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Packed-column supercritical fluid chromatography for the purity analysis of clevidipine, a new dihydropyridine drug ☆

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Abstract

In this paper we describe a packed column supercritical fluid chromatography method that can be used for the analysis of a new dihydropyridine substance. The method is based on methanol-modified carbon dioxide as the mobile phase and Hypersil bare silica as column support at a column temperature of 50°C and 150 bar as back pressure. Using an adjusted methanol gradient the most likely by-products can be separated and detected (240 nm) within 13 min. Occasionally the column needed treatment with 4 m*M* citric acid in the methanol modifier in order to give a narrow peak of an acidic analogue. The present method can detect analogues at the 0.1% (w/w) level. The precision at this level for one of the analogues was 5.9% RSD. This method shows a higher selectivity than a corresponding reversed-phase liquid chromatographic method.

Keywords: Purity analysis; Clevidipine

1. Introduction

With the re-introduction of commercial dedicated packed column supercritical fluid chromatography (SFC) instruments [1,2] the technique has found a new and increased interest that is likely to generate a wider field of use and the presentation of interesting and useful practical applications. With a laboratory-built packed column SFC system we have shown that it is possible to perform fast separations for the purity analysis of omeprazole substance from possible impurities within 10 min [3]. The system was similar to a normal-phase liquid chromatographic one and for some impurities the selectivity was superior to that obtained by the ordinary liquid chromatographic system [3,4]. Recently, similar applications for salbutamol [5] and paclitaxel [6] have been published. The comparison of liquid chromatography (LC), capillary electrophoresis and SFC for the determination of losartan potassium has been reported [7]. A literature survey on the use of SFC in the analysis of drugs has been published [8], which covers both capillary and packed column SFC applications and includes references until March 1994.

Dihydropyridines are widely used as drugs in order to reduce the blood pressure and to prevent angina. For bioanalytical purposes the method of choice for many of these drugs is gas chromatography (GC) with electron-capture detection (ECD) due to the high separation efficiency and good

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detection limits of this chromatographic technique [9]. For purity analysis milder chromatographic conditions are preferred since the thermal stability of impurities are seldom fully known in advance. Thus, for such determinations LC is almost exclusively used. A packed column SFC method has been presented for the determination of the dihydropyridine felodipine and its corresponding pyridine oxidation product [10]. This work focused on the analysis of felodipine tablets and showed the utility of the ECD system as a complement to UV detection commonly used in packed column SFC.

The new dihydropyridine, clevidipine, studied in this report (Fig. 1, solute I) is somewhat different in chemical nature to most dihydropyridines used as drugs today due to the labile ester group in one of the side chains (Fig. 1, solute I) which will result in the corresponding acid as degradation product. The purpose of this work was to develop a fast and selective packed column SFC method that simultaneously can separate and detect the dihydropyridine and analogues. Meanwhile, a method for the assay of this drug in a formulation has been published by our group [11].

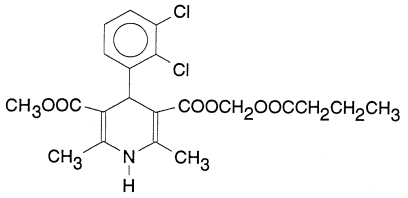
2. Experimental

2.1. Instrumentation

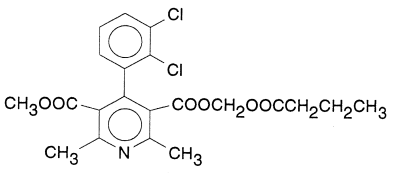
The SFC instrument used was a HP G1205A equipped with dual pumps for carbon dioxide and the modifier, a variable-wavelength UV detector and an autosampler (Hewlett-Packard, Little Falls, Wilmington, DE, USA). The instrument was controlled by the HP ChemStation software. The backpressure was 150 bar except in the chemometric experiments. The UV detector was set to monitor λ 240 nm. The instrument was equipped with a Rheodyne injection valve and a 5-μl loop.

2.2. Columns

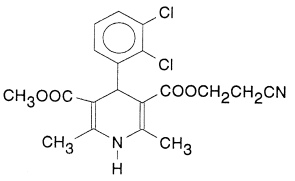
Standard liquid chromatographic columns were used throughout. 125×4 mm I.D. Superspher Si-60, LiChrosorb CN, LiChrosorb RP 18 and LiChrospher 100 Diol were all from E. Merck (Darmstadt, Germany). Kromasil 100-5 Sil 150×4.6 mm I.D. was from Eka Nobel (now Akzo Nobel, Bohus, Sweden) and Hypersil bare silica (Hypersil, formerly



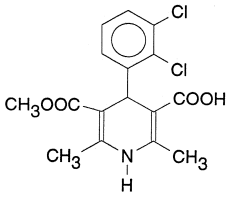
Solute I (clevidipine)



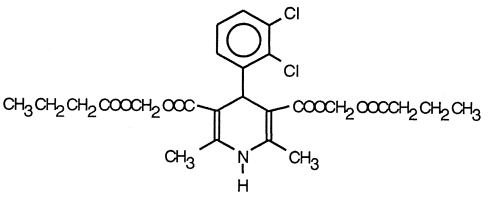
Solute II



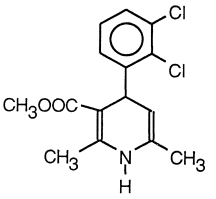
Solute III



Solute IV



Solute V



Solute VI

Fig. 1. The structure of clevidipine and the analogues studied in this work.

Shandon, Astmoor, UK) 200×4.6 mm I.D. was from Hewlett-Packard. The size of the silica particles was 5 μm in all columns.

2.3. Chemicals and reagents

Dihydropyridines were available from the Department of Medicinal Chemistry, Astra Hässle. The dihydropyridine was from Astra Chemical Production, (Södertälje, Sweden). Methanol and dichloromethane of HPLC-grade were from Fisons (Loughborough, UK) and citric acid of analytical-reagent grade was from E. Merck. The carbon dioxide used was 3.0 grade with a dipper tube from AGA (Lidingö, Sweden).

2.4. Methods

Solutions of the appropriate dihydropyridines were prepared in dichloromethane in a concentration range of 0.1–1.0 mg/ml. The dihydropyridine-substituted acid required some 10% of methanol in the dichloromethane in order to dissolve completely.

For analysis of the dihydropyridine the following gradient was used: 5% (v/v) of methanol at the time of injection followed by an increase of 0.6%/min to 10% and then 2%/min to 20%. The flow-rate was 1.5 ml/min and the column temperature 50°C.

Five mg samples of the dihydropyridine were dissolved in 1.5 ml dichloromethane to which was added 5 μl of stock solution containing 1.0 mg/ml of analogues by a 10-μl Hamilton syringe.

3. Results and discussion

3.1. Choice of chromatographic support

The neutral character of dihydropyridines under normal conditions makes them easy to chromatograph although due to the presence of an acid analogue (Fig. 1) acetic acid was added to the methanol modifier in order to improve the peak symmetry. Among four different silica supports the best chromatographic selectivity and column efficiency was observed with bare silica and diol-modified silica (Table 1). This is in line with discussions on column support by Berger [12] that matching stationary phase polarity to analyte polarity often improves peak shape. The bare silica was chosen as support since the range between the least retained compound (the pyridine) and the most retarded compound (the acid) was less than with diol as support (Table 1).

3.2. Chromatographic control of a weak acid

Although the pK_a of the weak acid in water is only 7.0 [13] the peak shape deteriorated with time even though acetic acid was present in the methanol modifier (cf. Fig. 2, upper trace). Since a modifier gradient is required in order to elute a wide range of different analytes, and 240 nm is monitored, the baseline is severely affected. Instead, citric acid was investigated as possible additive to the methanol modifier [14–16]. The presence of citric acid reduced the retention of, and improved the plate number, for the acid (Fig. 2, lower trace). Gradients were now possible although some minor baseline

Table 1
The separation factor (α) between clevidipine and some analogues on different silica supports[a]

Support	Solute[b]			
	Pyridine (II)	Clevidipine (I)	Nitrile (III)	Acid (IV)
Si 60	0.16	1.00	1.65	2.70
Nitrile	0.23	1.00	1.64	2.11
RP-18	0.40	1.00	1.21	1.60
Diol	0.16	1.00	1.59	4.45

[a] Conditions: 8% of methanol containing 0.35 *M* of acetic acid as modifier, 40°C, total flow 2.0 ml/min including CO_2. Details of the columns in Section 2.2.

[b] The Roman numerals refer to Fig. 1.

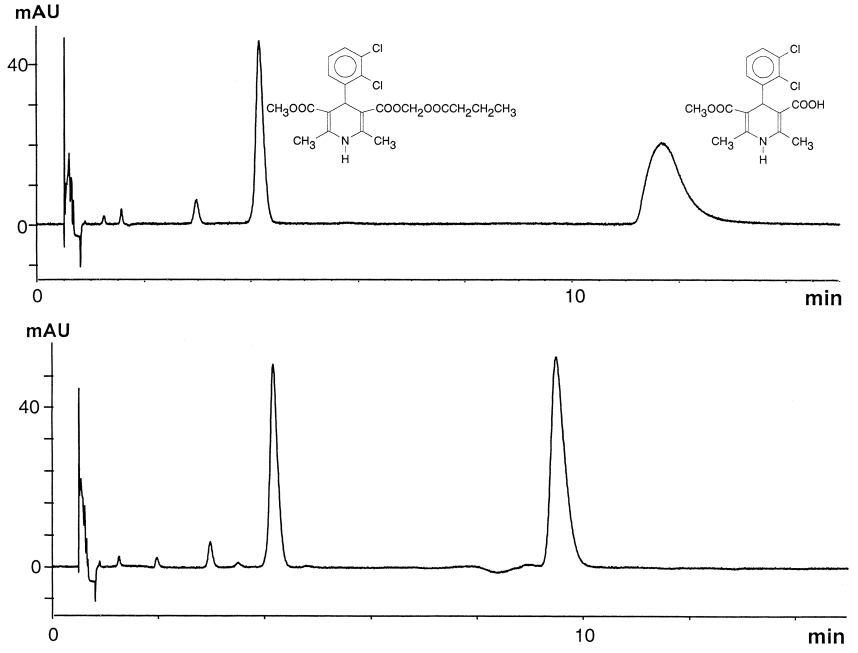


Fig. 2. Chromatogram of the dihydropyridine acid (solute IV) using methanol as modifier (upper trace) and methanol containing 0.004 *M* of citric acid (lower trace). Conditions: Si 60 column at 40°C, 10% methanol as modifier in carbon dioxide, flow-rate 2.0 ml/min, back pressure 150 bar. Sample: 200 μg/ml of the acid and 100 μg/ml of clevidipine.

unrest was noted as in Fig. 2, lower trace. We also observed that the positive effect of citric acid remained for a long time when pure methanol was used as modifier, which has been reported earlier in the literature [16]. Therefore a closer study on the presence of different acidic additives was undertaken. The results are shown in Table 2. From these data it is evident that polyvalent acids that also contain hydroxyl groups are the most effective additives to improve column efficiency and to reduce retention for the dihydropyridine substituted acid. Citric and tartaric, followed by lactic acid, are the best. It is interesting to note that the peak symmetry itself is little affected (Table 2). From the van Deemter curves, without and with citric acid present (careful washing before with water, buffer, pH 7 and methanol) show a higher level of the curve with methanol only as modifier (Fig. 3) whereas with citric acid present the curve is flatter and at a lower level (Fig. 3). The value of *H* is about two-times lower in the presence of citric acid. This indicates faster kinetics in the adsorption–desorption process when using an addition of citric acid in the mobile phase. We assume that these acidic additives acts as chelating agents on certain active sites on the silica surface that are responsible for the slower kinetic when using methanol only as the mobile phase

Table 2
Comparison of different acids as additives to the methanol modifier[a]

Acid	*N*	*k′*	*α*	Asf[b]	pK_a
–	2600	22.4	3.14	2.0	–
Cyclohexanoic	2700	22.1	3.06	2.1	4.8
Lactic	6900	18.2	2.48	2.1	3.8
Succinic	3600	19.3	2.69	2.2	4.21
Tartaric	8200	17.8	2.44	2.0	2.93
Citric	8100	17.8	2.46	2.0	3.14

[a] Conditions: Si 60 column at 40°C, flow-rate 2.0 ml/min of carbon dioxide with 10% of methanol containing 0.004 *M* of acid as modifier. The separation factor *α* was calculated from the capacity factor of the dihydropyridine acid vs. an aprotic dihydropyridine. A 10-min equilibration time between each acid, no washout, run order as in the table, 2–4 injections.

[b] Asymmetry factor.

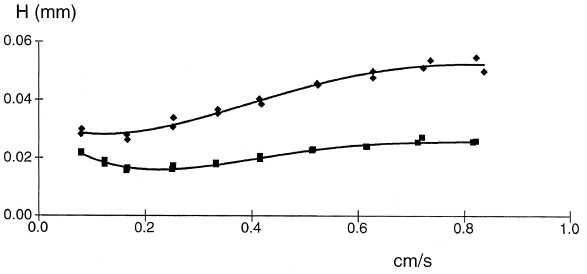


Fig. 3. Influence of mobile phase flow-rate on the efficiency for the dihydropyridine acid (solute IV) without (full diamonds, ♦) and with citric acid present (full squares, ■). Conditions: Si 60 column at 40°C, 10% methanol as modifier, 150 bar back pressure, sample 80 μg/ml in dichloromethane (solute IV). The carbon dioxide flow-rate was varied between 0.4 and 4.0 ml/min. Duplicate injections at each level. Manual evaluation of the peak width at half-height.

Table 3
The resolution between the dihydropyridine clevidipine and its symmetric ester on different bare silica supports[a]

Silica	α[b]	k'[c]	N	R_s
Hypersil	1.146	6.46	9500	5
Kromasil	1.099	8.90	9500	3
Si 60	1.083	25.9	4500	n.m.[d]

[a] Conditions: 5% of methanol as modifier, column oven 40°C, flow-rate 1.5 ml/min of carbon dioxide. Sample solution: 1.8 mg/ml of dichloromethane containing the dihydropyridine with 2% by area of the symmetric ester. Details of the columns in Section 2.2.

[b] α is the separation factor ($k'_{\text{symmetric ester}}/k'_{\text{dihydropyridine}}$).

[c] $k'_{\text{dihydropyridine}}$.

[d] n.m.=Not measured.

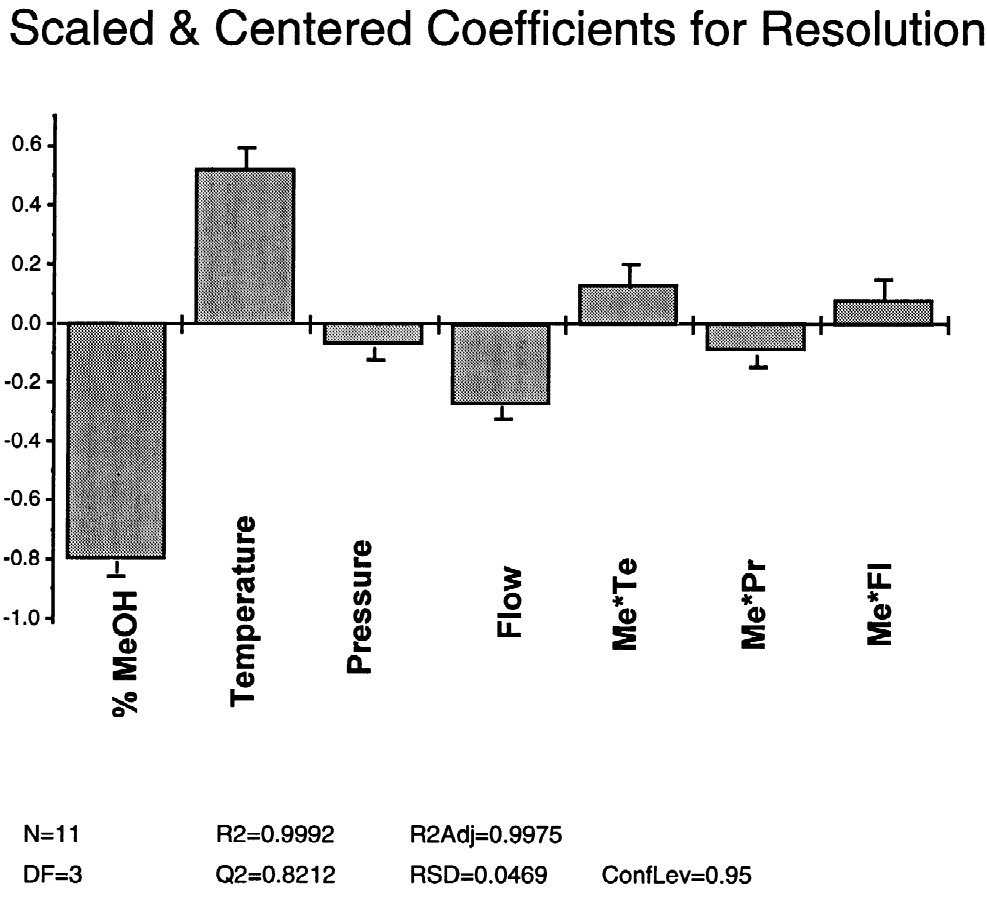


Fig. 4. Scaled and centered coefficients for resolution.

modifier. Thus, it is not a matter of suppressing the ionization of solutes [17] but of deactivating the silica surface. This assumption is also supported by the observation above that although the monovalent acids are at least two-orders of magnitude stronger than the analyte in question here, they have little effect on peak shape.

3.3. Choice of suitable bare silica

With Si-60 as support it was not possible to separate the symmetric dihydropyridine ester (Fig. 1). Other bare silicas from other vendors were investigated (Table 3) and Hypersil was chosen for further work owing to the superior resolution due to higher selectivity and plate number.

3.4. Chemometric optimization of the separation to a close analogue

With actual samples containing a thousand-fold excess of the dihydropyridine over the symmetric ester discussed above, the resolution, though a slow methanol gradient was used, must be better. Therefore a chemometric approach using a statistical experimental design was undertaken in order to optimize the chromatographic conditions further.

A fractional factorial design [18] including four descriptor variables was chosen. Three center points were included in the design in order to estimate the precision of the used chromatographic system. After screening experiments the following descriptor variable ranges were chosen, content of methanol 4–8%, column temperature 30–50°C, back-pressure 150–200 bar, and finally flow-rate 1.0–2.0 ml/min.

All the four linear terms as well as the three interaction terms Me*Te, Me*Pr and Me*Fl were included in the optimized model. The two most important variables to give highest possible resolution were the content of methanol and the column temperature, Fig. 4. The response surface plot of the response, the resolution, show that a low content of

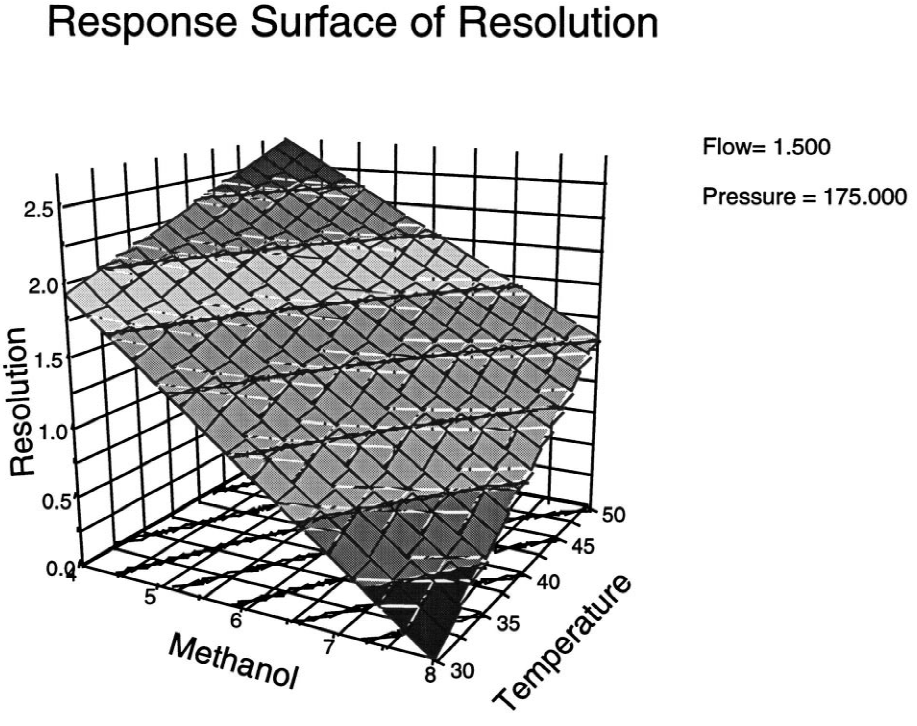


Fig. 5. Response surface of resolution. The pressure set to 175 bar and the flow-rate to 1.5 ml/min.

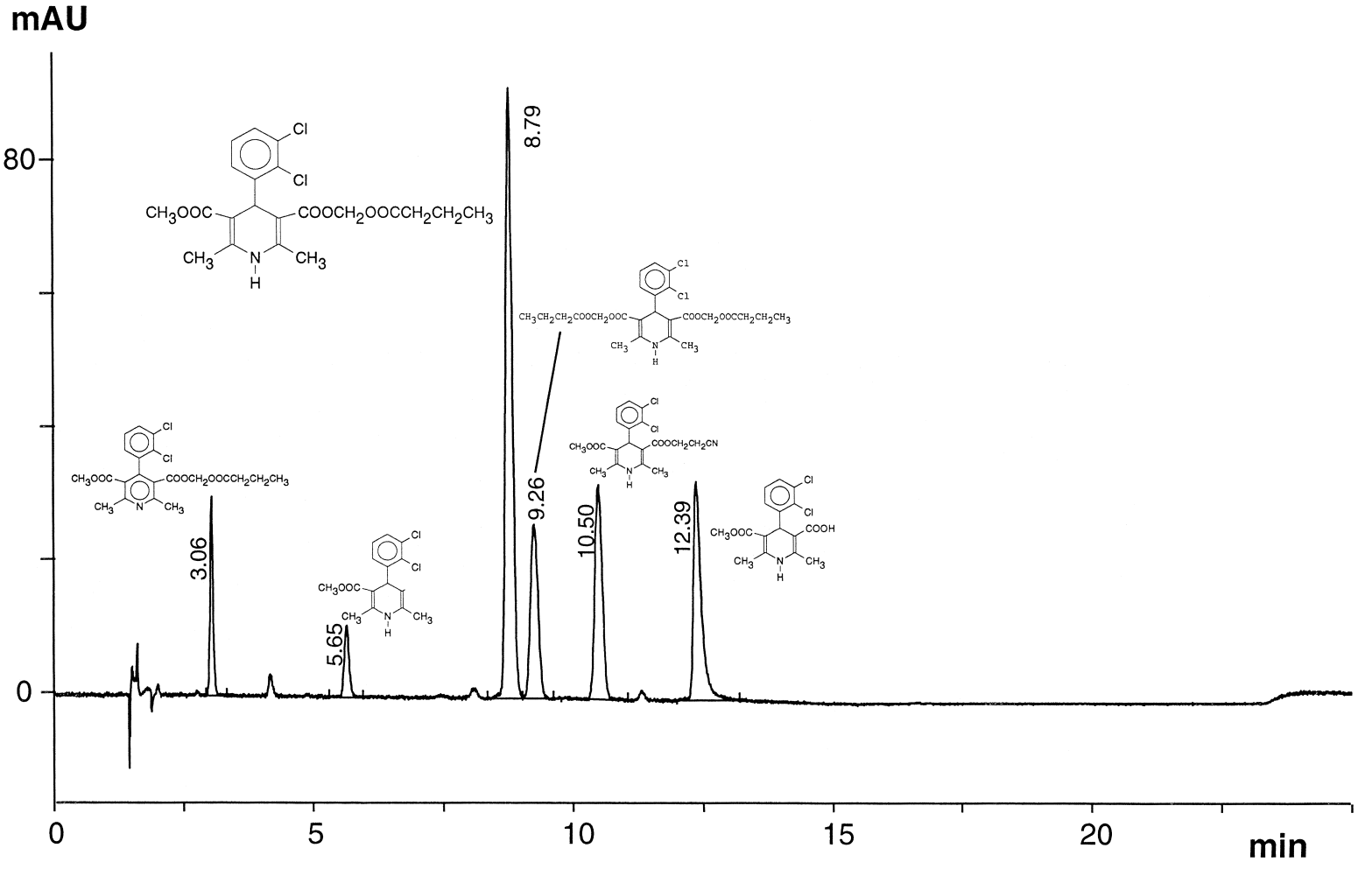


Fig. 6. Chromatographic test mixture analyzed on a Hypersil bare silica column. Chromatographic conditions as in Section 2.4.

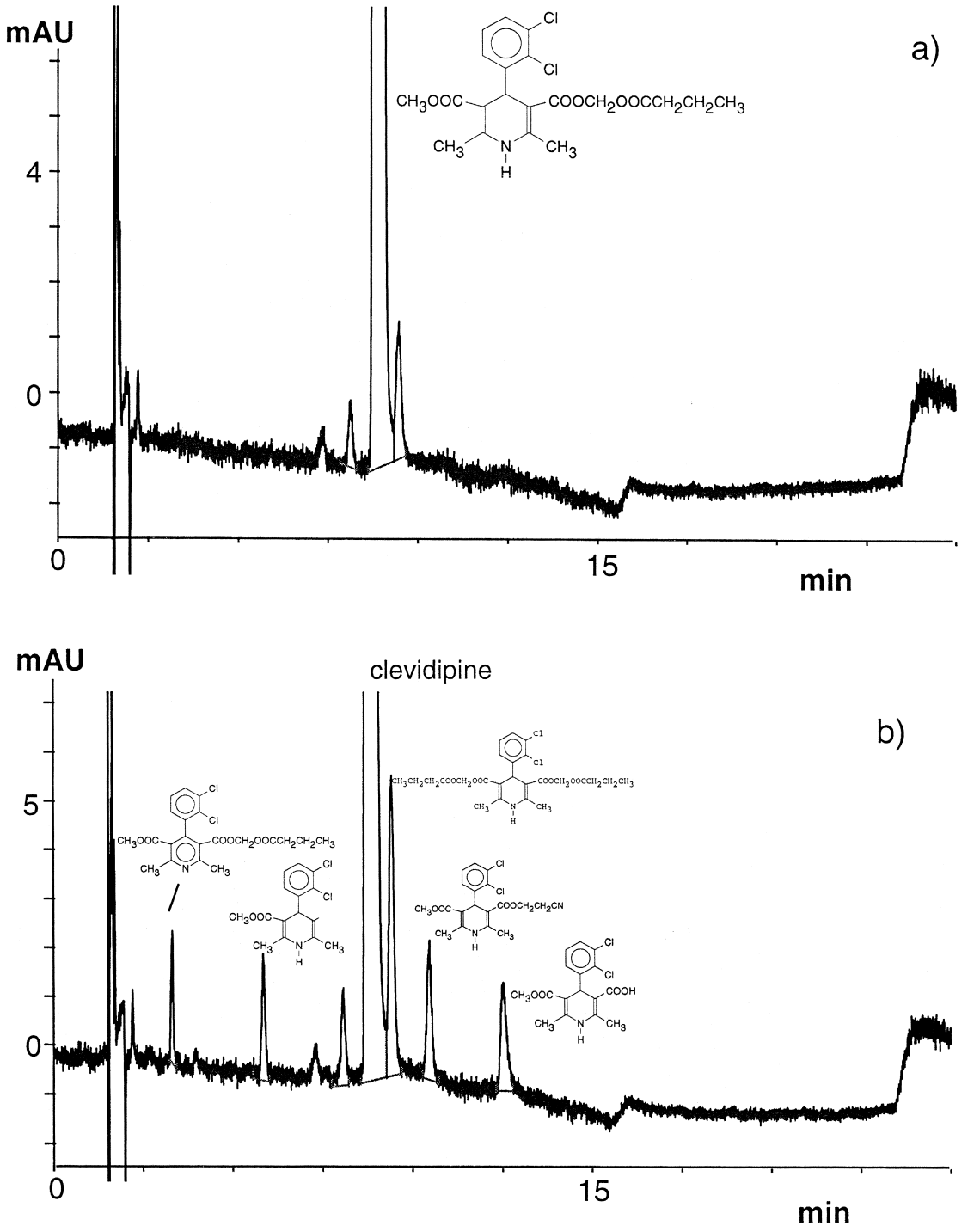


Fig. 7. Analysis of a clevidipine substance (a) and the same solution with 0.1% (w/w) of analogues added (b). Conditions: see Section 2.4.

methanol and high column temperature favor the resolution between the dihydropyridine and its symmetrical ester, Fig. 5.

However, if the column temperature was increased further, to 60°C and above, the peaks broadened and became distorted. We believe that this phenomenon is related to the sample introduction step, under the present conditions. Tuning the temperature has also been the important factor in the separation of phenothiazine antipsychotics [19], tricyclic antidepressants [20] and sulfonamides [21].

3.5. Final method

Fig. 6 shows a chromatogram with the dihydropyridine clevidipine and the analogues added. Before samples were analyzed, the plate number for the acid using isocratic conditions, 10% of methanol, was measured. Normally it was about 8000 or better, and if it was lower than 6000 the system was run for 1 h with 0.004 *M* of citric acid in the methanol modifier in order to restore the positive effect of the citric acid on the plate number. Cf. Section 3.2.

With the present method the linearity was good for area vs. concentration ($r=0.9998$, duplicate injections) in the range 38 μg/ml to 3.1 mg/ml. The data points of the 8.4 mg/ml sample were 9% below the constructed calibration line for the six lower levels, and these peaks were also 20% broader at half height than for the other concentrations.

Noteworthy, dual columns can be used in order to improve the resolution between the dihydropyridine and its symmetric ester further. The analysis time can be maintained if the flow-rate is doubled. Still there is a gain in resolution in spite of the decrease in column efficiency when using higher mobile phase flow-rates.

3.6. Application of the method

Fig. 7a shows the chromatogram of the dihydropyridine clevidipine using the final method. Addition of 0.1% (w/w) of analogues resulted in the chromatogram in Fig. 7b. The selectivity of this method is higher for the pyridine vs. the decarboxylated acid compared to the reversed-phase liquid chromatographic method [22] although with the latter method the symmetric ester is better separated and no mobile phase gradient is required. However, recent columns have shown better resolution of the previously mentioned difficult pair [22]. The time consumption is about the same although if no dimers are expected a new injection can be made after 13 min in SFC, versus 20 min in LC [22].

Since no nitrile (Fig. 1, solute III and Fig. 7b) was observed in the chromatogram Fig. 7a) the precision of the measured area ratio in the samples with 0.1% (w/w) of nitrile added could be calculated. For 11 samples the measured area % of duplicates injected twice was 0.12 with a precision of 5.9% RSD. Considering the noise in the SFC system and the accuracy in the volume of nitrile solution added, this is acceptable. The relative response measured at the mg/ml level was 1.1.

Acknowledgements

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References

[1] T.A. Berger, W.H. Wilson, in: The 4th International Symposium on Supercritical Fluid Chromatography and Extraction, Cincinnati, OH, May 1992, p. 7.
[2] F. Verillon, T. Marin Martinod, J.C. Robert, in: The 4th International Symposium on Supercritical Fluid Chromatography and Extraction, Cincinnati, OH, May 1992, p. 9.
[3] O. Gyllenhaal, J. Vessman, J. Chromatogr. 628 (1993) 275.
[4] A. Karlsson, J. Linqvist, K. Lövgren, L. Rosén, A.-M. Tivert, Int. Pharm. J. 10 (1996) 210.
[5] J.L. Bernal, M.J. del Nozal, J.M. Rivera, M.L. Serna, L. Torbio, Chromatographia 42 (1996) 89.
[6] N.K. Jagota, J.B. Nair, R. Frazer, M. Klee, M.Z. Wang, J. Chromatogr. 721 (1996) 315.
[7] R.C. Williams, M.S. Alessandro, V.L. Fasone, R.J. Boucher, J.F. Edwards, J. Pharm. Biomed. Anal. 14 (1996) 1539.
[8] A. Salvador, A. Jaime, G. Becerra, M. De La Guardia, Fresenius J. Anal. Chem. 356 (1996) 109.
[9] M. Ahnoff, B.-A. Persson, J. Chromatogr. 531 (1990) 181.
[10] J.T.B. Strode III, L.T. Taylor, A.L. Howard, D. Ip, M.A. Brooks, J. Pharm. Biomed. Anal. 12 (1994) 1003.
[11] L. Karlsson, O. Gyllenhaal, A. Karlsson, J. Gottfries, J. Chromatogr. A 749 (1996) 193.
[12] T.A. Berger, Packed Column SFC, R.M. Smith (Ed.), RSC Chromatography Monographs, The Royal Society of Chemistry, Cambridge, 1995.
[13] C. Östin, personal communication.

[14] A. Giorgetti, N. Pericles, H.M. Widmer, K. Anton, P. Dätwyler, J. Chromatogr. Sci. 27 (1989) 318.
[15] T.A. Berger, J.F. Deye, J. Chromatogr. Sci. 29 (1991) 54.
[16] L. Karlsson, T. Buttler, L. Mathiasson, J. Micrcol. Sep. 4 (1992) 423.
[17] T.A. Berger, J.F. Deye, J. Chromatogr. 547 (1991) 377.
[18] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experimenters, Wiley, New York, 1978.
[19] T.A. Berger, W.H. Wilson, J. Pharm. Sci. 83 (1984) 281.
[20] T.A. Berger, W.H. Wilson, J. Pharm. Sci. 83 (1984) 287.
[21] M.T. Combs, M. Ashraf-Khorassani, L.T. Taylor, J. Chromatogr. Sci. 35 (1997) 178.
[22] L. Tekenbergs-Hjelte, personal communication.