

QUANTITATIVE DETERMINATION OF ETHANOL IN WINE BY GAS CHROMATOGRAPHY

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ABSTRACT

A method is described for specific quantitative analysis of ethanol in wine by gas chromatography. This method, which uses an internal standard and flame ionization detector, is more accurate and more precise than methods commonly used. Statistical analysis of data from typical winery samples shows that the standard error of estimate for the method is 0.07% (v/v) over the range 7 to 24% (v/v). The estimate of the standard deviation of the chroma-

tography for duplicate injections is 0.02% (v/v). The gas-liquid chromatography method determines ethanol separately from the other wine components that interfere in other methods, and without distillation or chemical reaction. When large numbers of samples are analyzed, advantages include short analysis time per sample and potential for extensive automation.

Determination of ethanol is perhaps the most important routine analysis in a modern winery. Frequent, fast, and accurate results are needed to control the quality of the wine from grape to bottle, as well as for state and federal government tax and regulatory purposes. The alcoholic beverage industry and various regulatory agencies have devoted much effort in recent years to developing a faster, specific, more accurate, and automated method.

Gas-liquid chromatography is one of the most modern analytical techniques, dating from 1952. Even more recently, with the utilization of electronic digital data-processing equipment, gas chromatography has become increasingly preferred for accurate quantitative as well as qualitative analyses of many substances.

Traditional ethanol analysis methods, involving distillation and/or mass determinations, are known to include small inaccuracies due to the presence of interfering volatile or other components in wine (12). With an appropriately chosen column packing, gas chromatography is inherently specific, separating volatile compounds on the basis of compound-specific partitioning properties between a gas phase and a liquid (or a solid) phase. To evaluate the accuracy, precision, and specificity of the gas-liquid chromatography method described here, it was necessary to analyze ethanol standards and typical winery samples by a reference method. The reference

method used was the micro-dichromate procedure (9). Because of the high accuracy of the GC method, it was also necessary to develop a procedure for estimating the magnitude of the inaccuracy in the dichromate method caused by non-ethanol wine volatiles. The most significant non-ethanol wine components analyzed as "ethanol" by the dichromate method were determined to be methanol and the common fusel oils.

Wine industry researchers were among the early investigators of quantitative gas chromatography (GC) techniques, presenting data on ethanol analysis in 1961 (3). A 1961 industry paper included data on a relatively sophisticated GC ethanol analysis method utilizing an internal standard and electronic integrator for peak quantitation (8). A variety of GC methods for the analysis of ethanol are described in a recent review article by Jain and Cravey (6). Recent papers by Martin and Tenenbaum (7), Solon et al (10), and Goodman and Dorion (5) describe gas-solid chromatographic methods using the newer porous polymer column packings. Despite the extensive research on GC ethanol analysis in the past 15 years, no published method has been refined to the high accuracy and precision of the method described here. A nonlinearity in GC detector response to ethanol has been the main obstacle to high accuracy in previous work. Additional inaccuracies can be introduced into a GC method by inappropri-

ate selection of column packing and internal standard. Several combinations of column packing and internal standard were tested and rejected before the configuration described here was chosen. The method described, utilizing an internal standard, flame ionization detector, and electronic digital integrator, has achieved better results with gas-liquid chromatography than with gas-solid chromatography.

MATERIALS AND METHODS

Ethanol standards: Five ethanol standards were prepared by diluting 200-proof ethanol (Rossville Gold Shield) with distilled water to cover the range 6 to 20% (v/v) ethanol. Pure ethanol and distilled water were chosen to minimize the possibility of non-ethanol components interfering in determination of the ethanol content of the standards.

Winery samples: One hundred typical winery alcohol-analysis samples were selected for GC analysis. These samples included wine in all stages of production from fermentation through bottling. The ethanol content of these 100 samples ranged from 7 to 24% v/v (mean 13.5%) and included 36 samples in the range of 14 to 24%.

Dichromate analysis: The standards and wine samples were analyzed repeatedly for ethanol content by the micro-dichromate method to obtain an accurate (average) reference analysis for each sample. All samples were analyzed at 20°C and stored at 4°C.

Determination of interfering substances: Model solutions containing one non-ethanol wine volatile in distilled water or in a water-ethanol solvent of known ethanol concentration were analyzed for "ethanol" by the micro-dichromate method. Each model solution contained one non-ethanol volatile in a concentration between 0.5 and 5% (v/v) in the case of methanol, n-propanol, sec-butanol, isobutanol, isoamyl alcohol, acetaldehyde, ethyl acetate, 2-butanone, or glycerin. The 2,3-butanediol model solutions ranged from 10 to 15% v/v. Potassium sorbate and potassium metabisulfite model solutions were saturated.

Determination of methanol, fusel oils: Methanol, n-propanol, n-butanol, sec-butanol, isobutanol, and isoamyl alcohol were determined in the 100 winery samples by a GC method. For sample preparation, 25.0 ml of each sample plus 10.5 ml of 190-proof ethanol was steam-distilled, and 25.0 ml was collected. To 10.0 ml of the distillate, 1.0 ml of 3-pentanol was added as an internal standard. The chromatograph consisted of an Aerograph Hi-Fi GC with flame ionization detector, and a 6-ft. \times $\frac{1}{8}$ -inch stainless-steel column packed with 15% Hercoflex 600 on 80/100-mesh Aeropak 30. Column and de-

tector temperatures were 61°C, with an injector temperature of 170°C. Helium carrier flow was 30 cc/minute. Attenuation was 16 on the 10 range. Sample size injected was 2 μ l, and peak area counts were printed out on paper tape by a Varian 480 digital integrator. Standardization and calculations were similar to those in the GC ethanol method.

Internal standard and diluent: The internal standard was reagent grade n-butanol (Matheson Coleman & Bell Chromatoquality). A 15-liter diluent-internal standard solution was prepared by diluting n-butanol to 0.05% (v/v) with distilled water. The diluent solution was stoppered and stored at 20°C.

Sample preparation: With a York 2111 automatic diluter, 0.100 ml of wine (or ethanol standard) was diluted to 25.0 ml with the internal standard-diluent solution. The sample was diluted directly into a 30-ml serum vial and was immediately ready for injection into the GC.

Chromatography: A Varian 1400 GC with a flame ionization detector was used. A 6-ft. \times $\frac{1}{4}$ -inch copper column packed with 3% Carbowax 600 on 40/60-mesh Chromosorb T was used. (A fluidized drying technique was used to prepare the packing.) Column temperature was 80°C, with injector and detector temperatures of 120 and 125°C, respectively. Helium carrier flow was 110 cc/minute. Electrometer attenuation was 1 on the 10^{-11} range. Sample size injected was 0.5 μ l.

Integration: Peak areas and retention times were calculated and printed out in digital form on paper tape automatically by a Varian 480 electronic integrator. Integrator settings included slope sensitivity of 30 seconds (0.5) μ V/sec, slope filtering of 10 seconds (0.3 Hz), baseline shift of 50 μ V, and digital baseline correction of 9 (1.5 μ V/sec).

Standardization: The five ethanol standards were used to standardize the GC method as follows. Each of the five standards was diluted several times. The dilutions for one standard were each injected, and a "response factor" for each dilution was calculated by dividing the ethanol peak area counts by the n-butanol (internal standard) peak area counts. For the given standard, an average response factor for the dilutions of that standard was calculated. The dilution that produced the response factor closest to the average was retained for standardization purposes, and the other dilutions for that standard were discarded. This selection of an "average dilution" was carried out for each standard. The five average dilutions (corresponding to the five standards) were each injected once for the initial standardization of the GC system, and each injected once on subsequent days of the experiment. This set of five average dilutions of the standards was handled carefully to avoid evaporation and contamination, and stored at 4°C. The actual calculations used in

the standardization were linear regression calculations.

Linear regression calculations: Linear regression statistical analysis by least squares according to the usual method (1) was applied to the five response factors produced by the injection of the set of five standardization dilutions. Linear regression analysis of the response factors produced by the injection of the 100 winery samples was also performed. The average dichromate analysis of each sample provided the reference analysis for the calculations.

GC analysis of winery samples: The 100 winery samples were each diluted once and injected twice. Response factors were calculated for each injection. The ethanol content was calculated as (intercept) + (slope \times response factor), with slope and intercept determined by standardization and linear regression calculation as described above.

Diluter precision check: The precision of the York diluter used in the GC method was measured by diluting a ferroin dye solution and reading the absorbance of the diluted solution at 508 $m\mu$ on a Cary 15 spectrophotometer. Twenty separate dilutions were made and analyzed.

Standard-deviation calculations: The duplicate chromatography injections of each of the 100 winery samples were analyzed statistically for estimate of the standard deviation as described by Youden (11) for groups of duplicate samples. In addition, a standard deviation was calculated for the multiple injections of each ethanol standard, and for multiple ferroin dye dilutions.

Equipment: The equipment used is shown in Fig. 1. Analyst interaction with the equipment is minimal, consisting of positioning the sample for dilution, injecting the sample, and activating the diluter and integrator by foot switches.

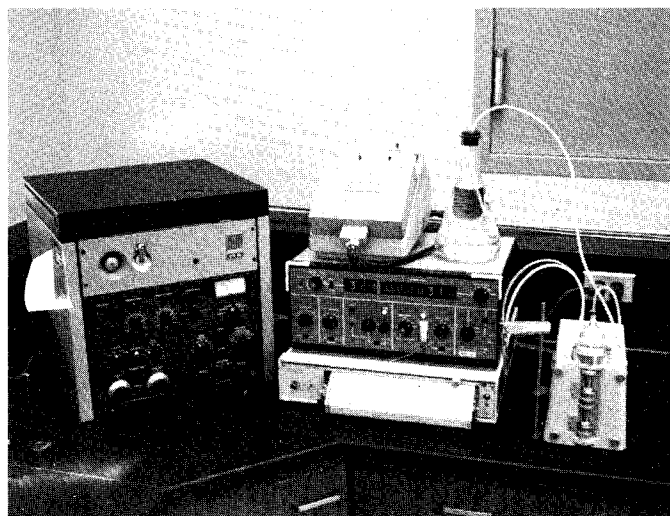
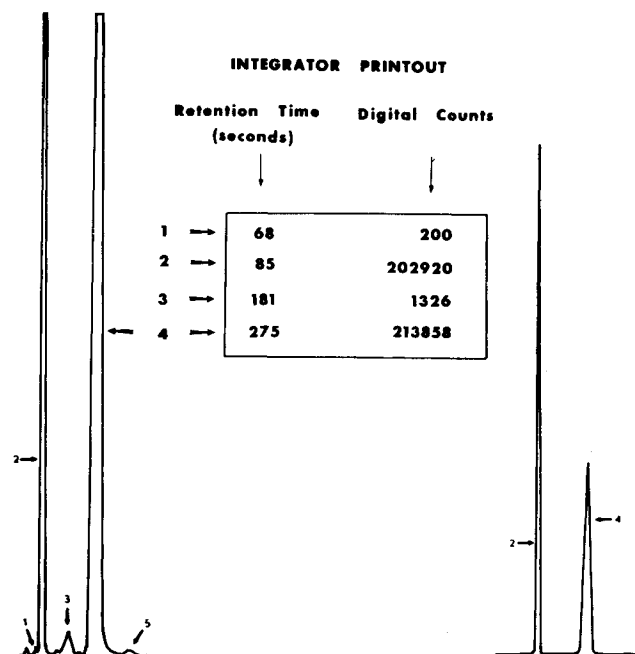


Fig. 1. Equipment used in GC ethanol analysis including GC, integrator and printer, recorder, and automatic diluter.



WINE COMPONENTS:

- 1, Methanol; 2, Ethanol; 3, "Water Peak";
4, n-Butyl alcohol; 5, Iso-amyl alcohol.

Fig. 2. Chromatograms, integrator printout of dry sherry.

RESULTS AND DISCUSSION

Fig. 2 shows GC chromatograms of a typical wine sample (dry sherry). Ethanol elutes with a retention time of 85 seconds, and the n-butanol internal standard elutes with a retention time of 275 seconds. The total analysis time of 5½ minutes (including the 15-second sample preparation-dilution) is short compared with that of other methods. As shown in Fig. 2-A, methanol, the fusel oils, other wine volatiles (2), and the "water peak" do not interfere with the elution of ethanol or n-butanol. The chromatogram in Fig. 2-B (higher recorder attenuation than Fig. 2-A) shows that the ethanol and n-butanol peak shapes are close to ideal. The copper column, inert Teflon support, liquid phase, column temperature, and carrier gas flow rate were selected to prevent wine volatiles from eluting at the same time as ethanol or the internal standard. The "water peak" (a change in the temperature of the flame detector caused by the elution of water vapor) was found to interfere directly with ethanol or internal standard elution, or by "tailing," when other chromatographic configurations were used.

Fig. 2 also shows the format of the electronic digital integrator paper tape printout, including peak retention times and area counts. The Varian 480 integrator does not calculate the response factor (divide the ethanol peak area counts by the n-butanol peak area counts), but GC digital data pro-

cessing equipment is commercially available that will perform this calculation. Some type of digital integration capability is necessary to obtain the accuracy and precision reported here. While not necessary for this method (and not used), one of the commercially available automatic GC sample injectors is desirable.

The ethanol standards were used to measure the precision and stability of the GC system, as well as for its standardization. Each of the five standards was injected once each day of the experiment to measure the variation in GC response to the standards from day to day. Table 1 shows that the GC system is remarkably stable. The relative standard deviation of the response factors for each standard is close to 0.1% over the entire 10-day period of the experiment. The GC system is so precise and stable that the initial injections of the standards (and only one injection of each standard) provided a standardization valid for 10 days.

The precision of the chromatography of the method was measured in terms of duplicate injections of the 100 winery samples. These samples, although injected twice, were diluted only once to eliminate any diluter imprecision from the measure-

ment of chromatography precision. Statistically, the estimate of the standard deviation of the duplicate injections was 0.021% (v/v), or 0.16% relative to the mean ethanol content of the 100 samples. The precision of the chromatography *per se* is substantially better than the precision reported for other methods (4,9). The precision of the chromatography is even more remarkable when it is considered that the duplicate injections of a given sample never occurred on the same day, and, in fact, were separated by 5 to 10 days. However, the relative standard deviation of the diluter was determined to be 0.17% (for ferroin dilutions using a spectrophotometer with a relative standard deviation of approximately 0.10%), so the overall precision of the GC method is not as good as the 0.021% (v/v) of the chromatography.

The accuracy of the dichromate reference analysis (and densitometric methods) is limited because non-GC methods cannot distinguish between ethanol and the small amounts of certain non-ethanol wine components. Table 2 shows the magnitude of the inaccuracy caused by each of the 12 non-ethanol wine components in model solutions. The first column shows how much "ethanol" is detected ("ethanol equivalent") for every 100 ppm of non-ethanol component. The second column shows how many ppm of the non-ethanol component will be detected as 0.01% (v/v) "ethanol" by the dichromate method. Methanol is the most significant interfer-

TABLE 1
Variation of GC Response to Ethanol Standards

Response factor					
Standard no.:	1	2	3	4	5
Dichromate ethanol analysis:	5.67% (v/v)	10.08% (v/v)	12.26% (v/v)	14.83% (v/v)	19.69% (v/v)
Day of expt.					
1	0.3141	0.5557	0.6791	0.8212	1.0927
2	0.3139	0.5550	0.6794	0.8217	1.0910
3	0.3148	0.5564	0.6790	0.8213	1.0924
4	0.3146	0.5570	0.6799	0.8214	1.0923
7	0.3148	0.5563	0.6784	0.8192	1.0905
8	0.3142	0.5560	not analyzed	0.8214	1.0931
10	0.3142	0.5560	0.6802	0.8240	1.0942
Mean	0.3144	0.5561	0.6793	0.8215	1.0931
Standard deviation	0.0003	0.0006	0.0006	0.0013	0.0011
Relative standard deviation	0.10%	0.10%	0.09%	0.16%	0.11%

TABLE 2
Non-ethanol Wine Components Analyzed as Dichromate "Ethanol"

Component	"Ethanol equivalent" per 100 mg/L (sample average)	Amount detected as 0.01% (v/v) ethanol (sample average)
Methanol	0.024% (v/v)	41 mg/L
n-Propanol	0.014	74
Sec-butanol	0.004	250
Isobutanol	0.014	71
Isoamyl alcohol	0.014	71
Acetaldehyde	0.011	108
2,3-Butanediol	0.00094	1080
Ethyl acetate	0.0089	113
2-Butanone	0.0012	850
Glycerin	None detected	—
Potassium sorbate	None detected	—
Potassium metabisulfite	None detected	—

TABLE 3

Summary of GC Method Linear Regression Analysis
Calculated with Reference to Micro-dichromate Method

	Five ethanol standards (6-20% v/v)	100 winery samples (7-24% v/v)	
		Uncorrected (for methanol and fusel oils)	Corrected (for methanol and fusel oils)
Standard error of estimate	0.028% (v/v)	0.072	0.070
Intercept	0.045% (v/v)	0.117	0.046
Slope	17.99	17.94	17.95
Correlation coefficient	0.99999	0.99986	0.99988

ing substance per ppm. The assumption is made in these calculations that the dichromate method responds linearly to the concentrations of each non-ethanol component, from the several percent concentrations actually measured to the ppm concentrations calculated for Table 2. (The titration step of the dichromate procedure is not accurate enough for direct determination of 100-ppm differences of any wine component, including ethanol.) The methanol and fusel oil analysis of each of the 100 winery samples, and the "ethanol-equivalent" conversion factors from Table 2, were used for calculating the total dichromate "ethanol equivalent" (for ethanol and the fusel oils) for each sample. The total dichromate "ethanol equivalent" ranged from 0.03% to 0.12% (v/v), with a mean of 0.07% (v/v). (n-Butanol was not detected in any of the winery samples.) The degree of interference with other methods depends on the density or refractive index of the non-ethanol components as compared with that of ethanol.

Table 3 shows the accuracy of the GC method as determined by linear regression analysis. The raw data for these calculations are the GC response factor and corresponding dichromate reference analysis for each sample. Table 3 also shows linear regression data for the 100 winery samples and for the ethanol standards. The winery sample data are calculated both for the usual "uncorrected" dichromate ethanol analyses and for dichromate analyses corrected for total methanol and fusel oil equivalents, as explained above. In both cases of winery sample data the standard error of estimate is 0.07% (v/v) ethanol (over the range 7 to 24%). The winery sample data are the average of statistics calculated separately for each of the two sets of injections of the 100 samples, while the ethanol standard data are the statistics from the original (single) injection of each of the five standards. The intercept (of the

line of regression) is 0.07% less for the corrected winery sample data (0.05%) than for the uncorrected winery sample data (0.12%) because the mean correction for methanol and fusel oil "ethanol equivalents" was 0.07%. It is presumed that a zero intercept would result if it were possible to correct the dichromate analysis of the wines for the "ethanol equivalent" of all the non-ethanol components, not just methanol and fusel oils. The standard error is better for the ethanol standards (0.03%) than for the wines (0.07%) because a dilution of each standard was selected to average out diluter variation from dilution to dilution, and because only five standards were injected.

Important parameters for GC ethanol analysis include sample size and sample concentration. Experience indicates that a common reason for less linearity of detector response (larger standard error) over the alcohol range of interest has been, very simply, samples not diluted to a low enough concentration. Larger diluter sample size results in better precision from dilution to dilution, but the more concentrated sample that results might cost linearity (accuracy) of flame detector response. Commercial diluters have definite limitations in terms of sample size and diluent size. Another advantage of injecting a low concentration sample onto the GC column is that non-volatile wine components do not build up on the column as quickly. After more than 5,000 injections over a several-month period, the original column continues to function with no detectable deterioration in performance. Even with the 0.1-ml to 25-ml dilution it is estimated that the method described here can detect ethanol concentrations as low as 0.1% (v/v). A modified dilution ratio might extend the range of the method from as low as 10 ppm to as high as 100% ethanol. The method has potential for determining the ethan-

ol concentration of most samples analyzed in the alcoholic beverage industry.

CONCLUSION

The GC ethanol analysis method described here is simple in concept, rapid, and highly accurate, determining ethanol specifically without interference from other wine components. Reagent preparation and expense (for 0.05% n-butanol in water) are minimal. Analyst manipulation is minimized through the use of an automatic diluter and automatic printout of raw data in digital form on paper tape. However, the method does require a gas chromatograph and a digital integrator, both moderately expensive and sophisticated pieces of equipment.

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