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Capillary supercritical fluid chromatography of aliphatic amines

Studies on the selectivity and symmetry with three different columns using carbon dioxide or nitrous oxide as mobile phase[a]

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ABSTRACT

The supercritical fluid chromatography of intact aliphatic amines with different columns is described. One group of amines was based on N,N-dimethyl-*n*-octylamine and related primary and secondary amines, and the other on the amino alcohol metoprolol and several of its analogues. Columns with three different phases were investigated, one non-polar coated with 5% phenyl methyl polysiloxane and two more polar with 25% cyanopropyl methylphenyl polysiloxane and Carbowax 20M. Generally, equal molar amounts were injected under splitless conditions and the peak symmetry was recorded.

The system with the non-polar silicone phase was more inert, followed by the wax-phase column. The cyanopropyl column gave severe peak tailing although it was loaded with five times more of the amines than the other columns. The selectivity was investigated and was found higher with the two polar columns. Both showed a marked increase in the retention of amines with free hydrogens. With nitrous oxide the selectivity was almost the same as that with carbon dioxide as mobile phase. The nature of the flame ionization detector changed, however, giving a negative baseline drift on pressure programming. An interesting conclusion is that the amines are chromatographed as such with carbon dioxide as the mobile phase.

INTRODUCTION

Many drugs contain aliphatic nitrogens. Primary amines do not occur so widely, although many tertiary amines are metabolized by dealkylation to primary amines

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or may contain trace amounts of the latter as impurities in drug substances. Today the method of choice for the monitoring of impurities is liquid chromatography with UV-detection. Accurate quantification requires a knowledge of the molar absorptivity, ε, which is not always the case with unknown impurities. Here, gas chromatography with flame ionization detection would be attractive, but the necessity to derivatize in order to prevent adsorption in the gas chromatographic system in principle precludes the use of this technique. The adsorption is often less pronounced with apolar stationary phases as in this instance the column walls can be highly deactivated in comparison with more polar phases, as these require some activity in order to be wetted before forming stable films. Still, the high boiling point of many amines often makes gas chromatography unattractive.

In supercritical fluid chromatography (SFC) there is a widespread conception that carbon dioxide can react with primary and secondary aliphatic amines. Few reports exist on the SFC of primary amines with carbon dioxide. Most chromatograms of tertiary amines have been recorded after split injection followed by density programming of the fluid, the actual amount introduced being virtually unknown. As with temperature programming in capillary gas chromatography, this mode of elution improves the peak shape. Under SFC conditions there are widely different opinions about the feasability of chromatographing primary amines without complications using carbon dioxide as the fluid. Except for several papers by Fields and co-workers[1–4], there are few publications that deal with the SFC of free amines in any detail. It has been suggested that a relationship between the basicity of an amine and its compatibility with carbon dioxide exists[5]. It was concluded that amines with pK_b below 9 would react[5], but these constants refer to aqueous solutions[2]. Fields and Grolimund[2] were able to show that this rule does not apply to tertiary alkylamines but for most secondary alkylamines except where the amine is sterically hindered. The presence of water may play a role but even under anhydrous conditions carbon dioxide reacted with diglycolamine and bis-2-propanolamine, as indicated by pressure measurements[6]. The use of alternative fluids such as dichlorodifluoromethane and sulphur hexafluoride has also been demonstrated[3,4].

David and Sandra[7] were not able to elute long-chain fatty acid amines by capillary SFC with nitrogen-selective detection. The column was ruled out as the cause as the amines could be gas chromatographed[7]. It is difficult to evaluate these observations as the amounts of the amines injected and the column temperatures were not given. As a conclusion the authors advocated derivatization of the amines with trifluoroacetic anhydride[7]. For the study of impurities derivatization will always be a drawback, the main reason being the possibility of different reaction kinetics of the components which might induce discrimination.

In a study on capillary and micropacked columns for SFC, Schomburg *et al.*[8] were unable to elute unspecified primary and secondary amines with carbon dioxide with several different supports. They suspected that if the amines do not react with carbon dioxide in the column itself to give insoluble carbamates, they might react at the elevated temperature in the restrictor zone. In this case the problem seems more likely to be due to the nature of the support materials investigated than to the mobile phase itself.

Nitrous oxide was selected as the mobile phase for basic compounds to eliminate the risk of reaction with analytes containing primary and secondary amine

functions, although mainly aromatic amines were studied[9]. A nitrogen-selective detector was chosen for its high selectivity and low detection limits, although it was found that the selectivity towards hydrocarbons was only *ca.* 200. Some adsorption in the column was also observed when free amines were analysed at 100 ppm or lower.

In capillary SFC the flame ionization detector is the most widely used. This detector offers good detectability and compatibility with some of the fluids most often used as mobile phases. Another important advantage is that equal amounts of hydrocarbons give the same signal independent of molecular weight. The signal is reduced when heteroatoms are introduced, but still the peak area can be used to calculate amounts with good accuracy.

The aim of this work was to investigate the selectivity for aliphatic amines using three capillary columns with different stationary phases available for SFC. We were also interested in observing the peak symmetry of the amines in the chromatographic system, as few data exist on this aspect in SFC. As discussed above, primary amines may react with carbon dioxide. Therefore, nitrous oxide was also studied in order to eliminate any artefacts with carbon dioxide and to see whether there were any significant selectivity changes.

EXPERIMENTAL

Apparatus

SFC was performed with an instrument constructed in our laboratories from an ISCO (Lincoln, NE, U.S.A.) μ-LC 500 syringe pump for fluid delivery, a Hewlett-Packard 5710A gas chromatograph as column oven and for detection and a Perkin-Elmer 56 recorder. The pump head was cooled to -15°C, when the pump was filled with liquid carbon dioxide or nitrous oxide, with an EK 12 cooling bath (Freon) from Hake (Karlsruhe, F.R.G.).

In all experiments the oven temperature was 100°C and the detector temperature 300°C. The inlet pressures of hydrogen and air to the flame ionization detector were 20 and 25 p.s.i., respectively. With nitrous oxide as the fluid it was necessary to lower the hydrogen pressure to 15 p.s.i. in order to reduce the detector noise.

The injection valve was a Valco (Houston, TX, U.S.A.) C14W.06 and all injections were made in the splitless mode. The column was attached to the bottom of this valve through a 5-cm piece of precut stainless-steel tubing of 0.01 in. I.D. and 1/16 in. O.D. (0.02 in. for the the DB-Wax column). The column extended *ca.* 0.2 mm from the stainless-steel tubing towards the rotor of the valve. At the other end of the tubing the column was sealed with a 15% graphite Vespel ferrule on a 1/16-in. stainless-steel Swagelock union. Ten seconds after injection the valve was returned to the load position. Frit restrictor (in 100 μm I.D. fused-silica tubing) (Lee Scientific, Salt Lake City, UT, U.S.A.) were shortened to *ca.* 8 mm, giving a linear velocity of 15 cm/s at 1500 p.s.i. carbon dioxide, the oven at 100°C and the detector at 300°C.

Columns

All capillary columns were 20 m $\times$ 100 μm I.D. fused-silica columns for SFC. They were SB-Phenyl-5, film thickness $d_f = 0.5$ μm, and SB-Cyanopropyl-25, $d_f = 0.25$ μm (both from Lee Scientific) and DB-Wax, $d_f = 0.10$ μm, from J&W (Folsom, CA, U.S.A.).

Fluids

The cylinder with carbon dioxide 3.5 (99.95% purity) and dipper tube was from AGA (Lidingö, Sweden), as was the nitrous oxide 2.0 (99.0% purity).

Chemicals

The simple aliphatic amines used are listed in Table I. They are available from Eastman Kodak (Rochester, NY, U.S.A.) and Fluka (Buchs, Switzerland). Metoprolol tartrate and analogues were from the Department of Organic Chemistry, AB Hässle.

TABLE I

ALIPHATIC AMINES STUDIED

Name	*Abbreviation*	*Structure*[a]
Simple aliphatic amines		
N,N-Dimethyl-*n*-octylamine	DMOA	$C_8H_{17}N(CH_3)_2$
N-Methyl-*n*-octylamine	MOA	$C_8H_{17}NHCH_3$
n-Octylamine	OA	$C_8H_{17}NH_2$
Tri-*n*-Butylamine	TBA	$N(C_4H_9)_3$
Di-*n*-Hexylamine	DiHxA	$NH(n\text{-}C_6H_13)_2$
Metoprolol and analogues		
Metoprolol	Meto	$ArCH(OH)CH_2NHCH(CH_3)_2$
H 105/29	tButyl	$ArCH(OH)CH_2NHC(CH_3)_3$
H 173/09	Ethyl	$ArCH(OH)CH_2NHC_2H_5$
H 98/52	PrimAmine	$ArCH(OH)CH_2NH_2$
H 170/64	Alcohol	$ArCH(OH)(CH_2)_3CH_3$
H 170/69	Amine	$ArCH_2CH_2NHCH(CH_3)_2$
Oxazolidone of metoprolol	Oxaz	$ArCH(O)CH_2N(CO)CH(CH_3)_2$

[a] Ar = 4-(2-methoxyethyl)phenoxy.

Sample solutions

The amines were dissolved in ethyl acetate and diluted to $2 \cdot 10^{-4}M$. In some instances a small volume of phosphate buffer (pH 12) was added to convert salts into free bases. This ethyl acetate stock solution was further diluted 1:5 (to $4 \cdot 10^{-5}M$). Owing to adsorption in the chromatographic system some amines were not diluted. The amounts loaded into the valve are given in the tables.

Calculations

After isobaric (isoconfertic) chromatography the capacity factor, k', was calculated. Ethyl acetate was assumed to be unretained. The asymmetry factor was calculated 10% up from the baseline and at as high a chart speed of the recorder as possible.

RESULTS AND DISCUSSION

Chromatographic system

As our aim was to study the SFC of aliphatic amines, maximization of the number of theoretical plates was not attempted. By using split or timed split injections, the column efficiency can be utilized better, but this injection mode is not practical when known, or reproducible, amounts are to be injected. In this work the molar amount injected was the same with a few exceptions. The amount is based on the nominal volume of the injection valve. This, however, can vary by as much as 30%[10]. With each column we studied the retention of the amines under constant conditions. The peak symmetry was also recorded and is given as the asymmetry factor. The three columns will be discussed in some detail under separate headings and then in comparison with their competitors. Thus the hold-up time in the chromatographic system will be different for the individual amines. Another approach would have been to obtain the same retention (capacity factor) for each amine, but then the actual density of the mobile phase would have been different. Representative chromatograms are given in Fig. 1 for (a) a neutral compound and (b) a secondary amine.

Capillary chromatography of amines

Chromatography of underivatized aliphatic amines is hampered by their polar nature, which causes interactions with polar and acidic sites in the chromatographic system. Generally the free silanol groups of the fused-silica column wall are responsible. In this study we chose one group of simple aliphatic amines as test compounds (Table I) and also one group based on the β-adrenoreceptor blocking agent metoprolol, which in addition to a secondary amine function has a secondary alcohol group in the molecule. Here compounds lacking either the amine or the alcohol were also available for comparison (Table I).

SB-Phenyl-5 column

This column was coated with methyl phenyl polysiloxane (substitution degree 5:95) and cross-linked for SFC use. In the group of simple aliphatic amines, with carbon dioxide as mobile phase, the symmetry is best for the two tertiary amines tributylamine and dimethyloctylamine (Table II). For the other amines in this group, with free hydrogens, the asymmetry factor is almost 2. The poor, and varying, symmetry for octylamine is not surprising. The superior symmetry of the dihexylamine peak can be explained by better shielding of the nitrogen of the *n*-hexyl group compared with the methyl group of N-methyloctylamine.

The retention as shown by the capacity factors are of the same magnitude for the octylamines. The dihexylamine is retained almost twice as much as tributylamine, both having the same number of carbon atoms. This is more likely to be a solubility effect than interaction of the free hydrogen with any silanol groups. The poor selectivity, and thus differentiating power, is even more evident with the metoprolol group. Here most capacity factors are about 0.5–0.6 and there is little difference if the alcohol is removed, 0.53 *vs.* 0.58. The symmetry improves with increased bulkyness of the alkyl substituent on the nitrogen atom. The *tert.*-butyl peak is symmetrical whereas the ethyl analogue has a marked asymmetry. The analogous compound without the nitrogen, the secondary alcohol, has a pronounced tendency to give leading peaks

TABLE II

RESULTS WITH SB-PHENYL-5 COLUMN AND CARBON DIOXIDE

Compound[a]	*Pressure (p.s.i.)*	*Capacity factor, k'*	*Separation factor, α (=k'/k')*	*Asymmetry factor*	*Amount injected on-column (in 60 nl) (ng)*
DMOA	1500	0.73	1.00	1.21	38
MOA		0.80	1.10	1.52	34
OA		0.65	0.89	1.80, 1.62	31
TBA		1.00	1.37	1.18	44
DiHxA		1.89	2.59	1.35	44
Meto	2500	0.58	1.00	1.17	64
tButyl		0.62	1.07	1.04	67
Ethyl		0.58	1.00	1.54	62
PrimAmine		0.55	0.95	n.m.[b]	56
Alcohol		0.53	0.91	0.88	67
Alcohol				0.82	22
Amine		0.52	0.90	1.16	60
Oxaz		0.93	1.60	0.98	23

[a] See Table I.
[b] Not measured.

with the amounts injected. It is also interesting that the oxazolidone derivative of metoprolol is strongly retained on this non-polar phase.

With nitrous oxide virtually the same observations can be made. The pressure was adjusted so that the capacity factors of tributylamine and metoprolol were almost the same as those with carbon dioxide. The data are listed in Table III. The

TABLE III

RESULTS WITH SB-PHENYL-5-COLUMN AND NITROUS OXIDE

Compound[a]	*Pressure (p.s.i.)*	*Capacity factor, k'*	*Separation factor, α*	*Asymmetry factor*	*Amount injected on-column (in 60 nl) (ng)*
DMOA	1250	0.74	1.00	1.21	38
MOA		0.77	1.04	1.83	34
OA		0.63	0.85	1.88	31
DiHxA		1.80	2.43	1.97	44
TBA		0.98	1.32	1.07	44
Meto	2150	0.55	1.00	0.99	64
tButyl		0.56	1.02	0.91	67
Ethyl		0.54	0.98	1.04	62
PrimAmine		0.53	0.96	1.53	63
Alcohol		0.47	0.85	0.65	67
Alcohol				0.61	22
Amine		0.48	0.87	1.07	60
Oxaz		0.95	1.73	0.67	69
Oxaz				0.74	23

[a] See Table I.

symmetry is perhaps slightly better for some of the compounds. This is more evident in the metoprolol group of compounds. The fact that the separation factor hardly changes on going from carbon dioxide to nitrous oxide means that nitrous oxide as fluid offers little from a selectivity point of view. This observation has also been reported by others[11,12].

The main interest here with nitrous oxide is that this fluid strongly suggests that the octylamine peak with carbon dioxide as the fluid is no artefact, *e.g.*, a carbaminic acid product. As the conditions of the flame ionization detector were not identical, comparison of peak areas may be misleading. A possible way to circumvent this problem would be to include markers with nitrogen atoms in the molecules.

The octylamines were also gas chromatographed at 100°C on an SE-54 capillary column (d_f = 0.25 μm). The capacity factors recorded were, in order of increasing molecular weight and substitution, 1.33, 1.85 and 1.91.

SB-Cyanopropyl-25 column

This column is coated with cyanopropyl phenyl methyl polysiloxane (substitution degree 25:25:50). For the chromatography of amines this column required five times more concentrated solutions to give any peaks that were useful for calculation of asymmetry and capacity factors. Polar compounds devoid of aliphatic amine groups posed no problem. With the simple aliphatic amines the symmetry is better for the tertiary than the secondary compounds but is still poor. The selectivity is more marked here than with the SB-Phenyl-5 column discussed above. The secondary amines are retained 4–5 times more strongly than the tertiary amines (Table IV). However, even in this system it is not possible to differentiate between metoprolol and its *tert.*-butyl analogue.

When nitrous oxide was available this column was not capable of giving any meaningful chromatograms of amines. The reason for the poor performance of this column with aliphatic amines is not clear. It may be related to incomplete deactivation and the acidic nature of the column wall silica. Another possible cause is the

TABLE IV

RESULTS WITH SB-CYANOPROPYL-25 COLUMN AND CARBON DIOXIDE

Compound[a]	*Pressure (p.s.i.)*	*Capacity factor, k'*	*Separation factor, α*	*Asymmetry factor*	*Amount injected on-column (in 60 nl) (ng)*
DMOA	1500	0.12	1.00	9	194
MOA		0.51	4.25	21	172
TBA		0.11	0.92	12.5	222
DiHxA		0.64	5.33	>28	222
Meto	3000	0.46	1.00	9.3	320
tButyl		0.45	0.98	20	335
Alcohol		0.22	0.48	0.78 0.83	67
Amine		0.22	0.48	30	300
Oxaz		1.77	3.85	1.12	68

[a] See Table I. OA, PrimAmine and Ethyl were not attempted.

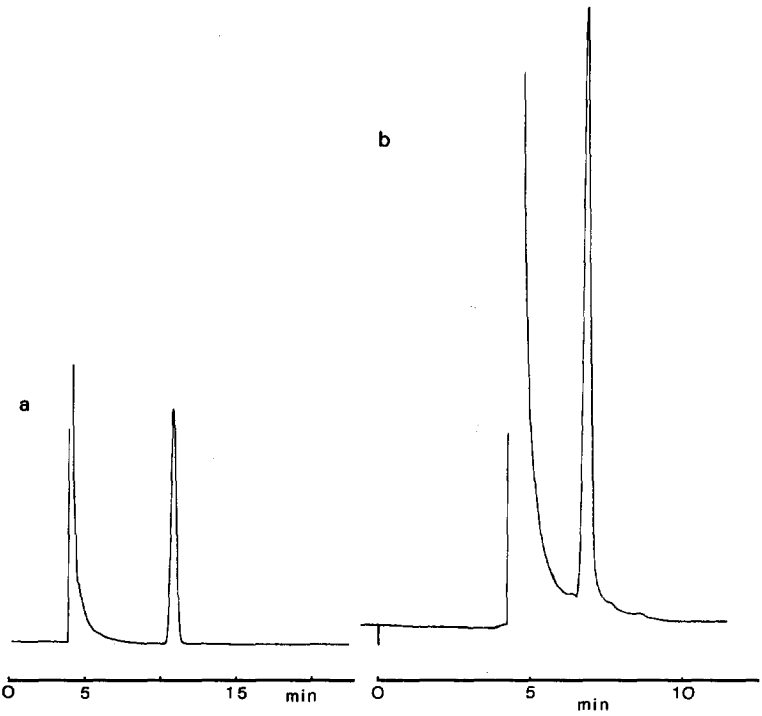

Fig. 1. (a) Chromatogram of metoprolol oxazolidone using carbon dioxide as mobile phase with an inlet pressure of 3000 p.s.i. The cyanopropyl column was kept at 100°C and 68 ng were injected. (b) Chromatogram of N-ethyl analogue of metoprolol using carbon dioxide as mobile phase with an inlet pressure of 2500 p.s.i. The 5% phenyl methyl column was kept at 100°C and 62 ng were injected.

phase itself. The cyanopropyl groups might have been oxidized to carboxylic groups to some extent. If so, the reasons why and how are unknown. Fields and Grolimund[4] described a poor performance of an old column as compared with a new column when analysing polar compounds with sulphur hexafluoride as mobile phase.

DB-Wax column

This column has a very thin film, only 0.10 μm thick. With the simple aliphatic amines the pressure used is below the critical pressure (P_c) of both carbon dioxide and nitrous oxide. The tertiary amines give symmetrical peaks whereas the secondary amines give severe tailing (Table V). Octylamine was difficult to chromatograph and it was necessary to increase the amount injected in order to obtain a peak. The capacity factors of the tertiary amines are low compared with those of the secondary amines, just as with the cyanopropyl phase, showing the interaction of the free hydrogens of the amines with the ether groups of the stationary phase. In the metoprolol group there is little difference in the symmetry between the three homologues. The retention differs considerably and from the pair with amine or alcohol only it is evident that the alcohol is more important for a high retention than the amine. It is perhaps not surprising that the oxazolidone requires a 400 p.s.i. higher inlet pressure for an acceptable elution time (Table V).

TABLE V

RESULTS WITH DB-WAX COLUMN

Compound[a]	*Pressure (p.s.i.)*	*Capacity factor, k'*	*Separation factor, α*	*Asymmetry factor*	*Amount injected on-column (in 60 nl) (ng)*
With carbon dioxide					
DMOA	950 (sub-P_c)	0.14	1.00	1.02	39
MOA		0.46	3.28	4.3	35
OA		0.74 ca	5.3	n.m.	104
TBA		0.14	1.00	0.81	44
DiHxA		0.78	5.57	5.0	44
Meto	2300	1.69	1.00	1.63	64
tButyl		1.42	0.84	1.58	67
Ethyl		2.48	1.47	1.78	62
PrimAmine		–			125
Amine		0.43	0.25	1.36	60
Alcohol		1.23	0.73	1.09	67
Oxaz	2700	1.99		0.85	68
With nitrous oxide					
DMOA	(800 sub-P_c)	0.14	1.00		
MOA		0.25	1.79		
TBA		0.11	0.79		
DiHxA		0.50	3.57		
OA		0.3	2.1		
Meto	1920	1.66	1.00		
tButyl		1.37	0.83		
Alcohol		1.2	0.73		
Amine		0.43	0.26		

[a] See Table I.

This column bled more than the silicone phase columns. The bleeding increased when the ethyl acetate solvent front had passed. Later this phenomenon was shown to be related to the nature of the solvent. On injecting ethyl acetate or toluene the bleeding and baseline instability were pronounced whereas this was not observed with hexane.

With nitrous oxide there was a marked difference in capacity factor for some of the simple aliphatic amines. Whether this is due to the nitrous oxide itself or to it being a liquid is not certain. In the metoprolol group the differences were small.

Choice of column

In this work only three columns were investigated. The most important criterion for selecting a certain column must be inertness. The support surfaces in capillary columns are not as inert as is usually presumed under SFC conditions[8]. The column surface deactivation is critical[4]. Owing to the poor peak symmetry of most amines and the large amounts required, the cyano column is out of the question for practical work. The second criterion is the selectivity. Although a large number of

TABLE VI

COMPARISON OF THE SELECTIVITIES WITH CARBON DIOXIDE AND NITROUS OXIDE AS THE MOBILE PHASE FOR AMINO ALCOHOLS

Compound[a]	*Column*			
	SB-Phenyl-5		*DB-Wax*	
	CO_2, 2500 p.s.i.	*N_2O, 2500 p.s.i.*	*CO_2, 2300 p.s.i.*	*N_2O, 1920 p.s.i.*
Meto	1.00	1.00	1.00	1.00
tButyl	1.07	1.02	0.84	0.83
Ethyl	1.00	0.98	1.47	–
PrimAmine	0.95	0.96	–	–
Alcohol	0.91	0.85	0.73	0.72
Amine	0.90	0.87	0.25	0.26

[a] See Table I.

plates can be achieved in SFC, this is at the expense of time. Therefore, the selectivity is of great importance. With this in mind, the Carbowax type of column might be of interest (*cf.*, Table VI). However, owing to the thin film this column will be of little practical value when looking for impurities at levels below 1% in, *e.g.*, drug substances, as the main component will easily overload and any separation of minor peaks in the close vicinity of the parent peak will be destroyed.

The capacity factors obtained with the SB-Phenyl-5 and the SB-Cyanopropyl-25 columns, using carbon dioxide as the fluid, are presented in Table VII. Although the film thickness differs it is striking how little hydrocarbons are retained on the polar column. Octanoic acid gave a good peak on the cyanopropyl column, which is as expected from the poor results with amines.

TABLE VII

COMPARISON OF CAPACITY FACTORS

Compound	*Column*	
	SB-Phenyl-5 (d_f = 0.5 μm)	*SB-Cyanopropyl-25 (d_f = 0.25 μm)*
Octanol	0.49	0.30
Octanoic acid	0.76	0.99
Undecane	0.57	–
Dodecane	0.72	–
Tetradecane	1.55	0.15
Octylamine	0.65	–
N-Methyloctylamine	0.80	0.51
N,N-Dimethyloctylamine	0.73	0.12

Carbon dioxide and amines

The results do not confirm the suggestion that carbon dioxide reacts with secondary and primary amines during capillary SFC. As the capacity factor of octylamine is the same with both carbon dioxide and nitrous oxide, and the peak areas are of the same order of magnitude, this suggests that the amines are chromatographed as such.

Changing the time in the system, with constant temperature and pressure, would give a change in peak area of the amine if it reacted to some extent during chromatography. Owing to the poor peak shape of octylamine this was studied with N-methyloctylamine. The detector temperature was varied between 150 and 350°C, as stopping the flow by keeping the valve in between inject and load gave broad peaks that were impossible to integrate properly. Although successful under certain circumstances[13], this mode would require a second valve in order to maintain a constant pressure in the column. The dead time thus varied between 8 and 13.4 min. The peak-area ratio *vs.* an inert marker was constant over the temperature range studied (1.21 ± 3.1%) and the area/height ratio changed only slighty. The last observation indicates that there is no significant peak broadening due to a low detector temperature and hence poor volatilization of the analyte in the frit restrictor used.

Nitrous oxide and flame ionization detector

With nitrous oxide as mobile phase, the noise of the flame ionization detector increased when the hydrogen and air flow-rates were as recommended by the manufacturer. The colour of the flame was distinctly white instead of faintly blue. The noise decreased when the hydrogen inlet pressure was reduced. On pressure programming the baseline decreased with increasing pressure. With carbon dioxide as the fluid it is normally the reverse and due to impurities. The difference in peak area for metoprolol oxazolidone was 20% between 2600 and 3200 p.s.i. in the isobaric mode (Fig. 2).

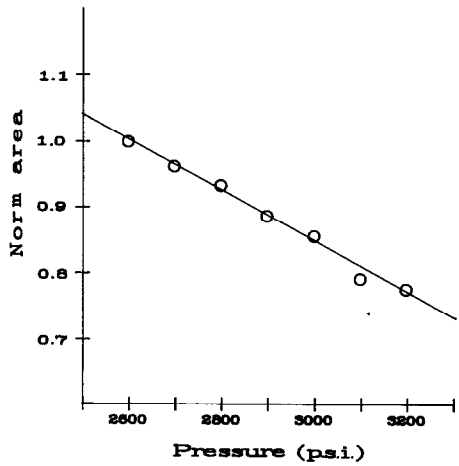

Fig. 2. Peak area *versus* pressure: SFC of metoprolol oxazolidone with nitrous oxide as mobile phase and flame ionization detection. Amount injected, 68 ng. Column: SB-Cyanopropyl-25. Each point is the average of three injections. For other conditions, see Experimental.

Apparently the flame is more oxidizing in nature and should perhaps be reoptimized for use with nitrous oxide as the mobile phase. A higher baseline level has been reported[12,14] and the detection limit was three orders of magnitude lower for hydrocarbons. Impurities have also been suggested to be involved[15]. The flame ionization detector response also changes with the density of carbon dioxide[16]. Hence, care should be taken when examining polymers where the individual oligomers elute at widely different densities, or erroneous results will be obtained.

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