A New Technique for GLC Sample Preparation Using a Novel Extraction Device

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Sample preparation is the first step in flavor and fragrance research. Numerous sample preparation techniques have been proposed to separate and concentrate the volatile chemicals from the matrix. Difficulties may result from the low concentration level of volatiles (frequently in the sub-ppm range), the presence of soluble and insoluble solids as well as lipid materials, and the presence of large amounts of water. These factors make it necessary to isolate a concentrated volatile fraction free of water and non-volatile material in order to obtain maximum qualitative and quantitative information from gas chromatographic analysis.

Weurman[1] reviewed isolation and identification techniques fifteen years ago; more recently, several monographs have covered isolation techniques in flavor research.[2–6] Steam distillation followed by solvent extraction is a technique which has been very extensively used. More recently, the Likens-Nickerson extractor (or a modification thereof) has come into wide use. In this apparatus the organic volatiles are continuously and concurrently steam distilled and solvent extracted to produce a volatile aroma concentrate. Other techniques which are employed include adsorption on carbon or on a porous polymer fol-

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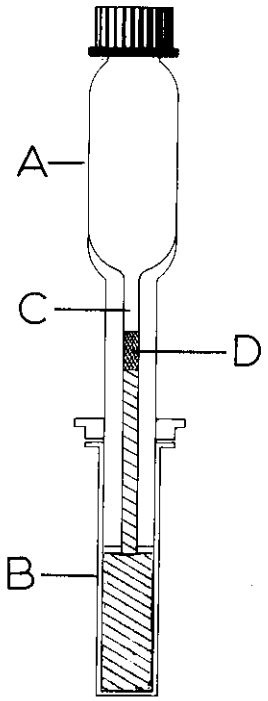

Figure 1. Mixing Device

lowed by thermal[7] or solvent[8] desorption. Innovative techniques such as critical gas extraction, trapping on a cooled gas chromatographic column and co-condensation by solvent refluxing have been reviewed.[9]

A number of workers have compared isolation procedures. Quite recently Leahy and Reineccius[10] compared direct head space analysis, head space concentration, Likens-Nickerson steam distillation extraction, and solvent extraction using an aqueous model system consisting of typical aroma chemicals. They concluded that direct extraction (using a separatory funnel) was the simplest and most efficient technique but was labor intensive since vigorous shaking (30 minutes) was required. One of the difficulties associated with separatory extraction of aqueous solutions is the quantity of solvent required and the fact that this solvent must normally be concentrated by several orders of magnitude before gas chromatographic analysis. For example, in their studies on grapefruit juice, workers at TNO extracted 25 ml of juice with 20 ml solvent and concentrated the latter to 200 microliters (factor of 100) prior to gas chromatographic analysis.[11] Concentrating solvents can lead to losses of low

boiling point compounds unless special precautions are taken, and it can also result in the concentration of solvent impurities. In addition, there is the possibility of sample decomposition or reaction as the solvent is removed.

One solution to these latter problems was addressed by Jennings.[12] He described a modified Babcock bottle for liquid/liquid extraction which permits the extraction of several tens of milliliters of sample with a few hundred microliters of solvent. Aqueous sample and solvent are combined in the bottle, the system is sealed and shaken and the solvent containing the sample can be removed with a microsyringe.

Recently a novel device for mixing and separating called a Mixxor™ has become available. This device utilizes a piston-cylinder principle for extraction and was described by Peleg and Vromen.[13] These authors studied the extraction of steroids from aqueous solutions into diethyl ether. They concluded that six piston movements were equivalent to forty shakes in a separatory funnel.

The purpose of the present study is to investigate the use of the separatory cylinder for direct extraction of aromas using only minimal amounts of solvent.

Procedure

A 10 ml Mixxor was used in these studies, as shown in figure 1. Eight ml of aqueous sample is placed in the upper Chamber A, along with 1.5 gm magnesium sulfate and a few drops of an FD&C color is added. The purpose of the sulfate is to aid in salting out the organics as well as to help break emulsions. The FD&C color assists in visualizing the interface between the colored aqueous material and the clear solvent. The cap is attached and the piston moved up and down several times to dissolve the salt. The extractor is cooled to room temperature and 300-350 microliters of 2:1 petroleum ether/diethyl ether is added. Again the piston is moved up and down five to ten times. The piston is raised completely out of the aqueous sample solvent mixture, which remains in Reservoir B. After phase separation occurs (normally two minutes) the piston is very slowly moved down into the graduated reservoir and liquid forced into Axial Chamber C. Several tens of microliters of organic phase is seen in the chamber resting above the colored bulk aqueous material. A standard 10 microliter syringe is used to remove an aliquot of the organic phase from the axial chamber of gas chromatographic analysis.

The first set of experiments were designed to measure the recovery of a series of ethyl esters from aqueous solutions. Dilutions in water were prepared and extracted as described above. Two microliters of sample were analyzed on a Varian 3700 gas chromatograph employing a glass column packed with 10% SP-1000 (carbowax-type liquid phase) using a column temperature of two minutes at 80°C, then a temperature program rate of 10°C/min to 200°C. The recoveries of these samples were compared to comparable dilutions of the esters in the solvent. Data analysis was performed by an IBM 9000 data system. Results of that study are shown in Table I.

The second set of experiments was designed to

Table I. Percent Recoveries of Ethyl Esters from Aqueous Solutions

	20 ppm	2.0 ppm	0.2 ppm
Ethyl C_5	97	112	*
Ethyl C_6	98	109	104
Ethyl C_7	104	112	106
Ethyl C_8	110	108	106
Ethyl C_9	114	100	112
Ethyl C_{10}	109	99	90

* Not resolved from solvent.

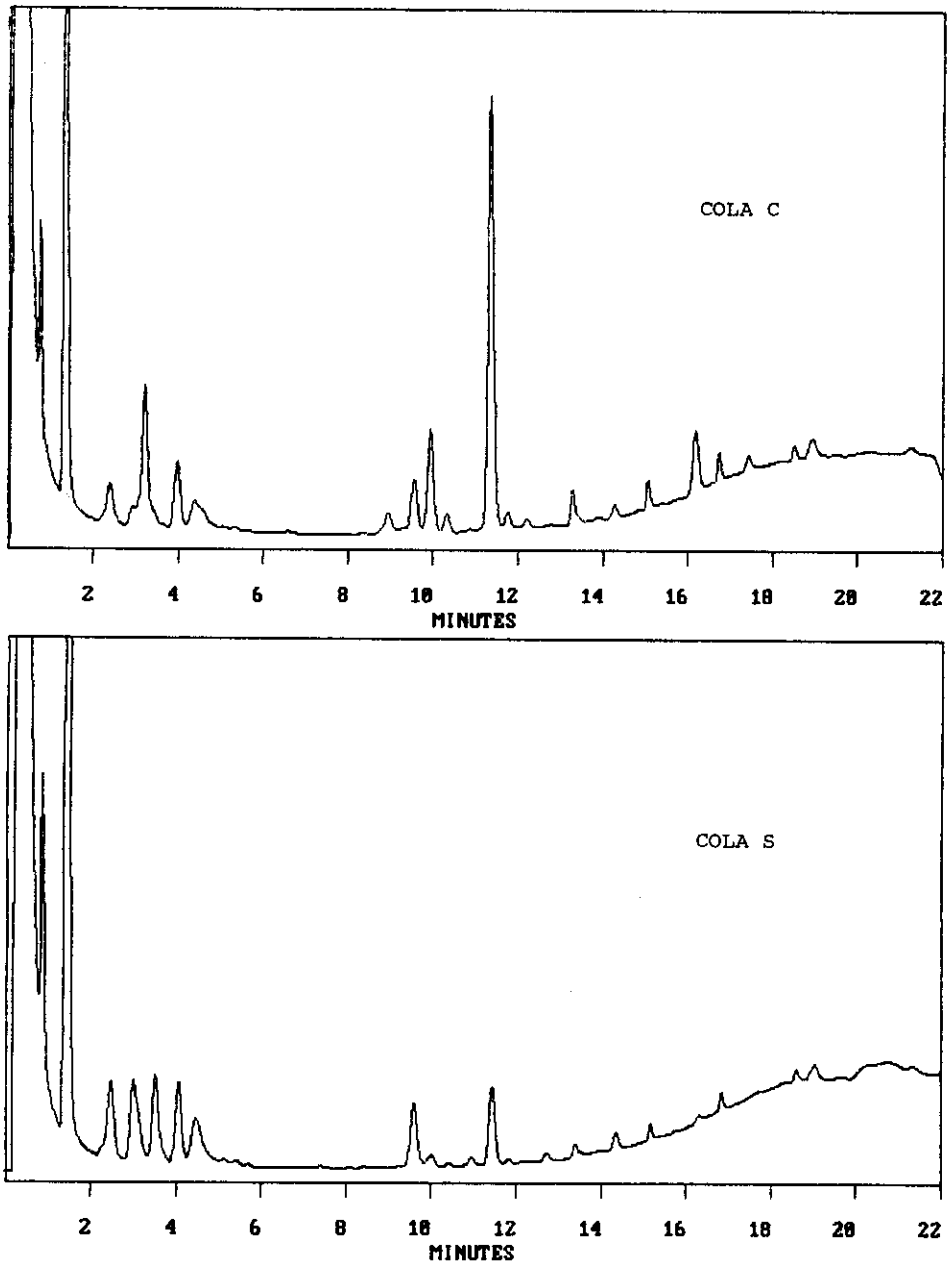

Figure 2. Gas Chromatographic Comparison of Two Cola Beverages

compare the volatile profiles of two commercial cola beverages. Ten ml portions of each beverage were extracted as described previously. The samples were analyzed using the same chromatographic conditions as for the ethyl ester studies, except that the temperature program rate was 8°C/min.

The final example represents the volatiles from a number of essential oils. One gram of the herb or seed was macerated wtih 10 ml water and the suspension was indirectly steam distilled until 8 ml of condensate was obtained. This aqueous material was extracted as described for the ethyl ester studies. The essence obtained was analyzed on a Perkin-Elmer Sigma II gas chromatograph using a 30m×0.32mm id DB-1 (methyl silicon) column. The gas chromatographic oven temperature was held for 5 min at 70°C, then temperature programmed to 200°C at 5°C/min.

Results and Discussion

The results of the extraction studies on low levels of esters are shown in Table I. It is apparent that the recoveries are quite satisfactory, even at the sub-ppm level.

Direct extractions were performed on two

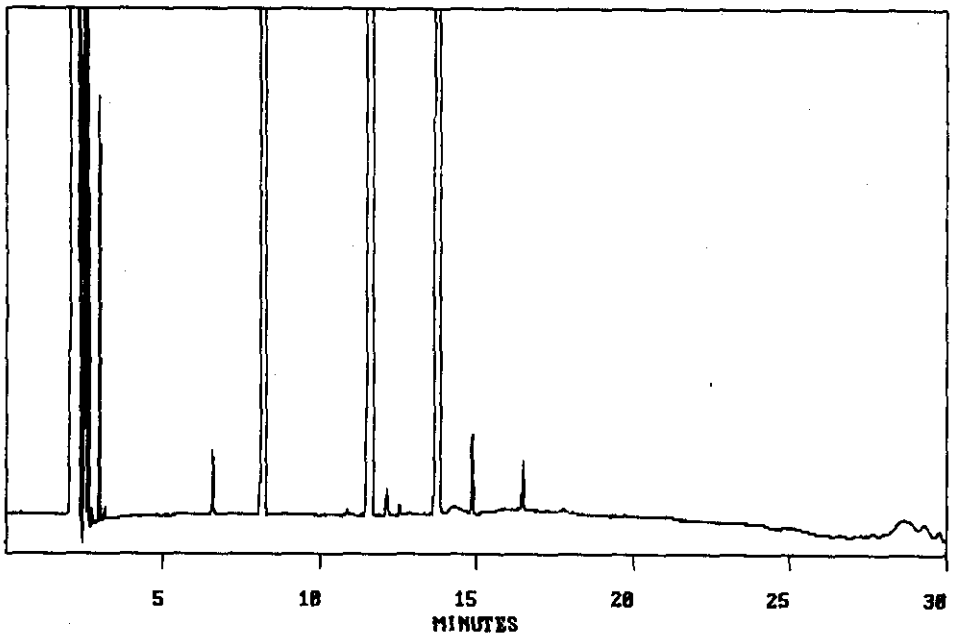

Figure 3. Gas Chromatogram of Rosemary

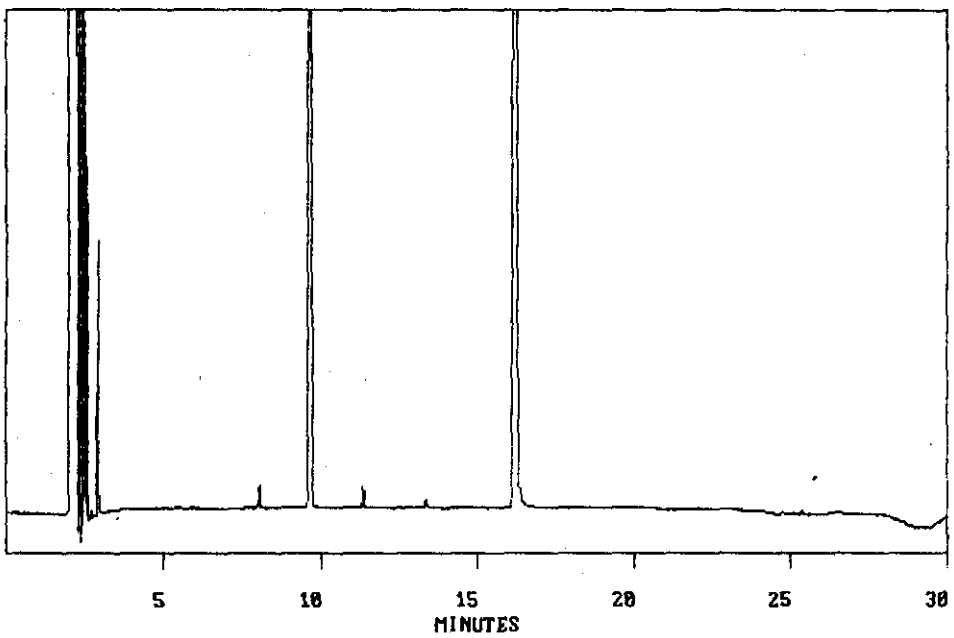

Figure 4. Gas Chromatogram of Fennel Seed

commercial colas. Cola C is a diet cola, while Cola S is a sugar sweetened product. The reconstructed chromatograms which were obtained are shown in figure 2. The patterns are quite dissimilar and readily distinguishable.

The capillary gas chromatograms of the essential oils are shown in figure 3, 4 and 5. Figure 3 is the pattern from the herb rosemary, a dense evergreen shrub, which has a characteristic camphoraceous odor. The large peak eluting at 8 minutes is 1,8-cineol, that at 12 minutes is camphor, while the peak at 14 minutes is borneol. The odor of borneol is often described as being similar to rosemary. Figure 4 is the chromatogram from fennel seed. The large component eluting at 10 minutes is fenchone; anethole is found at 16.5 minutes and is responsible for the characteristic anise character of fennel seed. The final chromatogram (figure 5) arises from celery seed. The compound eluting at 26.5 minutes is 3-butylphthalide, a lactone responsible for the celery-like aroma. These three gas chromato-

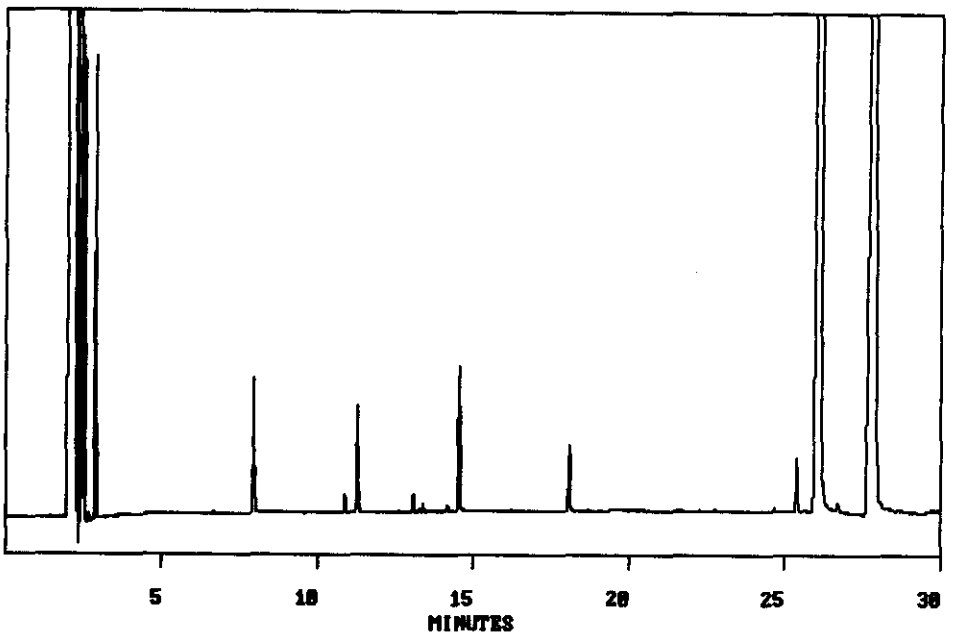

Figure 5. Gas Chromatogram of Celery Seed

graphic patterns are all quite characteristic. Total sample preparation time, including steam distillation and extraction was less than 20 minutes making this procedure quite convenient for spice analysis.

The advantages of this extraction system for low levels of organic compounds in water are several:

—The procedure is quite simple. No transfer of material is required.
—The procedure is quite rapid. It normally takes less than five minutes for extraction and phase separation to occur.
—Labor intensive separatory funnels are eliminated and no vigorous shaking is required.
—A concentration factor of 30 or more can be achieved.
—Several gas chromatographic analyses can be made from the same sample.
—The sample can be stored (in the extracted form) at least overnight since the system is well sealed.
—The axial chamber permits a very small quantity of a low density solvent to easily be separated from the aqueous phase.

Two potential problems can be due to emulsions and non-volatile materials.

Emulsions may be broken by the addition of salts or of an antifoam. Chilling the system after mixing may also aid.

Non-volatile materials, such as lipids, can be extracted into the solvent and, subsequently, transferred to the gas chromatographic column. In this case, some secondary isolation technique, such as steam distillation, can be employed as was done for the herbs.

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