Rapid, Quantitative, and Automated Headspace Analysis of Beer Volatiles[1]

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ABSTRACT

Beer volatiles were quantitatively analyzed by an automated dynamic nonequilibrium headspace gas chromatography technique. The system described consists of two Tekmar LSC-2 purge-and-trap systems connected in a master/slave configuration to a dual channel gas chromatograph. The operational procedure and the problems encountered are discussed. Modifications to the equipment were made to correct problems associated with both system design errors and the foaming and CO_2 content of the beer. The alcohols and esters of beer were quantitated with a coefficient of variation of better than 10% across two systems. The system can analyze over 6,000 samples per year.

Key words: Dynamic headspace, High-resolution gas chromatography, Multiplant brewing, Quantitative beer volatile analysis

Sophisticated gas chromatographic instrumentation (GC) has become an essential part of modern brewing. With the aid of the GC, the understanding and control of the production of very active flavor compounds such as dimethyl sulfide and diacetyl has been achieved.

Most modern GC methods that have been developed for the analysis of base flavor/aroma compounds of beer, i.e., volatile esters and alcohols, have proved less than ideal for routine quantitative analyses, mainly because of involved sample preparation (4–6). Headspace analysis of these compounds appears to be an attractive routine procedure because little or no sample preparation is required.

The quantitative analysis of beer volatiles by the purge-and-trap method (dynamic headspace) was recently described extensively by Chen (1). He showed that quantitative differences between various brands of beer can readily be shown. The same results have been observed in our own laboratory. However, the routine application of this technique to the brewing industry has not been demonstrated.

Our company operates 13 separate breweries, each producing up to seven different brands. The geographical locations vary considerably, e.g., from sea level to 6,000 ft. There are also major equipment differences ranging from 160-hl cylindro-conical flat bottom vessels through to kombi vessels (uni-tanks) to 3,000-hl cylindro-conical vessels. With today's increased population mobility, it is important to ensure product uniformity across such an environment. Hoff et al (3) demonstrated that analysis of beer volatiles can be useful in identifying and correcting problems associated with plant differences in multiplant brewing. With a total of 13 plants, the application of Hoff's procedures would require a rapid, routine method of analyzing beer volatiles. In this paper we report a fully automated method for the analysis of beer volatiles based on the purge-and-trap method, which allows up to 20 analyses in duplicate per day. The precision and quantitative calibration procedure is also discussed.

EXPERIMENTAL

Equipment

Two Tekmar LSC-2 purge-and-trap systems (Tekmar Co., Cincinnati, OH), each consisting of a liquid sample concentrator and an auto sampler were coupled in a master/slave configuration.

The sample (purge) vessels were water-jacketed, 10-ml tubes (15 × 110 mm) fitted at the bottom with a medium sintered glass frit to disperse the purge gas. The circulation water was held at 35° C. The purged beer volatiles were trapped on a porous polymer trap (300 × 3 mm) stainless steel tube containing 200 mg Tenax GC (60/80 mesh).

The gas chromatograph used was a Hewlett-Packard 5880 equipped with dual FID detectors, dual capillary inlet systems, and cryogenic cooling. Figure 1 shows the complete system.

Analytical Procedure

Quantitative information on the compounds of interest was obtained by comparing their integrated area to that of an internal standard. Response factors for the various compounds compared to that of internal standard were determined by standard addition of the compounds to beer.

One milliliter of internal standard (1.6% (w/v) *n*-butanol in 99.95% (v/v) ethanol) was introduced directly into varied volumes (corrected for by computer program) of cold beer (5° C) by way of a can/bottle piercing unit (Alltech Associates). The beer was inverted 10 times and allowed to stand 10 min to ensure mixing of the internal standard and minimize foaming when the sample was opened. The cold beer was carefully poured into a precooled glass syringe (5 ml, equipped with an on/off valve). The plunger was inserted, the valve opened, and the volume adjusted to 5 ml. The beer was then immediately introduced into the purge vessel through a syringe fitting. Polypropylene glycol 2025 (10 µl GC grade, E. Merck, volatile free) was introduced to the purge vessel before the sample.

The flow rate of the purge gas was set at 25 ml/min and passed through the sample for 6 min. After completion of the purge cycle, the trap was preheated rapidly to 180° C, and the trapped volatiles desorbed for 1.3 min. The volatiles were then transferred into the capillary inlet system of the GC (operated in the split mode) by way of a heated transfer line. The column temperature was held at 10° C during this procedure. During the GC run, the purge trap was baked at 200° C (28 min). After completion of the analysis, the GC oven and purge trap were cooled to the preset temperatures, the purged gas was automatically switched to the next sample, and the analyses sequence was repeated. After completing a series of samples, the purged vessels were washed with hot water (3 × 10 ml, 90° C) and purged (3 min).

Fig. 1. The two Tekmar LSC-2 purge-and-trap systems connected to the Hewlett-Packard 5880 gas chromatograph.

[1]Presented at the 51st Annual Meeting in Milwaukee, WI, June 1985.

RESULTS AND DISCUSSION

With the objective of maintaining brand integrities across a multiplant environment, rapid methods of obtaining analytical information are required. The analysis of beer volatiles by the purge-and-trap headspace technique is especially appealing because sample preparation is minimal, the method is suitable for high-resolution capillary gas chromatography, sufficient material is purged (concentrated) from the sample to make quantitation practical, and equipment is available to automate the procedure.

The Tekmar LSC-2 purge-and-trap system consists of a sample concentration unit and an automated sampler with a 10-sample capacity. Table I shows the basic functions of the system. When the system first comes on or is in an automated sequence it is held in the "standby" mode until the trap reaches the desired temperature. In this study a trap temperature of 32° C was selected. Once the trap temperature is obtained the system begins to purge the sample. The sample can be purged up to 99 min, but only 6 min were found to be necessary to concentrate sufficient volatiles for subsequent analysis.

On completion of the purge cycle, the system checks the GC oven temperature. If the desired initial GC oven temperature (10° C) has not been reached, the system is held in the "purge complete" state. When the desired oven temperature is reached, the system switches either to the "desorb ready" (a hold state) or "desorb preheat" state depending on the number of samplers in the system (discussed later). In the desorb preheat state, the trap is heated without a flow of purge gas to the desired desorb temperature (180° C). Once this temperature has been reached the system switches to desorb, which allows the purge gas to pass through the trap and into the GC injector for 1.3 min. On completion of desorb the purge gas is switched from the GC to vent and the temperature of the trap is increased to 200° C. This "bake" state is held for 28 min. From the bake state the system returns to standby and the sequence is repeated.

The Tekmar purge-and-trap system was primarily designed for routine trace analysis of water samples. The use of this equipment for carbonated beer analyses required several modifications. The problems encountered and solutions are shown in Table II.

The first problem encountered was excessive foaming of the sample during purging. The sample would foam out of the trap and into the common line to the purge trap. This necessitated a very difficult cleaning operation. This problem has been noted by the manufacturers of purge-and-trap systems and has generally been overcome by rapid stirring of the sample. With a total of 20 sample vessels in the system it was not practical to devise an automated sequence of stirring. The problem was overcome by the addition of 10 μl of GC-grade polyethyleneglycol 2025 to each sample. The polyethyleneglycol was stripped of volatiles in a vacuum oven before use, and no additional peaks were noted in the beer chromatograms with the use of this material.

Two design problems in the sampler were noted. First, the position and closure sequence of the valves allowed positive pressures to build up on the trap side of the sample, especially if the sample was not completely degassed by nitrogen purging. This positive pressure resulted in beer backstreaming through the purge line. Secondly, during the cleaning/baking cycle of the trap, the purge gas, used to sweep any remaining volatiles off the trap, first passed through the last purged sample. In practice, during its cleaning cycle the trap received a continuous stream of beer volatiles. Both of these problems were corrected by a Tekmar modification that placed an additional valve in the system that bypasses and vents the sample vessel during the cleaning/baking cycle.

With the water jackets of the 10 sample vessels connected in a series, it became time-consuming to remove sample vessels for cleaning. As much time was needed to clean the system for the next set of samples as was required to analyze the samples. To overcome this disadvantage a cleaning-in-place system was developed. It was found that flushing each tube with hot water followed by purging was sufficient to clean the tubes for the next set of samples.

After a few months of operation, it was found that a single Tekmar unit was not sufficient to handle the analytical load. The most economical way to increase the sample capacity was to install a second Tekmar unit on the same GC by utilizing the GC's dual injector/dual detector capability. The critical requirement of this configuration is that both samplers transfer their concentrated volatiles to the GC at the same time. To assure this step the samplers were modified by Tekmar to a master/slave configuration where one unit (master) triggers both units to transfer the sample to the GC. There is no feedback circuit from the slave telling the master that the sample is ready for transfer; therefore, the slave unit must always be started first, so that this unit is always ready to transfer the sample when the master unit signals.

The quantitation of a headspace method has been described by

TABLE I
Basic Functions and Selected Operational Parameters of the Tekmar LSC-2 Purge-and-Trap System

Indicated Function	Description	Selected Parameter
Purge ready	Preset temperature of trap reached. Purging can be started (start of run in auto mode)	32° C
Purge	Sample is purged up to 99 min (max 99 min)	6 min
Purge complete	Gas chromatograph oven temperature greater than starting oven temperature	10° C
Desorb ready[a]	Sampler on hold	System II only
Desorb preheat	Trap heated to preset desorb temperature	180° C
Desorb	Sample transferred from trap to gas chromatograph	180° C for 1.3 min
Bake[b]	Trap conditioned to clean	200° C for 28 min
Standby	Trap cooling to preset temperature	32° C

[a] System II gets signal from system I to proceed to desorb preheat.
[b] II starts gas chromatography program and integration at bake.

TABLE II
Problems Encountered and Solutions Developed for the Purge-and-Trap System

Problem	Solution
Excessive Foaming of beer	10 μl PEG 2025 (GC[a] Grade) added to purge vessels
Beer backstreams through purge line on completion of purge	Changed sequence of switching valve, i.e., close trap vent only after purge gas has been closed and pressure released
Purge gas passes through vessels during baking (28 min)	Solenoid valves installed to bypass vessel during bake cycle
Cleaning purge vessels	Rinse 3 times using hot water (3 × 10 ml at 90° C) with syringe
Synchronizing p/t units and GC[a]	Master/slave configuration introduced

[a] GC = gas chromatograph.

Hachenberg and Schmidt (2). Basically, it requires the determination of a response factor that relates the amount of an added internal standard to the concentration of the compound of interest. In the case of beer, where the compounds of interest are already present in unknown amounts, various concentrations of the compounds must be added and their response to that of the internal standard at each added concentration determined. From this information a linear regression can be calculated, the slope of the line being the required response factor. Figures 2 and 3 show the response curves for *n*-propanol and isoamyl acetate, respectively. Regression correlations (R^2) of 0.995 and 0.982 were obtained. These values are typical of all the regression curves. Slightly better regression curves have always been obtained for the alcohols. The response factors have remained very constant over a two year period.

Table III shows the percent recovery, the coefficient of variation from a single instrument, and the coefficient of variation obtained over both systems. Although the precision of analysis shown in Table III is acceptable, the use of an alcohol internal standard to quantify an ester is less than ideal. It has not been possible to find an appropriate ester to use as a second internal standard. All the esters tried to date have had overlapping retention times with compounds present in beer.

The high coefficient of variation shown for the two higher esters is mainly because of their low concentration in the beer. To obtain better quantitation of these compounds, a third internal standard of appropriate concentrations and type would be required. The slightly high percent recovery shown in Table III results from the nonlinearity of the calibration curves at very high concentrations. The linear ranges of all the calibration curves have not been determined, but fairly narrow ranges, especially for the esters, have been noted.

Figure 4 shows simultaneous chromatograms from the two purge-and-trap units. Peak 1 is acetaldehyde. All attempts to obtain acceptable quantitative data on this compound have failed; the reasons for this are not clear at this time. To obtain good quantitative data on *n*-propanol (peak 2), it is important that the ethanol peak be as sharp as possible. Attempts to take advantage of the ethanol as a solvent for splitless injection have been disappointing. However, the amount of research time available on the instrumentation has been insufficient to fully explore this mode. The chromatographic conditions used have allowed sufficient separation of the amylalcohols (peaks 6 and 7) for quantitation. All identified peaks are listed in Table III. The smaller peaks in the chromatograms have not yet been conclusively identified.

A BASIC program was written for the 3354 data system which, on completion of the analytical run, calculates the mean of the duplicate samples and prints the results as shown in Table IV. All duplicate analyses were obtained from the same sample, which was run on each of the two units. By inspecting the data from the duplicates, the operator can determine if any problems occurred during the analysis. All suspected analyses are repeated.

The system has been in use for over two years, running an average of 6,000 analyses (3,000 samples in duplicate) per year. Very few problems have been experienced with either the capillary

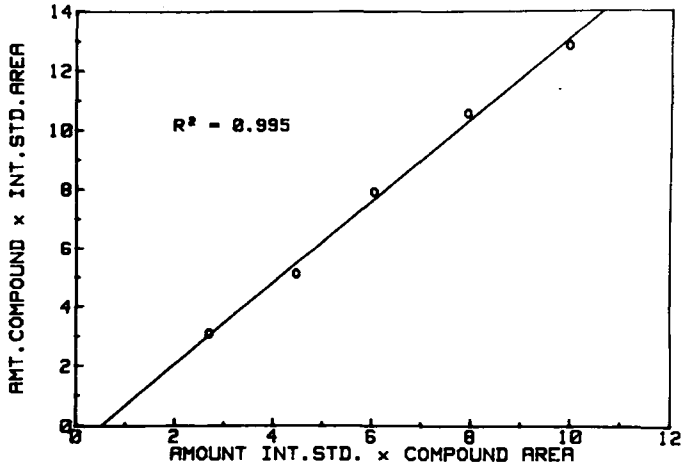

Fig. 2. Regression curve for determining response factor for *n*-propanol.

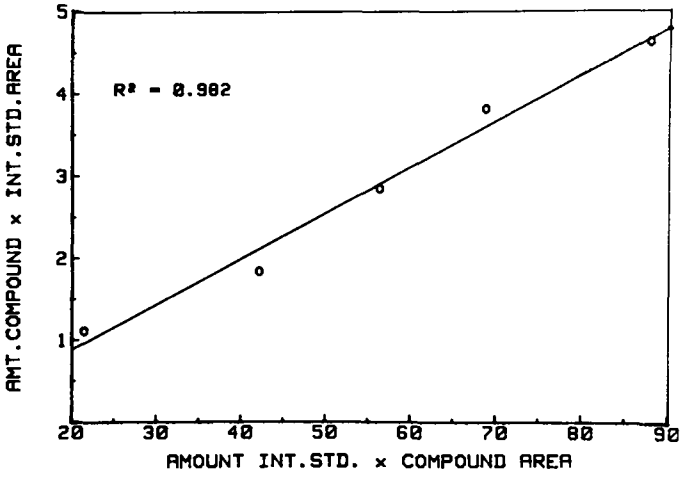

Fig. 3. Regression curve for determining response factor for isoamyl acetate.

TABLE III
Peak Identification, Percent Recovery, and Coefficients of Variation from a Single Sampler and from both Samplers for the Beer Volatiles Quantitated

Compound	Peak No.	% Recovery	c.v. System I (%)	c.v. System I & II (%)
Acetaldehyde	1	...	...	...
n-Propanol	2	103	0.6	6.4
Ethyl acetate	3	107	3.3	3.7
Isobutanol	4	101	2.3	7.3
+ *n*-butanol	5			
3-Methyl butanol	6	102	1.3	2.3
2-Methyl butanol	7	102	1.6	1.9
Isobutyl acetate	8	103	6.6	9.8
Ethyl butanoate	9	103	5.3	7.4
Isoamyl acetate	10	103	5.8	6.5
Ethyl hexanoate	11	...	10.6	12.8
Ethyl octanoate	12	...	19.2	31

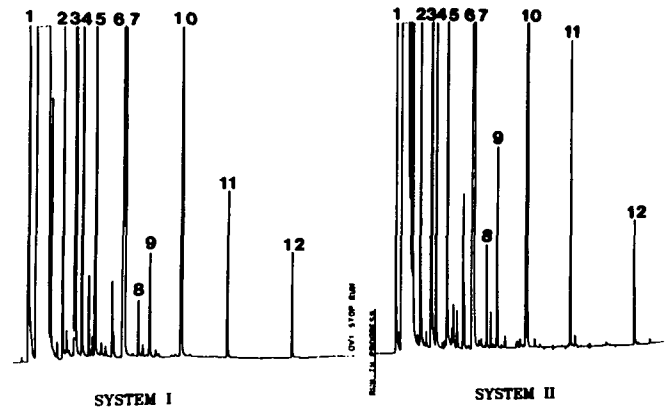

Fig. 4. Simultaneous chromatograms from the two purge-and-trap units.

TABLE IV
Example of Results Produced by the 3354 Data System on Completion of an Analytical Run

Compound	Mean	Sampler I	Sampler II
n-Propanol	12.82	12.97	12.67
Ethyl acetate	19.69	19.16	20.22
Isobutanol	11.82	11.92	11.71
+ *n*-butanol	21.39	21.39	21.39
3-Methyl butanol	46.08	45.57	46.58
2-Methyl butanol	18.78	18.25	19.31
Isobutyl acetate	0.03	0.03	0.03
Ethyl butanoate	0.08	0.08	0.08
Isoamyl acetate	1.97	1.95	1.98
Ethyl hexanoate	0.14	0.14	0.14
Ethyl octanoate	0.2	0.19	0.2

columns used or the Tenax GC trap. Periodically, peak broadening is observed on the capillary column, but this can generally be corrected by removing a small segment (±0.1 m) at the front of the column. Condensation and evaporation of large amounts of alcohol on the first part of the column appear to irreversibly damage the phase. Deterioration of the Tenax trap is generally observed by a steady increase in ester levels. The periodic analysis of samples of known concentrations can highlight problems of this nature.

Although most of the problems with the system have been solved, there still remains one unresolved area. In both Chen's work (1) and in this laboratory using the single-sample purge-and-trap system, peaks for 2-phenyl ethanol and 2-phenyl ethyl acetate were readily observable. With the automated system, no 2-phenyl ethanol and only a rather small variable peak for 2-phenyl ethyl acetate was observed. It is suspected that there is inadequate heating along the rather long transfer line (±2 m) between the sample and the GC. With trace analysis this would not be a problem, but for compounds in the parts per million range there would be sufficient material to condense on any cold spots in the system. Investigations in this area are continuing.

SUMMARY

A system and procedure was developed that allows the analysis of well over 3,000 samples in duplicate per year. All major problems experienced have been solved and the data produced by this method are used in pattern recognition techniques to identify plant difference (7) and yeast strains (A. W. Dercksen, *unpublished*).

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LITERATURE CITED

1. Chen, E. C. H. *J. Am. Soc. Brew. Chem.* 41:28, 1983.
2. Hachenberg, H., and Schmidt, A. P. In: Gas Chromatographic Headspace Analysis. Heyden & Son Ltd.: London, 1979.
3. Hoff, J. T., Chiroye, E., Herwig, W. C., and Helbert, J. R. Page 187 in: Analysis of Foods and Beverages, Headspace Techniques. G. Charalambous, ed. Academic Press: New York, 1978.
4. Home, S. E.B.C. Flavor Symposium, Copenhagen, 1981. p. 79.
5. Lindsay, R. C., Withycombe, D. A., and Micketts, R. J. *Am. Soc. Brew. Chem., Proc. 1972*, p. 4.
6. Stenroos, L. E., Siebert, K. J., and Meilgaard, M. C. *J. Am. Soc. Brew. Chem.* 34:4, 1976.
7. Torline, P. A. *Tech. Q. Master Brew. Assoc. Am.* 22:13, 1985.

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