Capillary GC:
HOT cold on-column injection

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In recent years, gas chromatographic analysis of triglycerides (TG) on capillary columns has become an important issue on a research level but even more on a quality control level.

Many efforts have been made in the last eight years to optimize and standardize TG analysis on capillary columns. One of the main obstacles to any gas chromatographic (GC) TG analysis is an appropriate injection technique. Flash evaporizing injectors of the split/splitless type or also the falling needle type require extremely high injection temperatures of near to or above 400° C. This induces an extremely high thermal shock, particularly dangerous for polyunsaturated TG.

In 1979, Monseigny et al. showed interesting results of capillary column application in TG analysis (1). They used a heated injector with injection temperatures as high as 400° C. Nonpolar columns of the dimethylpolysiloxane type were used in all cases. Although they could show an acceptable linearity of response factors of the different carbon number TG, resolution of different degrees of unsaturation was not achieved. In 1980, K. Grob Jr. et al. showed the application of a cold on-column injector to TG analysis and demonstrated a partial separation of TG of different degrees of unsaturation (2). Maximum oven temperature was above 300° C and TG of carbon number 52 and 54, respectively, eluted in this high temperature range between 300 and 330° C.

In 1981, Traitler and Prévôt published a base-line separation of TG differing in carbon number as well as in degree of unsaturation (3). Final oven temperatures in this case were 295° C and 50, 52 and 54 carbon TG, respectively, eluted during the isothermal hold period. Also, cold on-column injection was chosen and was proven to be the best technique for a damage-free sample transfer into the analytical column in the case of low-volatile substances such as TG.

Separation of compounds with different degrees of unsaturation only was achieved based on the total number of double bonds in one triglyceride molecule, but there was no distinction between triglyceride isomers with unsaturated acids in different positions. Under those conditions, separation of triglycerides with different isomers of unsaturated acids was not possible.

Separation of positional isomers of TG was shown by Traitler and Rossier in 1982 on specially prepared capillary columns containing inorganic salts such as $AgNO_3$, $CuCl_2$, $CoCl_2$ or other similar salts in the dimethylpolysiloxane matrix (4).

Retention times for TG analyses, which are already long under these conditions (40–50 min), become even longer in the application of mixed organic/inorganic phases and are around 80–90 min. This and the fact of an overlapping of different species in complex mixtures make clear-cut separations very difficult and disqualify this method for routine analyses of TG isomers. However, it can still be very helpful in the evaluation of preparations such as cocoa butter or palm midfraction in which the positional isomer is of prime interest; for melting quality reasons, symmetrical isomers of these triglyceride mixtures must be present and also must be controlled analytically. In all cases described thus far, dimethylpolysiloxane as such or mixed with inorganic salts has been used for the GC analysis of triglycerides.

Recently, other approaches with stationary phases were chosen. Instead of apolar stationary phases of the SE-30 of OV-1 type, phases of "quarter-polarity" were used, such as OV-17 or OV-17-like phases. OV-17 has a very high temperature stability and can be used at oven temperatures as high as 360° C. Due to its increased polarity, this phase resolves substances varying only very slightly in polarity (5).

For TG analysis, the use of the high temperature range between 300 and 360°C is necessary. Sample transfer onto the column at such an elevated temperature becomes a problem and requires a special injection technique. On-column injection technique is the method of choice, but conventional devices to cool injector ports will necessarily fail to cool the injection spot of the column so as to guarantee the sample transfer into the column in the liquid state. Therefore, it is appropriate, if not necessary, to use a movable on-column injector to completely pull out of the oven the part of the column in which the sample deposition takes place, or to keep the site of injection sufficiently cool (ca. 60° C) even with oven temperatures of around 300°C (6).

Movable injections have the disadvantage that one cannot or can hardly use them in autosampler mode; in addition, the timing of the whole injection procedure is relatively uncontrolled. For this reason, it is advantageous to use a device that allows a performant secondary cooling in the inlet zone (±60°C) (7) despite the fact that the initial oven temperature is at 300°C, for example. Such a device is shown in Figure 1.

The main features of this device include an insulation jacket at the column inlet of 15 cm length that efficiently can be "secondary-cooled" before the injection and for a given time after the injection. This cooling is necessary to avoid rapid evaporation of the sample solvent that would lead to sample material being pushed backward and possibly out of the column again. Therefore, it is imperative to pro-

vide sufficient time for sample evaporation, allowing the carrier to remove the generated vapor continuously. This can be achieved either by injection at low column temperatures (conventional on-column injection) or by slow introduction of the sample liquid from a cool zone into the heated zone. This latter alternative represents the technique described in this article.

Conventional on-column injection requires the column temperature during injection and subsequent solvent evaporation below the boiling point of the solvent at the given inlet pressure of the carrier gas, i.e., slightly above the boiling point of the solvent under atmospheric pressure. A typical oven temperature at injection for hexane or heptane could be around 80° C. This temperature provides controlled evaporation of the solvent and transfer along into the capillary column.

In the particular case of triglyceride analysis in which the actual analysis takes place at probably 300° C or above, this initial cooling-down step of the oven is very time-consuming and gives rise to an unstable baseline during the ballistic temperature programming up to the required temperature. Therefore, it was logical to investigate and find possibilities that would allow a high-oven-temperature (HOT) cold on-column injection. Two basically different concepts exist now and were defined by Grob in the following way (8,9):

Small sample volumes coating cooled column inlet. According to the first of two concepts, the sample liquid is coated as a film on the internal wall of the temporarily cooled inlet. The sample volume injected is kept small enough to prevent sample liquid from flowing into the oven-thermostated column. Solvent evaporation is carried out at a low temperature, and the inlet section is heated to or above the oven temperature only after the solvent has completely evaporated. This technique avoids the generation of large volumes of vapor through rapid heating.

Geeraert et al. applied this technique to the analysis of triglycerides by using a homemade movable on-column injector. Sample volumes between 0.2 of 0.3 μ l were injected into the column inlet kept in the atmosphere above the GC oven (5). A typical chromatogram is shown in Figure 2.

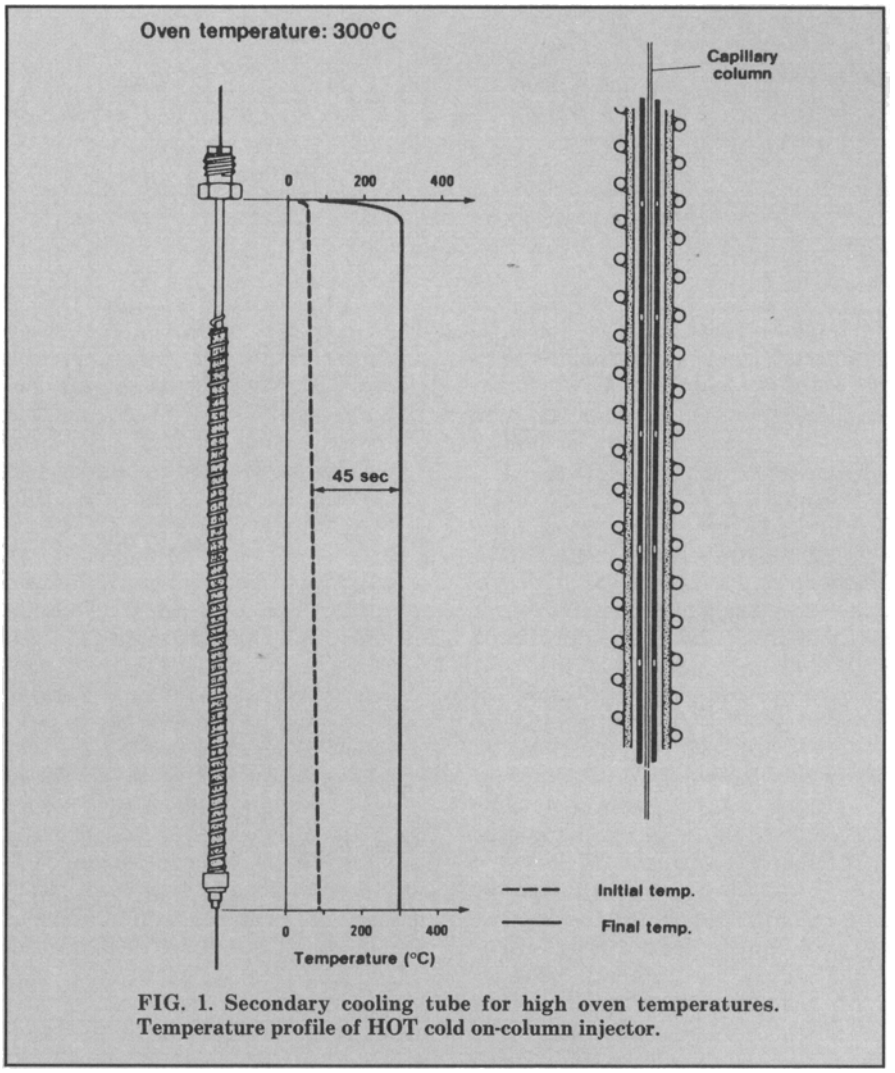

FIG. 1. Secondary cooling tube for high oven temperatures. Temperature profile of HOT cold on-column injector.

Cool buffer zone preventing rejection from column. The second concept tolerates larger sample volumes and accepts that sample liquid flows out of the cooled inlet into the entrance of the hot, oven-thermostated column. However, liquids cannot really penetrate into a column section at a temperature that far exceeds the boiling point of the sample because the sample liquid is stopped by its own vapor pressure. Therefore, the front of the sample plug remains in the zone where the column temperature changes from below to above the boiling point of the solvent, and solvent evaporation takes place there. After completion of solvent evaporation, the column inlet is heated, allowing the solute material to start the chromatographic separation process.

The first concept also can be applied by using commercially available movable on-column injections such as the J&W On-Column injector. As it was already mentioned, this type of injector is more difficult to run in automatic sampling mode.

Figure 3 shows a typical example of a triglyceride analysis done by HOT cold on-column injection and automated analysis (10).

The second concept of HOT cold on-column injection is based on the assumption that a volume of sample liquid is injected, causing some liquid to leave the tempo-

rarily cooled inlet and to flow toward the entrance of the oven-thermostated column, which is at high temperature (330° C or above). The sample liquid is rejected in the hot zone, flung backward into the cooled zone (the latter acting as a barrier against backflow into the injector) and pushed into the entrance of the heated column again by the carrier gas, resulting in a rapid movement backwards and forwards.

Solvent evaporation time

The solvent evaporation time is a key parameter in HOT cold on-column injection because the column inlet must be heated at the end of solvent evaporation. Solvent evaporation time can be roughly calculated from the width of the solvent peak; at high oven temperatures of around 300°C, the width of the solvent peak is determined primarily by the solvent evaporation time. Contributions from retention in the column itself are small. The peak width of the solvent gives a gross estimation of the solvent evaporation time and serves as a measure for starting the heating up of the column inlet either by pushing the movable injector into the oven or by stopping the intensive secondary cooling of the HOT-device.

Conclusion

The technique of HOT cold on-column injection in the analysis of low-volatile compounds becomes particularly interesting when applying not only high-temperature stable stationary phases but also column material, such as aluminum-coated fused silica, which withstands temperatures of 400° C and above.

The full potential of these columns, particularly concerning speed of analysis, can optimally be exploited by applying the technique of HOT cold on-column injection.

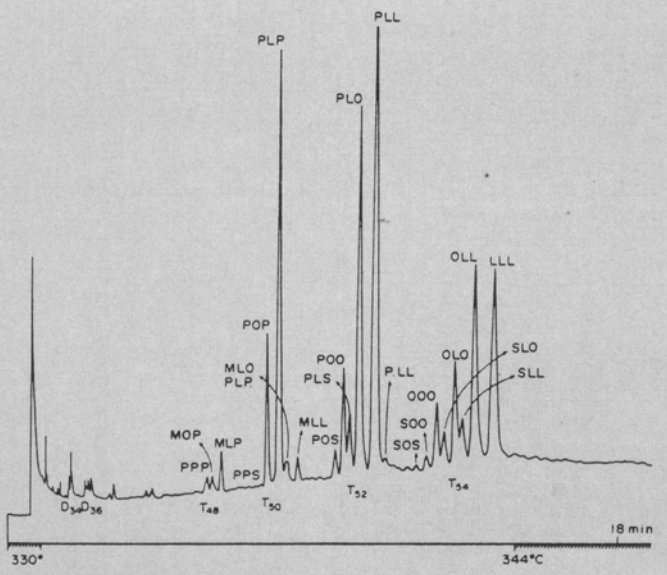

FIG. 2. Separation of triglycerides: cottonseed oil. For conditions and legend, see Figure 3, except 330° C, one min iso, 1°C/min to 344° C, hold.

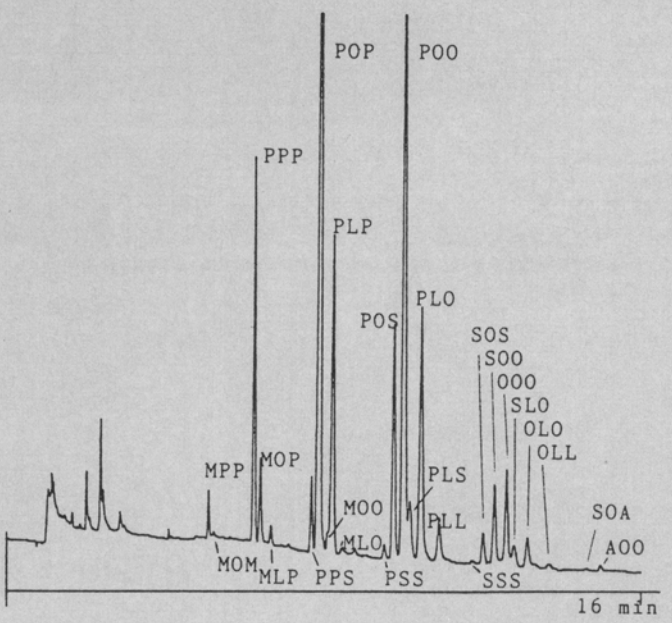

FIG. 3. Analysis of palm oil with HOT cold on-column injection. Column: 25 m × 0.25 mm SS armoured FSOT—phenyl methyl silicone, 0.1 μm film thickness. Sample: 0.3 μl 0.5% palm oil in iso-octane. Temperature 340° C (1 min) to 355° C at 1° C/min. Secondary cooling: three min activated before injection. Carrier gas hydrogen 10 psi.

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Publications

Book review

Nutritional Toxicology, Vol. 2, Nutrition: Basic and Applied Science, A Series of Monographs, edited by John Hathcock (Academic Press Inc., 6277 Sea Harbor Dr., Orlando, FL 32821-9989, 1987, 300 pp., $65).

This book covers a wide variety of topics that appropriately can be discussed under the title "Nutritional Toxicology." In the initial chapters, the detoxification process is explored and related to the field of nutrition. Other subjects addressed in this book include such diverse topics as toxicological effects of ethanol, caffeine and trace elements, mutagens in cooked food, allergic reactions to foods and nutritional aspects of pesticides.

Because this is a monograph, there is some overlap in terms of the subjects discussed. Some of the introductory chapters review the same detoxification processes; however, the approaches to these topics are quite different. One author provides a very molecular approach, whereas another discusses how various nutrients (fats, carbohydrates, proteins, vitamins and minerals) influence the metabolism and detoxification of drugs, carcinogens and foreign compounds.

Toxicological aspects of alcohol and caffeine are presented in separate chapters. Alcohol is discussed in relationship to a wide variety of diseases; however, emphasis is placed on the pathogenesis of alcoholic liver disease and its interaction with drug, vitamin and mineral metabolism. Effects of ethanol on hyperlipidemia and cardiovascular disease also are discussed, and an update is provided on the relationship between serum lipoproteins and ethanol consumption. Research regarding caffeine and its possible relationship with fibrocystic breast disease, sleep, anxiety and hyperactivity also is presented in detail. Some misconceptions about caffeine are addressed in this section.

A chapter also is devoted to a discussion about the mutagenic activity of cooked foods. Emphasis is placed on ways in which mutagens are activated and detoxified as well as methods of measurement. Mention is made of the carcinogenic effects of mutagens in cooked foods.

Food allergies and non-immunologic food sensitivities are presented nicely, with emphasis placed on the listing of common allergenic foods as well as methods of diagnosis and treatment. "Allergy-like" intoxications also are discussed, with particular emphasis placed on the role histamines play in this process.

This monograph ends with a two-chapter review of pesticides and foods. The main types of pesticides are listed, and their chronic and delayed effects on the systems of the body are discussed. Effects of pesticides on food absorption also are mentioned. These authors address how pesticides affect the food chain and mention what they believe will happen in the future regarding such areas as nonchemical pest control and pesticide monitoring.

This book is of value to people in a variety of professions. It would have been helpful if more information had been presented in tabular form for easier reference. Molecular biologists will be more interested in those chapters that have a strictly biochemical approach to toxicology and its relationship to food, whereas practicing nutritionists and physicians will be interested in the chapters on allergies, ethanol, caffeine and trace elements. Food scientists will gain the most from the chapters that deal with mutagens and pesticides in foods.

I believe this book can be a valuable resource to most of the readers of *JAOCS*. It provides numerous references that are not available elsewhere and deals with critical topics that are of current interest.

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New books

Alternatives to Animal Use in Research, Testing and Education, Office of Technology Assessment, Congress of the U.S., Marcel Dekker Inc., 270 Madison Ave., New York, NY 10016, 1988, 456 pp., $59.95 US and Canada, $71.50 elsewhere.

Supercritical Fluid Extraction and Chromatography: Techniques and Applications, ACS Symposium Series 366, edited by Bonnie A. Charpentier and Michael Sevenants, American Chemical Society, 1155 Sixteenth St., NW, Washington, DC 20036, 1988, 253 pp., $59.95 US and Canada, $71.95 elsewhere.

Polymer Modified Textile Materials, by Jerzy Wypych, John Wiley & Sons Inc., 1 Wiley Dr., Somerset, NJ 08875-1272, 1988, 317 pp., $79.95.