Journal of Chromatography, 97 (1974) 223–231

CHROM. 7521

INFLUENCE OF ALKALINITY IN THIN-LAYER CHROMATOGRAPHY SYSTEMS FOR THE SEPARATION OF IMPURITIES IN CHLORPROMAZINE, PROMAZINE AND PROMETHAZINE

W. N. FRENCH, F. F. MATSUI, D. L. ROBERTSON and S. J. SMITH*

Drug Research Laboratories, Health Protection Branch, Department of National Health and Welfare, Ottawa, Ontario K1A 0L2 (Canada)

(Received February 12th, 1974)

SUMMARY

A study of the influence of silica gel layer alkalinity and solvent-system variations on resolution and chromatospot definition in the thin-layer chromatography of high concentrations of chlorpromazine hydrochloride, promazine hydrochloride and promethazine hydrochloride is presented. The procedures utilized facilitate the determination of optimal thin-layer chromatographic systems for separating these compounds from each other and from low levels of extraneous material.

INTRODUCTION

The convenience and versatility of thin-layer chromatography (TLC) has led to a number of official pharmaceutical compendia utilizing the technique as a limit test for impurities present in drug substances and formulations (see *inter alia* refs. 1–3). In these applications, the large amount of parent drug required in order to visualize the low levels of impurities often gives rise to tailing of the main chromatospot with masking of impurities, poor separation, etc.

Typical of the difficulties is that found when large amounts (*ca.* 200 μg) of the phenothiazine derivatives chlorpromazine hydrochloride (CPZ), promazine hydrochloride (PRZ) or promethazine hydrochloride (PMZ), must be separated from low levels (*ca.* 1 μg) of impurities and impurity-comparison stand. ds. In order to overcome difficulties in scaling up analytical methods, a systematic study was made of some variables that are important to this type of separation.

EXPERIMENTAL

TLC layers

Four types of layer, 250 μm thick, were made with silica gel GF (Machery, Nagel & Co., Düren, G.F.R.: M-N), using standard equipment. Neutral layers and

* To whom correspondence should be adressed.

three strengths of alkaline layers were prepared with 0.1 *N*, 0.5 *N* and 1.0 *N* sodium hydroxide solutions, respectively. In addition neutral silica gel Type 60 (E. Merck, Elmsford, N.Y., U.S.A.) layers, 250 μm thick, were prepared in the standard manner. Layers were air dried for 1 h, dried at 105° for 2 h and stored in open racks. The layers were used as required without further precautions.

Commercially available pre-coated layers (250 μm thick), as obtained from suppliers, were also used.

Solvent systems

Dioxane (20 ml) was mixed with absolute ethanol (15 ml). Any required water or concentrated ammonium hydroxide was added, followed, with constant stirring, by cyclohexane (70 ml). Solvents were prepared immediately before use and utilized once in filter-paper-lined tanks. The plate was developed 15 cm from the origin.

Spotting solutions

Chlorpromazine·HCl, promazine·HCl and promethazine·HCl. Sufficient pharmaceutical-grade CPZ, PRZ and PMZ were each dissolved in methanol to afford individual solutions each containing 20 mg/ml. Ten-μl samples of each were spotted.

Trace-level components. Solutions of 2-chlorophenothiazine, phenothiazine, 4-chlorpromazine hydrochloride, isopromethazine hydrochloride, trimeprazine tartrate and levomepromazine maleate were prepared in methanol at concentrations of 0.20 mg/ml. Five-μl samples of each were applied to the layers as individual spots.

Visualization of chromatospots

Plates were sprayed with 1.0% (w/v) selenious acid in concentrated sulphuric acid.

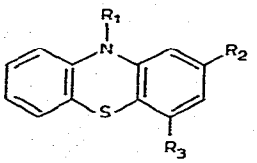
RESULTS AND DISCUSSION

Phenothiazine derivatives, in common with other organic bases, have a tendency to give rise to tailing during TLC on silica gel layers. To overcome this, workers have used alkaline solvents (see *inter alia* refs. 4–7), alkaline support layers[8], combinations of the two[8–10], pH gradient layers[11] and solvent systems containing relatively high concentrations of water[10] when chromatographing samples in the order of 5–10 μg. However, when 200-μg samples must be examined in order to detect low levels of impurities, tailing again becomes a serious problem because of the limited linear capacity of the layer[12]. Since the need to be able to examine pharmaceuticals at high chromatographic concentrations, in order to detect low-level impurities for quality control purposes, has become greater, facile procedures for scaling up analytical methods for this type of application are required. Because of their widespread use, importance and known high incidence of impurities[13], CPZ, PRZ and PMZ, together with impurities found in these drug substances and formulations as well as impurity-limit reference standards (Table I), were chosen to determine whether separation variables could be controlled so that scaling up could be readily accomplished.

A mixture of cyclohexane–dioxane–ethanol (70:20:15) can be used to separate low levels of these phenothiazine bases (Table I), and this was chosen as the fundamental or "datum" solvent system. This system, modified by the addition of water or

TABLE I

PHENOTHIAZINE COMPOUNDS EXAMINED

Name				*Designation in figures*
	R_1	R_2	R_3	
2-Chlorophenothiazine	H	Cl	H	A
Phenothiazine	H	H	H	B
Isopromethazine (as hydrochloride)	$CH(CH_3)CH_2N(CH_3)_2$	Cl	H	C
Trimeprazine* (as tartrate)	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H	H	D
Levomepromazine* (as maleate)	$CH_2CH(CH_3)CH_2N(CH_3)_2$	OCH_3	H	E
2-Chlorpromazine***** (as hydrochloride)	$CH_2CH_2CH_2N(CH_3)_2$	Cl	H	F
Promethazine** (as hydrochloride)	$CH_2CH(CH_3)N(CH_3)_2$	Cl	H	G
Promazine***§ (as hydrochloride)	$CH_2CH_2CH_2N(CH_3)_2$	H	H	H
4-Chlorpromazine	$CH_2CH_2CH_2N(CH_3)_2$	H	Cl	I

* Compound suitable as reference standard for impurity limit testing of promethazine.
** Utilized at levels of 200 μg drug compound.
*** Found present in promazine.
§ Found present in chlorpromazine.

concentrated ammonium hydroxide, was utilized for chromatographing 1-μg quantities of impurity compounds and standards along with 200-μg quantities of CPZ, PRZ and PMZ on silica gel layers varying in pH from neutral to strongly basic, in the combinations listed in Table II.

TABLE II

SOLVENT SYSTEMS AND LAYER COMBINATIONS USED

Addition to cyclohexane–dioxane–ethanol (70:20:15) system	*Silica gel (M-N) layer*			
	Neutral	*0.1 N NaOH*	*0.5 N NaOH*	*1.0 N NaOH*
Water, ml	0	0	0	0
	0.5	0.5	0.5	0.5
	1.0	1.0	1.0	1.0
	2.0	2.0	2.0	2.0
Concentrated ammonium hydroxide, ml	0.5	0.5	0.5	0.5
	1.0	1.0	1.0	1.0
	2.0	2.0	2.0	2.0

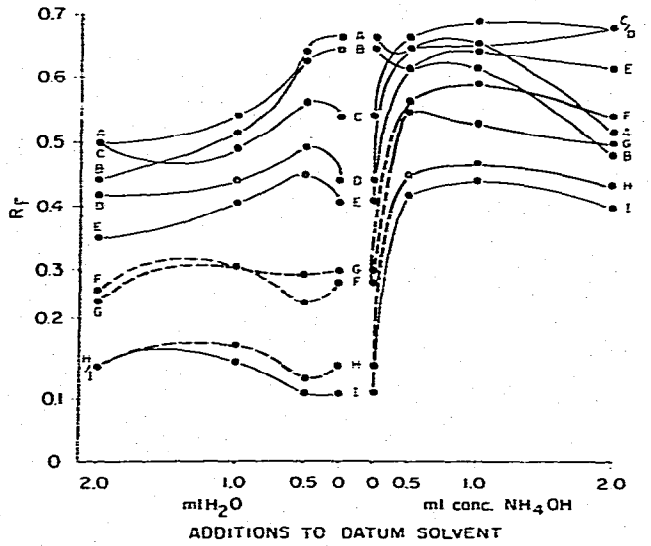


Fig. 1. Self-made silica gel (M-N) layers. Letters refer to the compounds listed in Table I. ●—●, No tailing; ●- - -●, extensive tailing.

Figs. 1–4 demonstrate R_F values for all compounds and variations in chromatospot character observed with CPZ, PRZ and PMZ, in the different TLC solvent and layer combinations examined. In all cases the low-level components afforded well-rounded chromatospots. However, variations in the definition of spots containing 200

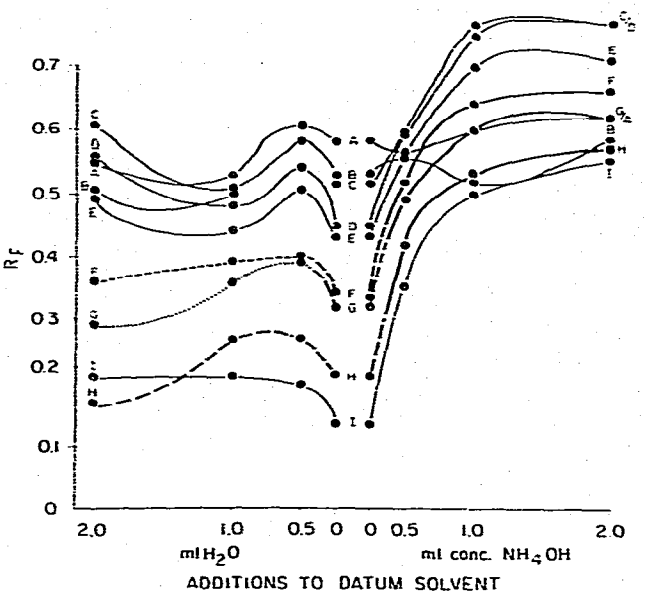


Fig. 2. Self-made silica gel (M-N) layers prepared with 0.1 *N* NaOH. Letters refer to the compounds listed in Table I. ●—●, No tailing; ●- - -●, extensive tailing; ●—●, tailing; ●···●, slight tailing.

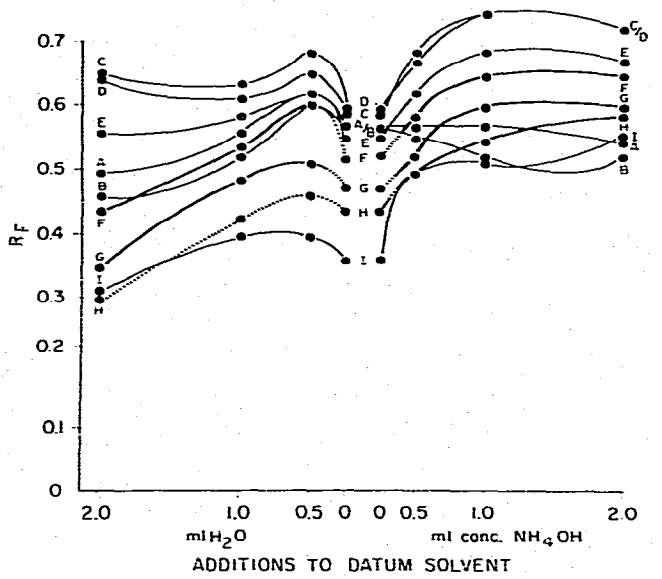


Fig. 3. Self-made silica gel (M-N) layers prepared with 0.5 *N* NaOH. Letters refer to the compounds listed in Table I. ●—●, No tailing; ●···●, slight tailing.

μg of compound were evident and the variations appeared to be more closely related to the nature of the solvent system than to the alkalinity of the silica gel layer.

Although strongly alkaline support layers prepared with 1.0 *N* sodium hydroxide utilized with the datum solvent did not give rise to tailing of chromatospots (Fig. 4).

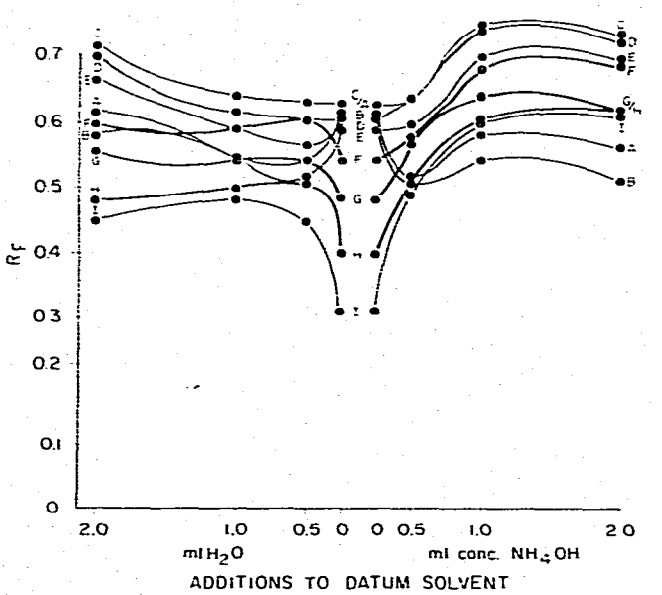


Fig. 4. Self-made silica gel (M-N) layers prepared with 1.0 *N* NaOH. Letters refer to the compounds listed in Table I.

less strongly basic layers, even with water in the datum solvent, did not eliminate tailing from all compounds (Figs. 2 and 3). On the other hand, the presence of 0.5 ml of concentrated ammonium hydroxide in the datum solvent with the neutral and alkaline layers eliminated tailing of the high-concentration chromatospots (CPZ, PRZ and PMZ). Tailing from chromatospots is not due to the spots being composed of mixtures of free bases and salts, owing to the presence of residual acidity in the layer or double spot formation[14], since TLC of the free bases from 200 μg of each of PCZ, PRZ and PMZ on alkaline (and neutral) layers in the datum solvent also gave rise to chromatospots with tailing.

The observed apparent increase in the capacity of TLC layers in the presence of ammonium hydroxide or appreciable quantities of water on alkaline layers appears to be the result of both deactivation of the layers and increased polarity of the solvent[15*].

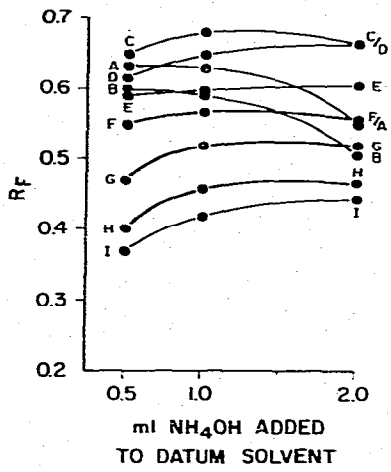
Water is not an effective layer deactivator in the cases being discussed here. TLC of free bases from 200-μg quantities of CPZ, PRZ and PMZ on neutral layers in the datum solvent containing water gives rise to chromatospots with extensive tailing which can be attributed to displacement of water from the silica gel active sites by the organic bases. Hydroxide and ammonia bind more strongly to active sites so that at sufficient concentration they extend the capacity of the layer[16]. These layer-deactivating species have Lewis basicity in the datum solvent similar to most of the compounds examined in this study so that compounds C to I inclusive exhibit similar R_F patterns. 2-Chlorophenothiazine and phenothiazine, compounds A and B respectively, which have pK_a values markedly below that of the other organic bases examined, exhibit R_F patterns clearly different from the stronger bases.

While ammonium hydroxide solution has proven to be an effective layer deactivator, water is not necessary in the solvent for the elimination of tailing in CPZ, PRZ and PMZ chromatospots. When the three compounds were chromatographed on neutral silica gel F60 (Merck) layers in the datum solvent into which ammonia gas had been bubbled, all compounds afforded precise round chromatospots without evidence of tailing or fronting. R_F values found were similar to those observed with aqueous ammonium hydroxide in the datum solvent.

In order to determine the generality of observations on chromatographic system variations, 200-μg samples of CPZ, PRZ and PMZ along with 1-μg samples of artifacts were chromatographed on four types of pre-coated layer and on self-made silica gel 60F (Merck) layers. The datum solvent containing 0.5, 1.0 and 2.0 ml of concentrated ammonium hydroxide was used. In all instances, CPZ, PRZ and PMZ (and low-level components) afforded chromatospots without visible tailing (Figs. 5–10). Although R_F patterns generally were similar to those observed with the self-prepared layers discussed above, there were differences on abrasion-resistant pre-coated layers.

Friable layers (self-made neutral and alkaline M-N, Merck Type 60 and Analtech (Newark, Del., U.S.A.) pre-coated silica gel G: Figs. 1, 5 and 6) that contained calcium sulphate as binder, all afforded similar R_F trend patterns, but abrasion-resistant pre-coated layers (Merck 60F, M-N Sil G254, and Analtech MH and HL)

* For a detailed discussion on factors influencing chromatographic behaviour of compounds, which is beyond the scope of this paper, refs. 12 and 16 are recommended.



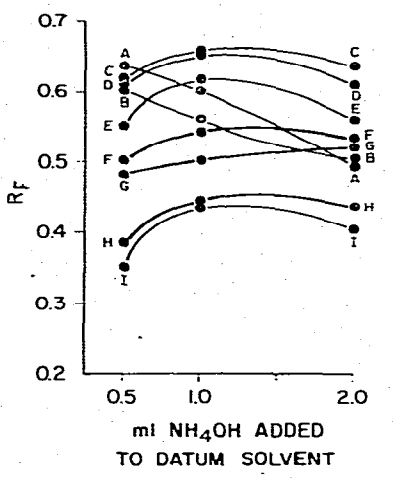
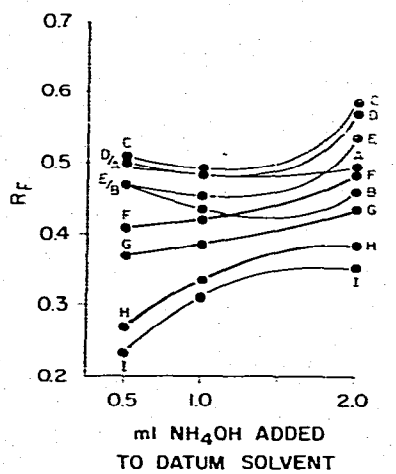


Fig. 5. Self-made silica gel Type 60 (Merck) layers. Letters refer to the compounds listed in Table I.

Fig. 6. Pre-coated silica gel G (Analtech) layers. Letters refer to the compounds listed in Table I.

with unknown proprietary binders gave rise to R_F trends different from soft layers but similar to each other (Figs. 7–10). Silica gel utilized in both the self-made and pre-coated Merck and M-N layers presumably are identical in each brand. Thus it would appear that the different R_F trends between these abrasion-resistant and soft layers are due to the effects of specialized binders and/or manufacturing processes for the preparation of abrasion-resistant pre-coated layers. Variations in these have been found to give rise to marked changes in layer characteristics[17].

CPZ was chromatographed on pre-coated Sil G-254 (M-N) layers to delineate the extension of linear capacity with solvent variations and the practical limits of use-



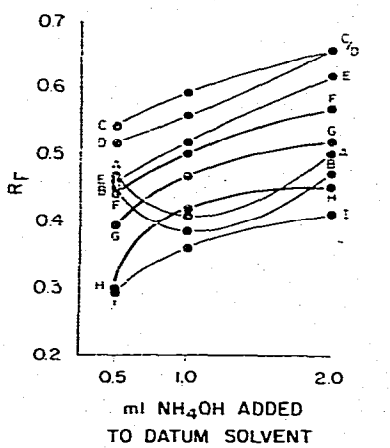
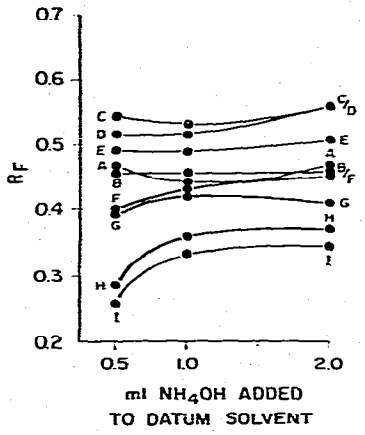


Fig. 7. Pre-coated silica gel F60 (Merck) layers. Letters refer to the compounds listed in Table I.

Fig. 8. Pre-coated Sil G-254 (M-N) layers. Letters refer to the compounds listed in Table I.



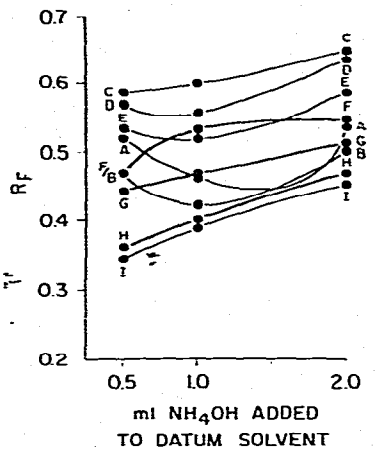


Fig. 9. Pre-coated silica gel MH (Analtech) layers. Letters refer to the compounds listed in Table I.

Fig. 10. Pre-coated silica gel HL (Analtech) layers. Letters refer to the compounds listed in Table I.

fulness* of the layer. Free base from CPZ was run on layers with the datum solvent containing 0.0, 0.5, 1.0 and 2.0 ml of concentrated ammonium hydroxide. In the datum solvent alone tailing was apparent at levels of 5 μg. However, in all solvents containing ammonium hydroxide the linear capacity and the limit of practical usefulness extended beyond 7500 μg. Trace-level impurities running behind the main chromatospot were not masked. The salt, CPZ, on layers with ammonium hydroxide in the solvent afforded lower sample capacities*. This probably is due to the effective concentration of ammonia being significantly reduced by hydrochloric acid released from CPZ. Thus in the datum solvent containing 0.5 ml of ammonium hydroxide 750 μg of CPZ was the maximum quantity that could be chromatographed without tailing although the linear capacity did not appear to have been exceeded. Datum solvent with 2.0 ml of ammonium hydroxide enabled over 4000 μg of CPZ to be run effectively.

CONCLUSIONS

It is apparent from this study that in the TLC of high levels of CPZ, PRZ and PMZ and related compounds in the datum solvent, there is no need to employ combinations of both alkaline silica gel layers and alkaline solvent systems to obtain good separations between high- and low-level components. These particular compounds can be readily chromatographed on neutral silica gel with datum solvent containing ammonium hydroxide. From the data presented one can rationally choose the optimum solvent combination for the analysis of the drug substance and related compounds.

Studies are being extended to ascertain the generality of this type of approach

* "Practical limit of usefulness" and "sample capacity" of the support layer here refer to the amount of compound that can be chromatographed without evidence of spot tailing or grossly asymmetric chromatospots.

for the separation of other basic drugs with emphasis on the separation of low-level components. Particular attention will be given to the role of ammonia as a silica gel deactivator[18].

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Poulenc Co., Montreal, Canada, in suppling samples of isopromethazine hydrochloride and 4-chlorpromazine hydrochloride and of Dr. N. J. Pound and Mr. A. G. Butterfield for most useful discussions.

REFERENCES

1 *British Pharmacopoeia*, Pharmaceutical Press, London, 1968, pp. 37, 135, 186, 293, 733.
2 *European Pharmacopoeia*, Vol. II, Maisonneuve, Paris, 1971, pp. 197, 206, 238, 242, 308, 324, 348.
3 *United States Pharmacopoeia*, Mack Publishing Co., Easton, 18th rev. ed., 1970, pp. 113, 123–125, 579.
4 J. Baümler and S. Rippstein, *Pharm. Acta Helv.*, 36 (1961) 382.
5 W. Paulus and R. Keymer, *Arzneim.-Forsch.*, 13 (1963) 609.
6 A. Noirfalise, *J. Chromatogr.*, 19 (1965) 68.
7 C. K. Fabierkiewicz and G. Cimbura, *J. Chromatogr.*, 53 (1970) 413.
8 I. Zingales, *J. Chromatogr.*, 31 (1967) 405.
9 E. Grusz-Harday, *Pharmazie*, 26 (1971) 562.
10 T. J. Mellinger and C. E. Keller, *J. Pharm. Sci.*, 51 (1962) 1169.
11 Lj. Kraus and E. Dumont, *Z. Anal. Chem.*, 252 (1970) 380.
12 L. R. Snyder, *Principles of Absorption Chromatography*, Marcel Dekker, New York, 1968, pp. 91–92.
13 W. N. French, F. F. Matsui, D. L. Robertson and S. J. Smith, submitted for publication.
14 B. Wesley-Hadzija, *J. Chromatogr.*, 79 (1973) 243.
15 E. Soczewinski and W. Golkiewicz, *Chromatographia*, 4 (1971) 501.
16 R. M. Scott, *J. Chromatogr. Sci.*, 11 (1973) 129.
17 W. N. French, F. Matsui and S. J. Smith, *J. Chromatogr.*, 86 (1973) 211.
18 A. Blomfield and L. H. Little, *Can. J. Chem.*, 51 (1973) 1771.