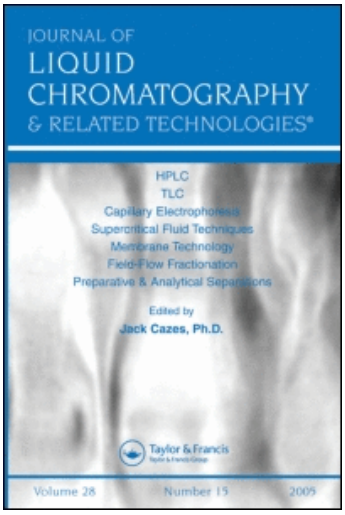

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS OF NITROSAMINES. I. CYCLIC NITROSAMINES

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ABSTRACT

The separation of cyclic nitros amines by HPLC was achieved using a cyclobond I column and a mobile phase of methanol/water. The effect of in-the-ring and on-the-ring substitutions on retention times were studied. The results show that the longer the chain of the substituent, the longer the retention time, which is consistent with the inclusion mechanism of separation. Also, the larger the ring, the longer the retention time. The results also show that the in-the-ring substitution has a dramatic effect on retention.

INTRODUCTION

The carcinogenicity of nitrosamines was first discovered in 1956 by Magee and Barnes (1). Since then increased research into the

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carcinogenicity of this group of compounds, the cyclic and acyclic compounds has been reported (2,3).

High performance liquid chromatographic separation of nitrosamines has been limited to small number of compounds and their metabolites (4,5). However, no concentrated effort into the separation of nitrosamines of different structures has been reported. In a literature search of Chemical Abstracts since 1975 no review on HPLC of nitrosamines was found. Due to their importance and the presence of some of them in the environment, work place and food chain, we felt it is important to produce reliable HPLC procedures for the separation and quantification of nitrosamines. Our work will be divided into separation of: (I) Cyclic nitrosamines; (II) Acyclic nitrosamines; (III) of positional isomers; and (IV) rotomers (syn and anti) of nitrosamines. A fifth project will concentrate on the extraction and separation of metabolites. This work is the first in the above series.

EXPERIMENTAL

Materials

The nitrosamines used in this study were synthesized in house and their structures were confirmed by mass spectrometry and nuclear magnetic resonance. Methanol was glass distilled uv grade (Burdick and Jackson, Muskegon, MI). Water was deionized glass distilled. The β-cyclodextrin bonded silica gel column (Cyclobond I) was purchased from Advanced Separations Technologies, Inc. (Whippany, NJ).

Apparatus

A Hewlett-Packard Model 1090 Liquid Chromatograph equipped with a photodiode array detector, an automatic injector, a strip chart recorder, a Hewlett-Packard Model 3392A integrator and a Hewlett-Packard Model 85 computer/controller was used. A Cyclobond I column, 250 mm x 4.6 mm, pre-packed with β-cyclodextrin bonded to 5 micron irregular silica gel particles

was used. Five ul of solution were injected and the absorption was monitored at 254 nm. The mobile phase was methanol/water as specified in the text, which was filtered and degassed before use and maintained under helium throughout the experiment.

RESULTS AND DISCUSSION

The separation of nitroso-1,3-oxazolidine, nitrosomorpholine and nitrosotetrahydro-1,3-oxazine was achieved on the Cyclobond I column using an isocratic mobile phase of 10% methanol: water (Figure 1). What is really interesting about this separation is not only the resolution of the 5 membered ring from the two 6 membered ring compounds but the separation of the nitrosomorpholine (a six membered ring) from the other 6 membered ring compound, (nitrosotetrahydro-1,3-oxazine), which are baseline separated by about 2.5 minutes, when the only difference between them is the position of the oxygen in the ring.

Figure 2 shows the separation of a group of cyclic nitroso compounds having a 5, a 6, a 7, and an 8 membered rings. The separation was achieved using a gradient of 25% methanol/water to 75% methanol/water in 20 min. using a convex curve, at a flow rate of 1 ml/min. Obviously, gradient elution was used in order to elute all the compounds in a reasonable period of time. As predicted, the smaller size molecules eluted first, using a lower percentage of methanol in the mobile phase.

Effect of substitution in the ring of cyclic nitrosamines on retention in the column was studied. The retention times (Table 1) of nitrosopiperidine (-CH_2-at the 4-position), nitroso morpholine (-O-at the 4-position), and piperazine (-N-N=O at the 4-position) show that substitution in the ring has a dramatic effect on the retention of cyclic nitrosamines. It seems that changing the methylene group by an oxygen atom, a retention time difference of 11 min., has a great effect on the structure, since we know that the retention mechanism using a Cyclobond I column is an inclusion

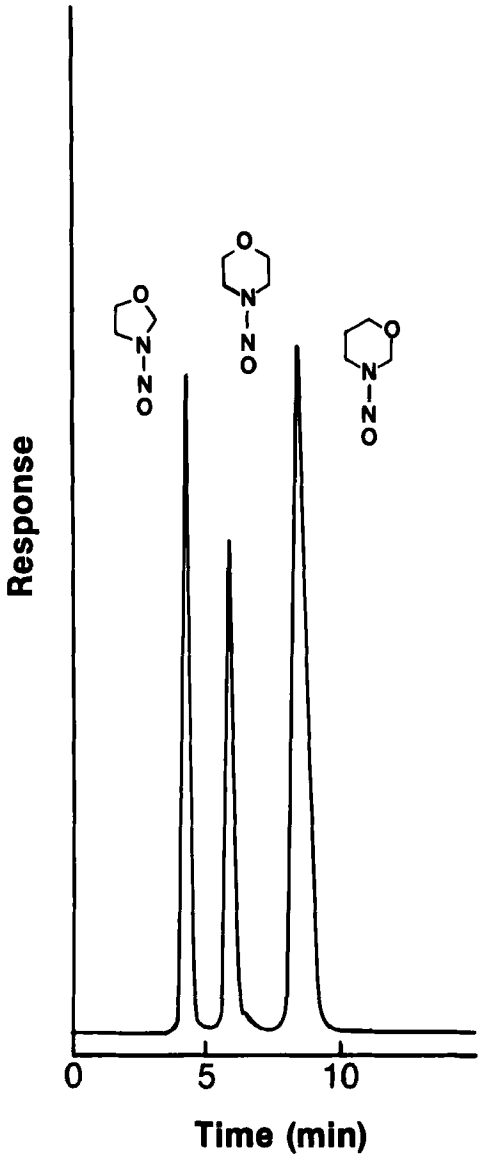


Figure 1. HPLC separation of nitroso-1,3-oxazolidine, nitrosomorpholine and nitrostetrahydro-1,3-oxazine using a cyclobond I column and a mobile phase of 10% methanol/water at a flow rate of 1ml/min. and detection at 254 nm.

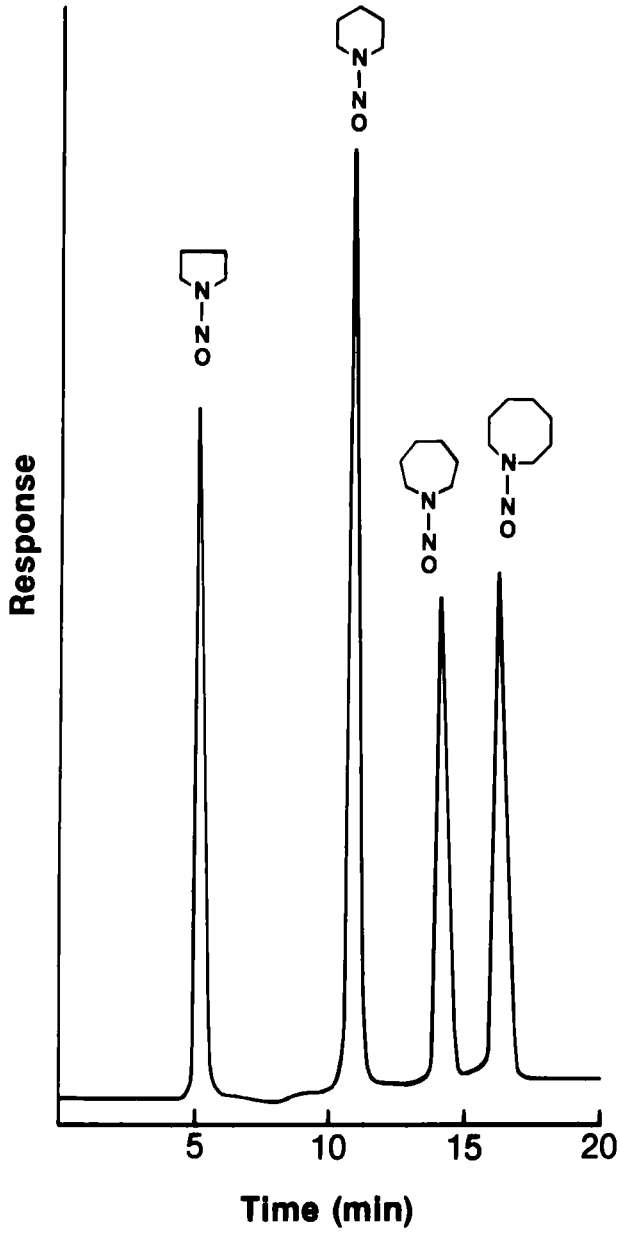
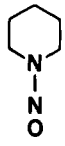
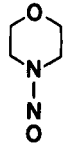
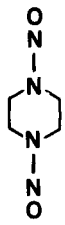


Figure 2. HPLC separation of a group of cyclic nitroso compounds having a 5, a 6, a 7, and an 8 membered rings using a gradient mobile phase of 25% methanol/water to 75% methanol/water in 20 min. at a flow rate of 1 ml/min. and detection at 254 nm.

Table I. Effect of in-ring substitution on the retention of cyclic nitrosamines using a 250 x 46mm cyclobond column and a mobile phase of 10% methanol/water at a flow rate of 1 ml/min. and detection at 254nm.

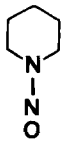
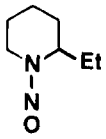
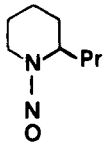
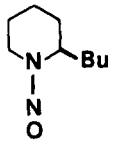
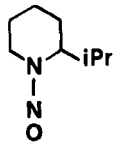
Compound	R_t* (min)
	15.7
	4.5
	3.9**, 4.2**

*10% methanol/water, 250 x 4.6 mm Cyclobond I column.
**Nitroso isomers.

one which is affected by the size and shape of the molecule. It is also possible that the polarity of the substituent (-N-N=O>-O->-CH_2-) may play a role in the retention of these compounds where the most polar is eluted first and the least polar is retained the most.

The effect of the chain length of the substituent on the ring on the retention of cyclic nitrosamines was also studied. The results are given in Table II. The results are consistent with the inclusion mechanism, where the smaller molecule should elute first. Note that the retention time difference between the n-propyl and isopropyl substituents is 9.3 min.,

Table II. Effect of chain length of substituent on retention of cyclic nitrosamines using a 100 x 4.6mm cyclobond I column and a mobile phase of 37% methanol/water at a flow rate of 1 ml/min. and detection at 254nm.

Compound	R_t* (min)
	3.8
	4.6
	5.5
	7.0
	14.8

*37% methanol/water, 100 × 4.6 mm Cyclobond I column.

which is again consistent with the effect of structure on the separation. It seems that the branching of the substituent, the isopropyl, has a larger effect on inclusion efficiencies than a straight chain. This and the effect of length to breadth ratios on retention are the topic of future research.

CONCLUSION

It is clear from the above that using a mobile phase of methanol/water and a cyclobond I column gives good separations of cyclic nitrosamines.

ACKNOWLEDGMENTS

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