Journal of Chromatography, 240 (1982) 243–246
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

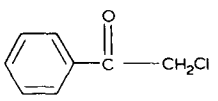
CHROM. 14,706

Note

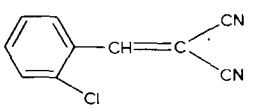
Reversed-phase high-performance liquid chromatography of some irritants

C. D. RAGHUVEERAN* and R. C. MALHOTRA
Department of Chemistry, Defence R&D Establishment, Gwalior-474002 (India)
(Received January 4th, 1982)

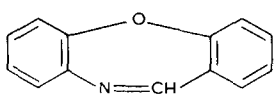
We report here the reversed-phase liquid chromatography of three commonly used irritants, *viz.*, ω-chloroacetophenone (CN), *o*-chlorobenzylidene malononitrile (CS) and dibenz[*b*,*f*]-1:4-oxazepine (CR).



CN



CS



CR

CN and CS have been used by military and police establishments for over two decades; CR is reported to be better than CN and CS in view of its mode of delivery and sustained irritant and lachrymatory action associated with low toxicity[1]. Sass *et al.*[2] reported a gas–liquid chromatographic (GLC) method of analysis of CN and CS. Blood levels of CS in animals exposed to aerosol sprays were determined by GLC by Leadbeater[3]. The same group[4] reported the detection and measurement in blood of CS H_2, a metabolite of CS, formed by the reduction of the olefinic bond in CS. No method for the determination of CR has previously been reported, and a high-performance liquid chromatographic (HPLC) procedure is described here.

EXPERIMENTAL

Apparatus

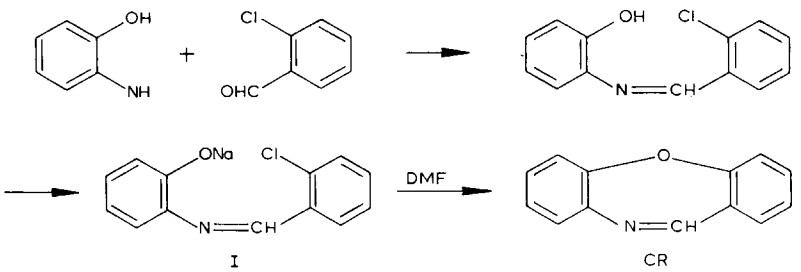
A Waters Model ALC-GPC-244 high-performance liquid chromatograph consisting of two Model 6000A pumps, a U6K injector, a Model 660 solvent programmer and a Model 440 absorbance detector was used. The separations were carried out with a μBondapak C_{18} column (Waters Assoc., Milford, MA, U.S.A.). Detection was carried out at 254, 280 and 313 nm.

Chemicals

Methanol and acetonitrile were of analytical reagent grade. Water, triply distilled in an all-glass distillation apparatus, was degassed by refluxing for 1 h and filtered over a 0.45-μm Millipore filter before use. CN was recrystallized from methanol. CS was synthesized by Knoevenagel condensation of *o*-chlorobenzaldehyde and

0021-9673/82/0000–0000/$02.75

malononitrile with alcoholic potassium hydroxide as catalyst and recrystallized from cyclohexane. CR was synthesized by a modification of the Higginsbottom and Suschitzky procedure[5] by condensing *o*-aminophenol with *o*-chlorobenzaldehyde. The sodium salt of the Schiff's base (I) so formed was cyclized to CR by refluxing in dimethylformamide (DMF):



I CR

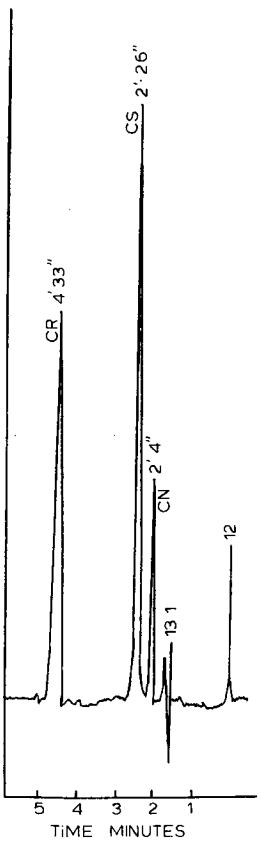


Fig. 1. Reversed-phase HPLC of CN, CS and CR. Column, μBondapak C_{18}; mobile phase, methanol–water (7:3); flow-rate, 2 ml/min; detection, 280 nm; 0.01 a.u.f.s.; chart speed, 1 cm/min; amounts, CN 40 ng, CS 24 ng, CR 30 ng.

The purities of the three compounds were established by spectroscopic methods, thin-layer chromatography and GLC.

Procedure

The mobile phase was methanol–water (7:3) mixed with a solvent programmer. Each solvent was pre-mixed with 10% of the other before use, as CR appeared to be unstable in methanol and water mixed as such, without pre-mixing. Acetonitrile was used for preparing solutions of all three compounds as CR tends to decompose in methanol. The flow-rate was maintained at 2 ml/min.

RESULTS

CN, CS and CR absorb at 254, 280 and 313 nm to different extents. Baseline separation of these compounds at 280 nm in the 10–100-ng range is shown in Fig. 1. Trace-level detection in the range 1–10 ng was carried out at 254 nm for CN and 313 nm for CS and CR. The latter two compounds absorb at 280 nm also, with the peaks of CR being larger than those at 313 nm for the same concentration and at the same sensitivity, *viz.*, 0.005 a.u.f.s. In spite of this, a calibration plot for CR shows better linearity at 313 than at 280 nm (see Fig. 2.). Calibration graphs for the three compounds are shown in Fig. 2 and are linear in the concentration range referred to.

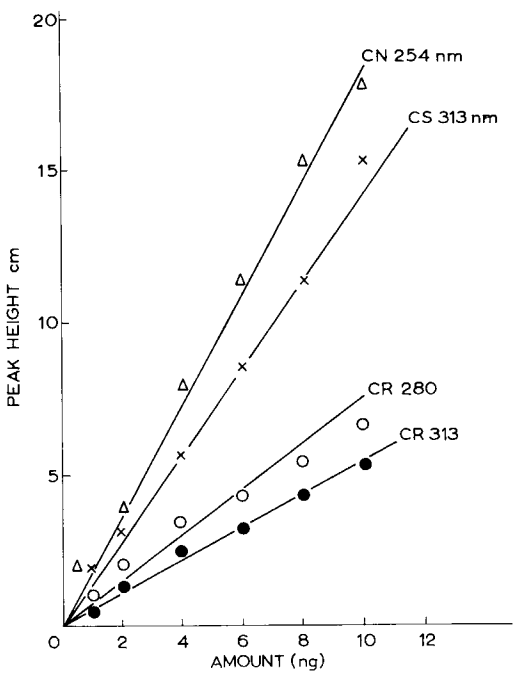


Fig. 2. Calibration graphs for CN (△), CS (×) and CR (○) at 280 nm and CR (●) at 313 nm.

The HPLC method reported here can serve as an adjunct to the GC electron-capture methods of Sass *et al.* and Leadbeater owing to its linearity in the 1–10-ng range, which is essential for quantitative determinations.

ACKNOWLEDGEMENTS

We are grateful to Dr. P. K. Ramachandran, Director, and to Dr. R. V. Swamy for their invaluable guidance and support, and to Dr. N. Raja for assistance with the instrumentation.

REFERENCES

1 B. Ballantyne, *Toxicology*, 8 (1977) 347.
2 S. Sass, T. L. Fisher, M. J. Jascot and J. Herban, *Anal. Chem.*, 43 (1971) 462.
3 L. Leadbeater, *Toxicol. Appl. Pharmacol.*, 25 (1971) 101.
4 L. Leadbeater, G. L. Salisbury and D. Utley, *Toxicol. Appl. Pharmacol.*, 25 (1971) 111.
5 Higginsbottom and H. Suschitzky, *J. Chem. Soc.*, (1962) 2367.