

# Determination of explosives residues in soils by micellar electrokinetic capillary chromatography and high-performance liquid chromatography

## A comparative study

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### ABSTRACT

Micellar electrokinetic capillary chromatography (MECC) is investigated for application as a complementary technique to reversed-phase high-performance liquid chromatography (HPLC) in the analysis of explosives residues. Separation efficiency, specificity, sensitivity, reproducibility, analysis time and calibration linearity are examined by comparing the suitability of HPLC and MECC in parallel analyses. Authentic soil samples from a former trinitrotoluene plant are examined, and the results show MECC to be a useful new technique in explosive analysis, yielding good sensitivity, high resolution and short analysis times.

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### INTRODUCTION

Concern about the environment has reached new heights in recent years, as evidenced by the 1987 Montreal Protocol and the 1992 Earth Summit in Rio de Janeiro. Many environmental guidelines, regulations and remedies are being initiated which require sound chemical monitoring techniques and analysis methods, in order to ensure that risks are accurately assessed and mandates are properly enacted. Environmental analytical chemistry provides the means to separate, identify and quantitate pollutants found in a variety of matrices including air, land and water [1]. Today, new challenges are being created in this rapidly growing discipline by a changing political climate and increasing environmental concern. One such challenge involves analysis of heavily contaminated soils at

military facilities associated with weapons production.

Since the end of the Cold War, various governments have begun to dismantle military installations and munition plants, in accordance with various non-proliferation agreements and disarmament treaties [2]. But as a new world peace and order are being created, major environmental problems are being discovered at many of these locations. Surrounding lands are found to be laden with explosives residues, the most common being 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-triazine and associated nitroaromatic and nitramine impurities and degradation products [3]. The highly toxic nature of many of these substances, coupled with their persistence in the environment, requires thorough characterization of contaminated areas.

Some work has been done to analyse these compounds in environmental matrices, including high-performance liquid chromatography (HPLC) [4–6], gas chromatography (GC) [7,8]

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and supercritical fluid chromatography (SFC) [9]. A forensic analysis has been developed for organic gunshot and explosive constituents using micellar electrokinetic capillary chromatography (MECC) to separate and identify species [10]. MECC has also been used to separate various nitroaromatics [11]. In this study, we investigate the suitability of MECC in routine environmental analysis of soils contaminated by explosive residues. The MECC method is compared with an established HPLC method in terms of separation efficiency, specificity, sensitivity, reproducibility, analysis time and calibration linearity.

## EXPERIMENTAL

### *Micellar electrokinetic capillary chromatography*

MECC analysis was accomplished using a commercially available capillary electrophoresis unit (Dionex, Idstein, Germany) and measurements were made at 20 kV (+polarity) with gravity injection (30 mm, 5 s), and absorbance detection (230 nm). A polyimide-coated fused-silica capillary [57 cm (51 cm to optical window)  $\times$  375  $\mu$ m O.D.  $\times$  75  $\mu$ m I.D.] was utilized in the separation. MECC runs were performed with forced nitrogen cooling of the capillary.

**MECC reagents.** Sodium borate, boric acid, sodium dodecyl sulphate (SDS), Sudan III, HPLC solvents, 2,4-dinitrotoluene and 3-nitrotoluene were purchased from various commercial suppliers. Other explosive components were obtained from Promochem (Wesel, Germany) or by private donation. Buffer was prepared using purified water (SG Reinstwasser system) and contained 2.5 mM sodium borate, 12.5 mM boric acid and 25 mM SDS. Buffer pH was adjusted to 8.5 with phosphoric acid before the solution was vacuum degassed and filtered through a 0.45- $\mu$ m Duropore membrane filter (Millipore, Eschborn, Germany). Stock solutions of explosives standards were prepared in the range 10–1000 ppm in HPLC-grade methanol (Baker). Serial dilutions were prepared for calibration curves. Prior to MECC analysis, the solutions were further diluted with borate buffer-methanol (95:5).

### *High-performance liquid chromatography*

LC separations were obtained on a modular system composed of a Hewlett Packard 1050 pump, a Rheodyne Model 7010A sample loop injector, a Hewlett Packard 1040M diode-array detector (230 nm) and a Hewlett Packard HPLC Chemstation. The components were separated on a Supelcosil LC-18 column (Supelco, 25 cm  $\times$  4.6 mm I.D.) using a linear solvent program at a flow-rate of 1.0 ml/min. The solvent system was water-methanol, with 20-min gradient from 40 to 60% methanol (Baker, HPLC grade). All explosives were dissolved in methanol-water (50:50) to prepare the stock standard. This standard was diluted with methanol-water to the final concentrations of interest.

### *Soil samples*

Authentic contaminated soil samples from Stadtallendorf, Germany, were extracted into organic solvents by Soxhlet extraction as detailed below. All soils were air dried to constant weight and ground with a pestle to pass a 30-mesh sieve [12]. Soils were subjected to exhaustive Soxhlet extraction with 200 ml of diethyl ether for 7 h. The soil extracts were filtered through a 0.45- $\mu$ m Duropore membrane filter before reducing the solvent to dryness by rotary evaporation. The residue was redissolved under sonication in 10 ml of the buffer solution for MECC analysis or in 50 ml of methanol-water (50:50) for HPLC analysis.

## RESULTS AND DISCUSSION

Two MECC methods for the analysis of nitro-compounds have been published [10,11]. The method of Northrop *et al.* [10] proved to be better suited to this application and was utilized in the work presented. In Table I the various explosive components analysed in the present study are listed.

Fig. 1 shows an MECC and an HPLC separation of explosive constituents in an eleven-component test mixture. Identification of individual components in the electropherogram was made by comparison with the elution order obtained by Northrop *et al.* [10] and by spiking the

TABLE I  
COMPOUNDS STUDIED

Name	Abbreviation
1,3,5-Trinitro-1,3,5-triazacyclohexane	RDX
2,4,6-Trinitrotoluene	TNT
4-Amino-2,6-dinitrotoluene	4-AMDNT
2,4,6-Trinitrobenzene	TNB
2,4-Dinitrobenzene	DNB
2,6-Dinitrotoluene	2,6-DNT
2-Nitrotoluene	2-NT
4-Nitrotoluene	4-NT
2,4,6,N-Tetranitro-N-methylaniline	Tetryl
1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane	HMX
Nitrobenzene	NB

mixture with single components on subsequent runs.

Surprisingly, the separation order obtained with both methods is nearly the same, although

the separation modes are dissimilar. In MECC the elution order of TNT and Tetryl is changed and, more importantly, the aminodinitrotoluene isomers are separated and eluted later than in HPLC. The quality of the MECC separation is readily apparent, as the important degradation products of TNT, the 2 and 4 isomers of aminodinitrotoluene, are baseline resolved and the complete separation required less than 7 min. The non-aromatic HMX and RDX heterocyclic systems should be solubilized to a lesser degree within the hydrophobic region created by the micellar aggregates than the nitrobenzene and toluene aromatic rings. These theoretical considerations are consistent with the results presented here, which show HMX eluting first, followed by RDX, then the various nitroaromatic substances.

In order to compare the efficiency of HPLC and MECC for the determination of explosives residues, the validation of the method is an

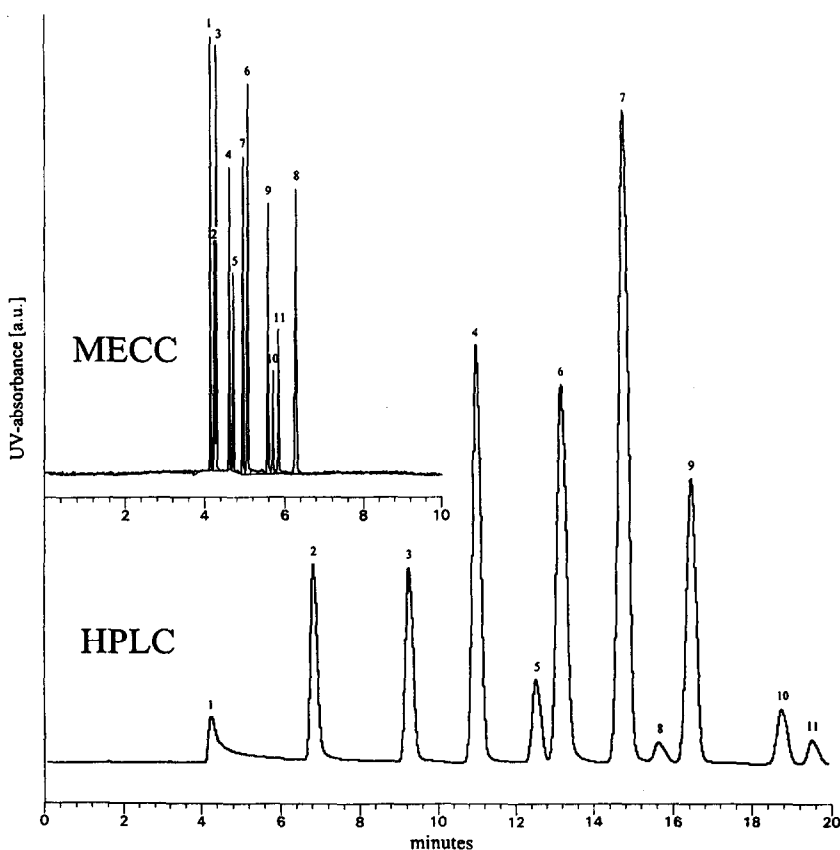


Fig. 1. MECC and HPLC separations of a mixture of explosives. For chromatographic conditions, see text. Peaks: 1 = HMX; 2 = RDX; 3 = TNB; 4 = DNB; 5 = NB; 6 = Tetryl; 7 = TNT; 8 = 4-AMDNT; 9 = 2,6-DNT; 10 = 2-NT; 11 = 4-NT.

TABLE II

AVERAGE VALUES AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF RETENTION TIMES ( $t_R$ ) AND PEAK AREAS (A) FOR STANDARD SOLUTIONS (St) AND SOIL EXTRACTS (E) WITH HPLC

n.q. = Reproducible peak detection but impossible quantification because peak height is below five-fold signal-to-noise ratio.

Component	Standard solution				Soil extracts			
	$t_{RSt}$ (min)	R.S.D. (%)	$A_{St}$ (AU)	R.S.D. (%)	$t_{RE}$ (min)	R.S.D. (%)	$A_E$ (AU)	R.S.D. (%)
RDX	6.94	0.07	29.2	3.0	6.97	0.35	29.1	9.61
TNB	9.41	0.05	47.6	5.8	9.50	0.18	728.5	5.27
DNB	11.07	0.03	37.4	1.8	10.93	0.23	12.6	7.96
TNT	14.78	0.20	65.4	2.4	14.78	0.50	5274.3	4.77
4-AMDNT	15.51	0.02	124.0	8.0	For coelution conditions, see text			
2-AMDNT	—	—	—	—				
2,6-DNT	16.25	0.40	32.5	3.6	15.41	0.23	103.6	3.10
2-NT	18.48	0.26	20.3	4.9	16.22	0.22	67.4	5.59
4-NT	19.15	0.43	25.1	5.9	18.44	0.16	9.7	11.23
					19.25	0.48	n.q.	n.q.

important task. The most important requirement for a validation include reproducible retention times and peak areas, minimal interferences, calibration linearity and specificity. In order to determine the within-day precision of the methods, replicate separations were made of standard mixtures of seven components and of soil extracts by HPLC and MECC. In Tables II and III the average values and standard deviations are given for the HPLC retention/MECC migration

times and peak areas. For both methods there is, as expected, no difference in elution order for standard solutions or soil extracts. While in HPLC for soil extracts only a slight deterioration in the standard deviations can be observed as compared with the standards, there is a large shift in migration times, and an increase in the standard deviations of migration times and peak areas by the MECC method. So in MECC the peak identification in real probes is impossible

TABLE III

AVERAGE VALUES AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF MIGRATION TIMES ( $t_M$ ) AND PEAK AREAS (A) FOR STANDARD SOLUTIONS (St) AND SOIL EXTRACTS (E) WITH MECC

n.d. = No reproducible peak detection because peak height is below threefold signal-to-noise ratio.

Component	Standard solution				Soil extracts			
	$t_{MSt}$ (min)	R.S.D. (%)	$A_{St}$ (AU)	R.S.D. (%)	$t_{ME}$ (min)	R.S.D. (%)	$A_E$ (AU)	R.S.D. (%)
RDX	3.39	0.34	647 141	0.6	4.12	1.84	For coelution conditions, see text	
TNT	3.39	0.34	—	—				
DNB	3.67	0.16	772 376	0.3	4.47	2.15	7 323	42.3
TNT	3.96	0.44	1 012 486	0.4	4.86	2.12	7 324 807	1.9
4-AMDNT	4.97	0.51	271 701	0.2	6.31	2.44	20 352	42.8
2-AMDNT	4.39	0.26	654 732	0.3	6.04	2.43	39 170	1.2
2,6-DNT	4.44	0.60	615 941	0.3	5.52	2.28	42 479	16.5
2-NT	4.51	0.44	31 210	7.9	5.61	3.10	8 887	14.9
4-NT	4.62	0.33	77 173	5.6	n.d.	n.d.	n.d.	n.d.

based on migration times of standards. Within a day, differences in migration times were usually less than 1%, and day-to-day or week-to-week variations of up to 4% were observed. This precision has been obtained by capillary temperature control by nitrogen cooling to reduce Joule heating effects.

#### Linearity and limit of detection

For a further comparison of the separation methods, we measured the peak areas for seven explosives from 0.145 to 50  $\mu\text{g/ml}$  for HPLC and from 0.600 to 50  $\mu\text{g/ml}$  for MECC samples. UV absorbance was plotted as a function of concentration, and calibration curves were observed to be linear over several orders of magnitude (for regression coefficients, see Table IV). The lower limit of detection of the MECC system was *ca.* 127 ng/ml for the strongly absorbing components, which corresponds to a value of nearly 1 ppm in soil samples. Based upon an injection volume of 2.4 nl for a 75- $\mu\text{m}$  capillary, a mass detection limit of 1.6 pg was calculated for TNT. To increase sensitivity, larger sample plugs may be introduced onto the capillary by choosing greater values of the sampling height and/or longer sampling times; however, prolonged injection decreases efficiency [10]. In Table IV the lower limit of detection, the regression coefficient and the standardized slope are given for the seven investigated compounds. Slopes were standardized at a single concentration to obtain

comparable values. With the exception of the 2 and 4 isomers of nitrotoluene in MECC, all calibration curves were observed to be linear over several orders of magnitude.

#### Residue analysis

Authentic soil samples were obtained from Stadtallendorf, Germany. During World War II, a munitions factory was located in Stadtallendorf situated near Marburg in central Germany, and today local and federal officials are developing guidelines to clean up this environmentally hazardous site. Efforts are under way to analyse the extent of environmental problems at this location and to monitor clean-up procedures [13]. Soil extracts prepared as previously described were analysed by HPLC and MECC, and the results are presented in Fig. 2. One advantage of the MECC technique discovered was that various humic substances that are co-extracted from the soil matrix along with the explosives residues were retained by the micelles. Thus, the micellar separation technique provided a simple and effective means of eliminating potential interferences. On the other hand, in the HPLC analysis of explosives in soils and sediments, these same substances might cause column fouling and require additional clean-up steps [12].

Separation, identification and quantification of several hazardous explosives in Stadtallendorf soil samples were accomplished by the MECC,

TABLE IV

SLOPE, REGRESSION COEFFICIENT AND LIMIT OF DETECTION FOR PEAK AREAS OF SOME SELECTED EXPLOSIVES MEASURED WITH HPLC AND MECC (UV-DETECTION AT 230 nm)

Component	HPLC				MECC			
	Corrected slope	Regression coefficient	Detection limit (ng absolute) <sup>a</sup>	Detection limit (ng/ml)	Corrected slope	Regression coefficient	Detection limit (pg absolute) <sup>b</sup>	Detection limit (ng/ml)
TNB	206.9	0.9999	4.8	240	n.d.	n.d.	n.d.	n.d.
DNB	182.7	0.9999	4.7	235	81.4	0.9997	1.6	676
TNT	3.0	0.9998	10.0	500	80.8	0.9970	1.6	676
4-AMDNT	988.0	0.9999	2.9	145	462.1	0.9971	0.3	127
2,6-DNT	182.5	0.9997	5.1	255	81.3	0.9974	1.6	676
2-NT	100.3	0.9999	4.9	245	28.3	0.7250	23.5	9940
4-NT	177.6	0.9999	6.3	315	60.6	0.9140	13.3	5626
TDX	147.5	0.9999	5.2	260	66.2	0.9969	2.0	846

<sup>a</sup> Injection volume 20  $\mu\text{l}$

<sup>b</sup> Injection volume 2.36 nl.

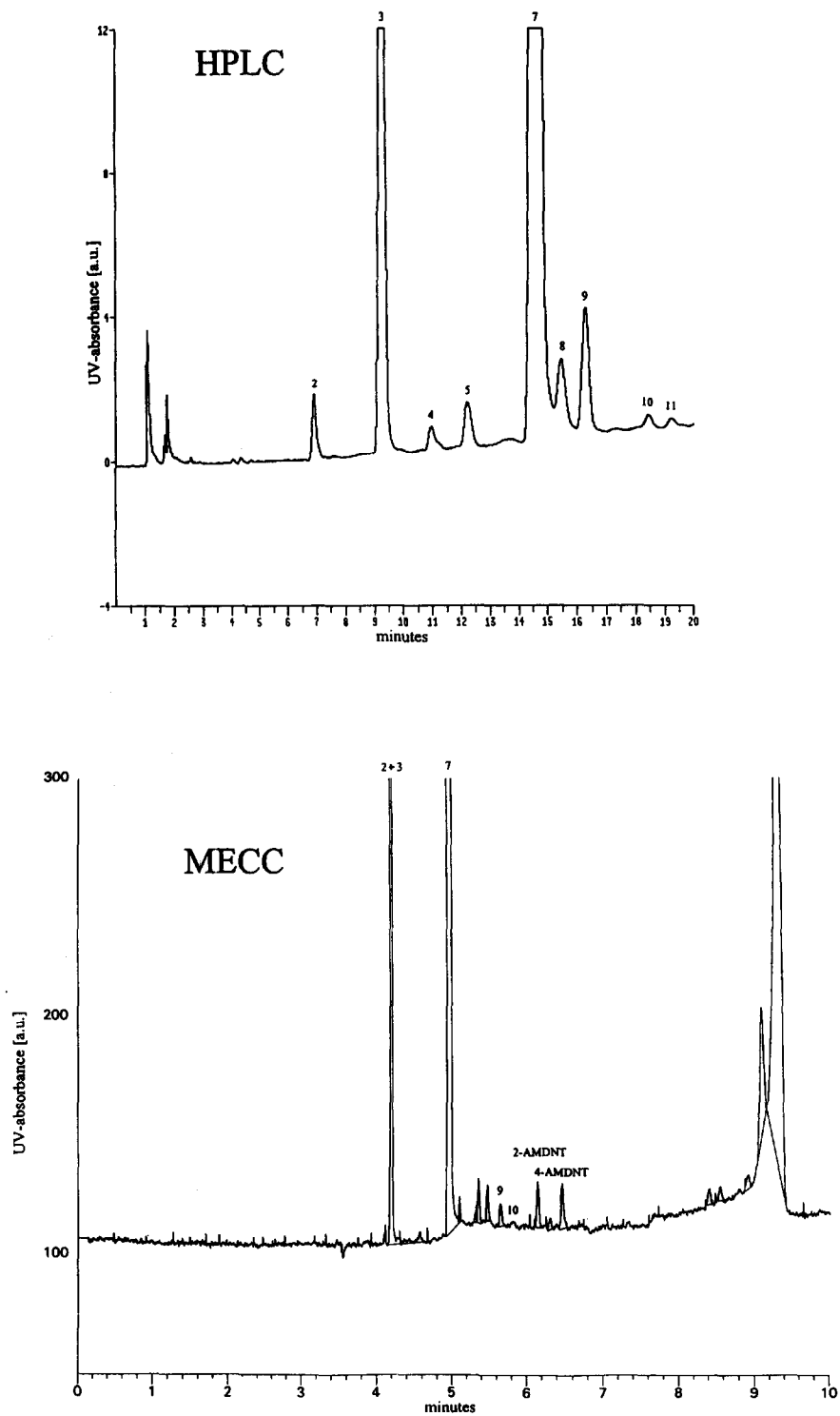


Fig. 2. MECC and HPLC separations of explosives extracted from contaminated soil at site undergoing environmental clean-up. For chromatographic conditions, see text. Peaks as in Fig. 1.

TABLE V

COMPARISON OF CONCENTRATIONS FOUND FOR NITROAROMATIC COMPOUNDS AND DEGRADATION PRODUCTS IN A SOIL FROM STADTALLENDORF (GERMANY), DETERMINED WITH HPLC AND MECC

Compound	HPLC		MECC	
	Concentration (mg/kg)	R.S.D. (%)	Concentration (mg/kg)	R.S.D. (%)
RDX	22.41	9.6	—	—
TNB	158.27	4.6	—	—
DNB	2.76	7.9	2.86	42.3
TNT	1409.90	4.7	1265.85	1.9
2,6-DNT	24.51	5.6	6.46	42.8
2-NT	—	—	—	—
2-AMDNT	—	—	4.59	1.2
4-AMDNT	14.14 <sup>a</sup>	3.3	4.97	16.5

<sup>a</sup> Sum of 2-AMDNT and 4-AMDNT.

as well as by the HPLC, method. Most of the signals in Fig. 2 were identified, but the presence of unidentified substances believed to be explosive degradation products was also noted in the soil samples using the MECC method, which offers higher efficiency than HPLC. Based upon calibration curves, concentrations of explosives in this case were found to range from 1265 ppm for TNT to 6.4 ppm 2,6-DNT in the original soil. Considering the toxicity of these substances [14], the value of this type of analysis for environmental assessment at military facilities cannot be overstated. In Table V concentrations of several nitroaromatic compounds in the soil determined with MECC and with HPLC are compared. The concentrations found for DNB and TNT are nearly the same for both methods. With MECC, RDX and TNB could not be evaluated, because in the soil probe both compounds had the same migration time. For the aminodinitrotoluene isomers the HPLC method can only give the sum of both amounts. However, considering the relative amounts of each isomer determined by MECC, the resulting sum is close to the HPLC value.

#### CONCLUSIONS

The MECC method can be successfully applied to analysis of environmental samples, such

as soil extracts, containing explosives residues. The method is fast, economic and generates little organic waste like the comparable HPLC method. Small sample sizes are easily handled, and detection limits are close to those obtained by HPLC. MECC offers high resolution and efficiency, allowing for "fingerprinting" of complex soil extracts. In addition, MECC provides easy removal of interfering humic substances extracted from complex soil matrices. The combined application of HPLC and MECC provides more information about the sample composition. In conclusion, it can be stated that MECC can compete with well-established techniques such as HPLC for the determination of explosives in complex matrices with regard to time of analysis and quantitation. However, better migration time reproducibility for the MECC method is needed. We are currently examining neutral flow markers to calculate MECC capacity factors and improve the precision of MECC results.

#### REFERENCES

- 1 R.E. Clement, M.L. Langhorst and G.A. Eiceman, *Anal. Chem.*, 63 (1991) 270R.
- 2 M. Reisch, *Chem. Eng. News*, 70 (26) (1992) 7.
- 3 R.F. Spaulding and J.W. Fulton, *J. Contam. Hydrol.*, 2 (1988) 139.

- 4 C.F. Bauer, St.M. Koza and T.F. Jenkins, *J. Assoc. Off. Anal. Chem.*, 73 (1990) 541.
- 5 J.B. Nair and J.W. Huber, *LC·GC*, 6 (1988) 1071.
- 6 J. Hirata and J. Okamoto, *J. Microcol. Sep.*, 1 (1989) 46.
- 7 M. Hable, C. Stern, C. Asowata and K. Williams, *J. Chromatogr. Sci.*, 29 (1991) 131.
- 8 M.H. Mach, A. Pallos and P.F. Jones, *J. Forensic Sci.*, 23 (1978) 446.
- 9 A. Munder, R.G. Christensen and S.A. Wise, *J. Microcol. Sep.*, 3 (1991) 127.
- 10 D.M. Northop, D.E. Martire and W. MacCrehan, *Anal. Chem.*, 63 (1991) 1038.
- 11 Y.F. Yik, H.K. Lee and S.F. Li, *J. High Resolut. Chromatogr.*, 15 (1992) 198.
- 12 Th.F. Jenkins, M.E. Walsh and P.W. Schumacher, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 890.
- 13 *Situationsplan, Umweltzentrum der Stadt Stadtallendorf, Regierungspräsidium Giessen, Landgraf, Giessen, 1989.*
- 14 R. Meyer, *Explosiv Stoffe*, Verlag Chemie, Weinheim, 1973.