Journal of Chromatography, 243 (1982) 1–8
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,793

QUANTITATIVE ANALYSIS OF COMPONENTS OF UNRESOLVED GAS CHROMATOGRAPHIC PEAKS BY ELECTRON-CAPTURE DETECTION WITH OXYGEN SENSITIZATION

J. A. CAMPBELL and E. P. GRIMSRUD*
Department of Chemistry, Montana State University, Bozeman, MT 59717 (U.S.A.)
(Received January 4th, 1982)

SUMMARY

A method for the quantitative analysis of unresolved gas chromatographic peaks is described which should be useful in many instances for the trace analysis of structural isomers which are sensed by the electron-capture detector (ECD). A determination of unseparated isomeric components of a peak is made by obtaining a chromatogram of the sample without and with added oxygen in the ECD. The method is shown to be successful if the magnitude of the response enhancement caused by oxygen is dependent on the structural differences within the set of isomers. The method is applied here to mixtures of the three isomers of chloroanthracene.

INTRODUCTION

For the analysis of organic mixtures, methods which first separate components by gas chromatography have been extremely successful. Nevertheless, in dealing with complex samples such as are typically encountered in environmental analyses, incomplete separation of some of the sample components can generally be expected. Even in cases where each of the peaks in a chromatogram appear to be caused by a single chemical substance, the possibility that two or more distinct compounds have eluted with the same retention times provides an ever present source of uncertainty. In many cases the problems associated with unresolved peaks can be solved by the use of detectors which are capable of differentiating between the coeluting compounds. Mass spectrometry is often used for this purpose and is almost always successful for the cases where the coeluting chemicals are structurally quite different and their similarity of retention times is due to a coincidence of the solute–stationary phase interaction. For the cases where the equality of retention times is due to similarity in structure, however, the combined powers of gas chromatography and mass spectrometry are frequently insufficient to solve these analysis problems. This is because the conventional forms of mass spectrometry are frequently unable to differentiate between a set of structural isomers having the same molecular formula. In addressing these challenging determinations, another means is provided by the use of multiple detectors, each of which responds to the sample by a fundamentally different interac-

0021-9673/82/0000–0000/$02.75

tion. This approach can be successful provided that the response of at least one of the detectors is unique to each of the various components of the peak and the measured response ratio of the multiple detectors is uniquely traceable to each component.

We will demonstrate here the use of a simple electron-capture detection scheme for the analysis of unresolved chromatographic peaks where the three isomers of chloroanthracene serve as the analyte. A desirable feature of this detection method is that the two detectors used are physically the same one which is made to respond differently in repeated analysis simply by the addition of a small amount of oxygen to the nitrogen carrier gas. The cause of electron capture sensitization by oxygen has been discussed several times previously[1–4]. Briefly, for cases where oxygen sensitization occurs, the response of the oxygen-doped electron-capture detector (ECD) is thought to be proportional to the rate of reaction of O_2^- with the sample molecule, while the normal ECD response is thought to be proportional to the rate of the reaction of the gaseous electron with the analyte. We have shown in previous studies that the ratio of response of the oxygen-sensitized and normal ECDs to various sets of geometric isomers are often varied and dependent on structural detail even for cases where mass spectra offer no detectable differences. In this report the quantitative analysis of the isomers of chloroanthracene is demonstrated by this method where absolutely no separation of the isomers is provided by the chromatographic function performed by a short packed column and where only partial separation is provided by a capillary column.

EXPERIMENTAL

The gas chromatograph used is a Varian 3700 with constant-current, pulse-modulated operation of its ^{63}Ni ECD. The carrier gas is prepurified nitrogen maintained at a flow-rate of 40 ml min. The packed column used was made from 1/8-in. stainless tubing of 1.5-ft. length and was packed with 4% OV-101 on Chromosorb W. Oxygen was added as a make-up gas after the column and immediately ahead of the detector. By combining the carrier gas with *ca.* 7 ml/min of nitrogen containing 2% oxygen, an oxygen concentration of *ca.* 3 parts per thousand is maintained in the doped detector. From one determination to another, a precise level of oxygen is fine-tuned by adjusting the make-up gas flow until a preselected magnitude of baseline frequency is observed. The temperature of the oven for packed column separations is 170°C. The injector is at 200°C.

All of the compounds studied were purchased from commercial suppliers. Standards were prepared by dilution into benzene. Aliquots of 1 μl were syringe-injected into the normal injection port of the instrument. Sample sizes sufficient to produce small, but easily measurable, peaks were chosen. These were from 1 to 10 ng per injection. Each prepared mixture of the isomers was analyzed at least three times without oxygen and at least three times with oxygen. Peak heights were reproducible to within 3–5%. The average of these repeated analyses were used for the response enhancements reported here.

An analysis is also reported here where an SE-52 capillary column (15 m × 0.25 mm I.D.) was used. The carrier gas is then He and the make-up gas is nitrogen. For the oxygen-sensitized response, the make-up gas is mixed with oxygen-containing nitrogen as described above so that the same predetermined level of baseline

frequency and oxygen concentration is selected. The oven is temperature-programmed as follows: 90°C for 3 min, 20°C increase per min to 200°C, hold at 200°C for 10 min. For capillary-column analyses, the injection port is modified to reduce its volume. A splitless injection of 0.4 μl is used where each injection contains at total of *ca.* 0.3 ng of each chloroanthracene isomer.

RESULTS AND DISCUSSION

The three isomers of chloroanthracene were chosen for this demonstration because their retention times on a short packed column will be identical and because these compounds are readily detected with an ECD. Also, this example is one where a distinction between isomers is not expected to be provided by the conventional forms of mass spectrometry. To verify this we have obtained the electron-impact mass spectrum of each of the chloroanthracene isomers. These are shown in Fig. 1. Indeed, the mass spectrum of each appears to be identical. In Fig. 2 typical chromatograms are shown from which the determinations to be reported here are obtained. The first three pairs of chromatograms are for the pure isomers of chloroanthracene and the last pair is for a mixture of all three chloroanthracenes. For each pair of chromatograms the lower one is obtained by normal electron-capture detection and the upper one is obtained with approximately 3.0 parts per thousand oxygen continuously present in the detector. It is seen that the response of each of the three pure isomers is enhanced by oxygen's presence, but by differing magnitudes. The response to the 1-isomer is enhanced by 4.0, to the 2-isomer by 6.9 and to the 9-isomer by 21.0. In addition, the data in Fig. 3 indicate that the magnitude of the response enhancements observed for each isomer is a relatively constant value over at least two orders of magnitude change in concentration above the lowest concentration levels used here.

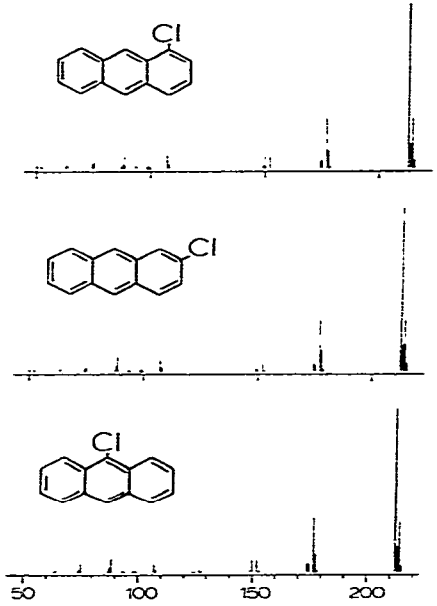

Fig. 1. Electron-impact mass spectra of three isomers of chloroanthracene.

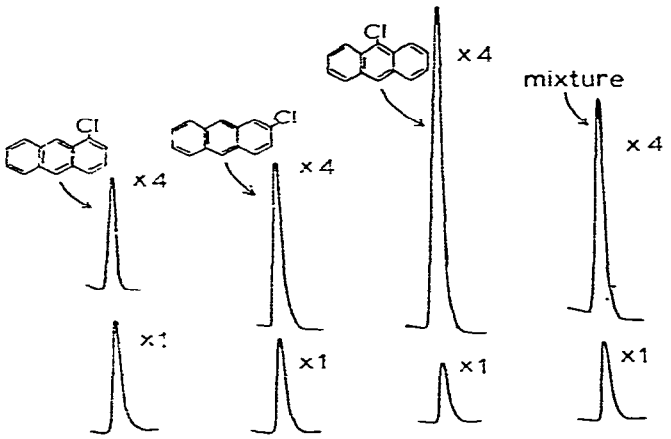

Fig. 2. ECD responses to pure isomers of chloroanthracene and a mixture of isomers without oxygen (lower chromatograms) and with 3 parts per thousand oxygen (upper chromatograms) in the detector at 300 C. Sample amounts are 2.9, 2.3 and 1.5 ng for the 1-, 2- and 9-isomers, respectively. The mixture contains molar fractions of these of 0.35, 0.28 and 0.37, respectively.

If the composition of mixed peaks are to be determined from the measured response enhancement it is necessary that the enhancement of a mixed peak, RE_{mix}, be expressable as the sum of the contributions of the individual components as shown in eqn. 1.

$$RE_{mix} = \sum_i X_i \, RE_i \tag{1}$$

where RE_i is the oxygen-caused response enhancement of the individual components, X_i is the molar fraction of substance i in the mixed peak and $\sum_i X_i = 1$. In order to test the validity of this relationship the response enhancements of many different

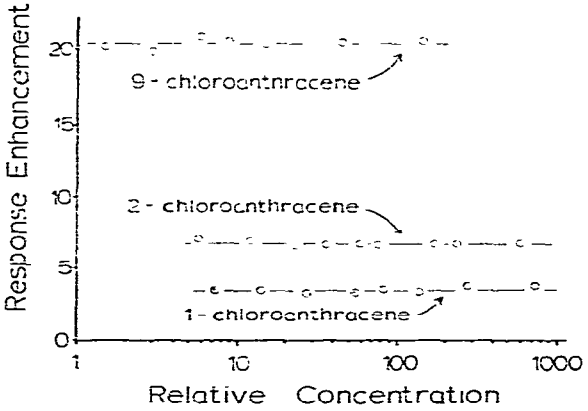

Fig. 3. Oxygen-induced response enhancements for the chloroanthracenes as a function of concentration. A relative concentration value of 1.0 corresponds to 10 pg injected.

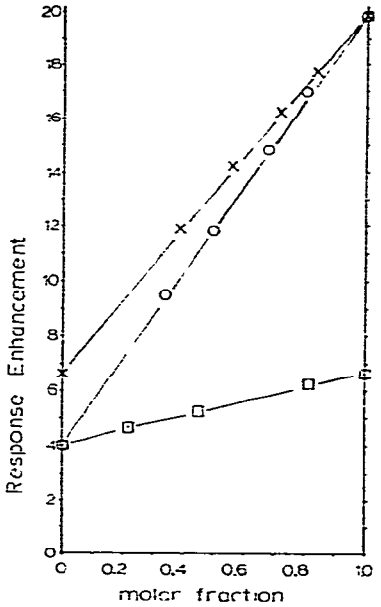

Fig. 4. Response enhancements of two-component mixtures as a function of the molar fraction of one of the components. The mixtures are composed of 1- and 2-chloroanthracene (□), 1- and 9-chloroanthracene (○), and 2- and 9-chloroanthracene (×).

mixtures of the chloroanthracenes have been measured. For the three possible sets of two-component mixtures, the results are shown in Fig. 4, where the measured enhancement is plotted as a function of the molar fraction of one of the sample components. These data indicate that the measured enhancements are linearly related to the molar fraction of each component and that eqn. 1 does, indeed, describe the response enhancement expected for a binary mixed peak. It would be relatively straightforward, therefore, to determine the relative amounts of two isomers known to be present in a mixed binary peak of unknown composition. For these cases the unknown quantities sought, X_1 and X_2, are obtained from application of eqn. 1 and the trivial relationship $X_1 + X_2 = 1$.

It is reasonable to expect that eqn. 1 will also be applicable to three component peaks. The last pair of chromatograms in Fig. 1 is seen to support this expectation, since response enhancement observed for this mixture is 11.2. From the enhancements measured for the individual isomers and from the known molar ratios of each in the mixture, a value of 11.1 is predicted ($0.35 \times 4.0 + 0.28 \times 6.9 + 0.37 \times 21.1 = 11.1$). Unfortunately, for the three-component system the relative composition of an unknown mixed peak is not uniquely determined by a single enhancement measurement as in the binary case. In this case a single RE_{mix} value has many possible solutions when applied to eqn. 1 since there are then three unknowns and only two equations relating them. For the chloroanthracenes under consideration here a solution to this problem is provided by repeating all enhancement measurements at a different detector temperature. The above measurements have indicated that at 300°C, the measured enhancements are $RE_1 = 4.0$, $RE_2 = 6.9$, $RE_9 = 21.1$ and $RE_{mix} = 11.2$. With a detector temperature of 350°C, these enhancement measure-

ments are found to be 2.5, 3.4, 15.6 and 7.6, respectively. With the data at 350°C eqn. 1 can be used a second time to provide the third equation necessary to determine uniquely the molar ratios in the three-component mixtures. Applying this procedure to the synthesized mixture of the three isomers, the data indicate that the molar ratios are $X_1 = 0.32$, $X_2 = 0.31$ and $X_9 = 0.37$. These values are in good agreement with the known composition of this sample (indicated in Fig. 2).

In solving the three-component problem it is essential to recognize that the new set of RE_i values obtained at a second detector temperature must not be proportionally related to the original set. That is, if the new set were simply half, for instance, of the original set, no additional information will be obtained by the analysis at the second temperature. The reason for this is made clear by inspection of eqn. 1 which is seen to be unchanged if all RE values are simply altered by a proportional factor. Also for this reason, no new information concerning the sample is expected by altering the amount of oxygen used in the detector for the enhancement measurement. We have previously shown[1,2] for the constant-current ECD that this change merely increases or decreases all RE values in proportion to the concentration of oxygen.

Also with this detection scheme, it is important to recognize the situations where peak height rather than peak area can be used as the measure of detector response. With the short packed column used here, the retention times of the three chloroanthracenes were indistinguishable. In this case their peak height or peak area could be used as a measure of response and the same values for RE_{mix} and RE_i are obtained (this must be as long as the normal and oxygen-sensitized responses are linearly related to sample concentration over the concentration range of interest).

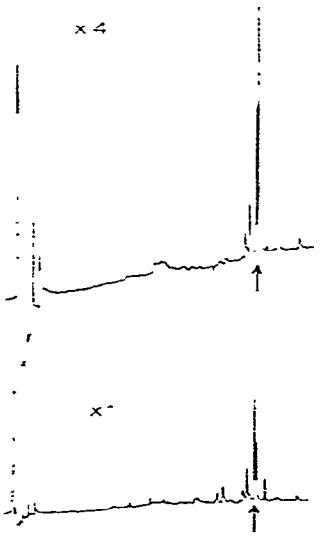

Fig. 5. Capillary column gas chromatograms of the three-component mixture described in Fig. 2. The arrows indicate the point of elution of the chloroanthracene isomers. The lower chromatogram is with normal electron-capture detection and the upper is with 3 parts per thousand oxygen in the make-up gas. Detector temperature is 300°C.

However, for those cases where the components of a mixed peak are partially separated by the column, care must be taken to use peak area, only, as the measure of response. Then the method as outlined above can be used for the quantitation of a partially resolved mixed peak. For these cases the use of peak height as the measure of response will cause systematic error because at the instant of the peak maxima, the molar ratio of components in the detector differs from the molar ratios of these components in the original sample.

For chromatographic separations where partial resolution of a mixed peak is observed, the use of oxygen sensitization can also be helpful in providing an indication of nature of that partial separation. Consider, for example, the two chromatograms shown in Fig. 5. The same mixture of the three chloranthracenes as was previously considered is now partially separated into a doublet by use of a capillary column. The question one might then ask is to which portions of this doublet do the individual isomers contribute. This question could be answered by very precise measurement of the retention times of the individual components or by the analysis of several prepared standards containing varied and known amounts of each of the three isomers. Alternatively, this information is provided very clearly and simply by repeating the analysis once with oxygen in the detector. By a comparison of the two chromatograms in Fig. 5, it is evident that the second peak of the doublet is due to the 9-isomer, alone, since it is enhanced by about 21 times by oxygen. The first peak is enhanced by 5.4 times and, therefore, is a composite of approximately equimolar quantities of the 1- and 2-isomers. Since the sample analysed here is the same three-component mixture shown previously in Fig. 1, the above deductions are correct.

It is anticipated that the detection scheme described here could be useful for the analysis of many electron capture-active compounds where the relative amounts of potentially present structural isomers is of interest. In these applications it will be necessary to determine whether the set of isomers under consideration meet certain minimum requirements of the method demonstrated here for the chloroanthracenes. Firstly, the measured response enhancements of the pure isomers must be unique for each isomer. Secondly, the normal and the oxygen-sensitized responses of the ECD must be linearly related to the concentration of each analyte over the concentration range of interest.

In the experiments described here, the precision of measurement was limited mainly by the necessity of measuring the enhancement by performing separate, paired chromatographic analyses, without and then with oxygen in the detector. Since our ability to reproducibly inject samples was estimated to be of the order of a few percent, the reproducibilities of the enhancement measurements reported here are about 5% (relative standard deviations). In order to increase the precision with which the enhancement measurements can be made, we intend to explore the use of two identical ECDs placed in series where the second detector contains added oxygen. Except for the most strongly responding molecules, the ECD can be considered a non-destructive detector. Therefore, with this tandem arrangement, one can expect that each detector will receive essentially the same quantity of analyte and the reproducibility of oxygen-induced enhancement measurements may be improved significantly. This improvement should similarly improve the quantitative accuracy of the analysis method described here.

ACKNOWLEDGEMENT

This paper is based on work supported by the National Science Foundation under grant number CHE-7824515.

REFERENCES

1 E. P. Grimsrud and D. A. Miller, *Anal. Chem.*, 50 (1978) 1141.
2 D. A. Miller and E. P. Grimsrud, *Anal. Chem.*, 51 (1979) 851.
3 D. A. Miller, K. Skogerboe, and E. P. Grimsrud, *Anal. Chem.*, 53 (1981) 464.
4 E. P. Grimsrud, in A. Zlatkis and C. F. Poole (Editors), *Electron Capture —Theory and Practice in Chromatography*, Elsevier, Amsterdam, Oxford, New York, 1981, ch. 5.