

## COMPARISON OF GAS CHROMATOGRAPHY TECHNIQUES FOR SUITABILITY IN CITRUS PRODUCT RESEARCH AND MONITORING

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**Abstract.** Hot versus cold on-column capillary gas chromatograph injection techniques were investigated to improve mass spectral identification and quantitation of components in aqueous citrus samples. The cold on-column injection techniques provided better resolution, less thermal degradation, fewer artifacts, and improved sensitivity of citrus-related compounds. The technique is currently being used to monitor aqueous citrus essence production by a thermally accelerated short time evaporation (TASTE) evaporator.

The search for a better method of essence and essential oil analysis for quality and quantity has been in progress for many years. A wide variety of methodologies have been tried with varying degrees of success (1). The gas chromatographic (gc) analysis of whole citrus essence offers the advantages of simplicity and speed (2). Each year improvements in instrument sensitivity and dynamic range make gc analysis of citrus products more reproducible and reliable. Sensitivity and reproducibility are vital to the success of any methodology. Simplicity and speed, although important, are secondary. The purpose of this paper is to present an improved gc technique as applied to a gas chromatograph-mass spectrometer (gc-ms) system for aqueous essence analysis.

### Materials and Methods

The analytical instrument used in this study is a Kratos gc-ms system consisting of a Carlo Erba Fractovap 4200 gas chromatograph with a modified interface connected to a Kratos MS25 magnetic sector, double focusing, mass spectrometer. The data was collected and processed with a Kratos DS55 data system. Two fused silica capillary columns were used. One was a 30 m J&W DB-5 of 0.25 mm inside diameter and the second was a 30 m J&W DB-1 of 0.32 mm inside diameter. Both columns are non-polar and are equivalent to SE52 and SE30, respectively. Two injectors were tested for performance with the DB-1 and DB-5 columns. The first was a Grob injector manufactured by Carlo Erba and is a standard accessory with the Fractovap 4200 gas chromatograph. The Grob injector was tested at both 10:1 and 100:1 split ratios and 240°C. The second injector was a capillary on-column injector manufactured by J&W Scientific, Inc., Rancho Cordova, CA., model OCI operated at room temperature. The gc program for this series of tests was: initial time of 4 min, ramp rate of 5°C/min and final temperature of 240°C. The gc interface to the mass spectrometer was modified to allow the capillary column to terminate at the mass spectrometer source in-

stead of in the interface region as originally designed. This arrangement prevents any sample from coming into contact with unsilanized glass at elevated temperatures which is a known cause of artifact production.

### Results and Discussion

*Development of procedure.* Our first attempts at gc-ms analysis of whole citrus essence with the Grob injector were not satisfactory. Large amounts of data reduction were required to achieve reliable identification of the mass spectra produced by the system. In addition, attempts to correlate the relative retention times produced by the gc-ms system with stand alone gc's equipped with flame ionization detectors were difficult, even when using the same column and operating parameters. It should be noted that when a gc is connected to a mass spectrometer, part of the column is under vacuum; this changes the flow characteristics of the carrier gas and vapor phases that are passing through the system. Recognizing this fact, lowering the temperature program by 5°C to 10°C aids in matching relative retention times with those produced by a stand alone gc equipped with a flame ionization detector, which is above atmospheric pressure throughout the entire system.

Gc peak resolution and reproducibility of relative retention times were improved by the lower temperature programs, but mass spectral identification (especially with the terpenes) was still very difficult due to high chemical background present during gc runs of various samples. Reviewing the operation of a Grob injector, the sample is introduced into a heated (200°C to 250°C) glass lined chamber by means of a stainless steel needle. The resulting chamber environment of steam, organic compounds and stainless steel could easily cause the production of artifacts. The extensive data reduction required, in the form of background subtraction to produce usable mass spectra for identification, is unacceptable.

At this point it was decided to test cold on-column injection techniques. A review of readily available on-column injectors indicated that the J&W OCI had 2 distinct advantages. A glass alignment piece facilitates the insertion of a 0.2 mm od fused silica needle into 0.25 mm id capillary columns. The second advantage is that the column is partially withdrawn from the oven. The injection point is totally outside of the oven for cold injection and, then, after a suitable period of time (typically 2 min for a 30 m column) the injection point is reinserted back into the oven for temperature programming and bake-out at the end of the analysis. It should be noted that the sample never comes in contact with any metal at elevated temperature until it is released into the ionization chamber of the mass spectrometer. The quality of the mass spectra obtained by using this injector is consistently better and background subtraction is rarely necessary to positively identify most compounds found in aqueous citrus essence. In addition, water, acetaldehyde and ethanol can be adequately resolved to provide quantitative data which would normally be determined by separate mechanical or chemical techniques. The dynamic range of detection of the mass spectrometer along with the quantitation of water, acetaldehyde and ethanol provide one step analysis of whole citrus essence. This technique is being developed to monitor the performance of essence production by the TASTE evaporator located at CREC, Lake Alfred, FL.

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Comparison of Fig. 1. and Fig. 2 demonstrate that system sensitivity with the J&W OCI is as good as with the Grob injector. The water peak area was not a linear function of the volume of sample injected when using the Grob injector, but was linear with injections up to 0.8  $\mu$ l for the 0.25 mm inside diameter column and 1  $\mu$ l for the 0.32 mm id column when using the J&W OCI injector. It should be noted that for 0.32 mm bore capillary columns to be effective with the J&W OCI injector the oven temperature should be as low as practical and no higher than 40°C for the initial temperature. Fig. 3 and Fig. 4 demonstrate the lost performance at higher initial temperatures. Note that at an initial temperature of 55°C most of the components have been swept away with the water and ethanol vapor. This effect is less pronounced with smaller bore (0.25 mm inside diameter) columns which can perform effectively with initial temperatures between 40°C and 50°C. It may seem that several peaks are missing from these chromatograms but they are actually very small compared to the initial peak consisting mostly of water, acetaldehyde and ethanol. These non-expanded chromatograms were chosen to emphasize the overall performance difference and the better resolution of the initial peak. Quantitation of the first peak produces a value related to the volume of sample injected. The quantitation of the rest of the resolved peaks can be corrected by applying response factors (correction of apparent signal to true quantity of material detected) and ratioed

to the first peak to produce true quantitation of each resolved component. This method negates errors during injection (0.05  $\mu$ l error on a 0.5  $\mu$ l injection produces a 10% error) and does not rely upon the use of internal standards (introduction of a reference compound of known concentration). The calculation of response factors for making the conversions can be performed by the data system utilizing standard software operating in conjunction with

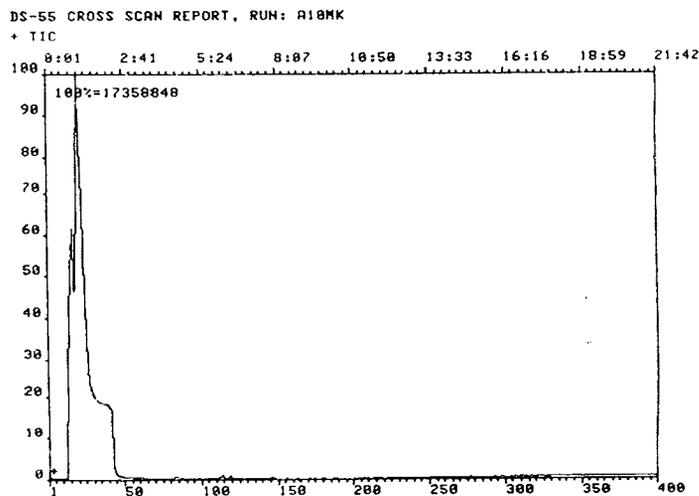


Fig. 3. Chromatogram of aqueous orange essence analyzed with J&W OCI injector. Initial oven temperature is 55° C. Virtually none of the sample is retained for analysis.

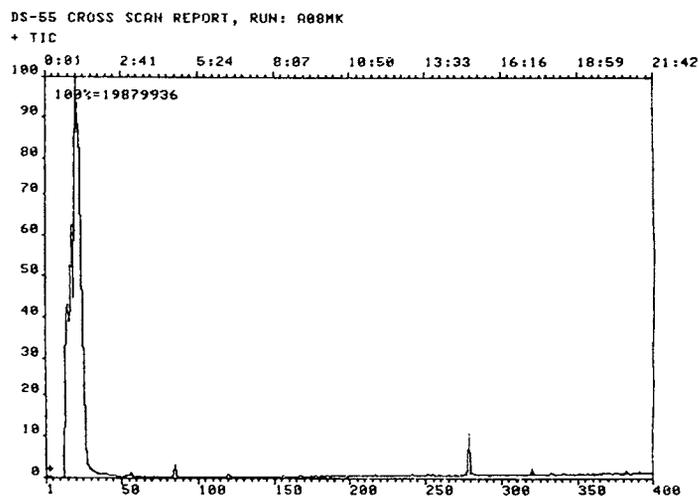


Fig. 4. Chromatogram of aqueous orange essence analyzed with J&W OCI injector. Initial oven temperature is 45° C. Some of the sample has been retained by the column for analysis. Initial oven temperature must be kept as low as possible for best results.

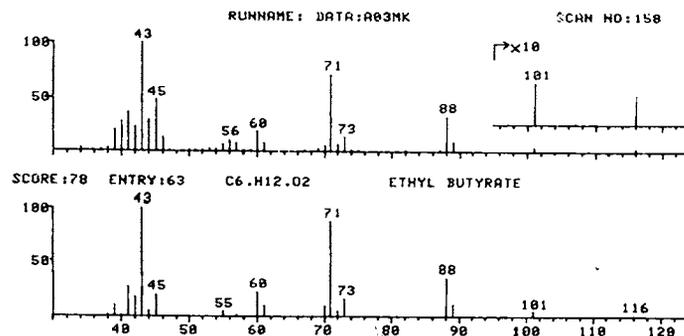


Fig. 5. Mass spectrum of ethyl butyrate compared with data system library entry. Sample was analyzed with the Grob injector and 30 m DB-1 column.

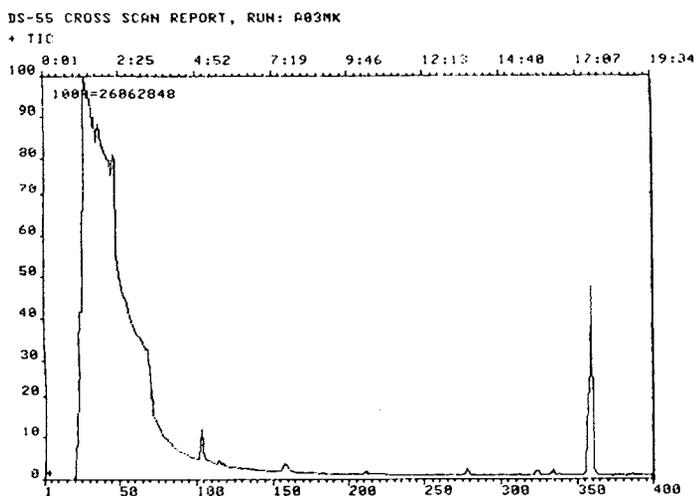


Fig. 1. Chromatogram of aqueous orange essence analyzed with Grob injector and 30 m DB-1 fused silica capillary column.

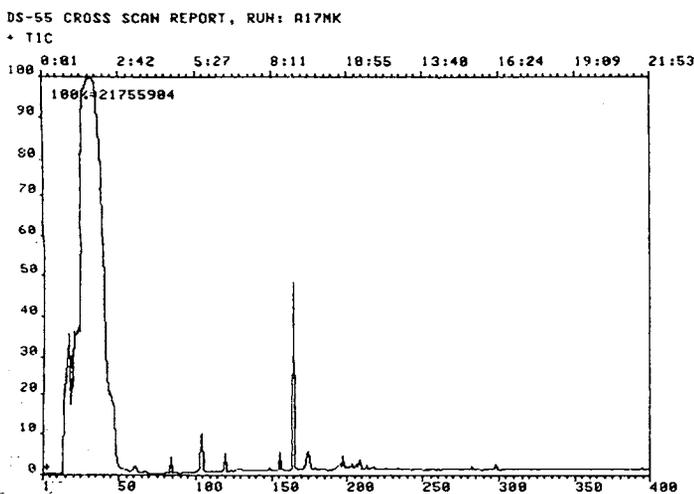


Fig. 2. Chromatogram of aqueous orange essence analyzed with J&W OCI injector and 30 m DB-1 fused silica capillary column.

the library of compounds used for identification. Fig. 5 and Fig. 6 compare the quality of the mass spectrum of ethyl butyrate as obtained from the system with the Grob injector, J&W OCI injector and the data system library entry. The sample spectra are from the same sample and analyzed on the same column. The small differences in the spectra are vital when determining isomers such as carvone (Fig. 7) or terpenes (Fig. 8).

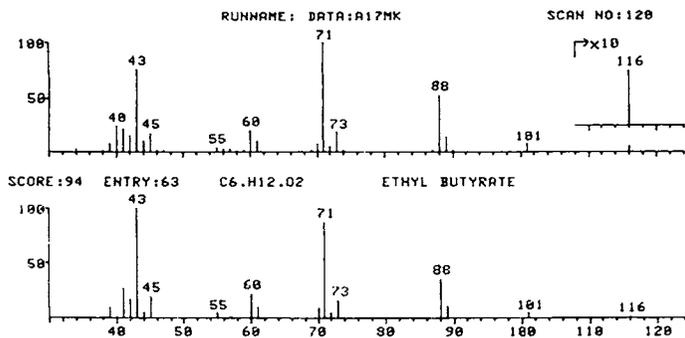


Fig. 6. Mass spectrum of ethyl butyrate compared with data system entry. Sample was analyzed with the J&W OCI injector and 30 m DB-1 column. The reduced chemical background results in a much higher match score.

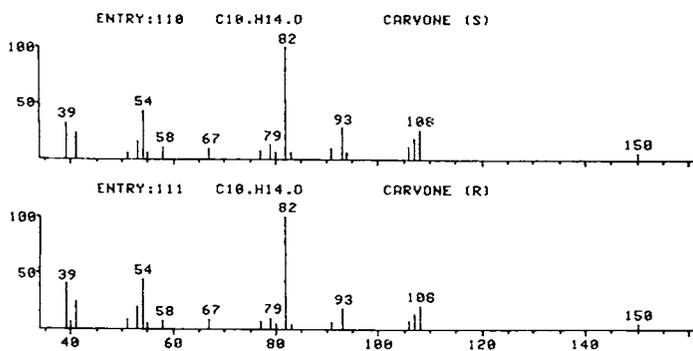


Fig. 7. Comparison of library entries of isomers of carvone. The intensity ratios of the masses are nearly identical. Successful identification of isomers depends heavily upon minimal artifact production which changes the intensity ratio of the resolved component in a gc analysis.

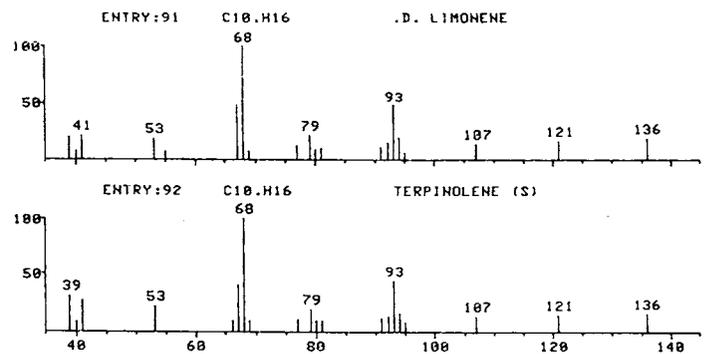


Fig. 8. Comparison of library entries of two common terpenes. The intensity ratio of masses 39 and 41 and the presence or absence of mass 66 are vital for correct identification. Artifact production with the Grob injector adds to these masses resulting in an inability to provide positive identification.

### Summary

The cold on-column injection technique has clearly demonstrated its superiority when used in a gc-ms system for analysis of aqueous essence samples. The mechanical design of the injector tested provides simple retrofit installation on any gas chromatograph that is using fused silica capillary columns of 0.32 mm id or smaller. The reduced temperatures that the sample is subjected to lowers the probability that artifact production occurs. The elimination of steam passing through the column should result in extended column life. Further improvements in sensitivity and dynamic range of the detector used are needed to fully utilize the potential of cold on-column injection techniques.

### Literature Cited

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## CITRUS JUICE TRACE METAL ANALYSIS BY AUTOMATED SEQUENTIAL MULTIELEMENT ICAP-AES

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*Abstract.* Major (P, Mg, Ca, K, Na), minor (Ba, B, Mn, Mo, Ni, Zn, Cu, Al, Fe, Sr, Rb) and ultra trace elements (Sn, Co, Cr, Ti, V, Li) in frozen concentrated orange juice (FCOJ) and canned single-strength grapefruit juice (SSGJ) were determined by an automated fast sequential multielement

spectrometer combined with Inductively Coupled Argon Plasma (ICAP) Atomic Emission Spectrometry (AES). The mean values for Ba, B, Rb, and Ti in canned SSGJ were lower than in the commercial FCOJ. Also, canned SSGJ mean values for P, K, Mn, and Li were lower than in FCOJ samples prepared at the Citrus Research and Education Center (CREC). All the other elements of canned SSGJ mean values were higher than the FCOJ. The mean value for Sn in canned SSGJ was significantly higher than in the CREC and commercial FCOJ samples.

There have been relatively few references on the comparison of trace metals in orange and grapefruit juices. Roberts and Gaddum (6) reported the elemental concentrations for several cultivars of Florida oranges and grapefruit in 1937. McHard et al. (1) compared some modern approaches to orange juice analysis in a recent

Florida Agricultural Experiment Stations Journal Series No. 6019.

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