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OLFACTORY, GAS CHROMATOGRAPHIC AND MASS-SPECTRAL ANALYSES OF FECAL VOLATILES TRACED TO INGESTED LICORICE AND APPLE

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Volatile components of two foodstuffs with characteristic aromas, apple and licorice, and fecal samples obtained from subjects on high-apple and licorice diets, were analyzed by head-space gas chromatography and gas chromatography-mass spectrometer. The volatile compounds characteristic of the odors of apple and licorice were detected by "sniffing" the GC effluent. The aromatic component of licorice was identified by GC-MS as anethole [1-methoxy-4-(2-propenyl) benzene]. The aromatic component of apple could not be chemically characterized by our GC-MS system even though readily detected by the nose. Head-space, GC-MS analysis of fecal volatiles offers a means of tracing odorous and non-odorous components to ingested foodstuffs with potential applications to clinical and forensic medicine and anthropology.

The detection of diet related odorous compounds in human fecal samples by vapor-phase (head-space), gas-chromatographic analysis has recently been applied to the partial reconstruction of ancient and modern diets (1). Fecal samples release several volatile, odorous components and some of these can be directly traced to ingested dietary substances that are readily identified by their characteristic aromas. The olfactory organs of animals including man are sensitive and specific vapor-phase analysers although the human sense of smell is not nearly as

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developed as, for example, the tracking dog. However, in combination with the technique of head-space gas-chromatographic analysis the human "sniffer" analyst is a selective and reasonably sensitive detector of a wide number of volatile, odorous compounds (2).

GC-MS chromatograms have been used to fingerprint normal and pathological samples of body effluents (3-7) and even without complete interpretation of individual chromatographic peaks the profiles have been used for medical diagnosis (8-13). Zlatkis, Liebich, and coworkers in a series of systematic studies (8-12) trapped volatile components of biological samples on hydrophobic porous polymers (9) prior to gas chromatography. The head/space volatiles produced complex chromatograms which could be further analyzed in combination with mass spectrometry to yield chemical characterization (14).

Gas-chromatographic analysis of archeological and modern fecal samples revealed a variety of volatile components that could be traced to ingestion of food items with characteristic odor (1). As many as 18 different dietary odors were detected in a single stool sample obtained from a healthy individual on an ad libitum, unrestricted diet. Some of the dietary derived odors are quite durable. For example, the odors of corn and licorice were recovered from coprolites (ancient, dessicated fecal samples) 700 and 6400 years old (1). The present study traces and identifies volatile components characteristic of the odors of apple and licorice obtained from fresh food sources as well as in fecal samples obtained from healthy human subjects after being placed on diets containing high amounts of apple and licorice.

MATERIALS AND METHODS Volatile components of homogenized samples of fresh apple and licorice as well as samples of stool collected from two healthy volunteers after ingestion of these foodstuffs were concentrated on Tenax traps and then separated by gas chromatography. The component(s) responsible for the characteristic aromas of apple and licorice, in either the foodstuff or stool, were detected by "sniffing" the peaks as they were eluted. Gas chromatographic/mass spectrometric analysis of the peaks was carried out.

Head-space Volatile Collections and Gas-Chromatography (GC) Approximately 2 grams of homogenized stool, apple or commercial Nibs

licorice were placed in separate vials containing 15 ml of 0.5% Na_3PO_4 for 24-72 hours at room temperature prior to analysis. Each solution was then transferred to a 40 ml purging apparatus and purged of volatile compounds by bubbling nitrogen gas through the solution at 20 mls per minute for 15 minutes. The volatiles were trapped on a Tenax GC trap constructed from a stainless steel tube 2 mm in diameter and 100 mm in length packed with 100 mg of absorbent (Tenax). The trap was connected to the GC column and thermally desorbed for trapped components at 175°C for 2 minutes. The eluted compounds were swept into the head of the GC column. The packed glass column was 1800 mm by 6 mm (O.D.) by 2 mm (I.D.) and contained 5% SP-2100 on 80/100 Supelcoport. The column outlet was provided with a 2:1 splitter; one branch of the splitter (2/3) connected to a flame ionization detector, the other (1/3) to a sniffing port. Column temperature was kept isothermal at 40°C for four minutes and then programmed at 10°C/minute to 250°C and held at the upper temperature for 10 minutes. The retention time and peak area of each sample component were recorded while the odor characteristics of the components emerging from the sniffing port were described by the operator. Analytical instrumentation included a Hewlett-Packard 5700 gas chromatograph and 3380 A strip chart recorder/integrator. The above analytical procedures were adopted from the method of Jarke et al (15).

Mass Spectrometry (MS) For mass spectrometric analysis, the same initial procedures as with headspace GC were performed. The Tenax trap was 4 mm in diameter and 70 mm in length packed with 200 mg of Tenax. The packed glass column was 1500 mm by 6 mm (O.D.) by 2 mm (I.D.) containing 10% SP2100 on 80/100 Supelcoport. Column temperature began at 50°C and programmed at 10°C/min to 250°C and held at the upper temperature for 10 minutes. Analytical instrumentation included a Dupont DP-102 gas chromatograph/mass spectrometer with a scan range/rate of 33 to 350 AMU at 250 AMU/sec and 70 EV electron impact ionization. A Hewlett-Packard 1000 computer contained software for instrument control and search capabilities for an EPA/NIH data base of 37,000 compounds.

Stool Sample, Apple Diet A single stool sample was obtained from a healthy male after five days of a daily diet consisting of Roman Beauty apples, high-fiber cereal and ad libitum water. From 3-7 apples were ingested daily. Approximately 2 grams of homogenized stool were immersed in 15 ml of 0.5% Na_3PO_4 and allowed to remain at room temperature for 72 hours prior to analysis.

Stool Sample, Licorice Diet A single stool sample was obtained from a healthy male subject after three days of a daily diet consisting of 180-300 grams of Nibs licorice, commercial corn bran breakfast food, beans, toast, noodles, popcorn, peanut butter and ad libitum water. Approximately 2 grams of the frozen sample were immersed in 15 ml of 0.5% Na_3PO_4 and allowed to remain at room temperature for 72 hours prior to analysis.

Apple Sample Approximately 5 grams of an homogenized fresh Roman Beauty apple slice were immersed in 15 ml of 0.5% Na_3PO_4 and remained at room temperature for 72 hours prior to analysis.

Licorice Sample Approximately 5 grams of commercial Nibs licorice were immersed and treated as above before analysis. In addition, varying known amounts of anethole, the licorice-flavoring chemical of anise, were injected into the headspace GC and GC/MS columns.

RESULTS AND DISCUSSION Figure 1 shows the GC-odorgram (Figure 1A) and GC-MS spectra of oil of anise, the chief constituent of which was found

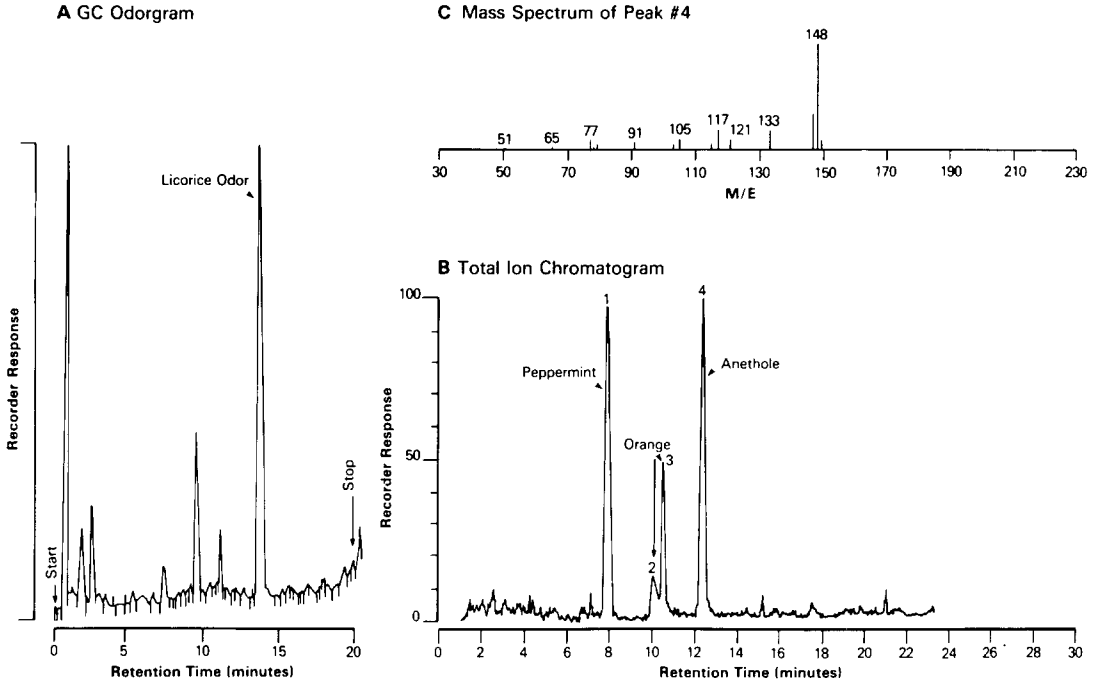
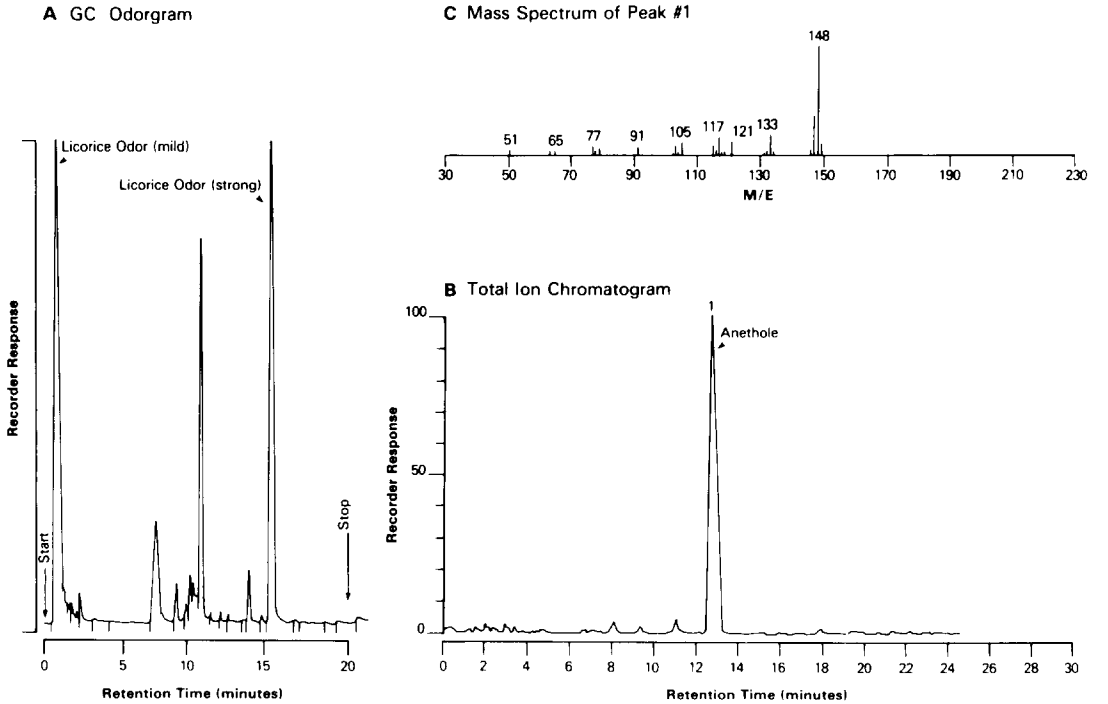


FIGURE 1: Oil of anise. Note detection and retention times of licorice odor with GC-odorgram analysis (A). Total ion chromatogram (B), and mass spectrum (C) demonstrate that the licorice odor detected in (A) and represented by peak number 4 in (B) is a property of anethole [Benzene, 1-methoxy-4-(2-propenyl)], mol wt = 148. Fit = 97.6%. Purity = 94.0%.



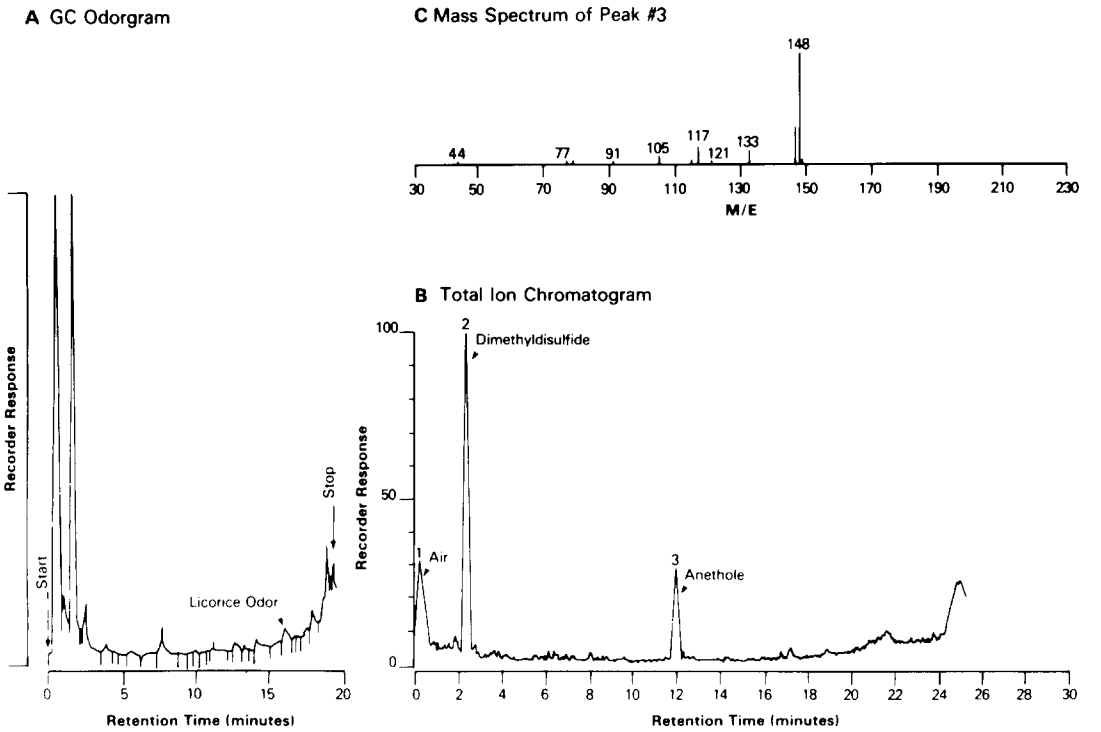


FIGURE 3: Licorice stool. Note detection and retention times of licorice odor in stool with GC-odorgram analysis (A). Licorice odor was distinct even though recorder response was small. Total ion chromatograph (B) and mass spectrum (C) demonstate that the licorice odor detected in (A), and represented by peak number 3 in (B) is a property of anethole [Benzene, 1-methoxy-4-(2-propenyl], mol wt = 148. Fit = 96.4%. Purity = 95.3%.

to be anethole. Figure 2 shows the GC-odorgram (Figure 2A) and GC-MS spectra of commercial Nibs licorice. Figure 3 shows the GC-odorgram (Figure 3A) and GC-MS spectra of the stool sample obtained after a Nibs licorice diet. The aroma of licorice was distinct in the headspace-GC effluent in all samples at the indicated retention times in each odorgram (Figures 1A, 2A, 3A). The GC-MS spectra identified the licorice odor in all samples as corresponding to the structure of anethole, 1-methoxy-4-(2-propenyl) benzene, the chief constituent of anise.

FIGURE 2: Nibs licorice. Note detection and retention times of licorice odor with GC-odorgram analysis (A). Total ion chromatogram (B) and mass spectrum (C) demonstrate that the licorice odor detected in (A) and represented by peak number 1 in (B) is a property of anethole [Benzene, 1-methoxy-4-(2-propenyl], mol wt = 148. Fit = 96.5%. Purity = 96.5%.

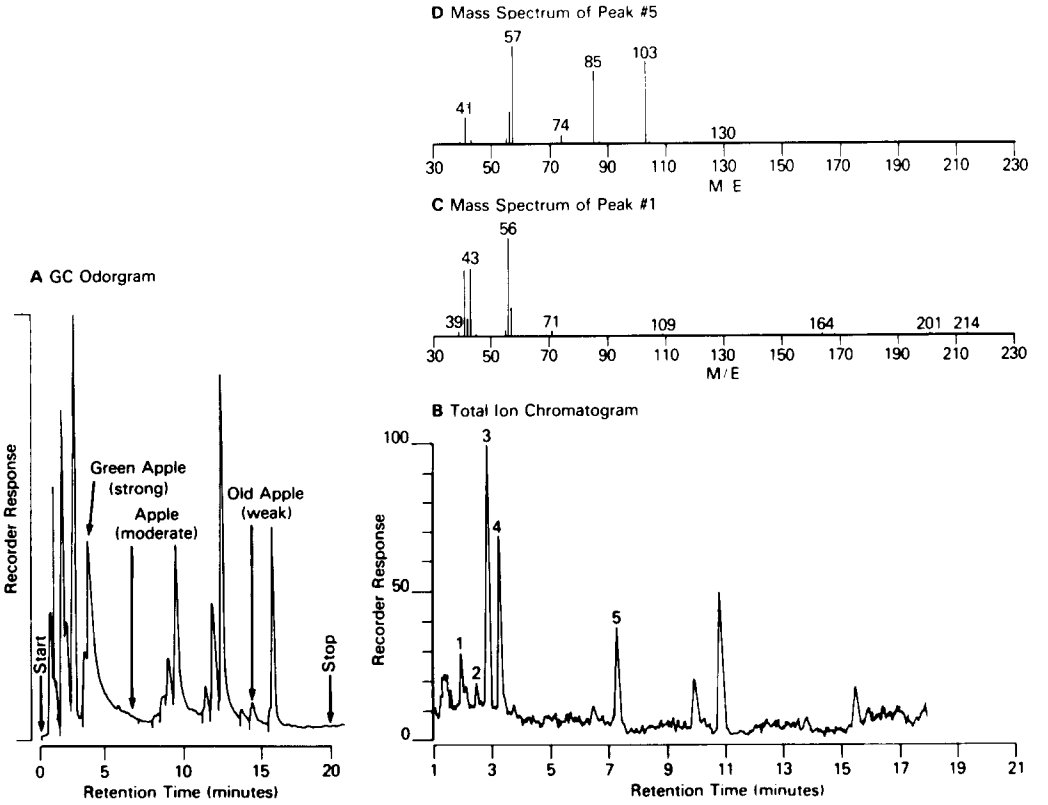


FIGURE 4: Fresh apple slice. Note detection and retention times of apple odor with GC-odorgram analysis (A). Apple odors were perceived as "strong" for green apple, "moderate" for apple and "weak" for "old" apple odor. Recorder responses were of ranging magnitude. Total ion chromatograph (B) and mass spectrum (C) demonstrate that the green apple odor detected in (A), and represented by peak number 1 in (B) is, in part, a property of pentane, 2,3-dimethyl, mol wt = 100. Fit = 89.1%. Purity = 62.6%.

Injection of 10 ng and 50 ng of anethole onto the GC column indicated a threshold level for anethole by odor detection at least as low as 10 ng.

Figures 4 and 5 show the GC-odorgrams and GC-MS spectra for the fresh apple slice and high-apple diet stool sample, respectively. The odor of apple was present and distinct in both samples at the indicated retention times. However, in contrast to licorice, the material giving the odor of apple recovered from stool could not be detected by GC-MS spectral analysis. This reflects the greater degree of sensitivity of the human olfactory system for detecting some odors, compared to the relative

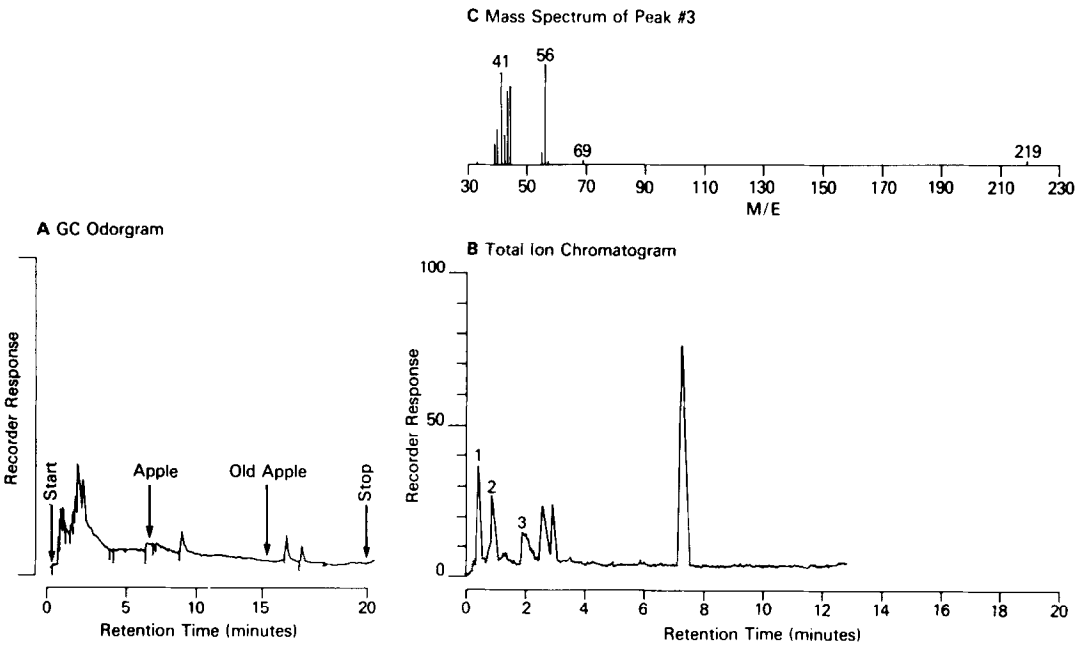


FIGURE 5: Apple stool. Note detection of apple and 'old' apple odors with GC-odorgram analysis (A) corresponding to odors detected with fresh apple slice in Figure 4. 'Green' apple odor detected with fresh apple slice not detected in stool sample. Note small or absent recorder responses at retention times where apple odor was detected. Total ion chromatogram (B) did not identify compounds corresponding to apple odors. However, the mass spectrum of peak number 3 in this figure strongly indicates the presence of the compound represented by peak number 5 in Figure 4 obtained from the fresh apple. The best fit for this compound was butanoic acid, 2-methyl-, 2-methyl propyl ester, mol wt = 158. Fit = 93.1%. Purity = 80.9%. Thus, a shared compound was detected with both the fresh apple slice and the stool obtained while on a high-apple diet which did not emit an apple odor.

insensitivity of our current GC-MS system to detect the odor-producing chemical.

These results indicate that it is possible to recover foodstuff volatile components, including those responsible for food aroma, by head-space, GC-MS analysis of stools. Within limits, diet components of a single individual may be reconstructed. These limitations include 1) the inability to detect food items ingested prior to 3-5 days before stooling, i.e. the normal mouth-to-anus intestinal transit time; 2) complete absorption of some food items which are then not excreted and thus escape fecal detection; 3) an insufficient amount of a specific food

item ingested such that a threshold concentration for detection in stool will not be attained; and 4) the inherent aromas of foods, some of which lack characteristic odor. Head-space, GC-MS analysis allows for tracing of specific volatile (odorous and non-odorous) dietary compounds through the intestinal tract. Intestinal transit times of many common food items may be determined with a sensitivity not possible with available techniques that employ charcoal or radionuclide markers. In addition, GC-MS stool analysis allows for the possible detection of novel compounds that may be unique to specific intestinal tract diseases. This technique may be useful for archeological, forensic, and clinical studies.

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REFERENCES

1. Moore, J.G., Krotoszynski, B.K., and O'Neill, F.J. (1984) Dig. Dis. 29, 907-911.
2. Dravnieks, A. (1982) Science 218, 799-801.
3. Larsson, B.R., and Widmark, G. (1969) Acta Pharm. Suecica 6, 479-488.
4. Teranishi, R., Mon, T.R., Robinson, A.B., Cary P., and Pauling, L. (1972) Anal. Chem. 44, 18-20.
5. Matsumoto, K.E., Partridge, D.H., Robinson, A.B., Pauling, L., et al. (1973) J. Chromatogr. 85, 31-34.
6. Keith, L., Stromberg, P., Krotoszynski, B.K., Shah, J., and Dravnieks, A. (1975) Arch. Gynat. 220, 1-10.
7. Stafford, M., Horning, M.G. and Zlatkis, A. (1976) J. Chromatogr. 126, 495-502.
8. Zlatkis, A., Bertsch, W., Lichtenstein, A., et al. (1973) Anal. Chem. 45, 763-707.
9. Zlatkis, A., Lichtenstein, H.A., and Tishbee, A. (1973) Chromatographia 6, 67-72.
10. Liebich, H.M. and Al-Babbili, O. (1975) J. Chromatogr. 112, 539-541.
11. Liebich, H.M., Al-Babbili, O., Zlatkis, A., and Kim K. (1975) Clin. Chem. 21, 1294-1299.
12. Liebich, H.M. and Woll, J. (1977) J. Chromatogr. 142, 505-516.
13. Politzer, I.R., Dowty, B.J., and Laseter, J.L. (1976) Clin. Chem. 22, 1775-1788.
14. Cronin, D.A., and Stanton, P. (1976) J. Sci. Fd. Agric. 27, 145-151.
15. Jarke, F, Dravnieks, A., and Gordon S.M. (1981) Am. Soc. Heating, Refrigeration Air Conditioning Engineer, Trans 87(1), 153-166.