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Short communication

Evaluation and optimisation of separation buffers for the determination of corticosteroids with micellar electrokinetic capillary chromatography (MECC)

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Abstract

Separation buffers for the determination of the corticosteroids cortisone, hydrocortisone, prednisone and prednisolone with micellar electrokinetic chromatography were developed with respect to separation efficacy and the migration times, depending on the type and the concentration of the organic modifier acetonitrile as well as on the addition of γ-cyclodextrin. The buffer containing 50 mM SDS and 16% (v/v) acetonitrile enables the rapid profiling of prednisolone together with cortisone and prednisone. Addition of γ-cyclodextrin alters the elution sequence, but does not further enhance resolution of the corticosteroids. Baseline separation at long migration times for cortisone, hydrocortisone, prednisone and prednisolone is achieved with a buffer containing 50 mM each of SDS, dehydrocholic acid sodium salt and glycodeoxycholic acid sodium salt.

Keywords: Prednisolone; Prednisone; Hydrocortisone; Cortisone; Cyclodextrin; Bile salt; Capillary electrophoresis

1. Introduction

Corticosteroids in various dosage forms are widely used as part of the treatment of immunological disorders. For dermatological treatment, corticosteroids are used in ointments or are administered perorally as tablets. In a life-threatening immunological status, e.g. anaphylactic shock, water-soluble corticosteroid prodrugs are applied to the patient intra venam. In immunosuppressive drug therapy, corticosteroids are used to prevent rejection episodes after solid organ transplantation [1–3].

Various methods for determining steroids are already at hand and approved in pharmaceutical analysis. Quality control of corticosteroids in dosage forms is mainly performed by using HPLC separation methods [4]. Hyphenated HPLC-MS is established for the determination of corticosteroids and their metabolites in pharmakokinetic studies. Low serum levels of corticosteroids in

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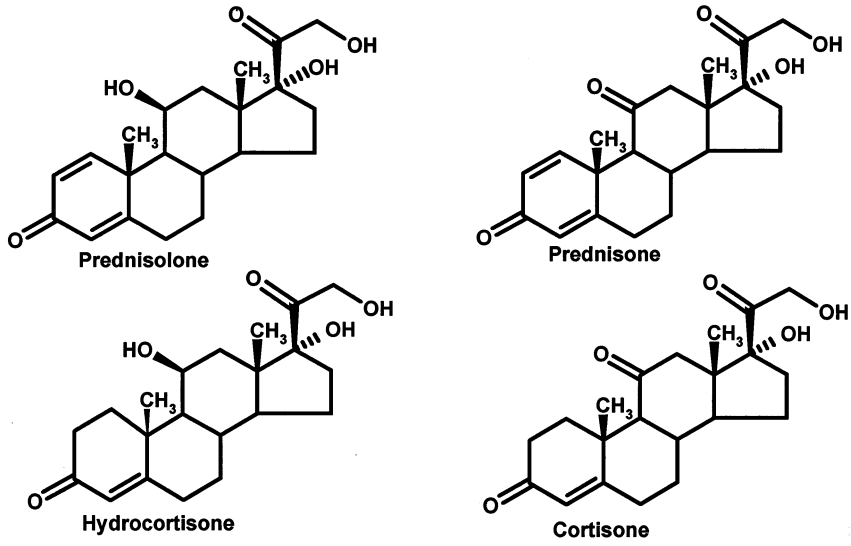

Fig. 1. Structures of the corticosteroids prednisolone, prednisone, hydrocortisone, and cortisone.

biological media can be measured with immunoassays, but a number of these assays show significant cross reactions between endogenous steroids and administered steroidal drugs.

This paper deals with the evaluation and optimisation of separation buffers for the determination of the corticosteroids prednisolone, prednisone, hydrocortisone, and cortisone (Fig. 1) with micellar electrokinetic chromatography (MECC).

MECC is a rapid, solvent saving, highly sensitive separation technique for the determination of uncharged analytes with capillary electrophoresis. Up to date only a few papers described the separation of corticosteroids with the MECC technique [5–15].

2. Experimental

2.1. Chemicals

Prednisone and prednisolone were gifts from E. Merck KG (Darmstadt, Germany), γ-cyclodextrin from Wacker Chemie GmbH (München, Germany), and hydrocortisone from Schering AG (Berlin, Germany), respectively. Cortisone, dehydrocholic acid sodium salt (DHC), and taurocholic acid sodium salt hydrate (TC) were purchased from Fluka Biochemika (Neu-Ulm, Germany), sodium dodecyl sulfate (SDS) from BioRad (Richmond, USA). Methanol (Rotisolv®, Roth Karlsruhe, Germany) and acetonitrile (Janssen, Geel, Belgium) were of HPLC grade. Stock solutions of steroids were prepared in methanol to concentrations of 1000 μg ml^{-1} and stored at ca 7°C.

2.2. Equipment

MECC was performed on a Prince Crystal CE 310 Capillary Electrophoresis System (ATI Unicam, Kassel, Germany) with autosampler and automatic outlet buffer changer. Separations took place in fused-silica capillaries (Laser2000, Weßling, Germany) with i.d. 50 μm, total length 66 cm, and effective length 49.5 cm. Detection was effected with an Unicam 4225 UV-detector (ATI Unicam, Kassel, Germany). Migration time acquisition was performed by ATI Unicam 4880 chromatography data handling system (version 2.04). Data evaluation and calculation were performed by Microsoft-Excel 7.0.

2.3. Buffer solutions

2.3.1. Buffer I

Phosphate–tetraborate buffer (pH 8.0; 20 mM): 275 mg sodium dihydrogenphosphate hydrate ($Na_2H_2PO_4 \cdot H_2O$) were solved in 100.0 ml of water (solution 1). 762 mg sodium tetraborate decahydrate ($Na_4B_4O_7 \cdot 10H_2O$) were solved in 100.0 ml of water (solution 2). Solution 2 was added to solution 1 until the pH value reached 8.0.

2.3.2. Buffer I.A–I.E

Phosphate–tetraborate buffer (pH 8.0, 20 mM) containing 50 mM SDS and various concentrations of acetonitrile. 720 mg SDS were dissolved in a small volume of buffer I, acetonitrile was added: Buffer I.A: 0% (v/v); I.B: 5% (v/v); I.C: 10% (v/v); I.D: 16% (v/v); and I.E: 20% (v/v) and made up to 50.0 ml with another portion of buffer I.

2.3.3. Buffer I.1–I.4

Phosphate–tetraborate buffer (pH 8.0; 20 mM) containing 16% acetonitrile and various concentrations of SDS. 8.0 ml acetonitrile were made up with buffer I to 50.0 ml (solution 3). SDS was solved in and finally made up to 50.0 ml with solution 3 as follows:

Buffer I.1: 12.5 mM SDS (180 mg)
Buffer I.2: 25 mM SDS (360 mg)
Buffer I.3: 50 mM SDS (720 mg)
Buffer I.4: 75 mM SDS (1080 mg)

2.3.4. Buffer II

Phosphate–tetraborate buffer (pH 8.0; 20 mM) with SDS (50 mM) and acetonitrile (16% v/v) containing γ-cyclodextrin (15 mM): 973 mg γ-cyclodextrin were solved and made up to 50.0 ml with buffer I.3.

2.3.5. Buffer III

Phosphate–tetraborate buffer (pH 9.0, 50 mM) with 50 mM of SDS, TC, and DHC each: 690 mg $Na_2H_2PO_4 \cdot H_2O$ were solved in 100.0 ml of water (solution 4). 1345 mg $Na_4B_4O_7 \cdot 10H_2O$ were solved in 100.0 ml of water (solution 5). Solution 5 was added to solution 4 until the pH value reached 9.0 (solution 6). 720 mg SDS, 1345 mg TC, and 1061 mg DHC were made up with solution 6 to 50.0 ml.

For conditioning, new fused-silica capillaries were rinsed with 0.1 M NaOH (30 min), demineralised water (10 min), and then with the buffer (10 min). Between run washes were performed by rinsing the capillary with 0.1 M NaOH (10 min), demineralised water (10 min) and then with the buffer (5 min).

2.4. Analysis

Methanolic solutions of the corticosteroids (100 μg ml^{-1} each) were injected hydrodynamically (20 millibar, 0.06 min) into the anodic capillary inlet using the autosampler. The autosampler was tempered at 20°C ($\pm$ 1°C) and the capillary oven at 25°C ($\pm$ 1°C). Automatic outlet buffer replenishment was performed using the butler. Detection was effected at $\lambda = 254$ nm. Separations using buffer I.A–E and I.1–4 were performed under constant voltage of 30 kV over a period of 15 min, with buffer II under 10 kV during 30 min, and with buffer III applying 15 kV within 40 min.

3. Results and discussion

Based on a separation buffer, published by Kobayashi and coworkers [10] for the measurement of endogenous steroids with MECC, we have developed separation buffers with different micelle forming agents and additives.

3.1. Various concentrations of acetonitrile (buffer I.A–I.E)

The influence concerning migration times and separation efficiency of the modifier acetonitrile added to a phosphate–tetraborate buffer (pH 8.0; 20 mM) with 50 mM SDS (buffer I.A) was investigated. In buffer I.A (without acetonitrile) only poor resolution of the four corticosteroids can be achieved. Adding 5% (v/v) acetonitrile to the buffer resulted in an increase of migration time of prednisone, cortisone, and of the co-eluting prednisolone and hydrocortisone. Further addition of acetonitrile (between 10–20% (v/v)) resulted in a

Table 1
Migration times of the corticosteroids prednisolone, prednisone, hydrocortisone, and cortisone in different buffer systems

Buffer	Acetonitrile	Predisone $n=5$		Cortisone $n=5$		Prednisolone+hydrocortisone $n=5$	
50 nM SDS	(%) (v/v)	Time (min)	RSD %	Time (min)	RSD (%)	Time (min)	RSD (%)
I.A	0	7.502	2.2	7.685	2.1	7.796	2.1
I.B	5	7.587	0.5	8.073	1.2	8.539	1.0
I.C	10	7.261	1.6	7.781	1.6	8.443	1.4
I.D	16	6.513	2.6	6.917	2.7	7.391	2.8
I.E	20	5.372	2.7	5.555	3.1	5.707	3.3
Buffer	SDS	Prednisone $n=5$		Cortisone $n=5$		Prednisolone+hydrocorisone $n=5$	
16% MeCN	(mM)	Time (min)	RSD (%)	Time (min)	RSD (%)	Time (min)	RSD (%)
I.1	12.5	4.093	2.2	4.093	2.2	4.093	2.2
I.2	25	4.895	0.7	5.078	0.8	5.250	1.4
I.3	50	6.509	1.2	6.922	1.4	7.419	2.7
I.4	75	6.850	0.5	7.300	0.5	7.853	0.7
Buffer	Cortisone $n=4$		Prednisolone+hydrocorisone $n=8$		Prednisone $n=4$		
Cylodextrin	Time (min)	RSD (%)	Time (min)	RSD (%)	Time (min)	RSD (%)	
II	24.645	5.8	25.858	5.8	26.610	7.7	
Buffer	Prednisone $n=3$		Cortisone $n=3$		Hydrocortisone $n=3$		Prednisolone $n=3$	
Bile salts	Time (min)	RSD (%)	Time (min)	RSD (%)	Time (min)	RDS (%)	Time (min)	RSD (%)
III	24.989	5.2	26.367	5.4	35.794	7.1	36.433	7.1

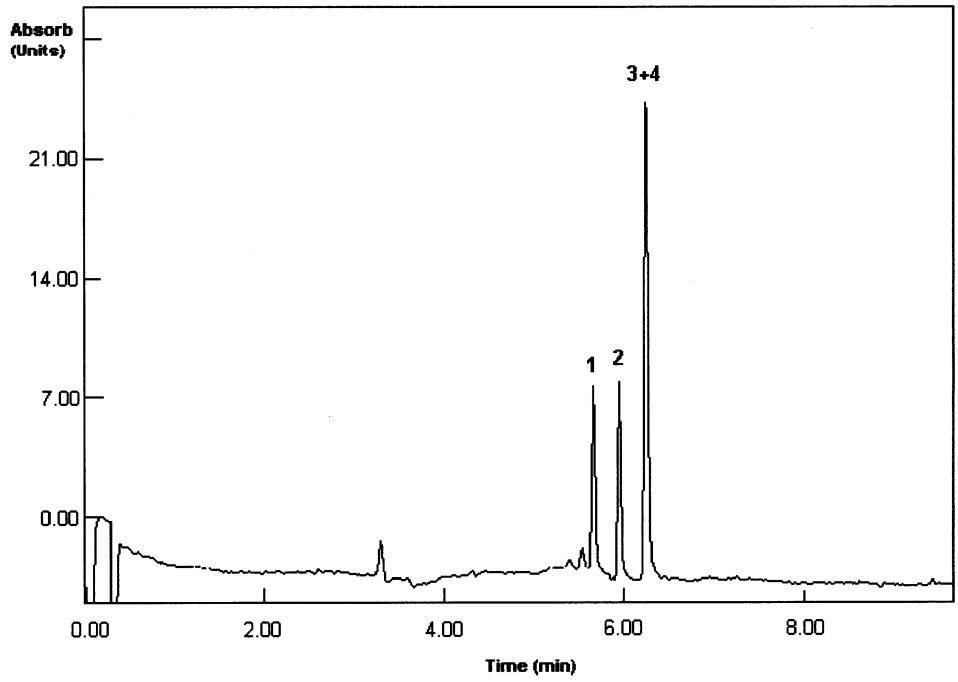

Fig. 2. Electropherogram of a mixture of prednisone (1), cortisone (2), prednisolone (3), and hydrocortisone (4) (100 μg ml^{-1} each), separated in buffer I.D.

decrease of migration times of the three separated peaks.

The results are given in Table 1. Changing the concentration of acetonitrile has no any positive effect on resolving hydrocortisone and prednisolone in this series. Good separation of the three peaks (prednisone (1); cortisone (2); and the co-eluting hydrocortisone (3); and prednisolone (4)) at short migration times resulted from using a phosphate–borate buffer (pH 8.0; 20 mM) with 50 mM SDS and 16% (v/v) acetonitrile (buffer I.D). The result is shown with the electropherogram of Fig. 2.

3.2. Various concentrations of SDS (buffer I.1–I.4)

The influence of the SDS concentration in the optimized phosphate–tetraborate buffer (pH 8.0; 20 mM) with 16% (v/v) acetonitrile (buffer I.D) was further evaluated for the resolution of the co-eluting hydrocortisone and prednisolone.

The concentration of the micelle forming agent SDS was varied from 12.5 to 75 mM. The results are given in Table 1. With the SDS concentration of 12.5 mM the four corticosteroids co-elute with the electroosmotic flow (EOF), since the critical micellar concentration (CMC) is not reached. Raising the concentration of SDS in the buffers results in an increase of migration times for the four steroids. At a concentration of 50 mM SDS, three peaks are baseline separated. Further addition of SDS up to 75 mM does not enhance the separation of the three peaks. Again, resolution of hydrocortisone and prednisolone fails independent of the concentration of SDS.

3.3. Cyclodextrin-supported micellar electrokinetic chromatography (buffer II)

γ-cyclodextrin was added to the optimized separation buffer I.D. to assess its influence on peak resolution. Micelles and cyclodextrins compete for the analytes and this may result in an

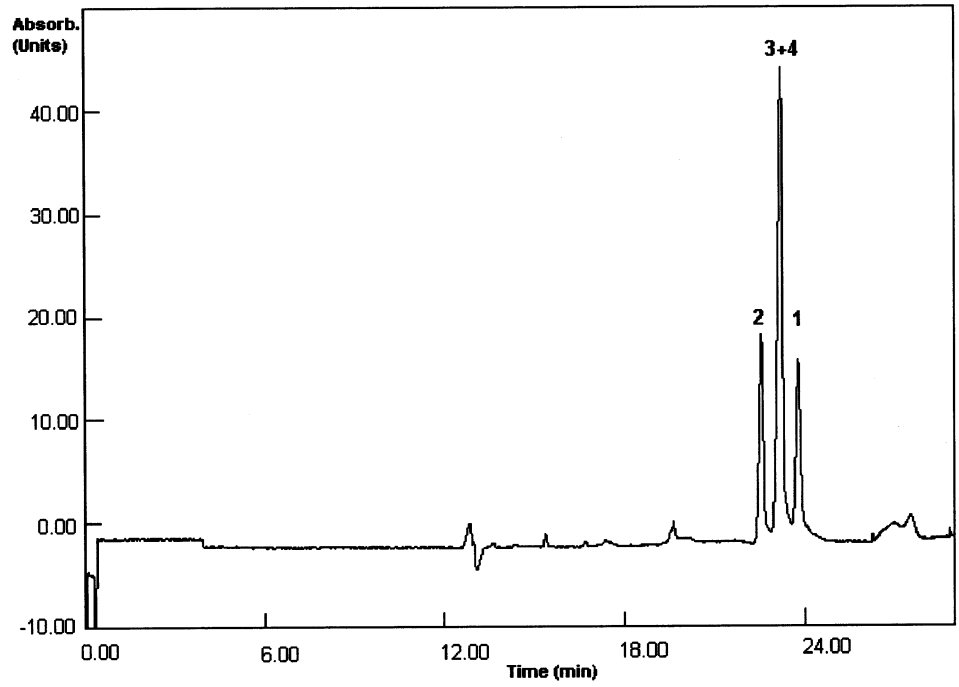

Fig. 3. Electropherogram of a mixture of prednisone (1), cortisone (2), prednisolone (3), and hydrocortisone (4) (100 μg ml^{-1} each), separated in buffer II.

enhancement of the separation of structural similar substances [7,16]. γ-cyclodextrin was chosen because of its large cavity, which makes inclusion of the steroids possible.

The addition of γ-cyclodextrin had a marked influence on the migration times of the analytes. Cortisone strongly interacts with γ-cyclodextrin and therefore elutes as first substance. Prednisone, which only differs by one additional double-bond, shows least interference with gamma-cyclodextrin and elutes as last. Although hydrocortison and prednisolone differ as well by one double-bond only, they show no significant difference in the interaction with the cyclodextrin's cavity and co-eluted between cortisone and prednisone.

This resulted in an alteration of the elution sequence, compared to those attained with the buffer systems I.A–E, I.1–4, and III, respectively. The results are given in Table 1. An electropherogram is shown in Fig. 3.

3.4. Bile salts as micelle forming agents (buffer III)

Bile salts can be used as micelle forming agent solely or in combination with other micelle forming substances [5,8,13–15]. A phosphate–tetraborate buffer (pH 9.0; 50 mM), containing 50 mM each of SDS and of the bile salts sodiumtaurocholate and sodiumdehydrocholate, was investigated. The results are shown in Table 1. All four corticosteroids prednisone (1), cortisone (2), hydrocortisone (3), and prednisolone (4) were separated in this buffer within 40 min. Fig. 4 shows a respective electropherogram.

4. Conclusion

The paper describes the development of a MECC method by evaluating separation buffers for the determination of the four corticosteroids

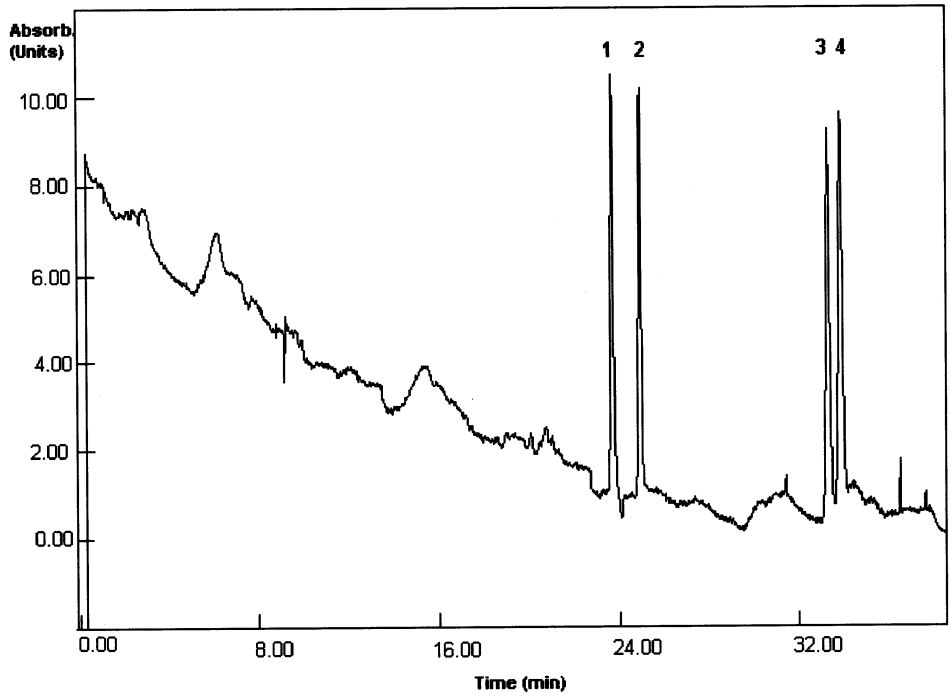

Fig. 4. Electropherogram of a mixture of prednisone (1), cortisone (2), prednisolone (3), and hydrocortisone (4) (100 μg l^{-1} each), separated in buffer III.

prednisolone, prednisone, hydrocortisone, and cortisone. The optimized buffer I.D, containing SDS and acetonitrile, can be used for rapid profiling of specimens containing corticosteroids. Addition of γ-cyclodextrin had a marked influence on the elution sequence of the analytes but can not improve the peak resolution. The buffer containing bile salts gives on the one hand further enhancement of the separation, on the other hand leads to increased migration times.

The limit of detection was assessed by a dilution sequence and found at 20 μg ml^{-1} corticosteroid for all three buffer types, which is sufficient for the identification of steroids either in quality control of bulk chemicals or in formulations.

For the determination of corticosteroids in biological samples, MECC has to be combined with special preconcentration procedures. The determination of prednisolone in human serum with presolid-phase-extraction using buffer I.D has already been published [17,18]. Therein quantification has been achieved using external standard calibration with the limit of detection at 250 ng ml^{-1} steroid [18]. The application of a special UV-Detector with a 'z-shaped' flow cell gives further improvement of the limit of detection (60 ng ml^{-1}). Thus, MECC with UV detection can be considered as an effective, solvent saving technique for the determination of corticosteroids in pharmaceutical and biomedical analysis.

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References

[1] A.S. Shoker, Drugs Today 33 (1997) 169–174.
[2] A.S. Shoker, Drugs Today 33 (1997) 221–236.
[3] M.S. Shaefer, Pharmacotherapy 11 (1991) 136–141.
[4] W.J. Jusko, J.Q. Rose, Ther. Drug Monit. 2 (1980) 169–176.
[5] H. Nishi, T. Fukuyama, M. Matsuo, J. Chromatogr. 513 (1990) 279–295.
[6] S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, K. Otsuka, J. Chromatogr. 545 (1991) 359–368.
[7] H. Nishi, M. Matsuo, J. Liq. Chromatogr. 14 (1991) 973–986.
[8] J.G. Bumgarner, M.G. Khaledi, Electrophoresis 15 (1994) 1260–1266.
[9] J.H. Jumppanen, S.K. Wiedmer, H. Siren, M.L. Riekkola, H. Haario, Electrophoresis 15 (1994) 1267–1272.
[10] Y. Kobayashi, J. Matsui, F. Watanabe, Biol. Pharm. Bull. 18 (1995) 1614–1616.
[11] M.A. Abubaker, M.G. Bissell, J.R. Petersen, J. Cap. Elec. 3 (1995) 105–110.
[12] M.A. Abubaker, M.G. Bissell, J.R. Petersen, J. Chromatogr. B 674 (1995) 31–38.
[13] S. Noé, J. Böhler, E. Keller, A.W. Frahm, Sci. Pharm. 64 (1996) 591–600.
[14] S. Yang, J.G. Bumgarner, L.F.R. Kruk, M.G. Khaledi, J. Chromatogr. A 721 (1996) 323–335.
[15] S.K. Wiedmer, H. Siren, M.L. Riekkola, Electrophoresis 18 (1997) 1861–1864.
[16] M. Kunkel, J. Böhler, E. Keller, A.W. Frahm, Pharmazie 52 (1997) 109–121.
[17] S. Noé, J. Böhler, E. Keller, A.W. Frahm, J. Pharm. Biomed. Anal. 18 (1998) 471–476.
[18] S. Noé, J. Böhler, E. Keller, A.W. Frahm, Pharmazie 53 (Suppl. 1) (1998) 29.