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Note

Thin-layer chromatographic identification of nineteen benzodiazepine derivatives

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Many papers dealing with the thin-layer chromatography of benzodiazepines and derivatives have been published. An important part of this literature treats the separation of a particular benzodiazepine from its impurities, related compounds or metabolites, but the methods described are less suitable for identification purposes. In most of the thin-layer chromatographic techniques used for toxicological analysis, the benzodiazepines and metabolites are first hydrolysed to the corresponding benzophenones, which are then identified by chromatography. These methods are not specific, as different benzodiazepines can give the same benzophenone. The hydrolysis into benzophenones also is not a general method because benzodiazepines such as triazolam, alprazolam and clobazam do not form benzophenones when treated in the usual way. Only a few papers have described the separation of intact benzodiazepines for identification purposes.

Earlier work up to 1974 has already been reviewed extensively[1,2]. Several methods involving the separation and identification of benzodiazepines have been published more recently[3–7]. Some of these use a two-dimensional technique[5,6], and one other uses a reaction chromatography technique[7].

We consider it more convenient to check the identity of a product or of a mixture of products by the use of two or three chromatographic systems. This method is the fastest as the three systems can be run at the same time and many samples can be put on the same plate. Methods involving two-dimensional chromatography are time consuming as only one unknown can be chromatographed on one plate. Furthermore, the interpretation is difficult and it is hard to obtain reproducible results. Thin-layer reaction chromatography has the disadvantage that the identification of mixtures is often impossible.

In this paper we describe the identification of nineteen benzodiazepines by the use of three mobile phases. Table I lists the benzodiazepines examined.

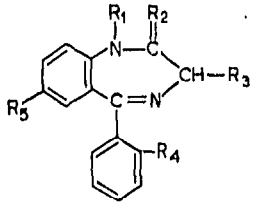
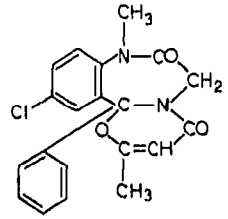
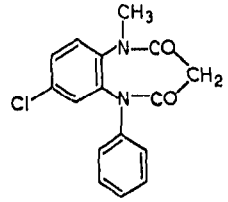
EXPERIMENTAL

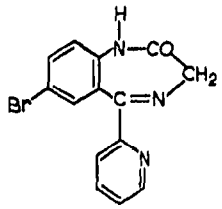
Samples

Prazepam (I) was kindly provided by Parke Davis Bornem, Belgium; VII and XVIII were obtained from Upjohn, Puurs, Belgium. XI was obtained by degradation

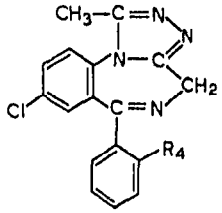
TABLE I

BENZODIAZEPINE STRUCTURES

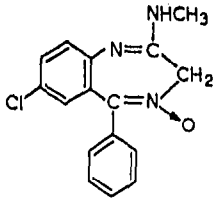
Structure	R_1	R_2	R_3	R_4	R_5	*Name*	*Trade name*
	CH_2—△	O	H	H	Cl	Prazepam (I)	Lysanxia
	CH_3	H_2	H	H	Cl	Medazepam (II)	Nobrium
	CH_2-CH_2-$N(C_2H_5)_2$	O	H	F	Cl	Flurazepam (III)*	Dalmadorm
	CH_3	O	H	H	Cl	Diazepam (IV)	Valium
	CH_3	O	H	F	NO_2	Flunitrazepam (V)	Rohypnol
	CH_3	O	$OCON(CH_3)_2$	H	Cl	Camazepam (VI)	Paxor
	CH_3	O	OH	H	Cl	Temazepam (IX)	Levanxol
	H	2OH	COOH	H	Cl	Chlorazepate (X)**	Tranxene Belseren
	H	O	H	H	Cl	Desmethyldiazepam (XI)	Madar
	H	O	H	Cl	NO_2	Clonazepam (XIII)	Rivotril
	H	O	H	H	NO_2	Nitrazepam (XIV)	Mogadon
	H	O	OH	Cl	Cl	Lorazepam (XV)	Temesta
	H	O	OH	H	Cl	Oxazepam (XVI)	Seresta
						Ketazolam (VII)	Unakalm
						Clobazam (VIII)	Frisium



Bromazepam (XII) — Lexotan



Cl	Triazolam (XVII)	Halcion
H	Alprazolam (XVIII)	Xanax



Chloordiazepoxide (XIX) — Librium

* As the monohydrochloride.
** As the dipotassium salt.

of X. For this purpose, 25 mg of X were dissolved in 10 ml of 4 *N* hydrochloric acid and, after 10 min, the solution was brought to pH 10 with 10 *N* sodium hydroxide and extracted twice with 15-ml volumes of chloroform. The combined organic layers were washed with water, dried over anhydrous sodium sulphate, filtered and evaporated to yield chromatographically pure XI. The structure was confirmed by mass spectrometry. All other samples were obtained from Mr. E. Denis, Instituut voor Hygiëne en Epidemiologie, Brussels, Belgium.

Stationary and mobile phases

Silica gel was used as the coating material. To check for the influence of the binder of the chromatoplates, three different brands of ready-made plates were used, *viz.*, DC-Fertigplatten Kieselgel 60 F 254 (E. Merck, Darmstadt, G.F.R.), Stratochrom Si F 254 (Carlo Erba, Milan, Italy) and DC-Fertigplatten Si F (Riedel de Haën, Hannover, G.F.R.). Home-made plates were also used: 0.25-mm layers were prepared with a suspension of 30 g of Kieselgel GF 254 (Type 60) (Merck) in 60 ml of water. After drying in the laboratory atmosphere the home-made plates were activated at 105° for 1 h and stored without further precautions. The ready-made plates were used without prior activation.

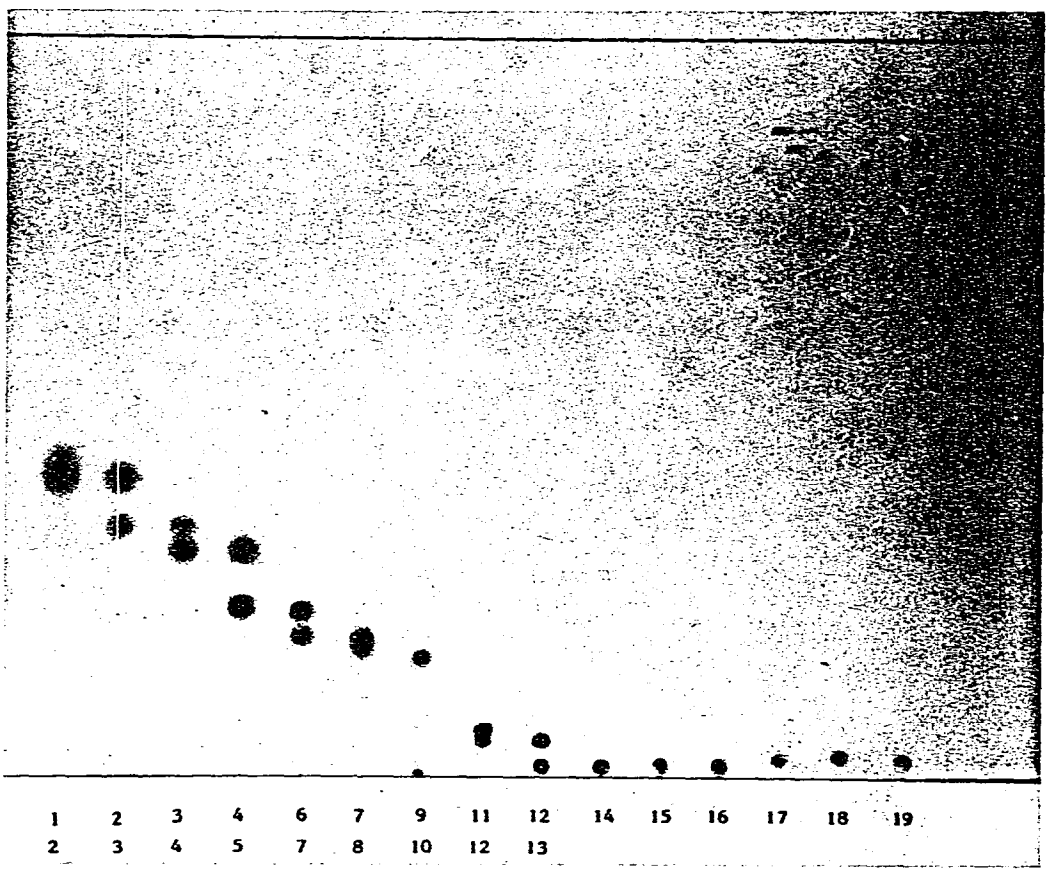


Fig. 1. Chromatogram obtained using toluene–*n*-hexane–diethylamine (55:36:9) as the mobile phase The numbers of the spots correspond to those listed in Table I.

Three mobile phases were used: A, toluene–*n*-hexane–diethylamine (55:36:9); B, ethyl acetate–diethylamine–ethanol (95:3:2); C, nitromethane–ethyl acetate (85:15). The ratios are expressed in volumes. Mobile phase C has been proposed for controlling the purity of nitrazepam[8].

Chromatographic procedure

Solutions (0.4%, w/v) were prepared using methanol as the solvent except for X, which was dissolved in water and then diluted with methanol to a final concentration of 80% (v/v) of methanol. Amounts of 0.5 μl (2 μg) were spotted on the chromatoplate.

Paper-lined chromatographic chambers were equilibrated with the mobile phase for at least 1 h. The plates were developed over a distance of 15 cm, dried in a stream of warm air and examined under an ultraviolet lamp having a maximum output at about 254 nm. The detection limit was less than 1 μg when a Sylvania G 15 T8 A lamp was used as the light source. All experiments were carried out at temperatures between 20° and 25°.

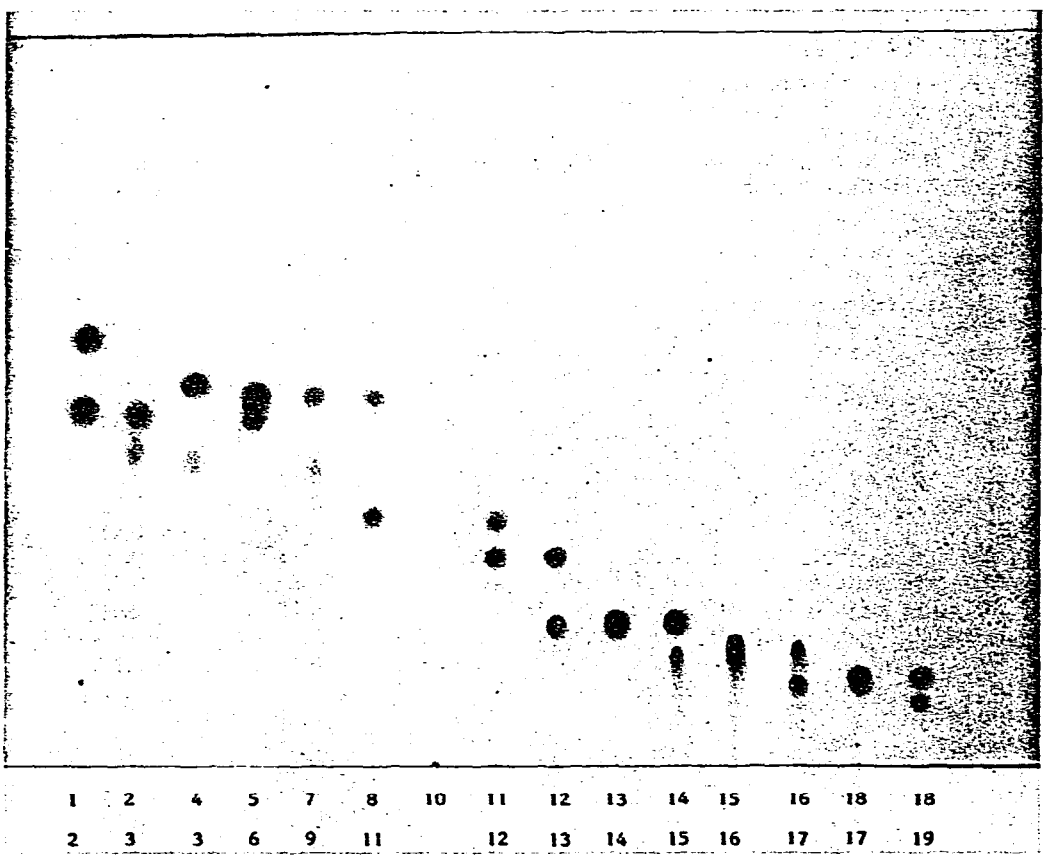


Fig. 2. Chromatogram obtained using ethyl acetate–diethylamine–ethanol (95:3:2) as the mobile phase. The numbers of the spots correspond to those listed in Table I.

Photography

Photographs of the chromatoplates were taken with Agfa Ortho 25 Professional film; the camera was equipped with a UV filter. Two Sylvania G 15 T8 A lamps were used as the light source.

RESULTS AND DISCUSSION

Figs. 1–3 show photographs of the chromatograms obtained with mobile phases A, B and C, respectively, on Merck ready-made plates. Chromatography with mobile phase A allows a good spread of the R_F values for benzodiazepines I–XII. The N-substituted benzodiazepines I–IX show the highest R_F values. The pairs I and II, V and VI, VIII and IX, and XI and XII are not or poorly separated, but each of these pairs is clearly separated by both mobile phases B and C. The dipotassium salt X does not migrate and is converted into XI on the plate as well as in solution and therefore always gives two spots. The tailing observed between the two spots is due to decomposition on the plate[9].

TABLE II

R_F VALUES OF BENZODIAZEPINES

Mobile phases: A = toluene–*n*-hexane–diethylamine (55:36:9); B = ethyl acetate–diethylamine–ethanol (95:3:2); C = nitromethane–ethyl acetate (85:15); D = nitromethane–methanol (90:10); E = *n*-heptane–chloroform–ethanol (50:50:10); F = ethyl acetate–1,2-dichloroethane–25% ammonia (80:20:10); G = chloroform–acetone (4:1); H = ethyl acetate–methanol–25% ammonia (85:10:5); I = ethyl acetate. Values in parentheses are taken from the literature.

Drug	*Mobile phase*					
	A		*B*		*C*	
	Merck plate	*Home-made plate*	*Merck plate*	*Home-made plate*	*Merck plate*	*Home-made plate*
I	0.44	0.56	0.59	0.68	0.23	0.39
II	0.43	0.52	0.49	0.61	0.13	0.34
III	0.37	0.42	0.43*	0.57*	0.0	0.01
IV	0.33	0.39	0.56	0.66	0.19	0.38
V	0.25	0.32	0.51	0.65	0.31	0.52
VI	0.25	0.30	0.47	0.62	0.26	0.45
VII	0.22	0.27	0.51	0.59	0.18	0.39
VIII	0.20	0.24	0.51	0.63	0.33	0.53
IX	0.18	0.20	0.42*	0.51*	0.25*	0.41*
X	0.0 + 0.07	0.0 + 0.09	0.0 + 0.35	0.0 + 0.47	0.0 + 0.16	0.0 + 0.36
XI	0.07	0.09	0.34	0.47	0.16	0.36
XII	0.05	0.06	0.29	0.41	0.02	0.12
XIII	0.01	0.02	0.19	0.30	0.29	0.50
XIV	0.01	0.02	0.20	0.30	0.24	0.45
XV	0.02	0.02	0.16*	0.17*	0.20*	0.36*
XVI	0.01	0.02	0.17*	0.21*	0.17*	0.32*
XVII	0.03	0.04	0.11	0.21	0.01	0.03
XVIII	0.03	0.03	0.12	0.21	0.0	0.03
IXX	0.02	0.04	0.09	0.17	0.03	0.09

* Spot with tailing.

Benzodiazepines XII–XIX hardly move in system A but they migrate well with mobile phase B. The components of the pairs XIII and XIV, and XV and XVI, which are not separated by mobile phase B, are easily identified after chromatography with mobile phase C. In the literature on the thin-layer chromatographic analysis of benzodiazepines, the separation of XV and XVI has always been mentioned as a problem[3,7,10]. An analogous problem is encountered with benzodiazepines XVII and XVIII, which have the same structure except for the presence of an additional chlorine substituent in the former. As can be seen in Fig. 2, a partial separation is obtained with mobile phase B, but in mobile phase C they migrate so poorly that no separation could be expected. The use of the more polar mobile phase nitromethane–methanol (90:10) (D) resulted in a better migration and good separation on pre-coated plates; on home-made plates, the spots were too diffuse to allow separation. In this system flurazepam (III) shows two spots; the upper one is the monohydrochloride and the lower one the base. The presence of a small amount of the base probably causes the slight tailing of III in system B.

D, *Merck plate*	*E*, *Merck plate*	*F*, *Merck plate*	*G*, *Merck plate*	*H*, *Merck plate*	*I*, *Merck plate*
0.52	0.45	0.88	0.43 (0.60)	0.70 (0.77)	0.47 (0.65)
0.37	0.43 (0.61)	0.86 (0.75)	0.36	0.65	0.29
0.03 + 0.08*	0.11* (0.22)	0.76 (0.63)	0.02*	0.57	0.23*
0.49	0.39 (0.55)	0.80 (0.68)	0.37	0.65	0.39
0.60	0.35	0.79	0.36	0.65	0.37
0.57	0.39	0.77	0.39	0.62	0.26
0.51	0.37	0.74	0.35	0.60	0.37
0.62	0.36	0.75	0.38	0.61	0.39
0.52	0.33	0.60	0.31* (0.48)	0.51 (0.58)	0.38* (0.56)
0.0 + 0.45	0.0 + 0.31	0.0 + 0.63	0.0 + 0.21	0.0 + 0.57	0.0 + 0.38
0.45	0.31	0.63	0.21	0.57	0.38
0.17*	0.15*	0.51	0.06*	0.51	0.12*
0.55	0.28	0.56	0.23 (0.34)	0.57 (0.64)	0.35 (0.53)
0.53	0.29 (0.38)	0.54 (0.47)	0.22	0.55	0.35
0.41	0.20* (0.24)	0.31* (0.20)	0.13* (0.20)	0.36* (0.40)	0.24* (0.48)
0.40	0.20* (0.24)	0.29* (0.20)	0.13* (0.18)	0.36* (0.38)	0.23* (0.44)
0.14	0.13	0.29	0.03	0.34	0.02
0.12	0.13	0.29	0.03	0.33	0.02
0.26	0.26 (0.33)	0.34 (0.25)	0.57	0.40	0.05

Several systems mentioned in the recent literature as suitable for the separation of benzodiazepines were also checked, *viz.*, *n*-heptane–chloroform–ethanol (50:50:10) (E), ethyl acetate–1,2-dichloroethane–25% ammonia (80:20:10) (F), chloroform–acetone (4:1) (G), ethyl acetate–methanol–25% ammonia (85:10:5) (H) and ethyl acetate (I)[3,11].

In Table II all the R_F values are listed. For systems A, B and C a comparison was made between different brands of pre-coated plates. No noticeable difference was observed. The R_F values given are those obtained with Merck DC-Fertigplatten. The same three systems were tried on home-made plates, and different R_F values were obtained. With mobile phase A the pair VIII and IX is better separated, but the pair III and IV forms one spot. With mobile phase B a better separation of XIV and XV is obtained. Products XVI and XVII are not separated but can be distinguished from each other with mobile phase C. More important differences between ready-made plates and home-made plates are observed by the use of mobile phase C. However, the specific aim of this mobile phase is still reached, *i.e.*, to separate those spots that are not or poorly separated by mobile phase A or B.

For systems E, F, G, H and I, the R_F values from the literature, if available, are given in parentheses. A comparison between the recorded and literature values

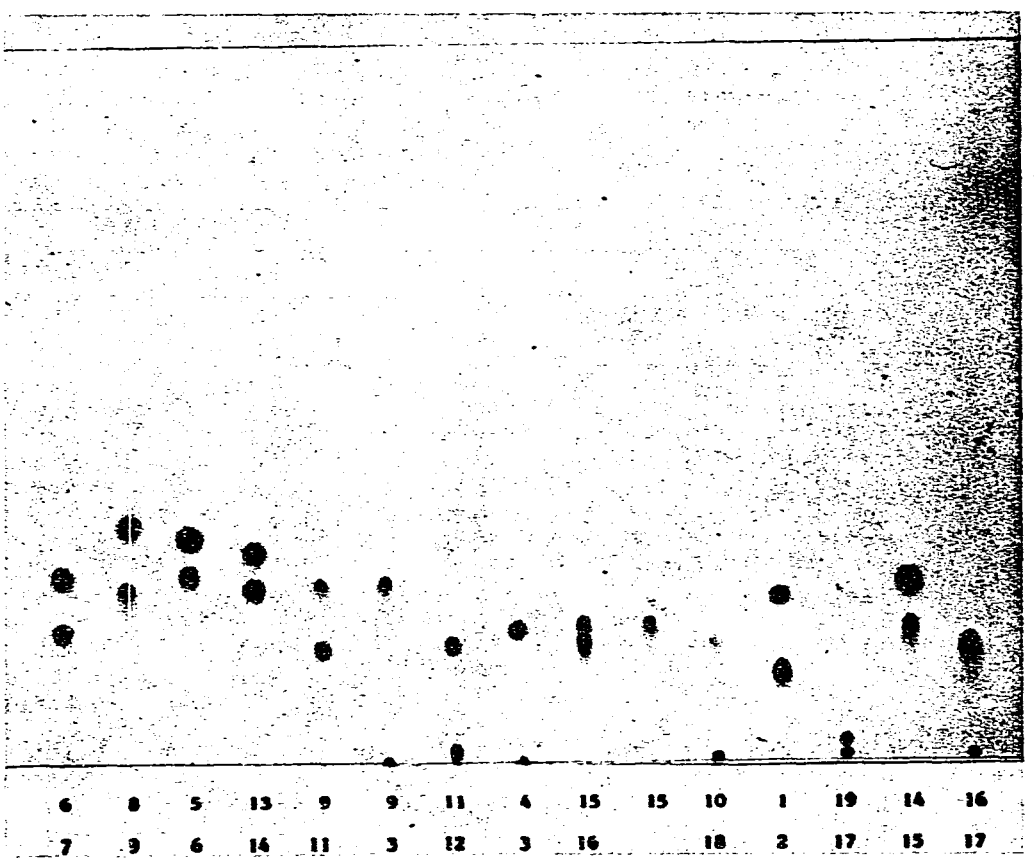


Fig. 3. Chromatogram obtained using nitromethane–ethyl acetate (85:15) as the mobile phase. The numbers of the spots correspond to those listed in Table I.

is difficult to make. It is not certain whether the thin layers used for systems E and F were of the same quality as those cited in the literature. Although the absolute R_F values are different, the ratios between them are comparable in most instances. This indicates that the same pattern of separation occurs. Literature values suggest the separation of XV and XVI in system I, which was not confirmed by our investigation.

Combination of the results obtained with systems E, F, G, H and I does not allow the separation of all nineteen benzodiazepines. None of the pairs VII and VIII, XIII and XIV, XV and XVI or, XVII and XVIII are separated. Mixtures consisting of more than two compounds are often incompletely resolved with those systems.

We consider that the method presented here, using systems A, B and C, permits an easy and rapid identification of a wide range of benzodiazepines currently in use.

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REFERENCES

1 D. M. Hailey, *J. Chromatogr.*, 98 (1974) 527.
2 J. M. Clifford and W. F. Smyth, *Analyst (London)*, 99 (1974) 241.
3 M. Th. H. A. Meeles, S. M. Dreyer-Van Der Glas and G. L. Kho, *Pharm. Weekbl.*, 110 (1975) 1.
4 G. Gräfe and W. Schmeling, *Deut. Apoth.-Ztg.*, 116 (1976) 163.
5 Z. Soljic, V. Grba and J. Besic, *Chromatographia*, 10 (1977) 751.
6 S. N. Tewari and S. K. Shukla, *Pharmazie*, 22 (1977) 536.
7 D. Dekker and P. M. C. Parel, *Pharm. Weekbl.*, 113 (1978) 465.
8 *British Pharmacopoeia 1973*, H. M. Stationery Office, London, 1973.
9 J. A. Raihle and V. E. Papendick, in K. Florey, *Analytical Profiles of Drug Substances*, Vol. IV, Academic Press, New York, 1975, p. 110.
10 C. Schütz and H. Schütz, *Z. Klin. Chem. Klin. Biochem.*, 10 (1972) 528.
11 P. Owen, E. Pendlebury and A. C. Moffat, *J. Chromatogr.*, 161 (1978) 187.