Journal of Pharmaceutical & Biomedical Analysis
Vol. 1, No. 3, pp. 321–329, 1983
Printed in Great Britain

0731-7085/83 $3.00 + 0.00

Development of a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography — I. Philosophy and selection of extraction technique

G. HOOGEWIJS and D. L. MASSART*

Farmaceutisch Instituut, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussel, Belgium

Abstract: The first of a series of papers on the development of a standardized analysis strategy for basic drugs explains the possible advantages and philosophy of the strategy. The scheme uses ion-pair extraction with direct injection of the extracts into an HPLC system emanating from two previously-selected systems. The extraction efficiency of sodium-*n*-octylsulphate as the ion-pairing reagent is compared with that of di(2-ethylhexyl) phosphoric acid (previously shown to be generally applicable to the extraction of basic drugs). Direct injection of the ion-pair extracts into an HPLC system is possible because retention behaviour is independent of whether the basic drugs are injected as an ion-pair or as a base.

Keywords: *Standardized analysis strategy; basic drug analysis; ion-pair extraction; octylsulphate; di(2-ethylhexyl)phosphate; direct injection HPLC.*

Introduction

For the analysis of basic drugs, whether in pharmaceuticals, cosmetics or biological samples, a wide variety of extraction conditions, chromatographic systems and so on are used. This is in part due to the variety of drugs and matrices to be analysed, but it is also due to the favour with which individual analysts regard particular extraction and chromatographic techniques. The wealth of existing analytical methodology for drug determinations is encouraging, but often hampers a systematic choice of initial investigation conditions for the development of an analytical procedure. In laboratories where routine drug analyses are increasing in volume and variety, and where time is a crucial factor, it is essential to make a proper choice of method and to avoid a lot of 'trial and error' experiments. The time needed for the investigation and development of the entire analytical procedure should be as short as possible. It is thus essential to have a standardized analysis scheme at hand, versatile enough to allow the analysis of most

* To whom correspondence should be addressed.

basic drugs in most matrices, with a minimum of additional experimental work needed for optimization of the experimental conditions for a particular problem.

The systematic approach to the selection of separation conditions in high-performance liquid chromatography (HPLC) described by Glajch *et al.* [1], Snyder *et al.* [2] and Schoenmakers [3] shows an increasing interest in optimization strategies. However, a systematic approach to the entire analysis procedure, including sample preparation, has never been attempted. This series of papers describes the development of a standardized analysis strategy for basic drugs using ion-pair extraction and HPLC. The present paper describes the philosophy of the strategy and deals with the extraction step, the following paper reports on the selection of the preferred HPLC systems, while further papers will describe the evaluation of the strategy for the analysis of pharmaceutical dosage forms, hair dye products, and blood plasma.

Philosophy

A standardized analysis strategy is considered to be a combination of a standardized extraction procedure which is as generally applicable as possible, with a limited set of standard HPLC systems, applicable to the analysis of as many basic drugs as possible. Optimization experiments for a particular problem would then be reduced to a minimum. The time needed for the development of the method and the analysis time should both be as short as possible, implying a minimum of manipulations.

Conventional extraction procedures for drugs, based on the extraction of the uncharged form of the analyte at an appropriate pH and using an appropriate organic solvent, have been shown to be very useful, but suffer from several drawbacks. It is frequently necessary to optimize the pH and the composition of the solvent for a particular problem; this often requires a lot of experiments. For polar drugs the extraction efficiency is often low and multiple extractions are needed. Furthermore, when applied to bioanalysis, rather impure extracts are obtained and clean-up of the extracts by back- and re-extraction has to be carried out, implying supplementary manipulations and even lower extraction yields. In 37 papers [4]–[40] in which conventional extraction techniques for basic drugs were employed, 18 different extraction solvents or solvent mixtures were used, and in 17 instances, back- and re-extraction or another clean-up step was necessary. In the remaining 20 papers, the clean-up step was avoided by the use of specific detection methods (fluorescence detection, dual or specific wavelength monitoring, N-selective detection, chemical ionization and electron capture detection). In almost one-fifth of the papers multiple extraction was needed, and more than one-third of the quoted extraction recoveries were lower than 80%. These findings, while not conclusive, indicate that conventional extraction techniques suffer from several problems.

In order to obtain suitable extraction recoveries even for hydrophilic drugs, ion-pair extraction techniques using organic counterions were studied. Two organic ion-pairing reagents were selected, di(2-ethylhexyl) phosphoric acid (HDEHP) which is soluble only in organic solvents, and the water soluble sodium-*n*-octylsulphate (NaOS). Hence an ion-pairing reagent which is added directly to the (aqueous) matrix (NaOS) and a reagent present in the extracting solvent (HDEHP) can be compared.

A systematic study of HDEHP was reported previously [41], while the results with NaOS are described in the present paper. This ion-pairing reagent has been used in ion-pair chromatography [42]–[44] but not so far for extraction purposes.

The standard HPLC-systems were originally developed for identification purposes [45] and their selection is reported in detail in the following paper. Out of 16 possible systems the following two mobile phases were selected:

Mobile Phase I (= MI):	acetonitrile	90
	water	10
	propylamine	0.01
Mobile Phase II (= MII):	*n*-heptane or *n*-hexane	50
	dichloromethane	50
	acetonitrile	25
	propylamine	0.1

Although the standardized strategy is to be used for quantitative rather than qualitative analysis, these two HPLC systems were adopted in our scheme. Indeed, the principal property of these HPLC systems, their discriminating power, is an indispensable factor. In contrast to classical ion-pair extraction techniques, in which the conditions are chosen to be more or less selective for the analyte, this work uses a generally applicable extraction step, the selectivity of the analytical procedure being achieved in the chromatographic step. The chromatographic system should therefore have the ability to isolate the analyte from co-extracted excipients, degradation products, metabolites, plasma constituents etc. This should be possible by optimizing MI or MII for the specific problem. By using one stationary phase (CN-bonded phase) and two standard mobile phases, general applicability of the strategy to basic drugs is maintained. The two preferred mobile phases are complementary in that both the normal and the reversed phase mode can be applied; if, for example, MI produces insufficient or excessive retention, then MII can be used as an alternative. Furthermore, the preferred eluents, particularly MII, are very versatile and should allow a fast optimization of the mobile phase composition. In summary, the rather neglected CN-column in combination with the listed mobile phases is inspired by the possibility of obtaining general applicability and sufficient selectivity simultaneously.

Experimental

Apparatus

UV-spectrophotometric determinations were performed with a Perkin–Elmer/Hitachi 200 spectrophotometer. pH values were measured with an Orion Research Ionalyser 601 and a combination glass electrode. Chromatography was performed using a Varian 5020 liquid chromatograph equipped with a Valco loop injector (volume 100 μl) and fitted with a fixed wavelength 254 nm UV-detector and a Varian 9176 recorder. The following columns were used: MicroPak CN-10 column (dp = 10 μm), 300 × 4 mm; Lichrosorb CN column (dp = 10 μm) 250 × 4 mm; MicroPak Si-5 column (dp = 5 μm) 250 × 4 mm; and Lichrosorb RP-18 column (dp = 10 μm) 250 × 4 mm.

Chemicals and reagents

Sodium *n*-octylsulphate (for tenside tests) was purchased from E. Merck (Darmstadt, FRG). Di(2-ethylhexyl) phosphoric acid from Koch-Light Laboratories Ltd. (Colnbrook, Bucks, UK) was 96.9% pure as determined by potentiometric titration with NaOH. It was purified by shaking twice for 30 min with an equivalent volume of 1M H_3PO_4. After phase-separation by centrifugation, the reagent was dried (at least 48 h)

and stored in a H_2SO_4-desiccator. Potentiometric titration showed it to be 99.9% pure after this treatment. All drugs were of pharmacopoeial or equivalent purity. *n*-Hexane, dichloromethane and acetonitrile were HPLC grade and purchased from Fluka AG (Buchs, Switzerland) or from E. Merck (Darmstadt, FRG). All other reagents were analytical reagent grade and obtained from E. Merck (Darmstadt, FRG), except propylamine which was purchased from Fluka AG (Buchs, Switzerland).

Procedures

The partition experiments were carried out in centrifuge tubes equipped with PTFE-covered screw-caps. To 5 ml aqueous solution of a salt of the drug was added:

(A) 5 ml phosphate buffer pH 3.0 (μ = 0.1) containing octylsulphate (0, 0.005 or 0.05 M) and 10 ml chloroform;
or (B) 5 ml phosphate buffer pH 5.5 (μ = 0.1) and 10 ml HDEHP in chloroform (0, 0.01, 0.1 or 0.5 M);
or (C) 5 ml phosphate buffer pH 10.0 (μ = 0.1) and 10 ml chloroform.

Both organic and aqueous phases were equilibrated with each other before use. The tubes were gently shaken along their long axes for 30 min in a thermostat bath at 25°C. After centrifugation at *ca.* 2500 rev/min for 15 minutes, the phases were separated, and the content of the aqueous phase analysed by UV-spectrophotometry. Each experiment was carried out at least in triplicate.

Results and Discussion

Extraction

On the basis of the results obtained using HDEHP as ion-pairing reagent [41] it was thought that general applicability and acceptable extraction yields for basic drugs might also be obtained using NaOS. Hence chloroform was chosen as the extracting solvent and all extractions were carried out at the same pH. To ensure ionization of both the analyte and the ion-pairing reagent a pH of 3.0 was chosen. The extraction efficiency of NaOS was evaluated by extracting a number of probes, each representative of a group of structurally related compounds [41]. The entire test set was representative of the whole basic drug population. The extraction recoveries are presented in Table 1, which also includes the results obtained for a number of other compounds. It can be seen that the addition of octylsulphate enhanced the extraction of each drug tested. Excellent extraction recoveries, even for rather hydrophilic compounds such as the quaternary ammonium derivatives and mephentermine, could be obtained if a sufficient excess of counterion was present. The reproducibility was also very satisfactory with a mean standard deviation of 0.1%. In Table 2 the extraction efficiencies of the ion-pair technique (using HDEHP or NaOS), and a classical extraction are compared for very hydrophilic compounds: the HDEHP-extraction was superior to the NaOS-technique and far superior to the classical extraction. HDEHP is thus a more generally applicable ion-pairing reagent for basic compounds and consequently better suited to the present work.

Chromatography

To allow routine application of the standardized method and to enhance the total analytical recovery and reproducibility, it was necessary to reduce the number of

Table 1
Recoveries (%) for the extraction of various basic drugs from aqueous solution at pH 3.0, using octylsulphate (OS^-) as counterion and chloroform as the solvent

Drug	Drug concentration (M)	% Extraction with		
		$CHCl_3$	0.0025 M OS^- *	0.025 M OS^- *†
Imipramine	1×10^{-4}	42.7	99.9	99.9
Naphazoline	1×10^{-4}	1.3	74.8	94.4
Yohimbine	1×10^{-4}	1.1	77.9	95.7
Methapyrilene	2×10^{-4}	0	94.0	99.7
Promethazine	2×10^{-4}	42.6	100.0	100.0
Carbetapentane	5×10^{-3}	65.4	83.1	99.4
Fluphenazine	2×10^{-4}	27.6	98.0	100.0
Heroin	5×10^{-4}	6.1	98.6	99.7
Thonzylamine	4×10^{-4}	0	97.8	98.2
Cocaine	1×10^{-4}	0	92.7	98.1
Chlordiazepoxyde	1×10^{-5}	86.7	95.7	100.0
Aminopyrine	1×10^{-4}	56.0	83.6	95.9
Metoclopramide	5×10^{-5}	2.6	96.1	98.8
Procaine	5×10^{-5}	0.7		92.1
Diphenhydramine	1×10^{-3}	7.2	99.9	100.0
Mephentermine	5×10^{-4}	1.0	80.3	89.4
Domperidone	5×10^{-5}	2.8	93.4	97.2
N-Cetylpyridinium chloride	1×10^{-4}	2.5	96.3	
Benzalkonium chloride	1×10^{-3}	60.7	97.9	
Benzethonium chloride	5×10^{-4}		99.6	
Pyrimethamine	2×10^{-5}	6.9		96.9
Aprindine	5×10^{-4}	1.1	98.8	92.7
Nomifensine	5×10^{-5}	2.0	87.4	95.9

* Actual concentration in the aqueous phase.
† The mean standard deviation ($n = 3$) was 0.1%.

Table 2
Recoveries (%) for the extraction of very hydrophilic basic compounds from aqueous solution using chloroform

Amine	Amine concentration (M)	OS^- (pH 3.0)			$DEHP^-$ (pH 5.5)*				
		0	0.0025M	0.025M	0	0.01M	0.1M	0.5M	pH 10
Ephedrine	2.5×10^{-4}	0.0	12.1	77.6	0.1	88.6	100.1		58.2
Amphetamine	1×10^{-4}	7.9	97.4	99.7					14.3
m-Aminophenol	1×10^{-4}	15.0		13.2	8.9		59.6	84.3	6.6
o-Aminophenol	4×10^{-4}	5.4		6.4	18.3		90.3	95.5	21.1
Hydralazine	1×10^{-4}	0.1	5.8	42.3	7.5		88.5	92.4	58.8
Dihydralazine	1×10^{-4}	2.1		5.3	3.7		71.3	91.4	17.0

* Previous HDEHP extractions were carried at the pH of the aqueous phase [41]: here a pH of 5.5 was used to ensure the ionization of drugs with pK_a values ≤ 7.

Table 3
Comparison of retention times on CN-bonded phases of some basic drugs injected as ion-pairs with OS (A), as ion-pairs with DEHP (B), and as free base (C)

Mode	Column	Mobile phase composition	Flow rate (ml/min)	Drug	Retention time (min) A	B	C
Normal phase	Micropak CN	Hexane–dichloromethane–acetonitrile–propylamine 50 : 25 : 25 : 0.1	2	Papaverine	3.4	3.4	3.4
			2	Mepyramine	4.0	4.0	4.0
			2	Methapyrilene	3.7	3.7	3.7
			2	Diphenhydramine	4.2	4.2	4.2
		10 : 45 : 45 : 0.1	2	Melperone	2.4	2.7	2.4
	Lichrosorb CN	70 : 15 : 15 : 0.1	4	Ketotifen	1.7	1.7	1.7
		80 : 10 : 10 : 0.1	4	Oxomemazine	2.3	2.3	2.3
			4	Bamipine	1.7	1.7	1.7
			4	Diphenhydramine	1.6	1.6	1.6
Reversed phase	Lichrosorb CN	Acetonitrile–water–propylamine 90 : 10 : 0.01	2	Melperone	5.1	5.1	5.2
			2	Imipramine	4.7	4.7	4.7
			2	Desipramine	23.1	23.1	23.1
			2	Pindolol	7.1	7.1	7.1
			2	Chlorpheniramine	10.7	10.7	10.7
	Lichrosorb CN	90 : 10 : 0.1	2	Ephedrine	4.0	4.0	4.0
			2	Metoclopramide	2.0	2.0	2.0

Table 4
Comparison of retention times on Micropak Si-5 and Lichrosorb RP-18 columns of basic drugs injected as ion-pairs with OS^- (A), as ion-pairs with $DEHP^-$ (B), and as free base (C)

Stationary phase	Mobile phase composition	Flow rate (ml/min)	Drug	Retention time (min)		
				A	B	C
Si	Hexane–dichloromethane–isopropanol–propylamine 25 : 75 : 3 : 0.3	0.5	Imipramine	2.3	2.6	2.2
			Procaine	2.5	2.5	2.6
			Methapyrilene	2.3	2.1	2.5
C_{18}	Acetonitrile–water–propylamine 90 : 10 : 0.1	2	Promethazine	4.7	4.7	4.8
			Emetine	2.2	2.2	2.2

manipulations by avoiding back- and re-extraction. This would imply direct injection of the extract onto the column, after concentration by evaporation and reconstitution of the residue in a minimal amount of solvent. It was thus necessary to show that the proposed ion-pair extraction techniques were compatible with the preferred HPLC systems, i.e. to show that the retention behaviour of a substance injected as an ion-pair with octylsulphate or di(2-ethylhexyl) phosphate was the same as that of the same compound injected as a base or as a salt. Direct injection of ion-pair extracts into an HPLC system has not been reported, except in a few publications [46]–[47] in which such an extract is injected into an ion-pair chromatographic system. In such a system, both the injected ion-pair (with counterion X^-) and the standards (bases or salts) are chromatographed as ion-pairs with counterion Y^-, because of the presence of excess Y^- in the stationary or mobile phase. In the chromatographic systems used here, the situation was quite different. Because of the propylamine in the mobile phase, a free base was liberated from its salt, and an identical retention time was observed for the salt and the base. Similarly, it was expected that an ion-pair would be converted to its components. Furthermore, ion-pair formation is an equilibrium reaction which only occurs if the counterion is present in sufficient excess. Since this was not the case in the present work, decomposition of the ion-pair was expected.

The results presented in Table 3, in which the retention times of drugs injected as ion-pairs or as the base are compared, show this to be true. It was concluded that ion-pair extracts could be chromatographed directly in both the polar and the apolar mobile phases used. Results of similar experiments but with a silica column and an octadecyl-silica column are presented in Table 4. The octadecyl-silica column was used in order to make decomposition of the ion-pair more readily observable: on a hydrophobic stationary phase the ion-pair would exhibit a clearly observable retention enhancement relative to the base. Furthermore it was demonstrated that both ion-pair extraction techniques are also compatible with normal phase chromatogrpahy on a silica column and reversed phase chromatography on a C_{18}-column.

Acknowledgements: We thank Mrs M. De Vreese for skilful technical assistance, and the Belgian affiliations of Ciba-Geigy, Parke-Davis, Delagrange, Janssen Pharmaceutica, Wellcome, Hoechst and A. Christiaens for gifts of drug standards. The financial support of FGWO (Fonds voor Geneeskundig Wetenschappelijk Onderzoek) is gratefully acknowledged.

References

[1] J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.* **199**, 57–79 (1980).
[2] L. R. Snyder, J. L. Glajch and J. J. Kirkland, *J. Chromatogr.* **218**, 299–326 (1981).
[3] P. J. Schoenmakers, Ph.D. Thesis, Technische Hogeschool Delft (1981).
[4] S. E. Tsuei, J. Thomas and R. L. Nation, *J. Chromatogr. Biomed. Appl.* **182**, 55–62 (1980).
[5] H. Nichol, J. Vine, J. Thomas and R. G. Moore, *J. Chromatogr. Biomed. Appl.* **182**, 191–200 (1980).
[6] R. R. Brodie, L. F. Chasseau, L. M. Walmsley, H. H. Soegtrop, A. Darragh and D. A. O'Kelly, *J. Chromatogr. Biomed. Appl.* **182**, 379–386 (1980).
[7] J. Vasiliades and C. Owens, *J. Chromatogr. Biomed. Appl.* **182**, 439–444 (1980).
[8] T. M. Jaouni, M. B. Leon, D. R. Rosing and H. M. Fales, *J. Chromatogr. Biomed. Appl.* **182**, 473–477 (1980).
[9] E. Bailey and E. J. Barron, *J. Chromatogr. Biomed. Appl.* **183**, 25–31 (1980).
[10] N. Bernard, G. Cuisinand, C. Jozefczak, M. Seccia, N. Ferry and J. Sassard, *J. Chromatogr. Biomed. Appl.* **183**, 99–103 (1980).
[11] L. M. Ross-Lee, M. J. Eadie, F. Bochner, W. D. Hooper and J. H. Tyrer, *J. Chromatogr. Biomed. Appl.* **183**, 175–184 (1980).
[12] P. Hartvig, U. Åhs and G. Wickström, *J. Chromatogr. Biomed. Appl.* **183**, 229–233 (1980).
[13] T. Aaes-Jørgensen, *J. Chromatogr. Biomed. Appl.* **183**, 239–245 (1980).

[14] T. Sadanaga, K. Hikida, K. Tameto and M. Nakanishi, *J. Chromatogr. Biomed. Appl.* **183**, 246–249 (1980).
[15] M. Bangah, G. Jackman and A. Bobik, *J. Chromatogr. Biomed. Appl.* **183**, 255–259 (1980).
[16] K. Møller Jensen, *J. Chromatogr. Biomed. Appl.* **183**, 321–329 (1980).
[17] P. J. Meffin and K. J. Smith, *J. Chromatogr. Biomed. Appl.* **183**, 352–356 (1980).
[18] H. K. L. Hundt, L. W. Brown and E. C. Clarck, *J. Chromatogr. Biomed. Appl.* **183**, 378–382 (1980).
[19] E. Antal, S. Mercik and P. A. Kramer, *J. Chromatogr. Biomed. Appl.* **183**, 149–157 (1980).
[20] N. Van Den Bosch and D. De Vos, *J. Chromatogr. Biomed. Appl.* **183**, 49–56 (1980).
[21] T. Kaniewska and W. Weijman, *J. Chromatogr. Biomed. Appl.* **182**, 81–87 (1980).
[22] T. Agoh, M. Konishi and Y. Mori, *J. Chromatogr. Biomed. Appl.* **182**, 171–177 (1980).
[23] C. P. Quaterman, M. J. Kendall and D. B. Jack, *J. Chromatogr. Biomed. Appl.* **183**, 92–98 (1980).
[24] H. R. Angelo, J. M. Christensen, M. Kristensen and A. McNair, *J. Chromatogr. Biomed. Appl.* **183**, 159–166 (1980).
[25] G. M. Schier and I. Eng Tho Gan, *J. Chromatogr. Biomed. Appl.* **182**, 232–236 (1980).
[26] K. M. Wolfram and T. D. Bjornsson, *J. Chromatogr. Biomed. Appl.* **183**, 57–64 (1980).
[27] J. T. Ahokas, C. Davies and P. J. Ravenscroft, *J. Chromatogr. Biomed. Appl.* **183**, 65–71 (1980).
[28] P. A. Reece and P. E. Stanley, *J. Chromatogr. Biomed. Appl.* **183**, 109–114 (1980).
[29] C. Van Der Meer and R. E. Hass, *J. Chromatogr. Biomed. Appl.* **182**, 121–124 (1980).
[30] L. J. Lesko, J. Ericson, G. Ostheimer and A. Marion, *J. Chromatogr. Biomed. Appl.* **182**, 226–231 (1980).
[31] M. Ervik, K. Kylberg-Hanssen and P. O. Lagerström, *J. Chromatogr. Biomed. Appl.* **182**, 341–347 (1980).
[32] V. Rovei, M. Sanjuan and P. D. Hrdina, *J. Chromatogr. Biomed. Appl.* **182**, 349–357 (1980).
[33] M. Van Boven, P. Daenens and G. Vandereycken, *J. Chromatogr. Biomed. Appl.* **182**, 435–438 (1980).
[34] S. R. Gautam, A. Nahum, J. Baechler and D. W. A. Bourne, *J. Chromatogr. Biomed. Appl.* **182**, 482–486 (1980).
[35] A. Sioufi and F. Pommier, *J. Chromatogr. Biomed. Appl.* **183**, 33–39 (1980).
[36] E. L. Crampton, R. C. Glass, B. Marchant and J. A. Rees, *J. Chromatogr. Biomed. Appl.* **183**, 141–148 (1980).
[37] R. C. Boselt and S. Franck, *J. Chromatogr. Biomed. Appl.* **183**, 234–238 (1980).
[38] E. D. W. Moore and G. Powis, *J. Chromatogr. Biomed. Appl.* **183**, 372–377 (1980).
[39] V. A. Raysis, P. N. Friel, P. R. Graaff, K. E. Opheim and A. J. Wilenksy, *J. Chromatogr. Biomed. Appl.* **183**, 441–448 (1980).
[40] T. W. Guentert, A. Rakkit, R. A. Upton and S. Riegelman, *J. Chromatogr. Biomed. Appl.* **183**, 514–518 (1980).
[41] G. Hoogewijs and D. L. Massart, *Anal. Chim. Acta* **106**, 271–277 (1979).
[42] G. Hoogewijs, J. Matterne and D. L. Massart, *Anal. Lett.* **13 (B8)**, 691–704 (1980).
[43] I. M. Johansson, K. G. Wahlund and G. Schill, *J. Chromatogr.* **149**, 281–296 (1978).
[44] J. H. Knox and R. A. Hartwick, *J. Chromatogr.* **204**, 3–21 (1981).
[45] D. L. Massart and M. R. Detaevernier, *J. Chromatogr. Sci.* **18**, 139–143 (1980).
[46] B. M. Eriksson, B. A. Persson and M. Lindberg, *J. Chromatogr.* **185**, 575–581 (1979).
[47] K. T. Muiz, J. H. G. Jonkman, D. S. Tang, M. Kunitani and S. Riegelman, *J. Chromatogr.* **221**, 85–95 (1980).

[First received 31 January 1983]