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QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS ON SUPPORT-COATED OPEN TUBULAR CAPILLARY COLUMNS

I. ANALYSIS OF ISOMERIC ETHYLPHENOLS

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SUMMARY

Gas chromatographic methodology has been developed for quantitative analysis of samples of *o*-, *m*-, and *p*-ethylphenols. Samples are dissolved in a dichloromethane solution of the internal standard, anisole, and are then derivatized with N-trimethylsilylimidazole to form the silyl ethers. The samples are chromatographed with similarly prepared standards on a stainless-steel OV-17 support-coated open tubular (SCOT) column.

Specificity, linearity, precision and stability of the samples are discussed, and chromatograms obtained on the SCOT column are compared to those obtained on a packed analytical column.

This report describes one of the first quantitative gas chromatographic analytical methods to use a SCOT (capillary) column.

INTRODUCTION

The great value of gas chromatography (GC) for highly reliable quantitative analyses of a wide variety of compounds is immediately apparent to all who familiarize themselves with its literature. GC is one of the most practical and widely used techniques for determinations at almost any concentration, including trace levels. However, the majority of applications involve conventional packed columns which have a limited separating efficiency.

Although successful high-efficiency capillary column separations were achieved more than a decade ago, the versatility and application (especially for quantitative analyses) of such columns is subject to argument. Highly satisfactory chromatograms are normally obtainable in roughly the same time on packed or capillary columns, but quantitative capillary column applications are often dismissed due to reluctance to work with somewhat more sophisticated (and more expensive) instrumentation. The latter usually includes an inlet splitter which may itself be a source of error in quantitative work[1].

Because of insufficient accuracy and precision often related to unreliable

sampling methods, and due to instrument installation problems, relatively few quantitative analyses have been reported using capillary GC techniques. Most work is qualitative, dealing with petrochemical[2,3], biomedical[4-6] and environmental[7-9] applications. Mattsson and Nygren[10] have reported on the determination of a variety of polychlorinated biphenyls and chlorinated pesticides in sewage sludge by use of a capillary column, but results were reported with relatively wide variation. McCallum and Cairns reported conditions for quantitative analysis of cannabinoids using a support-coated open tubular (SCOT) column without stream splitting[11] but reported no numerical results. Only very recently have the quantitative determination of chlorophenols[12] and the determination of the anti-depressant psychotropic drug Nomifensine in human plasma[13] been reported.

Handled properly, capillary columns can be a powerful tool for quantitative analyses of substances (especially isomers) not satisfactorily handled on packed analytical columns usually due to incomplete resolution. The ethylphenols are used as starting materials and intermediates in a wide variety of organic syntheses but suffer from incomplete resolution of the *meta* and *para* isomers on typical packed columns[14]. Precise and accurate quantitation is difficult if not impossible under such conditions. This paper reports the quantitative analysis of the ethylphenols on an OV-17 SCOT column.

EXPERIMENTAL

Reagents

The "distilled in glass" dichloromethane was obtained from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Anisole (used as internal standard) was "certified" grade from Fisher Scientific (Fair Lawn, N.J., U.S.A.). N-Trimethylsilyl-imidazole (TMSI) was purchased from Pierce (Rockford, Ill., U.S.A.). Regisil was from Regis (Morton Grove, Ill., U.S.A.). Commerical samples of *m*- and *p*-ethyl-phenols were purified by distillation to 98% or more. *o*-Ethylphenol was used "as is" from Aldrich (Milwaukee, Wis., U.S.A.).

Ethylphenol samples for analysis were prepared as follows. 50 mg of ethyl-phenol (for linearity, 0–100 mg) were weighed into a test tube (Cat. No. 99447; Corning, Corning, N.Y., U.S.A.) to which 10.0 ml of 2.5 mg/ml anisole in dichloro-methane were added. The tube was closed with a screw cap and a PTFE-lined septum (Microsep F-138, Canton Bio-Medical Products, Boulder, Colo., U.S.A.) and 0.5 ml of TMSI (or Regisil for packed-column experiments) was added. After heating the tubes for 30 min at 80°, they were cooled and the contents were diluted to approx. 50 ml with dichloromethane. Solutions were mixed and a portion of each was trans-ferred to auto-injector vials (Hewlett-Packard No. 5080-8712) for GC.

Equipment

The gas chromatograph was a Hewlett-Packard Model 5711A dual-flame instrument equipped according to Hewlett-Packard instructions with a Model 18704 inlet splitter and the appropriate injection port and detector connections. A Brooks dual GC mass-flow controller Model 5840 calibrated for helium was used to control the column carrier flow (set at 10.0 ml/min) and the flame make-up gas flow (30 ml/min) (Brooks Instrument Div., Emerson Electric, Hatfield, Pa., U.S.A.). A split ratio of

about 2 was used. The capillary column was a standard SCOT column (No. 008-0218), coated with OV-17, from Perkin-Elmer (Norwalk, Conn., U.S.A.). Its dimensions were 50 ft. × 0.02 in. I.D. and it was of stainless steel with 1/16-in. female fittings. The oven was operated at 115°, the injection port at 200° and the detector at 250°. A Hewlett-Packard Model 7671A automatic injector was used to inject 0.5-μl samples.

For packed-column chromatograms, a Hewlett-Packard Model 402 high-efficiency dual-flame gas chromatograph was used with an oven temperature of 70°, an injection port temperature of 70°, a detector temperature of 125°, and a helium flow of 60 ml/min. The column was a 4 ft. × 1/8 in. I.D. glass U-tube packed with 3.8% UC-W-98 on 80–100 mesh Gas-Chrom Z prepared by the funnel coating method[15].

Peak areas were calculated using an on-line calculation program from the expanded RTE 2100 computer system (Hewlett-Packard, Avondale, Pa., U.S.A.). Statistical evaluation of the results was accomplished by use of a program[16] available through a time-shared DEC-10 computer system (Digital Equipment, Marlborough, Me., U.S.A.).

RESULTS AND DISCUSSION

Ethylphenol isomers are very useful starting materials and intermediates in a variety of organic reactions. For a highly selective, reproducible assay, complete resolution of the isomers on the GC column is necessary. When a routine screening program[17] failed to yield conditions which would completely resolve the *ortho, meta,*
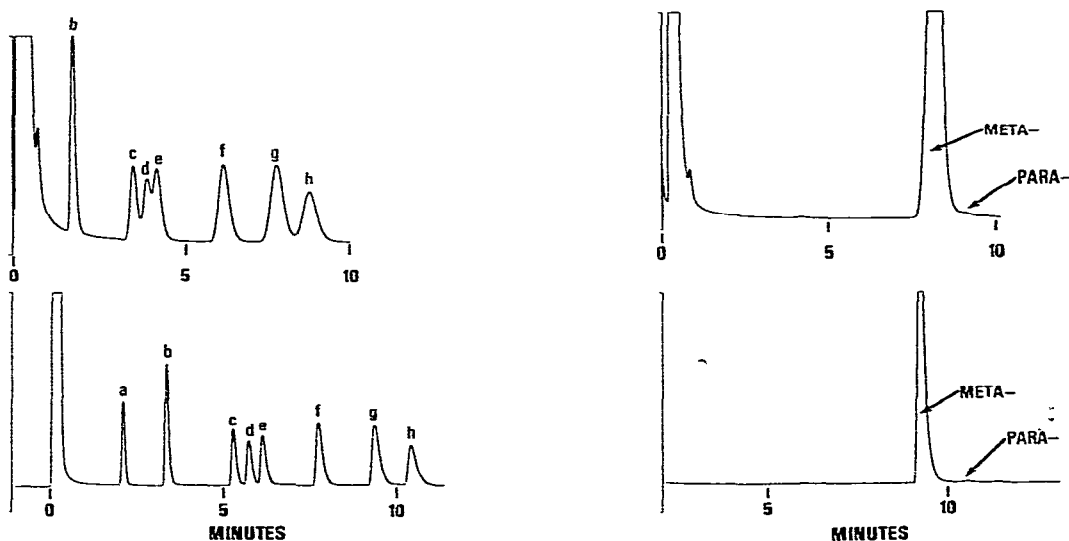
Fig. 1. Gas chromatograms of phenol mixture. Top, packed column; bottom, SCOT capillary column Peaks: a = anisole (in solvent front in upper trace), b = phenol, c = *o*-cresol, d = *m*-cresol, e = *p*-cresol, f = *o*-ethylphenol, g = *m*-ethylphenol, h = *p*-ethylphenol. For chromatographic details see text.
Fig. 2. Gas chromatograms for approx. 0.5% *p*-ethylphenol in *m*-ethylphenol. Top, packed column; bottom, capillary column. For chromatographic details see text.

and *para* isomers, attention was turned to SCOT columns for a quantitative analysis.

Chromatograms in Figs. 1 and 2 illustrate results for the packed and capillary columns and demonstrate some problems that are overcome by use of the capillary column for assay. Fig. 1 shows comparative chromatograms of the ethylphenols mixed with some potential impurities: phenol and *o*-, *m*- and *p*-cresol. Note that resolution of the *m*- and *p*-ethylphenols on the packed column is incomplete but that they are totally resolved on the capillary column in about the same analysis time. This *meta–para* resolution problem is very critical when *m*-ethylphenol samples are to be assayed for low levels of the *para* isomer. Fig. 2 illustrates the problem and it is obviously much easier to get good accuracy and precision of determination in the case of the capillary column. With the bad overlap on the packed column, the analyst encounters difficulty in determining the area of the *p*-ethylphenol peak and has reproducibility problems in determining the *m*-ethylphenol peak area. Since *m*-ethylphenol is on the leading side of the *p*-ethylphenol peak, determination of small quantities of it in the presence of the latter is not a problem on either column.

For each ethylphenol isomer the linearity of response to concentration from 0 mg/ml to about 2 mg/ml was tested. In all cases the coefficient of determination was greater than 0.999 and the log–log slope was between 0.995 and 1.010. The maximum percent deviation from a least squares line was 1.6 for *o*-, 0.4 for *m*- and 1.1 for *p*-ethylphenol.

Table I shows the results of precision testing for each of the separate ethylphenol isomers. Over twenty separate replicates were prepared of each isomer to determine the number of replicates necessary to assure that the assay results are $\pm 2\%$ of the true value at a confidence limit of 95%. For an assay, equal numbers of replicates of each sample and of the appropriate standard are prepared.

TABLE I

PRECISION DATA FOR ETHYLPHENOL POSITIONAL ISOMERS

Parameter	Ortho	Meta	Para
No. of samples tested	24	23	23
Standard deviation	4.36×10^{-2}	3.78×10^{-2}	3.75×10^{-2}
Mean normalized response	3.15	3.17	4.67
RSD	1.38	1.19	0.80
Method RSD*	1.96	1.69	1.14
Replicates for 95% confidence ratio of 2%	7	5	2

* Method RSD = RSD $\times \sqrt{2}$ to compensate for variation from samples *and* standards in assay[16].

Calculations for a typical assay are done as follows. For each replicate standard or sample, the peak area of the ethylphenol is divided by the peak area of the anisole (internal standard). These are then divided by the respective weights to give a normalized response. For each sample and standard the normalized replicate responses are averaged and the final purity of the sample (using *p*-ethylphenol as an example) is calculated:

$$\% \text{purity of sample} = \frac{\text{avg.norm. response } p\text{-ethylphenol sample}}{\text{avg.norm. response } p\text{-ethylphenol standard}} \times \% \text{purity standard}$$

Since a large sample load, computer or other instrumental problems may occasionally cause delay in chromatographing the prepared solutions, the stability of *m*-ethylphenol solutions prepared as described was tested. The solutions were chromatographed, then allowed to stand in the auto-injector vials for 24 h, and then re-chromatographed. The average response for ten samples was 99.5% of the original response. The results obtained after a delay of 24 h indicated that the solutions were acceptably stable under the described conditions. It was assumed that the stabilities of the *o*- and *p*-ethylphenol solutions would be similar.

No quantitative results were generated from the packed-column work due to inadequate resolution. Therefore, attention was focused on capillary column experiments.

Several lots of *m*- and *p*-ethylphenol were assayed and, in instances where possible, results were compared to those found using a UV method based on hydrogen-bonding differences. The results compared favorably.

CONCLUSIONS

This paper describes one of the first reported quantitative analyses done on GC SCOT capillary columns. The method is accurate, highly precise, and selective for the determination of ethylphenol positional isomers. Futher work is in progress to test applicability of capillary columns to quantitative analysis in GC.

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