of this two-phase system proved to be largely a consequence of the unique resolution afforded by the Bondesil C18 column: complete separation of the 14 explosives could not be achieved using other CN/C18 column combinations. Additionally, while Method 8330 calls for a 100-μl injection loop, separation on the Bondesil CN/C18 system was possible only using a 20-μl injection loop. The loss of resolution with larger injection volume appears to be a result of the injection solvent, methanol, modifying the composition of the mobile phase both in the CN guard column and in the initial portion of the C18 column. The current method nevertheless represents an improvement over Method 8330, which calls for both a screening and confirming analysis. By separating the 14 Method 8330 analytes in a single run, the two-phase approach can increase sample throughput by decreasing analysis times.

Keywords: EPA method 8330; Nitroaromatics; Nitramines

1. Introduction

The analysis of explosives has several applications, including forensic analysis of post-explosion residues, detection of regulated compounds in munitions wastewater, and analysis of soils and groundwater contaminated with explosives residues. Analysis of explosives residues is nevertheless complicated by the wide range of primary and secondary contaminants found in those residues. For example, because the explosives RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) are often used in explosive formulations together with TNT (2,4,6-trinitrotoluene), they are normal constituents of samples containing TNT [1–5]. Residues of TNT are also often accompanied by co-contaminants that represent manufacturing impurities, including 24DNT and 26DNT (2,4- and 2,6-dinitrotoluene), DNB and TNB (dinitrobenzene and trinitrobenzene), and all three nitrotoluenes (2NT,3NT,4NT) [1,2,5]. In environmental matrices, degradation products and metabolites also frequently occur as co-contaminants of explosives [2,4]. The common occurrence of 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT) in soil and water contami-

*Corresponding author. Tel.: +1-616-387-2878; fax: +1-616-387-2909.

E-mail address: burnss@wmich.edu (S.E. Burns)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00591-9

nated with TNT, for example, is a consequence of the wide-ranging ability of bacteria, fungi and plants to transform TNT into these compounds [6–8].

A variety of hyphenated chromatographic techniques have been evaluated for their ability to separate and detect multiple components in explosives residues. The chromatographic portion of these techniques includes gas chromatography [9–11], supercritical fluid chromatography [12], thin layer chromatography [13], micellar electrokinetic capillary chromatography [14–16] and high-performance liquid chromatography [1,2,4,7,17–21]. The favored method of these for routine analyses appears to be high-performance liquid chromatography (HPLC), with ultraviolet detection favored for its ability to detect nitroaromatic and nitramine analytes at concentrations down to 2.5 ppb in extracts of water, soil, or sediment.

Reported first by Kaplan [18] and Bratin et al. [17] in the early 1980s, HPLC methodology for the analysis of explosives residues is now standardized in US Environmental Protection Agency (EPA) Method 8330, AOAC International (formerly the Association of Official Analytical Chemists) Official Method 986.221, and American Society for Testing & Materials (ASTM) Method D5143-90. Even so, explosives analysis using HPLC methodology continues to be problematic. The simplest methodologies, developed mostly for use in routine analysis of large numbers of samples, all make use of isocratic mobile phases. EPA Method 8330, for example, calls for 1:1 methanol–water as a mobile phase while AOAC Official Method 986.22 calls for 50:30:12 water–methanol–acetonitrile. Isocratic elution, however, is best suited for the analysis of only small subsets of the common components of explosives residues. In EPA Method 8330, which is the most comprehensive of the standardized methods, analyses must be performed on both normal and reversed-phase HPLC columns because of analyte co-elutions on both phases [2]. And while there are several gradient elution methods capable of separating analytes that co-elute using isocratic mobile phases, the complexity of the gradients [21], or analyte retention times approaching an hour [20], makes these methods somewhat prohibitive for large volume analyses.

In the current work, all of the 14 compounds in the EPA Method 8330 analyte list were resolved using a single HPLC separation. While the EPA Method 8330 HPLC conditions and calibrations were retained in the current method, the separation was improved using a two-phase column system.

2. Experimental

2.1. Apparatus

HPLC measurements were carried out on a Varian Star HPLC system which included a 9012 Gradient Solvent Delivery System, a 9050 UV–VIS Detector, a 9300 refrigerated autosampler, and a Star Chromatography Workstation. Reversed-phase (5-μm particle size) columns evaluated in the current work were Varian Bondesil C18 (150 and 250×4.6 mm), Varian Nucleosil C18 (250×4.6 mm), Pickering C18 (250×4.6 mm), Shandon Hypersil Green PAH (150×4.6 mm), and Varian Res Elut C18 (30, 150 and 250×4.6 mm). Cyano columns evaluated were Varian Res Elut CN (30 and 250×4.6 mm) and Varian Bondesil CN (250×4.6 mm). Flow rates evaluated were from 0.5–2.0 ml min^{-1} and injection loop volumes evaluated were 20, 50 and 100 μl. The mobile phase was 1:1 methanol–water and the detector wavelength was 254 nm.

2.2. Chemicals

Water and all organic solvents were Burdick and Jackson (Muskegan, MI) HPLC grade. Method 8330 analytes were obtained from AccuStandard (New Haven, CT) as solutions in 1:1 methanol–acetonitrile and stored at 4°C in the dark.

2.3. System performance

System performance was judged based on results from 8-point calibration curves. Standards in calibration curves were mixed standards containing all 14 Method 8330 analytes. Mixed standards were prepared in methanol to give analyte concentrations spanning 1–1000 μg ml^{-1} (1 ppb–1 ppm). All standards were analyzed in triplicate, and analyte quantification was by peak area.

3. Results and discussion

3.1. Performance of EPA method 8330

A total of 14 nitroaromatic and nitramine compounds are targeted in EPA Method 8330 (Table 1), which calls for both a screening and confirming chromatographic separation for their analysis. Primary screening is performed using a 250×4.6 mm, 5-μm particle size, reversed-phase octadecyl (C18) column. Sample chromatograms exemplifying results obtained with the screening analysis are shown in Fig. 1a–b. The chromatogram in Fig. 1a was obtained using a Bondesil C18 column and shows co-elution of TNT with 4ADNT. The chromatogram in Fig. 1b was obtained using a Res Elut C18 column and shows co-elution of the DNTs, co-elution of the ADNTs, co-elution of TNT with NB, and co-elution of DNB with tetryl. While co-elutions of Method 8330 analytes on C18 columns varies with column properties, in general, it is co-elutions of the ADNTs and DNTs on C18 columns that makes the confirming separation in Method 8330 necessary [2,5]. Confirmational analysis is performed using a 250× 4.6 mm, 5-μm particle size normal-phase cyanopropyl (CN) column. Typical of the confirmation run is separation of the ADNT and DNT isomers, but co-elution of the NTs as well as DNB and TNB [2,5].

3.2. Performance of the two-phase approach

Fig. 2a shows the baseline separation of the 14 target compounds in the EPA 8330 analyte list. This separation was achieved using a 30×4.6 mm Res Elut CN guard column (5-μm particle size) in series with a 250×4.6 mm Bondesil C18 column (5-μm particle size) and was reproducible across C18 column lots (column serial numbers 073227-11 and 072137-18). Consistent with EPA Method 8330, the mobile phase used in the separation was 1:1 methanol–water and the mobile phase flow-rate was 1.5 ml min^{-1}. Column temperature was 20–25°C.

Analyte calibration curves (1 ppb–1 ppm) obtained using the Bondesil CN/C18 system all had zero intercepts and correlation coefficients >0.99. Analyte calibration curves were constructed from triplicate analyses of eight standards containing all 14 Method 8330 analytes (24 data points). Retention times for the compounds, relative to the retention time of NB, appear in Table 1. NB was used as the standard for calculating relative retention times in part because it is the first eluting of the 14 compounds on CN columns, and its retention time on the

Table 1
Compound relative retention times on one-phase and two-phase systems

Compound	Relative retention time				
	Bondesil C18	Bondesil CN/C18	Bondesil CN/C18 100 μl inj. loop	Res Elut C18	Res Elut CN/C18
HMX	0.184	0.354	0.300	0.278	0.492
RDX	0.390	0.499	0.474	0.508	0.602
TNB	0.538	0.595	0.508	0.692	0.721
TETRYL	0.804	0.852	0.692	0.872	0.888
DNB	0.872	0.926	0.897	0.872	0.888
NB	1.00	1.00	1.00	1.00	1.00
TNT	1.11	1.10	1.06	1.07	1.15
4ADNT	1.14	1.18	1.21	1.22	1.19
2ADNT	1.24	1.30	1.38	1.22	1.22
26DNT	1.49	1.45	1.44	1.47	1.47
24DNT	1.59	1.57	1.47	1.47	1.47
2NT	2.06	1.91	1.75	1.77	1.72
4NT	2.20	2.06	1.90	1.92	1.86
3NT	2.41	2.24	2.05	2.06	1.99

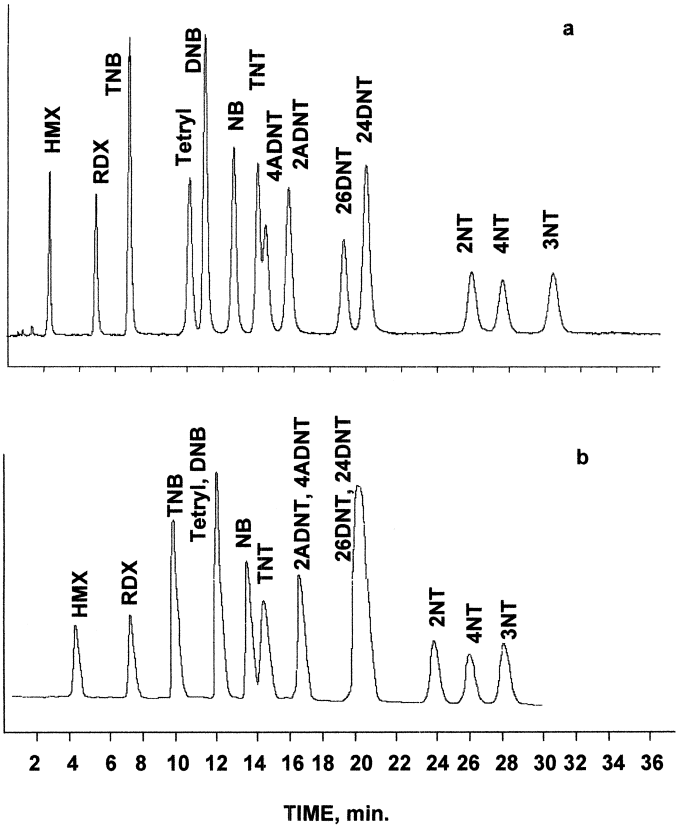

Fig. 1. HPLC separation of EPA Method 8330 compounds using 5-μm particle size, 250×4.6 mm Bondesil C18 (a) and Res Elut C18 (b) columns. Mobile phase was 1:1 methanol–water and flow-rate was 1.5 ml min^{-1}.

CN/C18 system therefore is least affected by the presence of the CN guard column. NB also was chosen because, unlike tetryl and the ADNTs and DNTs, selectivity for NB appears to be relatively unaffected by differences in C18 column properties (see below). Day-to-day variation in analyte retention time and response was <5%.

3.3. Phase contribution to the separation

The contribution of the CN guard column to the baseline separation of the 14 Method 8330 analytes is relatively small. A comparison of Figs. 1a and 2a shows that insertion of the CN guard column in front of the Bondesil C18 column does not affect the elution order of any of the 14 compounds. A comparison of relative retention times in Table 1 also shows that the CN guard column has little apparent affect on the resolution of the NTs, the DNTs or tetryl and DNB. Instead, the most significant changes induced by the CN column appear to be small shifts in the retention times of the ADNTs. These shifts do not significantly change the resolution between the ADNTs, but they do nearly double the separation between the ADNTs and TNT. Nevertheless, since this is the only separation that needs to be affected, the minimal influence of the CN column is adequate when coupled with the Bondesil C18 column.

The contribution of the Bondesil C18 column to the separation is unique: of the C18 columns tested in CN/C18 series, only the Bondesil column completely separated the 14 Method 8330 analytes. A sample chromatogram of a CN/C18 series using

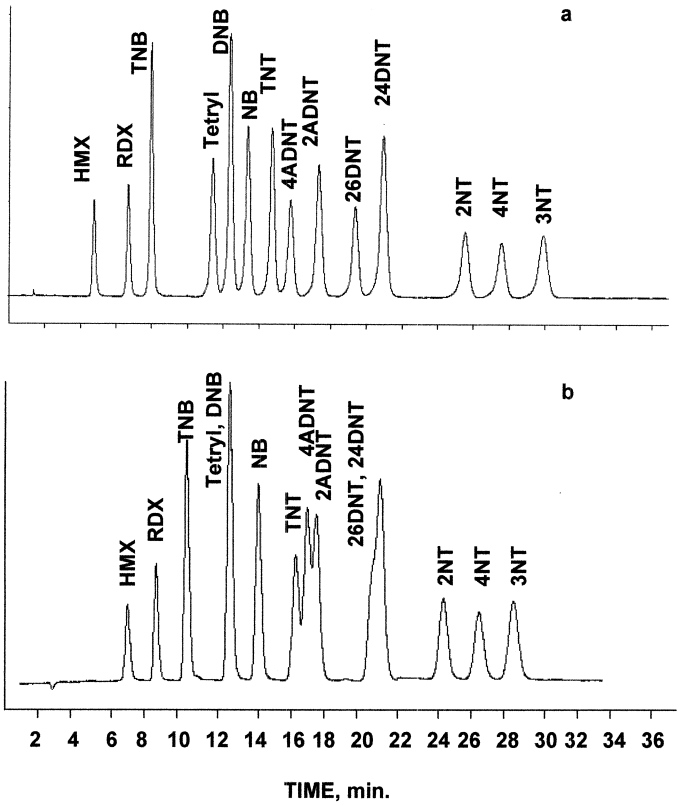

Fig. 2. HPLC separation of EPA Method 8330 compounds using (a) Bondesil and (b) Res Elut two-phase systems composed of 30×4.6 mm CN guard columns in series with 250×4.6 mm C18 columns (5-μm particle size). Mobile phase was 1:1 methanol–water and flow-rate was 1.5 ml min^{-1}.

another C18 column is shown in Fig. 2b. This chromatogram was obtained using a 30×4.6 mm Res Elut CN guard column in series with a 250×4.6 mm Res Elut C18 column (both columns have 5 μm particles). As with the Bondesil CN/C18 series, the separation afforded by the Res Elut CN/C18 system largely reflects that of the C18 column alone (Fig. 1b). As a consequence, insertion of a CN guard column in front of the Res Elut C18 column does not resolve the DNT and ADNT co-elutions that occur on that column.

Other C18 columns (250×4.6 mm, 5 μm particles) unable to resolve the DNTs and ADNTs given Method 8330 conditions include Supelco Supelcosil LC-18 [2], Restek Pinnacle ODS [22], and Alltech Platinum C18 [23] columns. These columns consequently are poor candidates for incorporation into a CN/C18 system applied to Method 8330. C18 columns able to resolve the DNTS and ADNTs under Method 8330 conditions include, in addition to the Varian Bondesil C18 column, the 250×3 mm, 5-μm particle size Allure C18 column [22]. Comparison of properties of these columns (Table 2) suggests that the ability to resolve the DNTs and ADNTs is a consequence of column retentivity or capacity factor rather than selectivity. For example, the four columns unable to affect the resolution all have particle phase ratios (specific surface area/specific pore volume) <35 dm^{-1} as compared to 50–60 dm^{-1} for the Bondesil and Restek Allure columns. Co-elution or resolution of other Method 8330 analytes on C18 colums, such as tetryl and DNB on the Res Elut vs Bondesil columns (Fig. 1a–b), appears to be a result of column selectivity

Table 2
Properties of C18 columns

Column	Manufacturer	Pore diameter (Å)	Surface area ($m^2 g^{-1}$)	Carbon load (%)
Bondesil	Varian	50	200	17
Allure	Restek	60	540	27
Res Elut	Varian	90	220	12
Platinum	Alltech	100	200	6
Supelcosil	Supelco	120	170	11
Pinnacle	Restek	120	170	9.5

differences, as they cannot be correlated to column properties (Table 2).

3.4. Flow rate and injection volume effects

EPA method 8330 specifies a mobile phase flow-rate of 1.5 ml min^{-1}. For the Bondesil CN/C18 system, a mobile phase flow-rate of 1.3 ml min^{-1} proved optimal. Complete separation was nevertheless possible at the prescribed flow-rate (Fig 2a).

EPA method 8330 also specifies use of a 100-μl injection loop. However, the resolution evident for the Bondesil CN/C18 series in Fig. 2a could only be obtained with a smaller, 20-μl injection loop. Use of a 100-μl injection loop resulted in co-elution of the 2ADNT with the DNTs as well as co-elution of RDX and TNB (Fig. 3a). The loss of resolution at the higher injection volume appears to be largely a consequence of using methanol, rather than mobile phase, as the injection solvent. At 100 μl, the injection volume is ~1/6 of the volume of the short CN guard column (0.632 ml). The result, using an injection solvent different from the mobile phase, is a complete change in the separation afforded by that column. Comparison of Figs. 2a and 3a, as well as comparison of relative retention times in Table 1, also indicates that the larger injection volume affects the separation afforded by the C18 column.

Using a 20-μl injection volume instead of the prescribed 100-μl injection volume, does not decrease method sensitivity because sample preparation in explosives methodology uses a concentration step. Additionally, the sensitivity of the UV detector makes it possible to detect nitroaromatic and nitramine compounds at concentrations down to 2.5 ppb, which is below the minimum sensitivity required by method 8330.

3.5. C18 column length effects

Use of C18 columns shorter than 250 mm proved inadequate to affect complete separation of the 14 Method 8330 analytes. This is illustrated by a comparison of Figs. 2b and 3b. In both figures, chromatograms were obtained using the same short CN column in series with a Res Elut C18 column. However, the chromatogram in Fig. 3b was obtained using a 150 mm column instead of a 250 mm column. Use of the 150 mm column resulted in partial co-elution of DNB with NB and incomplete separation of the NT isomers. Results obtained with four other 150 mm C18 columns were similar. This suggests that complete separation of these compounds requires the longer 250 mm C18 column.

3.6. Comparison with previous two-phase systems

The use of two phases in series has been investigated previously for the analysis of method 8330 analytes. These investigations, however, all used a reversed order for the phases, with a small C18 column preceding a larger CN column in the series [24,25]. A sample chromatogram showing the separation achieved with a C18/CN system appears in Fig. 3c. This chromatogram was obtained using a Res Elut C18 guard column (30×4.6 mm) in series with a Res Elut CN column (250×4.6 mm). A comparison of Figs. 1b and 3c shows that the C18/CN system improves on the C18 column by resolving the ADNT and DNT isomers. However, like results obtained with CN columns alone [2,5], the

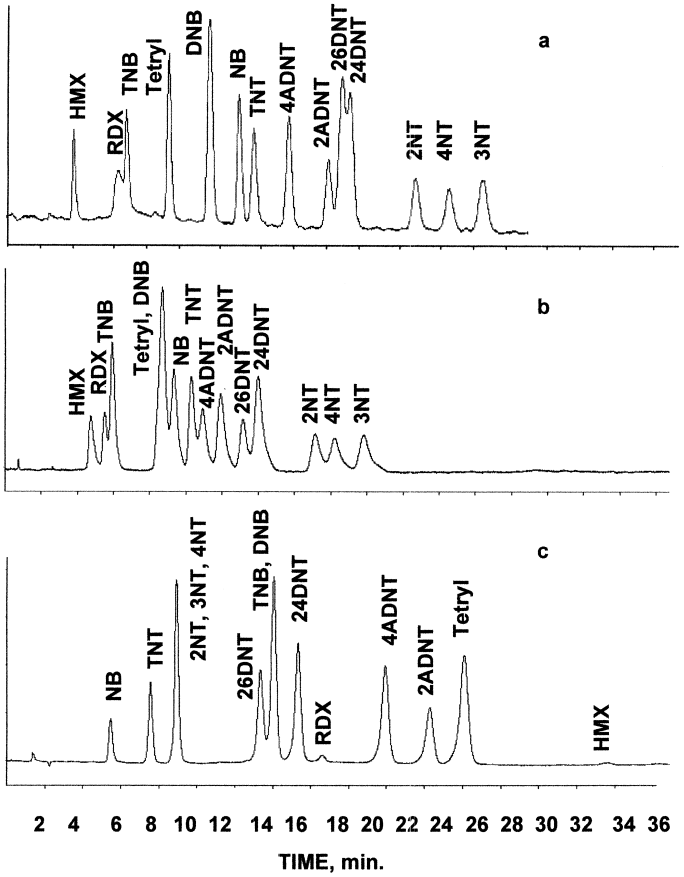

Fig. 3. HPLC separation of EPA Method 8330 compounds using (a) a 100-μl injection loop on the Bondesil CN/C18 system (250×4.6 mm C18 column), (b) a Bondesil CN/C18 system utilitizing a 150×4.5 mm C18 column. Mobile phase was 1:1 methanol–water and flow-rate was 1.5 ml min^{-1}, and (c) a Res Elut 30×4.6 mm C18 guard column in series with 250×4.6 mm CN column (5-μm particle size).

C18/CN series results in TNB/DNB co-elution as well as co-elution of the NT isomers.

4. Summary

Using 1:1 methanol–water as the mobile phase, the combination of a 30×4.6 mm Bondesil CN column in series with a 250×4.6 mm Bondesil C18 column (5 μm particles) produces complete separation the 14 EPA Method 8330 analytes in a single run. The resolution of the 14 compounds by the CN/C18 system is predominantly a result of the performance of the Bondesil C18 column. Since this performance differs from that obtained with other C18 columns, the current application of the two-phase approach specifically requires the use of a Bondesil C18 column. Even so, this application represents an improvement over the current EPA Method: by eliminating the need to repeat injections on a second column, the two-phase system can decrease analysis times and, therefore, improve sample throughput. The two-phase system also eliminates the additional time and effort required to maintain and calibrate two separate columns.

As a general approach to analytical separations, the two-phase system is likely to be most useful as an alternative to screening and confirming analyses, or as an alternative to some analyses using mobile phase gradients, since the two-phase approach also

affords the analyst with a way to modify the capacity factors of mixture components. In the current method, the amount of modification is small precisely because the modifying column, a guard column, contains only a small amount of phase. The possibility of coupling fast-separation columns either with each other or with more traditionally-sized columns means potentially greater modification of capacity factors. However, because a two-phase system can increase resolution only by increasing retention, useful application of the approach requires balancing increased resolution against increased retention. In general, this means targeting the choice of modifying phase at earlier-eluting compounds.

References

[1] T.F. Jenkins, D.C. Leggett, C.L. Grant, C.F. Bauer, Anal. Chem. 58 (1985) 170.
[2] T.F. Jenkins, M.E. Walsh, P.W. Schumacher, C.F. Bauer, C.L. Grant, J. Assoc. Off. Anal. Chem. 72 (1989) 890.
[3] R.M. Smith, C.M. Burr, J. Chromatogr. 475 (1989) 57–74.
[4] M.E. Walsh, T.F. Jenkins, Identification of TNT Transformation Products in Soil, CRREL Report 91-2, 1991.
[5] M.E. Walsh, T.F. Jenkins, P.S. Schnitker, J.W. Elwell, M.H. Stutz, Evaluation of SW846 Method 8330 for Characterization of Sites Contaminated with Residues of High Explosives, CRREL Report 93–5, 1993.
[6] N.G. McCormick, F.E. Feeherry, H.S. Levenson, Appl. Environ. Microbiol. 31 (1976) 576.
[7] S.D. Harvey, R.J. Fellows, D.A. Cataldo, R.M. Bean, J. Chromatogr. 518 (1990) 361.
[8] T. Gorontzy, O. Drzyzga, M.W. Kahl, D. Bruns-Nagel, J. Breitung, E. von Loew, K.-H. Blotevogel, Crit. Rev. Microbiol. 20 (1994) 265.
[9] T. Welsch, H. Block, Fresenius J. Anal. Chem. 357 (1997) 904.
[10] G.C. Slack, H.M. McNair, S.B. Hawthorne, D.J. Miller, J. High Res. Chromatogr. 16 (1993) 473.
[11] E.S. Francis, M. Wu, P.B. Farnsworth, M.L. Lee, J. Microcolumn Sep. 7 (1995) 23.
[12] J. Via, L. Taylor, Anal. Chem. 66 (1994) 1385.
[13] C. Steuckart, E. Berger-Preiss, K. Levsen, Anal. Chem. 66 (1994) 2570.
[14] W. Kleibohmer, K. Cammann, J. Robert, E. Mussenbrock, J. Chromatogr. 638 (1993) 349.
[15] S. Kennedy, B. Caddy, J.M.F. Douse, J. Chromatogr. 726 (1996) 211.
[16] B.G. Belen'kii, Y.V. Belov, G.E. Kasalainen, M.I. Medvedeva, S.T. Khor, J. Anal. Chem. 53 (1998) 291.
[17] K. Bratin, P.T. Kissinger, R.C. Briner, C.S. Brunlett, Anal. Chim. Acta 130 (1981) 295.
[18] D.L. Kaplan, A.M. Kaplan, Anal. Chim. Acta 136 (1982) 425.
[19] J. Yinon, D.-G. Hwang, J. Chromatogr. 268 (1983) 45.
[20] M. Emmrich, M. Kaiser, H. Ruden, S. Sollinger, J. Chromatogr. 645 (1993) 89.
[21] F. Ahmad, D.J. Roberts, J. Chromatogr. A 693 (1995) 167.
[22] Restek, c-gram #7045.
[23] Alltech Associates, Chromatogram 8279.
[24] M.E. Walsh, T.F. Jenkins, Anal. Chim. Acta 231 (1990) 313.
[25] J.E. Caton, W.H. Griest, J. Liq. Chromatogr. & Rel. Technol. 19 (1996) 661.