Journal of Chromatography, 138 (1977) 41–45

CHROM. 10,024

PHENONES: A FAMILY OF COMPOUNDS BROADLY APPLICABLE TO USE AS INTERNAL STANDARDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

APPLICATION TO THE ANALYSIS OF CARBOFURAN

E. J. KIKTA, Jr. and A. E. STANGE

FMC Corporation, Agricultural Chemical Division, 100 Niagara Street, Middleport, N.Y. 14105 (U.S.A.)

(Received February 28th, 1977)

SUMMARY

Phenones have been employed in high-performance liquid chromatography (HPLC) as a series of compounds forming a rational series of internal standards. It is shown that they are useful in systems as polar as methanol–water (1:1) and in systems as non-polar as methylene chloride–hexane (1:9). The potential in using a stable series of internal standards for many HPLC systems leading to improved quantitative precision and accuracy is discussed. An application to the analysis of carbofuran, a broad-spectrum insecticide–nematicide, is described and discussed.

INTRODUCTION

Leitch[1] described the advantages of using internal standards for precise and accurate quantitative analysis using high-performance liquid chromatography (HPLC). One of the important advantages in using an internal standard for quantitative analysis is that this technique allows for minor variation in column conditions. In HPLC, unlike gas chromatography (GC), work is still most often performed under ambient conditions. As an example, on a hot summer day, the temperature in our laboratory can typically vary from 20° in the morning to 32° by midafternoon. This has led to observed peak height variations of $\pm 6\%$ in many to the analyses performed. Since most of our assay work is performed via peak height calibration, such variation in height can lead to similar variation in both precision and accuracy. It is well known that internal standards, if properly chosen, can help eliminate this type of problem. A quick survey of the HPLC literature which involves quantitative analysis shows that most workers are still reluctant to employ internal standards, relying heavily on absolute quantitation. This is surprising since it has been shown[1,2] that the utilization of internal standards can lead to improved quantitation. Other references can be cited but it is not the purpose of this paper to review the use of internal standards.

In several publications, Grushka and Kikta[3–5] have used phenones as probe molecules for studying the dynamics of liquid systems. The solutions of phenones

used in these studies were prepared in solvents ranging from the polar methanol to the non-polar *n*-heptane. These solutions, which were generally prepared so that the concentration of the phenone was 1 $\mu g/\mu l$, showed excellent storage stability. Working samples were often stored at room temperature for periods extending from six months to a year with excellent stability and quantitative reproduction of peak height with little or no sign of decomposition products evident in the test chromatograms. Considering these observations and the fact that phenones ranging from the quite polar acetophenone to the much less polar myristophenone are readily available in a high purity state, typically 98–99%, from several manufacturers, they were chosen as an ideal series of compounds to be evaluated as internal standards for HPLC. This paper describes the range of use of phenones as internal standards in HPLC and shows an application to the analysis of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranylmethylcarbamate), a widely used insecticide–nematicide.

EXPERIMENTAL

Chemicals and reagents

Phenones were obtained as follows: acetophenone through hexanophenone (Aldrich, Milwaukee, Wisc., U.S.A.); heptanophenone through nonanophenone (Eastman-Kodak, Rochester, N.Y., U.S.A.); decanophenone through myristophenone (Pfaltz & Bauer, Stamford, Conn., U.S.A.).

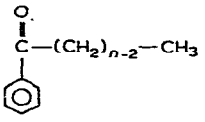
Hexane, methylene chloride and methanol for mobile phases were obtained from Burdick & Jackson (Muskegon, Mich., U.S.A.). Water for mobile phases was distilled and passed through a Barnstead (Boston, Mass., U.S.A.) combination column for ion exchange and organic removal.

Liquid chromatography

The liquid chromatograph employed consisted of equipment obtained from Waters Assoc. (Milford, Mass., U.S.A.). Two 6000 A pumps were controlled by a Model 660 solvent programmer. Injections were performed via a Model U6K injection valve. The detector was a Model 440 equipped for simultaneous detection at 254 and 280 nm. Data were collected on an Omniscribe® dual-pen chart recorder. Two columns (Waters Assoc.) were employed in this study: for normal phase work, a 30 cm × 3.9 mm I.D. μPorasil column was used while for reversed-phase systems, a 30 cm × 3.9 mm I.D. μBondapak C_{18} column was employed.

RESULTS AND DISCUSSION

Phenones typically show a broad absorbance with $\lambda_{max.} \approx 240$ nm with log $\varepsilon > 4$ making them detectable down to at least nanogram quantities with the common UV detector employed in HPLC. The general structural formula for a phenone is:



As mentioned before, phenones ranging from $n = 2$ (acetophenone) to $n = 14$ (myristophenone) are readily available. The variation in solubility properties within

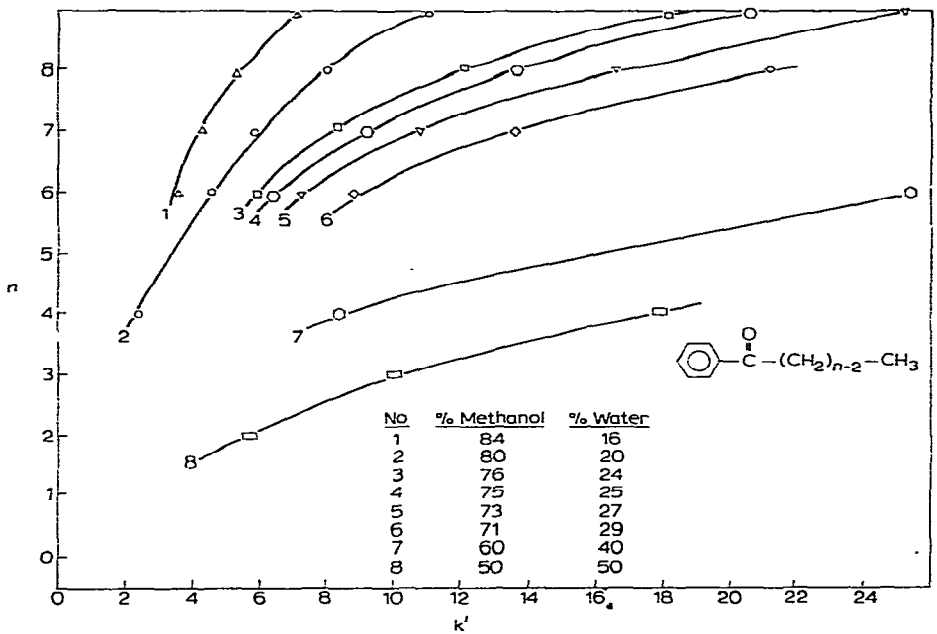


Fig. 1. Internal standard capacity ratio k' calibration for reversed-phase systems. n = Carbon side-chain length for various phenones. Column, Waters Assoc. μBondapak C_{18}; temperature, ambient ((≈ 27°).

this family of compounds is quite interesting and applicable to HPLC analysis. Acetophenone is quite soluble in methanol while myristophenone shows enhanced solubility in non-polar solvents such as hexane or heptane. Fig. 1 shows an internal standard calibration chart for reversed-phase systems. It should be noted that for a wide variation in the water–methanol ratio, excellent adjustment of the capacity ratio, k', is obtained. Fig. 2 illustrates a similar normal phase calibration using higher phenones. The data presented in Figs. 1 and 2 bracket the polarity ranges used in more than 95% of all HPLC systems. Thus, phenones form a widely applicable series of compounds suitable for use as internal standards. The phenones probably should not be used with compounds containing primary amine residues since the possibility of reaction does exist, especially if a temperature-controlled system is employed above 50°. Yet even with this limitation, we suggest that phenones will be useful internal standards for at least 90% of all HPLC systems employed today. Data precision in our laboratory, which was estimated to be poor (±6%), has improved using the internal standard technique to a maximum veriation of ±1% with ±0.5% being more typical.

We have applied this internal standard system to the analysis of carbofuran. Carbofuran has been widely analyzed by GC[6,7]. A reported LC method does not show wide applicability to real samples[8]. Many metabolic and decomposition products of carbofuran have been identified[6,7,9]. Fig. 3 shows an analytical separation of some of these compounds employing the internal standard of choice, *n*-butyrophenone. Samples are prepared so that each injection contains 0.6–6 μg of carbofuran and 2–3 μg of internal standard. Over this range, a calibration curve is set up so that

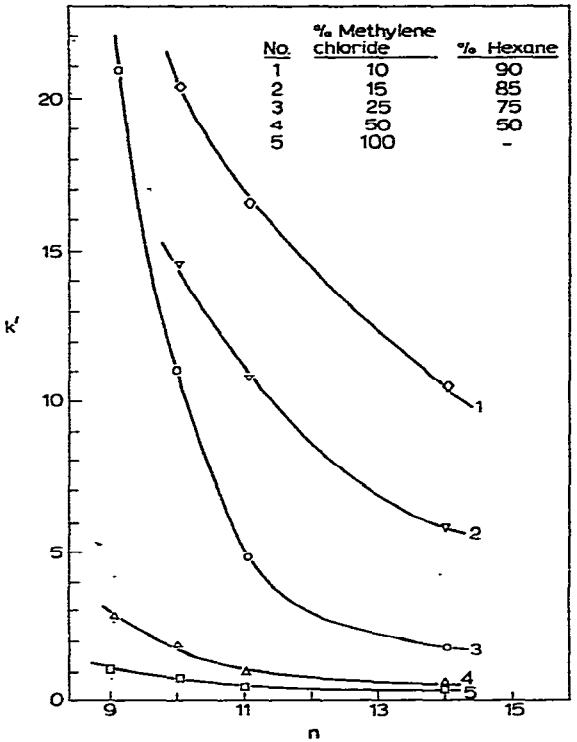


Fig. 2. Internal standard capacity ratio calibration for normal phase systems. n = carbon side-chain length for various phenones. Column, Waters Assoc. μPorasil, temperature, ambient ($\approx$ 27°).

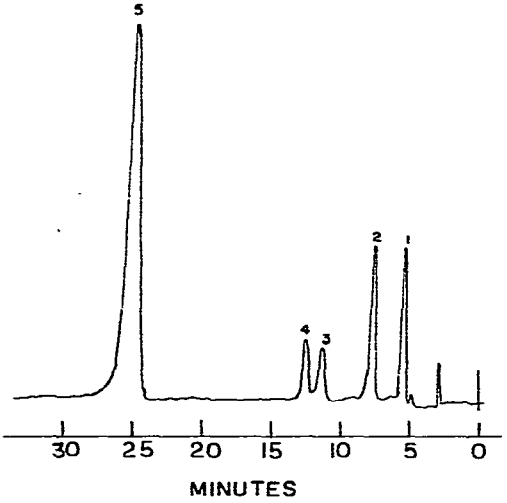


Fig. 3. Analytical separation of carbofuran and some common impurities. Column, μBondapak C_{18}, 30 cm × 3.9 mm I.D.; temperature, 31.5°; solvent system methanol–water (1:1); flow-rate, 1 ml/mm. Detection at 280 nm, 0.02 a.u.f.s. Peaks: 1 = 2,3-Dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate; 2 = 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl methylcarbamate; 3 = carbofuran; 4 = 2,3-dihydro-2,2-dimethyl-7-benzofuranol; 5 = n-butyrophenone. Pressure, 700 p.s.i.

the amount of sample (μg) is plotted *versus* relative height. (There is no point to showing a straight line plot.) We have observed no deviations from linearity in preparing such a calibration curve within the previously detailed limits of experimental error. The results obtained via the LC method are found to be of equal quality to those obtained in our laboratory using GC methods[6,7].

The use of phenones as a rational series of internal standard compounds shows great promise. We are currently exploring their utilization in other analytical systems.

REFERENCES

1 R. E. Leitch, *J. Chromatogr. Sci.*, 9 (1971) 531.
2 C. D. Chandler, G. R. Gibson and W. T. Bolleter, *J. Chromatogr.*, 100 (1975) 185.
3 E. Grushka and E. J. Kikta, Jr., *Anal. Chem.*, 46 (1974) 1370.
4 E. Grushka and E. J. Kikta, Jr., *J. Amer. Chem. Soc.*, 98 (1976) 643.
5 E. J. Kikta, Jr. and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
6 R. F. Cook, R. P. Stanovick and C. C. Cassil, *J. Agr. Food Chem.*, 17 (1969) 277.
7 R. F. Cook, in G. Zweig (Editor), *Analytical Methods for Pesticide and Plant Growth Regulators*, Vol. VII, Academic Press, New York, N.Y., 1973, pp. 187–210.
8 *Liquid Chromatography of Carbamate Pesticides*, Technical Report EPA-R2-72-079, U.S. Environmental Protection Agency, Covallis, Ore., 1972.
9 Metabolism of Pesticides, an Update, U.S. Department of the Interior, Fish and Wildlife Service, Special Scientific Report, Wildlife No. 184, 1974, pp. 79–81.