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Ion-pair high-performance liquid chromatographic determination of morphine and pseudomorphine in injections

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Abstract: A stability-indicating HPLC procedure has been developed for the determination of morphine and its degradation product pseudomorphine in injection solutions. The reversed-phase chromatographic procedure uses an octadecyl-bonded column with a mobile phase of acetonitrile and aqueous buffer containing an ion-pair reagent. Chromatographic conditions are identified that enable the complete separation of morphine and pseudomorphine without peak deformation or splitting. The only sample preparation required is a simple dilution. The method is found to be precise and linear. The devised method has been applied to the determination of the content of morphine and pseudomorphine in injection solutions stored under normal conditions and after exposure to daylight or heating at 60°C.

Keywords: *Ion-pair reversed-phase chromatography; stability-indicating assay; morphine injection solution; pseudomorphine, peak-splitting.*

Introduction

Morphine (MR) is the most significant opiate analgesic, the introduction of which in pharmacotherapy is far from recent. Many analytical techniques have been applied to its determination in various matrices including pharmaceuticals and body fluids. High-performance liquid chromatographic methods have become increasingly important, especially in the latter field because of their specificity, sensitivity and simplicity. Moreover, MR, as a polar substance is readily amenable to reversed-phase liquid chromatography.

By the use of HPLC methods for the analysis of morphine containing pharmaceutical preparations the tedious and time-consuming sample preparation procedures employed in pharmacopeial methods [1] can be avoided. Furthermore additional information on the presence of other substances, such as preservatives, degradation products and impurities can be obtained.

A gradient reversed-phase liquid chromatographic [2], an ion-exchange liquid chromatographic [3] and two different ion-pair reversed-phase liquid chromatographic methods using heptanesulphonic acid [4] or pentanesulphonic acid [5] as an ion-pair reagent have been described for the determination of MR in injection solutions and bulk material.

MR is known to decompose in aqueous solution on storage to yield pseudomorphine (PMR) as the major degradation product [6, 7]. This degradation product, however, was determined in injection solutions of MR in only one of the above papers [3]. The content of PMR was measured in injection solutions of MR stored for many years in various containers and the published data used to show that the content of the degradation product PMR can be used as a sensitive indicator of MR stability.

The present communication describes an ion-pair liquid chromatographic method suitable for studying the effect of temperature and daylight on the stability of commercially available injection solutions containing MR, stabilizers and isotonic agents. Emphasis is put on the stability-indicating capability and on the possibility of the simultaneous determination of MR and the degradation product PMR.

Experimental

Reagents and chemicals

Morphine hydrochloride was supplied by Slovakofarma (Hlohovec, Czechoslovakia) and the degradation product pseudomorphine was prepared as described previously [8]. Its identity was confirmed by mass spectrometry.

1-Pentanesulphonic acid sodium salt (PSA) was purchased from Sigma (St Louis, MO, USA) and 1-octanesulphonic acid sodium salt (OSA) from Fluka (Buchs, Switzerland). Acetonitrile (ACN) analytical reagent grade (VEB Laborchemie, Germany) and distilled water used in the mobile phase were distilled in glass before use. All chemicals were of analytical reagent grade supplied by Lachema (Brno, Czechoslovakia).

The mobile phase was prepared by mixing appropriate volumes of ACN and phosphate buffer, adding an ion-pair reagent and passing the mixture through 0.45 μm Millipore filter. The optimum mobile phase compositions for the various C18 columns used are given in Table 1.

Standard solutions for quantitative measurement were prepared by dissolving MR and PMR in distilled water containing aminoacetic acid (0.30 mg ml^{-1}), disodium edetate (0.10 mg ml^{-1}) and sodium chloride (7.50 mg ml^{-1}). The concentrations of MR and PMR were in the ranges of 0.75–2.47 mg ml^{-1} and 0.006–0.614 mg ml^{-1}, respectively.

Injection solutions (declared content of MR 10.00 mg ml^{-1}) were diluted 1:5 prior to quantitative analysis and a 8 μl aliquot was injected into the chromatograph (16 μg of MR).

Chromatography

All measurements were performed by means of a HPLC system consisting of a Waters Model 590 pump, a Waters U6K injector, a Varichrom UV50 variable wavelength UV detector operating at 225 nm and 0.05, 0.5 or 1.0 a.u.f.s. or a Waters Model 990+ diode array detector.

Analytical HPLC columns used and corresponding flow rates are given in Table 1. The column Nova-Pak C18 was used only for comparison of retention data.

Results and Discussion

Ion-pair chromatography of MR and PMR

PMR tends to yield tailing peaks on normal reversed-phase liquid chromatography and for this reason an ion-pair technique was adopted. PSA and OSA were tested as ion-pair reagents.

Since with increasing retention peak height decreases and consequently it is more difficult to detect small amounts of separated degradation products, the chromatographic conditions were optimized to achieve the best possible separation of MR and PMR.

The retention of PMR was found to decrease with increasing concentration of phosphate buffer in the mobile phase and decreasing concentration of an ion-pair reagent. In contrast the retention of MR (see Fig. 1) was less dependent upon these variables so that the separation coefficient α_{MR-PMR} became lower.

However, peak-splitting appeared when the concentrations of PSA and OSA in the mobile phase were below 3×10^{-3} M and 2×10^{-3} M, respectively. Clearly when analysing samples in which partial degradation is expected, such peak-splitting is misleading.

Peak-splitting

Retention of MR and PMR and the peak-splitting phenomena were studied with respect to the ion-pair reagent concentration, phosphate buffer concentration and ACN content in mobile phases and on the amounts of solutes injected.

An example of the dependence is shown in Fig. 1 which demonstrates the effect of increasing phosphate buffer concentration in a mobile phase containing 2×10^{-3} M PSA. As indicated in Fig. 1, the peak due to MR was split into two peaks in case the mobile phase contained 0.07 M phosphate buffer. If higher amounts of MR (30 μg) were injected, the peak-splitting occurred at phosphate buffer concentration of 0.08 M. Outside this concentration range one sharp and symmetrical peak was obtained with the exception of low phosphate buffer concentration (0.01–0.02 M), under which conditions the peak is found to be rather broad. The more retained PMR gave a tailing peak under low phosphate buffer concentration but no peak-splitting was observed over the entire concentration range.

When the concentration of PSA was decreased to 1×10^{-3} M, a much wider range of phosphate buffer concentrations was found to produce peak-splitting and again this concentration range was affected by injected amounts of MR and PMR. For injection of 10 μg of MR splitting into two peaks was observed at phosphate buffer concentrations of 0.03–0.06 M. The splitting patterns varied with the phosphate buffer concentration as shown in Fig. 2. The most resolved split peaks were obtained at 0.04 M phosphate buffer concentration. In this case the peak-splitting occurred even for

Table 1
Chromatographic conditions and corresponding characteristics of peak shape and retention

HPLC column	Mobile phase* and flow rate (ml min^{-1})	Retention time (min) MR	PMR	Efficiency N_{PMR}	Asymmetry AF_{PMR}	Separation coefficient α_{MR-PMR}
Ultrasphere ODS II 5 μm; 150 × 4.6 mm i.d.	A; 1.15	4.6	11.5	6000	1.5	2.9
Ultrasphere ODS I 5 μm; 150 × 4.6 mm i.d.	B; 1.00	5.4	8.0	3000	1.9	1.7
Ultrasphere ODS II 5 μm; 150 × 4.6 mm i.d.	B; 1.00	5.3	7.9	3500	1.8	1.7
Nova-Pak C_{18} 4 μm; 150 × 3.9 mm i.d.	C; 0.80	4.5	6.9	2500	2.2	1.7

*Mobile phase composition — A: ACN–0.02 M phosphate buffer (pH 2.2) (15:85, v/v) containing 1×10^{-3} M OSA; B: ACN–0.04 M phosphate buffer (pH 2.5) (8:92, v/v) containing 2×10^{-3} M PSA; C: ACN–0.04 M phosphate buffer (pH 2.5) (7:93, v/v) containing 2×10^{-3} M PSA.

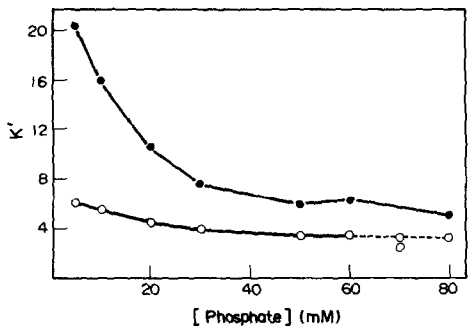

Figure 1
Dependence of the capacity factors, k', of MR (○) and PMR (●) on phosphate buffer concentration. -----, Peak splitting region. Mobile phase: 2×10^{-3} M PSA in ACN–phosphate buffer, pH 2.55 (7:93, v/v). Flow rate: 1.0 ml min^{-1}, 10 μg MR and 8 μg PMR on column.

lowered amounts injected (down to 0.8 μg). With a phosphate buffer concentration of 0.02 M a skewed peak of MR was recorded (see Fig. 2) and with an increasing amount injected the splitting was more pronounced. A significant improvement in the peak shape was achieved with the use of the mobile phase containing 0.07 M phosphate buffer. A symmetrical sharp peak was obtained even for high injection amounts of MR (up to 50 μg).

The curve representing the dependence of the capacity factors of PMR upon the concentration of phosphate buffer approached the curve for MR at a concentration of 0.05 M and starting from this point both solutes behaved in the same way with a higher phosphate buffer concentration. Thus, below a phosphate buffer concentration of 0.05 M the peak of PMR was split in a similar way to the MR peak, however both compounds gave sharp and symmetrical peaks when the mobile phase containing 0.07 M phosphate buffer was used.

For mobile phases containing OSA as an ion-pair reagent, the retentions of both solutes were found to be greater and more strongly dependent on the phosphate buffer concentration. For OSA concentration of 1×10^{-3} M only PMR was found to give a split peak. The range of the phosphate buffer concentration producing peak-splitting was 0.05–0.06 M, the amount injected being 8 μg. The splitting pattern differed from that obtained for PMR with PSA as an ion-pair reagent, as shown in Fig. 3.

When the effect of the ACN content of the mobile phase was examined, peak-splitting also was found at some critical compositions of the mobile phase.

Peak-splitting phenomena in ion-pair reversed-phase liquid chromatography already have been reported, various solutes being

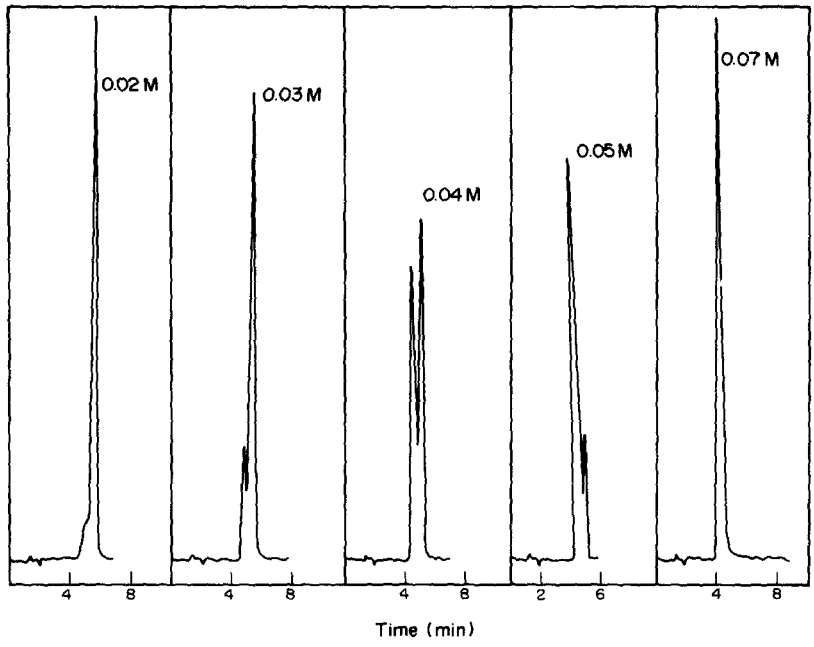

Figure 2
Peak-splitting of MR in dependence on phosphate buffer concentration. Mobile phase: 1×10^{-3} M PSA in ACN–phosphate buffer, pH 2.55 (7:93, v/v). Flow rate: 1.0 ml min^{-1}. Injected amount: 10 μg of MR. Numbers alongside peaks indicate phosphate buffer concentration.

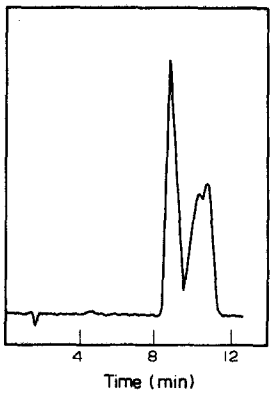

Figure 3
Peak-splitting of PMR. Mobile phase: 1×10^{-3} M OSA in ACN–0.05 M phosphate buffer, pH 2.55 (15:85, v/v). Flow rate: 1.15 ml min^{-1}. Injected amount: 10 μg of PMR.

investigated [9–12]. These observations can be interpreted on the basis of the formation of migrating zones having a decreased or an increased concentration of one of the mobile phase components. Such zones are created if the column equilibria are disturbed, for example after sample injection [13–16]. When mobile phases are used that contain a UV-absorbing component these zones are visualized as positive or negative system peaks [13, 14]. Formation of UV-transparent zones cannot be detected with UV detection but in case such zones coelute with a solute, peak compression, broadening or splitting appears [13, 15, 16].

Deformation and splitting of MR and PMR peaks were only observed for rather low ion-pair reagent concentrations. Injected amounts of both solutes were high enough to influence significantly the distribution of an ion-pair reagent in the column. As a consequence a UV-transparent system peak due to changes in the concentration of PSA or OSA originated and moved with the rate characteristic of the ion-pair reagent and dependent on the composition of the mobile phase. If such a system peak coelutes with MR or PMR, deformation or even splitting of the solute peak can occur. Obviously only in the case of MR and PMR which are similarly retained, the simultaneous splitting of both peaks could occur.

If peak-splitting of MR and PMR was viewed as a sign of coelution of a system peak and a solute peak, an increase in the retention of PSA and OSA system peaks with increasing phosphate buffer concentration in mobile phases could be traced from the measured data. This increase was slight for a small phosphate buffer concentration becoming more significant with an increasing phosphate buffer concentration. However, the retention of MR and PMR decreased with increasing phosphate buffer concentration, coelution of system and solute peaks could be easily affected by changes in the phosphate buffer concentration.

The effect of OSA and PSA concentration on the system peak retention could not be followed as the peak-splitting only occurred at a small ion-pair reagent concentration.

With an increasing content of ACN in the mobile phases the retention of system peaks decreased similarly as the retention of MR and PMR.

As has been already indicated, the observed peak-splitting phenomena also depended on the amounts of solutes injected. Injecting very small amounts of the solutes (10^{-1} μg), no deformation or splitting of peaks was observed. Clearly at this concentration the injected solutes have a negligible influence on column equilibria.

With a higher quantity of the solutes injected, peak deformation and peak splitting were observed under certain chromatographic conditions. The phenomenon gets more severe with the increasing amount of solutes injected as Fig. 4 illustrates.

The reason can be that injection of a higher amount of a solute increases the change of the mobile phase component concentration of a system peak (to complete depletion) and consequently, the system peak area increases to a constant value and also the retention time of the system peak changes [13, 15, 16]. Thus, for high sample volumes the system peak and the solute peak can coelute whilst for small amounts of sample they elute in close vicinity without any mutual influence.

Optimum chromatographic conditions

On the basis of the measurements discussed above, chromatographic conditions were chosen under which peak-splitting phenomena did not occur and the separation of MR and PMR was achieved within a short time. Table 1 summarizes the chromatographic parameters of MR and PMR for the three C18 columns listed and the corresponding optimum mobile phases.

The separation of MR and PMR achieved with the mobile phase A and B is depicted in

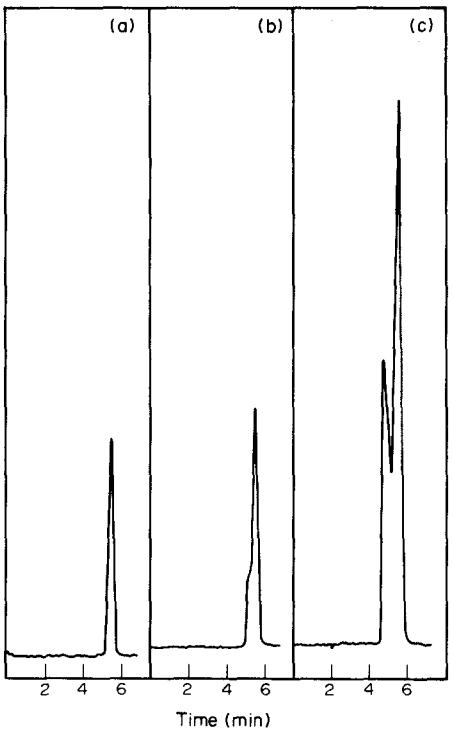

Figure 4
Effect of sample size upon peak-splitting of PMR. Injected amount and absorbance range: (a) 0.7 μg, 0.5 a.u.f.s.; (b) 2.0 μg, 1.0 a.u.f.s.; (c) 6.6 μg, 1.0 a.u.f.s. Mobile phase: 1×10^{-3} M PSA in ACN–0.05 M phosphate buffer, pH 2.55 (7:93, v/v). Flow rate: 1.0 ml min^{-1}.

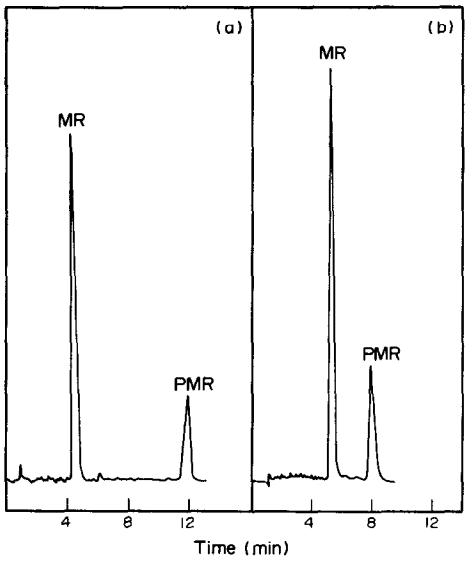

Figure 5
Chromatograms of injection solutions of MR exposed to daylight for 5 months. Injected amount: 8 μl of the injection solution diluted 1:5. (a) Mobile phase: 1.10^{-3} M OSA in ACN–0.02 M phosphate buffer, pH 2.2 (15:85, v/v). Flow rate: 1.15 ml min^{-1}; (b) mobile phase: 2×10^{-3} M in ACN–0.04 M phosphate buffer, pH 2.5 (8:92). Flow rate: 1.0 ml min^{-1}.

Fig. 5, which shows chromatograms of injection solutions of MR containing the degradation product PMR. The mobile phase with PSA as an ion-pair reagent was preferred as a smaller separation coefficient α_{MR-PMR} was obtained and the analysis time was shorter.

Stability-indicating capability

To simulate decomposition of MR in injection solutions contained in ampoules, two partially degraded samples were prepared. The first was obtained by exposing the injection solution to daylight for 4 years, the other by heating the injection solution to 95°C for 1 month. These two samples were analysed together with a synthetic mixture of MR and PMR to ensure the stability-indicating capability of the HPLC method.

PMR was identified in the degraded samples by comparison of its retention time and the UV spectrum obtained with the aid of a diode array detector with those of a standard sample.

To test the homogeneity of the peak of MR, UV spectra were taken on the up-slope and the down-slope of the peak over the range 220–350 nm for the degraded samples and also for an undegraded injection solution. The resulting overlaid spectra for the injection solution exposed to daylight is shown in Fig. 6.

PMR was found to be the main degradation product in both stressed injection solutions, its concentration being many times higher in the sample exposed to daylight than in the thermally degraded sample. Additionally several minor peaks were detected in both cases.

From all these experiments it was concluded that the suggested determination method is suitable for stability-indicating purposes.

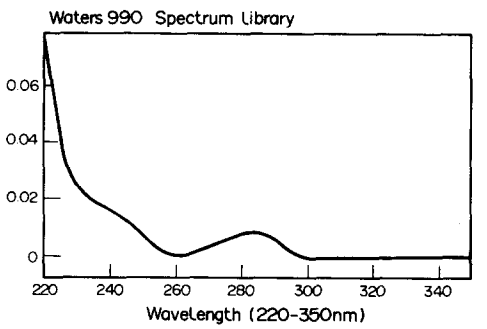

Figure 6
Overlaid UV spectra taken at ascending and descending slopes of MR peak in a chromatogram of an injection solution exposed to daylight for 4 years.

Table 2
Linearity and precision of the HPLC method

Compound	Amount injected (μg)	RSD (%) (n = 10) Within 1 day	Within 2 days	Conc. range (mg ml^{-1})	Correlation coefficient (r)
MR	12.8	0.8	1.8	0.75–2.47	0.9999
	14.0	0.8	0.8		
PMR	0.84	1.9	2.2	0.006–0.614	0.9987
	1.54	1.3	1.8		

Chromatographic conditions as given in the text.

Table 3
Stability of injection solutions (declared content of MR 10.00 mg ml^{-1})

Charge	Stored at	Time (days)	Content (mg ml^{-1}) MR	PMR
A	Room temperature and protected from light	840	9.86 ± 0.10	0.042 ± 0.004
B		480	9.80 ± 0.18	0.049 ± 0.009
C		1980	9.76 ± 0.14	0.031 ± 0.004
D		660	9.91 ± 0.13	0.029 ± 0.003
A	Daylight	19	9.39 ± 0.19	0.135 ± 0.007
A	Daylight	58	7.35 ± 0.53	1.103 ± 0.198
A	60°C	54	9.71 ± 0.11	0.055 ± 0.007

Chromatographic conditions as given in the text.

Quantitative evaluation

For the quantitative analysis the chromatographic conditions given in Fig. 5(b) were used along with the method of external standardization.

The linearity of the method was tested by analysing standard solutions containing MR and PMR at six concentration levels in the range listed in Table 2. The limit of quantitation of PMR was 0.003 mg ml^{-1}. Both the dependences of the peak area for MR and the peak height for PMR on the concentration were linear as shown by the values of the correlation coefficient r, given in Table 2. The peak height measurement was used for PMR as it gave more reproducible results than peak area measurement.

The precision of the method was evaluated on the basis of the results of 10 analyses of a sample of MR injection exposed to daylight for 1 month. The RSD given in Table 2 indicates a satisfactory precision.

The accuracy of the method was assessed by applying the method to the injection solutions incorporating precisely weighed amounts of MR. For the MR concentration of 6.80–10.5 mg ml^{-1} (68–105% of the labelled amount) a recovery of 99.1–101.2% was obtained.

The proposed HPLC method was used to assay commercially available injection solutions of MR. Some results of the quantitative analyses of the injection solutions stored under normal conditions and exposed to external effects are listed in Table 3.

Small amounts (0.3–0.5%) of the degradation product PMR were found in injection solutions of various samples stored under normal conditions. Light stress, in contrast with thermal stress, was found to increase significantly the content of PMR in the injection solutions.

References

[1] The United States Pharmacopeia, 21st edn, USP Convention, Rockville, MD (1985).
[2] K.L. Austin and L.E. Mather, *J. Pharm. Sci.* **67**, 1510–1511 (1978).
[3] P.O. Roksvaag, J.B. Fredrikson and T. Waaler, *Pharm. Acta Helv.* **55**, 198–202 (1980).
[4] R.K. Jhangiani and A.C. Bello, *J. Assoc. Off. Anal. Chem.* **68**, 523–527 (1985).
[5] I. Beaumont and T. Deeks, *J. Chromatogr.* **238**, 520–524 (1982).
[6] S. Yeh and J.L. Lach, *J. Pharm. Sci.* **50**, 30–42 (1961).
[7] S. Yeh and J.L. Lach, *Am. J. Hosp. Pharm.* **17**, 101–103 (1960).
[8] K.W. Bentley and S.F. Dyke, *J. Chem. Soc. London*, 2574–2577 (1959).

[9] G.K.O. Low, A.M. Duffield and T.R. Haddad, *Chromatographia* **15**, 289–296 (1982).
[10] H.U. Ehmcke, H. Kelker, K.H. König and H. Ullner, *Fresenius Z. Anal. Chem.* **294**, 251–261 (1979).
[11] W.S. Hancock, C.A. Bishop, J.E. Battersby, D.R.K. Harding and M.T.W. Hearn, *J. Chromatogr.* **168**, 377–384 (1979).
[12] Z. Plzák, J. Plešek and B. Štíbr, *J. Chromatogr.* **212**, 283–293 (1981).
[13] A. Sokolowski, *Chromatographia* **22**, 168–182 (1986).
[14] J.J. Stranahan and S.N. Deming, *Anal. Chem.* **54**, 1540–1546 (1982).
[15] L.B. Nilsson and D. Westerlund, *Anal. Chem.* **57**, 1835–1840 (1985).
[16] M. Johansson and D. Westerlund, *J. Chromatogr.* **452**, 241–255 (1988).

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