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DETERMINATION OF ALCOHOLIC STRENGTH IN ALCOHOLIC BEVERAGES BY GAS–SOLID CHROMATOGRAPHY

PRECISION AND ACCURACY

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SUMMARY

The influence of various parameters on the accuracy and precision of gas chromatographic analysis of alcoholic beverages was investigated; a procedure for chromatographic strength determination is presented.

High precision in gas chromatographic spirits analysis can be achieved using the internal standard method when the working solutions are prepared by weighing rather than by volume measurement. The between-injections precision is much higher than the between-specimens precision, so that repeated injections are not required when repeated specimens are used to enhance the reliability of the procedure.

On many occasions, particularly with liquids of lower alcoholic strength, the external standard procedure without sample pretreatment provides sufficient precision, in particular when the advantage of the simplicity of the procedure is utilized for multiple sample analysis. The accuracy of the procedure is determined by the accuracy of the standardization (calibration) process.

The linearity between the detector response and the amount of ethanol injected is good in a very broad range of injection amounts allowing for injection of straight, undiluted samples if necessary. Better reproducibility is achieved with high rather than low injection amounts.

INTRODUCTION

The determination of ethanol in alcoholic beverages is of great significance in beer production, in the wine industry, in distilleries and spirit handling establishments, as well as in the administration of excise and customs regulations. Traditionally, and to a large extent "officially"[1,2], alcoholic strength has been determined by density or specific gravity measurement and conversion of density values to alcoholic content with reference to alcoholometric tables. Although reasonably accurate —the determination of density by hydrometry has an accepted instrument scale deviation of 0.2%, and pycnometry is claimed to be reproducible to about 0.1%,

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expressed as standard deviation in % alcohol[1,3] with a standard error of the mean of 0.226% determined in a collaborative study[4]— the technique suffers from several serious drawbacks.

The method is applicable to pure water–ethanol mixtures only. Any impurity will render the correspondence between density and the tabulated value of strength inaccurate. Generally, the ingredients other than water and ethanol are relatively non-volatile, and a fairly pure water–ethanol mixture can be obtained by simple distillation and the density–strength determination then applied. Any error inherent in the distillation process compounds the error of the strength determination[1,5]. Particularly low results were reported in distillation–density procedure analyses of cocktails and specialty products, such as cordials, where "loss" of up to 0.7% proof was observed[6]. If the foreign components are volatile, the distillation does not remove them and the procedure produces incorrect results notwithstanding. Relatively large volumes of samples (100–1000 ml) are required for density and for distillation–density procedures, which is impractical on some occasions. The tables used for converting the density of specific gravity values to strength do not always agree with one another[7] and discrepancies of up 0.2% happen when different tables are applied to the same density value. Finally, because most alcoholic beverages, including beers, wines, liqueurs, and many of the distilled spirits require prior distillation, the procedure is quite tedious, time consuming and operator sensitive. It is also almost impossible to automate the distillation–density procedure.

It was expected, with reason, that these difficulties could be removed by the application of gas chromatographic (GC) techniques to the beverage analyses and indeed, spirits were analyzed by GC soon after the technique was discovered, in the fifties. The first attempts were mainly devoted to discerning minor ingredients of the spirituous beverages[8,9] with analyses for ethanol coming almost concurrently[10]. In the meantime, many procedures were described for ethanol determination in the area of trace amounts of ethanol detection (for a review see refs. 11 and 12). However, the limited accuracy of the GC procedure, generally about 5% (rel.), rarely approaching or exceeding 1%, restricted the use of GC in cases where the alcohol content was significant and the relative error would be unacceptable. Methods for ethanol determination in wines were proposed in 1961[3,10] and refined with the advent of electronic integrators and extensively used in wineries[13]. The method was collaboratively tested[14] and adopted as the "official final action" by the Association of Official Analytical Chemists (AOAC)[15,16]. Although one column packing only is recommended in the final action method (Carbowax 1500), several columns were used in the collaborative test, considered roughly equal[14] and the restriction was made mainly to simplify the written procedure[15]. High degree of dilution (1:250 or 1:100) and low injection volume (1 μl or less), resulting in an average injection amount of 0.4–1.4 nl ethanol, was considered crucial for attaining high reproducibility [0.16% R.S.D. (relative standard deviation) in duplicate injections, not considering the error in diluting]. The overall range of the R.S.D. established by the collaborative study is 0.50–1.50% with two collaborators out of fourteen excluded (the range extends to 2.6% with all included). Internal standard, *n*-butanol or 2-propanol, was used for quantitation without comment.

A similar procedure was developed for alcohol determination in beers[17,18]; after a collaborative study, the procedure was adopted as "official first action" by

the AOAC[19,15] and recommended as an alternate to the American Society of Brewing Chemists (ASBC) distillation method BEER-4 A by the ASBC[18]. This procedure uses a gas–solid chromatographic (GSC) technique with Chromosorb 103 as the column packing. A 1:1 dilution and 0.2 μl injection of the sample leads to a higher injection amount of 5 nl ethanol on an average. *n*-Propanol was used as the internal standard and the range of combined random and between-laboratory R.S.D. was found to be 1.1–2.1% (rel.) by the collaborative study[18].

A procedure using Chromosorb 101 with a comparatively high injection amount of 20–28 nl ethanol and with 2-propanol (20 nl) as the internal standard was described[20] for ethanol determination in liqueurs and distilled spirits; also, the original wine procedure[13] was adapted for spirits analysis[21] with injection values increased to 5–7.5 nl ethanol. Average deviation of the GC results from classical distillation–density results were 0.4% (range 0.01–0.98%) in the former, and very similar values were reported in the latter.

While the R.S.D. of 1–2% is quite acceptable in beer analyses (average alcohol content 5%, absolute deviation 0.05%) and wines (average alcohol content 12%, deviation 0.1%), the same R.S.D. in the case of spirits (average alcohol content 40%) becomes marginal, although comparable with combined errors due to density and distillation processes. Therefore no official standing has yet been granted to the procedures for alcohol in spirits and the study by the AOAC is being continued[15].

Our laboratory has been involved in the analysis of spirits for a long time, using classical and developing new GC methods. Therefore we have decided to investigate the parameters likely to influence the precision and accuracy of GC alcohol analysis in order to support the current endeavours in this field and to facilitate the tedious tasks of strength determination, eventually including automation of the procedure.

Several approaches were tested during these years; in the following, "standard" procedures are described as they evolved from this work. These procedures were used unless otherwise indicated.

EXPERIMENTAL

Materials

Ethanol (High Proof Alcohol; Hiram-Walker) 94.25% (v/v) (containing approximately 70 mg l^{-1} methanol and 300 mg l^{-1} propanol). Ethanol (Absolute; Aristar, BDH Chemicals) 99.71% (v/v) (no congeners detectable). Ethanol of lesser strength was prepared from one of the above by diluting with water as needed and strength determined by densitometry as described previously[22]. A series of commercially available spirits described in a previous paper[23] was also used in this study. Methanol, 2-propanol, propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and hexanol were analytical grade solvents (Baker, Analyzed; Fisher Certified; Anachemia, Anagraphic), and were used without further purification.

*Apparatus**

The gas chromatograph HP 5880A Level IV (Hewlett-Packard) equipped with

* Product names in this paper are mentioned only for information and do not constitute endorsement; any equivalent or better make could be used.

an electronic digital processor–integrator–plotter and with the autosampler HP 7672A was used throughout the study.

The empty space in the heated injector, between the septum and the beginning of the column, was loosely filled with silanized glass wool that was regularly replaced after approximately 500 injections. The flame ionisation detetor was automatically calibrated (at turn-on) by the instrument.

A stainless-steel, 180 × 0.32 cm I.D., column filled with Porapak Q (solid support), 100–120 mesh was conditioned according to the manufacturer's instructions. Gases: carrier was nitrogen, 400 kPa inlet pressure, 29.5–30 ml min^{-1} flow-rate; hydrogen, 245 kPa inlet pressure, 32 ml min^{-1} flow-rate and air, 312 kPa pressure and 400–450 ml min^{-1} flow-rate were used for the flame ionisation detector. Injections were made using a 10-μl Hamilton syringe with the sampler set for 1 μl delivery, unless otherwise indicated.

Instrument setpoints

Temperatures: oven, 150°C (isothermal); injector, 160°C; detector, 200°C. Chart speed, 0.5 cm min^{-1}; attenuation 11; offset 10%; threshold, 4; peak width, 0.04.

Observed retention times: methanol, 1.1 min; ethanol, 2.05 min; 2-propanol, 3.6 min; propanol, 5.1 min; 2-methyl-1-propanol, 10.5 min; 3-methyl-1-butanol, 27.0 min. An oven temperature of 160°C was also used with correspondingly shorter retention times.

A Parr DMA-55 calculating precision density meter with the temperature controlled by a Haake F3-C circulating ultrathermostat was used to determine densities as described in a previous paper[22]. The instrument was calibrated to indicate "densities-in-air", at 20°C, using nominal values for density-in-air of water (0.99715) and air (0.00000)[24] based on the tabulated values of the corresponding densities *in vacuo*[25] and recalculated for "standard" atmospheric conditions and "standard" densities of weights as recommended by L'Organisation Internationale de Métrologie Légale (OIML)[26]. Corresponding strengths (% by volume) were calculated using the OIML "general formula"[27].

Procedures

(1) Samples of 80–100% (v/v) strength. A volume of 500 μl of 2-propanol (internal standard) was transferred using an Eppendorf pipette into a tared 7-ml vial and weighed; 500 μl of sample was similarly added and weighed; finally, 4.50 ml water was added and the mixture mixed by shaking the closed vial. The volumes of internal standard and sample were calculated by dividing the corresponding predetermined densities-in-air into the respective weights.

(2) Samples of 10–85% (v/v) strength. (a) Procedure 1 was followed except that 1000 μl of the sample and 4.00 ml water was used. (b) Ingredients were mixed as in procedure 2a but the ingredients were not weighed; the volumes indicated by the Eppendorf pipettes (500 and 1000 μl, respectively) were used in the automatic calculations (default values).

(3) Samples of 2–18% (v/v) strength. Internal standard, 500 μl, and 5.00 ml of the sample were mixed without further dilution. The values of 500 and 5000 (μl) were used as the internal standard amount and sample amount, respectively, in the automatic calculations.

(4) External standard method. Samples were injected without any pretreatment, as received.

Calibration for determination of ethanol

Five solutions of ethanol in water, nominal strength 10, 25, 40, 75 and 95% (v/v), with exactly known density in air and corresponding[27] strength were analyzed as in procedure 2a to calibrate the apparatus in five levels, thus covering the whole range of amounts injected in any of the procedures. Three calibration runs in each level were combined and the mean values for each level used for sample analyses.

Retention times of expected impurities

A sample containing 580 ppm methanol, 2200 ppm propanol, 3700 ppm 2-methyl-1-propanol and 15700 ppm 3-methyl-1-butanol in 44.5% (v/v) ethanol was used, without quantitation, to determine the retention times, given above.

Calibration for concurrent determination of methanol

(a) A stock solution of methanol was prepared dissolving about 100 mg accurately weighed purest methanol in distilled water and filling up to 100.0 ml in a volumetric flask; 10.00 ml of this solution was diluted to 100.0 ml with water. The concentration of methanol in the final solution was expressed in mg l^{-1} (numerically equal to the original weight of methanol in mg).

(b) Following procedure 2a, five calibration specimens were prepared as for calibration for ethanol only, except in place of 4.00 ml water increasing amounts of final methanol stock solution and correspondingly decreasing amounts of water were used as follows:

Nominal alcoholic strength (%)	*10*	*25*	*40*	*75*	*95*
Volume of final methanol solution (ml)	0.0	0.5	1.0	2.0	4.0
Volume of water (ml)	4.0	3.5	3.0	2.0	0.0

Linearity of response

A primary solution of 512 ml/l ethanol and 256 ml/l 2-propanol in water was prepared by diluting 20.275 g absolute ethanol (99.71%, v/v) and 10.077 g 2-propanol (99.97%) to volume in a 50.0-ml volumetric flask with water.

A series of solutions with the same ratio of ethanol to 2-propanol of 2 was obtained by sucessively diluting 25.00 ml of a preceding solution to 50.0 ml with water, at 20°C. Eleven solutions with concentrations of 2^N ml/l were thus obtained, with N of 9 to -1 for ethanol and 8 to -2 for 2-propanol.

RESULTS AND DISCUSSION

Column selection and chromatography conditions

Several columns have been recommended for ethanol analysis; the porous polymers (Chromosorbs 101, 102, 103, Porapaks Q and QS) and the liquid packings with polyethyleneglycols (Carbowax 600, 1500, or 20M) are mentioned most widely in the literature. All meet the main requirement of spirit analysis, which is compati-

bility with water; the sample matrix is simple and no high resolving power is required. Therefore little difference has been found between the different packings[14] and the selection is mostly based on personal preference. Our selection of Porapak Q as given in the Experimental section is based on several years of experience where we have found the column to be stable over long periods of time, insensitive to "dirt" including congeners and sugars in the beverages, maintaining the activity as judged by the constancy of retention times and sustaining rather large injection amounts, which we found particularly important. We had similar good experience with other types of packing, *e.g.* Chromosorb 101 or Carbowax 20M on Carbopack but did not include them in the procedure for the sake of simplicity.

Although the optimum nitrogen carrier gas flow was found to be about 11 ml/min, sufficiently good (baseline) resolution for the system of ethanol–internal standard was still achieved at 30 ml/min; higher than optimum flow-rate was selected to help to accelerate the analysis. The instrument parameters then were accommodated to achieve a retention time for ethanol of about 2 min and a total analysis time below 6 min. It is likely that shorter times could be achieved without deteriorating the performance. The instrument was operated isothermally to eliminate the equilibration time; the matrix is sufficiently simple not to require temperature programming.

Injection amounts; specimen concentrations and injection volumes

As pointed out in the Introduction, the published method for wine analysis prescribes very low injection amounts (high dilution) based on the claim made in the original paper[10,13] from which the later "official final action" was developed (ref. 3 cited in ref. 14) that the detector response is directly proportional to the amount of ethanol only at very low concentrations (injection amounts). Low injection amounts are used in the official beer analysis method; the concurrently published procedure for spirits analysis[20] works with amounts about ten times higher, but still recommends considerable dilution, although there was actually little experimental evidence to support the claim, except for an earlier paper[28] reporting a very good linear relationship between ethanol concentration and ethanol–propanol peak ratio [correlation coefficient 0.9998, R.S.D. of slope 0.7%, standard error of y (ethanol %) on x (ratio) $S_{y,x}$ 0.10, that is 1.0% of the mean value; calculated from data of Table I in ref. 28] for a range of injection amounts 1.25 to 5 nl absolute alcohol. The paper states that at higher concentrations the plot is no longer linear.

Our preliminary experiments have shown that the detector response as indicated by the response factors (ratio of injection amount to area counts) is fairly linear for 2-propanol in the range of 30 to 120 nl injection volume (Fig. 1). The slope of the plot of the response factors against injection amounts was zero within the experimental error; the R.S.D. in these individually prepared samples was 0.9%, reflecting rather the variation of injection volumes and variation in sample preparation than the variation of the response factors. However, the linearity is not evident in responses when injections below 30 nl were made (Fig. 2). The plot shows a very distinct curvature, the slope of the best fitting line is significant and the scatter of values was 9% expressed as coefficient of variation.

To verify these findings, a series of solutions with constant ratio of ethanol and 2-propanol, was prepared (see Experimental) in a range of 512 to 0.5 ml/l ethanol and 128 to 0.25 ml/l 2-propanol, and analysed using 1 μl injection and the standard procedure.

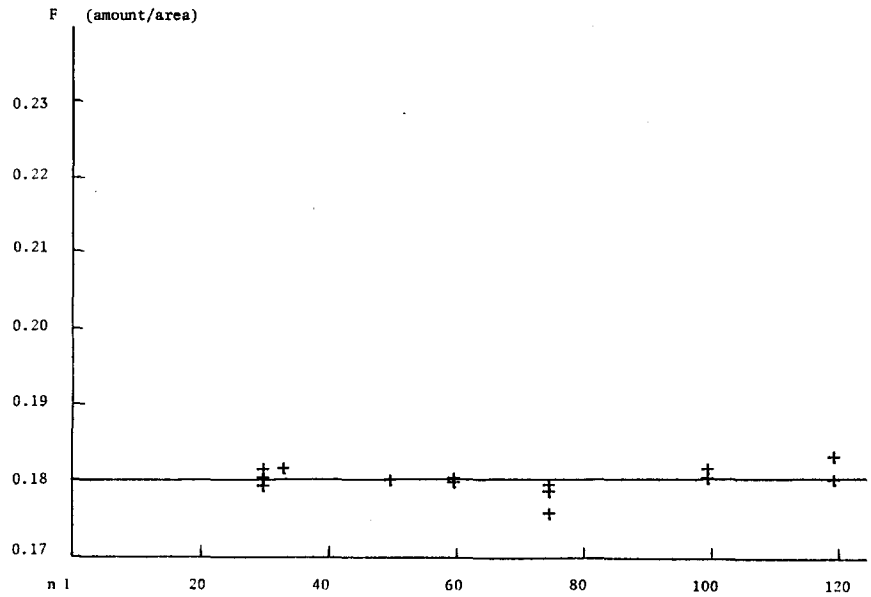

Fig. 1. Variation of the response factor (F) with the amount of 2-propanol. Range, 30–120 nl; slope, (5 ± 10) × 10^{-5}; mean, 0.1806 ± 0.0016; R.S.D., 0.90%.

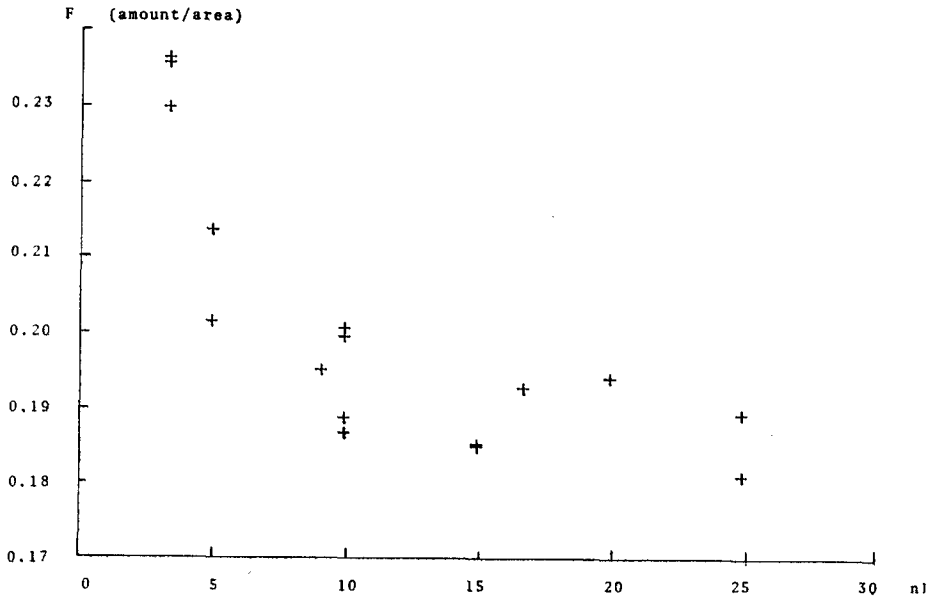

Fig. 2. Variation of the response factor (F) with the amount of 2-propanol. Range, 3–30 nl; slope, (−1.8 ± 0.4) × 10^{-3}; mean, 0.201 ± 0.018; R.S.D., 9.04%.

A good linear relationship was observed for the injected amount of ethanol and corresponding area (correlation coefficient better than 0.9999 and 0.9997 for 2-propanol). The slope of the plotted best fitting straight line had, however, slightly lower standard deviation in the concentration range 8 to 512 ml/l than in the range 0.5 to 16 ml/l for ethanol and a significantly lower standard deviation for 2-propanol in a similar division. The individual response factors (ratio of injection amount to corresponding area) were again fairly constant (see Table I) for ethanol and for 2-propanol in the higher injection amount ranges and have shown a very significantly larger scatter (total standard deviation) in the low injection amount ranges, even so much that plots of the factors against the injection amounts, that should in theory be a straight line with close to zero slope, have shown a very distinct curvature in the low ranges.

The situation was slightly ameliorated when the ratios of response factors (which are equivalent to the ratios of areas for ethanol and propanol, resp., in this experiment) were considered, but still a statistically very significant variance ($F = 13.4$, better than 99% confidence) was observed between the scatter of values in high injection amount ranges, (over 32 nl) and low ranges (below 32 nl). The rather abrupt change in the ratio of response factors is reflected in Fig. 3.

While it is possible to obtain a satisfactory relationship between injection amounts and corresponding areas with low injection amounts as stipulated in the papers cited above, these results demonstrate that high injection amounts are by no means in practice detrimental and that they actually show much less scatter than the former.

When the response factors (ratios of injection amounts to corresponding areas) in individual runs are compared (Table II), the difference between the two ranges of injection amounts is even more apparent. The factor is reasonably constant (3.3% R.S.D.) for ethanol in the range of 16 to 512 nl injection amount, but becomes as high as 11% for 0.5 to 16 nl ($F = 12.6$; 6, 6). The difference is even more significant

TABLE I

LINEARITY OF THE RELATIONSHIPS BETWEEN THE RESPONSE FACTORS OF ETHANOL AND 2-PROPANOL, OR THEIR RATIOS, WITH THE INJECTION AMOUNTS

Response factor *(y)* = Ratio of injection amount to area count; range = injection amount in nl series (see Experimental); N = number of injections (with two injections per solution); slope = least square linear regression: response factor as y, injection amount as x; S.D. = standard deviation of slope; $S_{y,x}$ = standard error of y on x; $RS_{y,x} = S_{y,x}$ as % of the mean response factor (y); F = variance ratio low range/high range; $F_{(N,N;0.05)}$ = tabulated value for F for N,N degrees of freedom and 95% confidence.

Compound(s)	*Range*	*N*	*Slope* $\times 10^5$	*S.D.*	$S_{y,x}$	*R.S.*$_{y,x}$ *(%)*	*F*	$F_{(N,N;0.05)}$
Ethanol	512–16	12	3.5	0.5	0.003	1.40	42.95	2.69
	16–0.5	12	−300	100	0.02	8.25		
2-Propanol	256–16	10	2.0	0.5	0.0013	0.66	446.7	2.98
	16–0.25	10	−800	300	0.027	11.22		
Ethanol–2-propanol	512–32	10	11.0	2.0	0.011	0.97	16.6	2.91
	32–0.5	12	200	100	0.043	4.30		

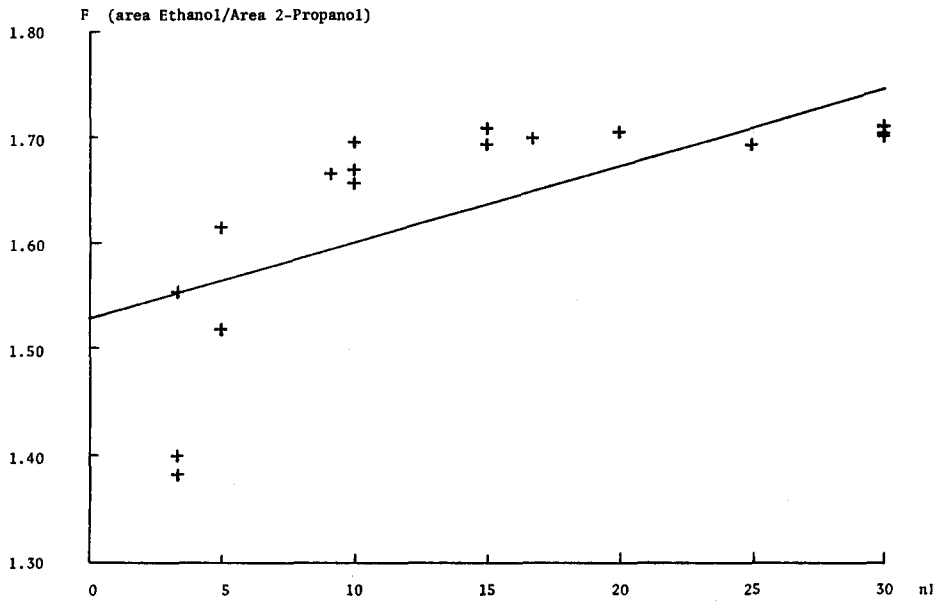

Fig. 3. Relationship between the area ratios (response factor ratios) of ethanol and 2-propanol and the injection amount of ethanol. Range, 3–30 nl ethanol; slope, (7.2 ± 1.9) × 10^{-3}; R.S.D. (slope), 25.8%.

for 2-propanol ($F = 287$ with $N = 5$). Also the mean values of the ratios of the response factors for ethanol and 2-propanol in individual runs show a difference characterized by $F = 5.0$, much less pronounced but still significant at the 95% confidence level ($F = 0.05$; 5, 5 is 5.05). The differences are clearly attributable to the differences between samples, that is to injection amounts, because the variation(s) between injections from the same solution are in all cases smaller by an order of magnitude in the simple response factors (external standard method) and by several orders of magnitude in the ratios of factors (internal standard method).

Injection volume

The examples discussed so far were based on experiments where an injection volume of 1 μl was used consistently. When 3 μl of the same series of solutions was injected, the difference between the high injection amount range (1536–96 nl) and low range (96–1.5 nl) was insignificant and the variations in response factors were comparable to those for the high injection amount range in 1-μl injection experiments. However, the repeatability of injection (R.S.D. between injections) was about ten times worse than the corresponding values for 1-μl injections when the ratios of response factors were compared (*viz.* 0.06% R.S.D. for 1 μl and 0.6% for 3 μl injections).

The R.S.D. of simple response factors between injections were comparable. The 1-μl volume injection was therefore adopted for routine analyses, and the injection amounts were controlled by changes in concentration only.

External standard calibration method (ESTD)

It has thus been demonstrated that the system described can sustain injection

TABLE II

MEAN VALUES AND SCATTER OF RESPONSE FACTORS IN DIFFERENT RANGES OF INJECTION AMOUNTS

Compounds(s)	*Range*	*N*	*Mean*	*Total*		*Random*		*F total/random*	*F (low/high)*	
				S.D.	*R.S.D. (%)*	*S.D.**	*R.S.D.*		*Total*	*Random*
Ethanol	512–16	12	0.212	0.007	3.3	0.0016	0.73	19.4	12.6	1.58
	16–0.5	12	0.223	0.025	11.2	0.002	0.88	155.0		
2-Propanol	256–16	10	0.194	0.002	1.2	0.0008	0.39	8.37	287	69.4
	16–0.25	10	0.222	0.037	16.8	0.007	3.0	31.0		
Ethanol–2-propanol	512–32	10	1.10	0.02	2.1	0.0007	0.062	1135	5.0	13.4
	32–0.5	10	1.03	0.05	5.0	0.0025	0.24	416		

* Random S.D. $= \sqrt{\Sigma \Delta^2/n}$

where Δ is the difference between response factors in two injections from the same solution and n is the number of pairs.

amounts well above 1000 nl without detriment to the linearity of the relationship between the alcohol amount and peak area count. Injection of 1 μl of sample up to 100% concentration and the application of the external standard method without any sample pretreatment should therefore be feasible. Indeed, in a calibration in three levels (5, 20 and 95% alcohol) with three 1-μl injections per level excellent linearity was observed (correlation coefficient 0.99998). The slope had a R.S.D. of only 0.15% with a standard error of y (% alcohol) on x (area) $S_{y,x}$ of 0.18. The precision (variability of results around mean, or standard deviation) in an external standard method without any sample pretreatment is determined by the repeatability of the injection amount from injection to injection in the same sample. It has been demonstrated earlier that the R.S.D. between injection for a range of 5–100% alcohol (50–1000 nl injection amount with 1-μl injection) using the autosampler and the conditions given in the Experimental is 0.4–0.8%. If this level of precision is satisfactory for the desired results, this method should be the method of choice, because of the extreme simplicity. The precision can be easily improved by repeated injections; no new working solutions are required and minimal operator involvement is necessary. Five repetitions should give a standard error of the mean of only 0.1%.

Internal standard calibration method (ISTD)

The above experiments indicated that the repeatability between injection of area ratios, (or response factor ratios) which is used in the internal standard method(s) is significantly better than the repeatability of the simple area for a single compound. A R.S.D. (between injections) of 0.06% was observed for the range of 32 to 512 nl ethanol injection amounts, as compared to 0.8% R.S.D. for the between injections ethanol area variations in the same range. Similarly low values were obtained by analyzing ten standard solutions of ethanol in water following one of the internal standard procedures described in the Experimental section, with four injections per specimen. The R.S.D. (%) varied form 0.005 to 0.05 with a mean of 0.03 ± 0.10% of the value (Table III).

TABLE III

ANALYSIS OF STANDARDIZED WATER–ETHANOL MIXTURES

Samples 1–4 are calibration standards.

Sample No.	*Expected (%, v/v)*	*Found*				*Mean*	*S.D.*	*R.S.D. (%)*	*Difference exp.–found*
1	9.339	9.351	9.343	9.355	9.353	9.351	0.005	0.05	−0.012
2	39.947	39.956	39.968	39.965	39.987	39.969	0.013	0.03	−0.022
3	74.978	75.004	75.005	75.073	75.056	75.034	0.035	0.05	−0.056
4	94.242	94.193	94.214	94.204	94.172	94.196	0.018	0.02	0.053
5	23.258	23.549	23.536	23.535	23.532	23.538	0.008	0.03	−0.280
6	19.858	20.271	20.268	20.268	20.269	20.269	0.001	0.005	−0.411
7	40.034	40.084	40.107	40.109	40.092	40.098	0.012	0.03	−0.064
8	60.872	60.961	60.991	60.989	60.995	60.984	0.016	0.03	−0.112
9	94.273	94.400	94.433	94.428	94.456	94.429	0.023	0.02	−0.156
10	99.710	100.226	100.241	100.248	100.275	100.247	0.020	0.02	−0.537
Mean								0.03	−0.16
S.D.								0.01	0.19

However, in a internal standard method, the sample cannot be injected without a preceding workup; the internal standard must be added in amounts reasonably close to the amount of the compound to be determined, and the mixture is usually further diluted to form a working solution or specimen. Therefore, the excellent repeatabilities reflect only the consistency of the procedure in analyzing the same specimens (working solutions), but not necessarily the variability of actual sample results on repeated analyses. Indeed, the between-specimens reproducibility in routine analysis of a series of eight commercial spirits by procedure 2b (see Experimental) with two specimens per sample was 0.6% (as R.S.D.), which is comparable to the R.S.D. of the external standard procedure (above). Even larger day-to-day between specimens variation (R.S.D. 0.9–1.1%) was registered in a series of 39 commercial liqueurs and spirits analyses. Nevertheless, the between-injection repeatabilities on the same specimen remained in the 0.05% (R.S.D.) range. Evidently, the results are the effect of volume measurements in the specimen (working solution) preparation.

Volume measurement

The specimens in this study were routinely prepared by pipetting using Class A glass pipettes, a Repipet dispenser, or Eppendorf pipettes. On many occasions, the delivered volumes were weighed to establish accurate proportions (*cf.* procedure 1 and 2a in Experimental) and the data provided a basis for evaluation of the repeatability of our volume measurement. The results are summarized, together with several indications gathered from the literature, in Table IV.

Although there are certainly diluting instruments available on the market which allow for higher precision in volume measurement, and one such instrument is specified in the Official method for alcohol in wine determination (ref. 16, Method 11.D01, paragraph 3b) these diluters are not yet generally available in routine analytical laboratories, and the existence of a R.S.D. of 0.5–1.0% on one routine volume determination must be accepted. While two volume measurements are required in an internal standard method specimen preparation, the variation between specimens might be expected in the range of 1% (R.S.D.), which is in reasonably good agreement with the figures reported above. This limits the usefulness of the internal standard procedure and makes it at best equivalent, if not inferior to, the external standard method in overall within-sample precision.

If any better precision is required, as might be the case in analyzing distilled spirits or high concentration alcohols, and in absence of a high precision diluter, the

TABLE IV

PRECISION OF VOLUME MEASUREMENT

Instrument	*R.S.D. (%)*	*Note*
1 ml Pipette	0.4	Experimental
5 ml Pipette	0.2	Experimental
Repipet	0.2	Experimental
Eppendorf 1000 and 500 µl	0.13	Short runs
	0.5–1.0	During a day
Automatic	0.5	Reference 17
Mohr	1.4	Reference 17

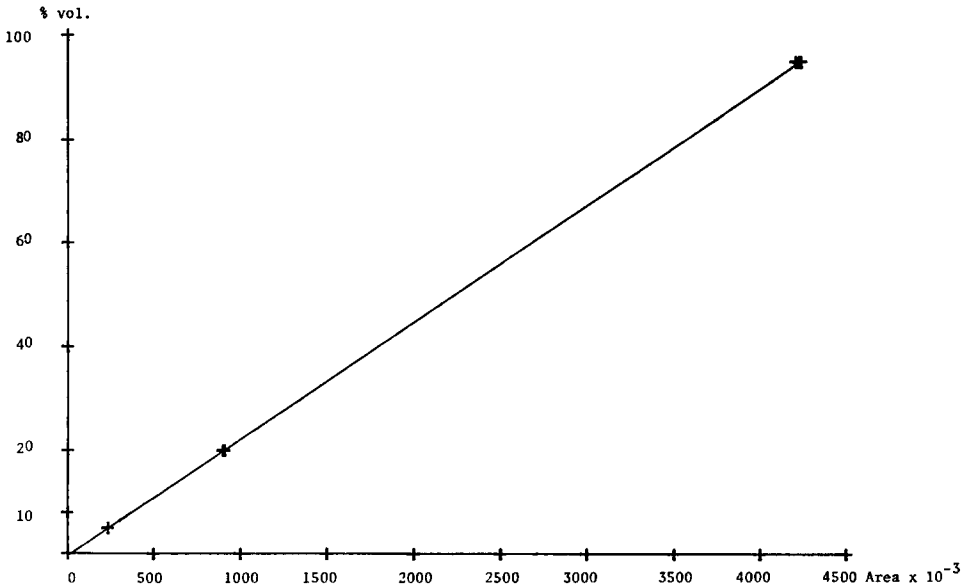

Fig. 4. Relationship between peak area and concentration of ethanol. Range, 5–95% (v/v); slope, (2261 ± 3) × 10^{-5}; intercept, 0.30 ± 0.08; Standard error of y,x, 0.18% (v/v).

specimen (working solution) preparation must be done by a procedure with higher reliability than by pipetting and volumetric flask measurements, such as by weighing, where the repeatability is better by an order of magnitude. The volumes of the ingredient can be calculated, if needed, using densities-in-air values that have to be determined concurrently, and with high precision as well, *e.g.* using digital density meters[24]. The procedure is described in the Experimental as procedure 1 and 2a.

Indeed, in an analysis of five specimens prepared from one synthetic (40% nominal strength) sample with five injections each, an overall R.S.D. of 0.08% was obtained. The precision achieved in routine analysis of eight commercial products using the procedure 2b (volume measurement) with two specimens per sample and three injections per specimen is still very good between injections (random standard deviation between replicate injections, 0.05) but the reproducibility between specimens was 0.6 which for a mean strength of 40% corresponds to R.S.D. of 1.5%. This is in line with the repeatability of the two volume measurements necessary in the specimen preparation, as discussed earlier. Sufficiently good precision, better than that of the ESTD method, can be achieved using the ISTD method only when the sample preparation is done by a correspondingly reliable method, that is, in the absence of a high precision diluter, using weighing as in procedure 1 or 2a (see Experimental).

Accuracy

Defined as the difference between the true and the found value, the accuracy in a chromatographic procedure is primarily determined by the correctness of the calibration, *i.e.* the runs with standards of known composition to which the sample runs are compared. Assuming perfect calibration with exactly known standards, the

mean accuracy value for a sample should approach zero with a variance approaching the value of precision, that is, the variance within a sample.

In our experiments using procedure 2a (with specimen preparation by weighing), the results of analysis of five specimens from one sample (39.95%, v/v) and four injections per specimen had a mean deviation from the expected (true) value of −0.08% with a standard deviation of 0.01% (v/v) (absolute), corresponding to a standard error of mean of 0.006% and a confidence limit of 0.03 at 99% confidence. Analysis of the four calibration standard solutions treated as "samples" and using procedure 2a and one specimen per sample, provided a mean difference from expected values of 0.01% with a standard deviation of 0.05% (abs.). When synthetically prepared alcohol–water mixtures covering the range of 10 to 100%, other than the calibration standards, were analyzed, the mean difference from the expected increased to 0.16% with a standard deviation of 0.10% (abs.), reflecting the additional error due to preparation and densitometric standardisation of the check solutions. Similar accuracy was observed when real (commercial) spirits (40–60%, v/v) were analyzed and the results compared to the values obtained by distillation–pycnometry; the mean difference was 0.14% with a standard deviation of 0.16% (v/v) (abs.). These latter values include the uncertainties due to the strength determination by the reference method that alone is estimated as 0.2% (v/v) in the distillation procedure[1,5] and a standard error of mean of 0.226% in pycnometry was reported in a collaborative study[4]. An earlier report on wine analysis mentions a R.S.D. in distillation–pycnometry of a similar magnitude, 0.5% (rel.)[3]. Considering the magnitude of these errors, the error due to the GC procedure becomes vanishingly small.

When procedure 2b was used (specimen preparation by volume) to analyze a series of commercial spirits[23], the means of two specimens per sample still showed an acceptable mean difference between found and expected values of 0.12% (v/v); however the standard deviation rose to 0.94% (v/v), demonstrating the lower precision of this procedure.

Influence of methanol and higher alcohols

Under the standard conditions (see Experimental), methanol was eluted before, and well separated from ethanol, and its determination would therefore not interfere with the ethanol assay at all. In view of the importance of the methanol presence in beverages, as a characteristic ingredient as well as a possible health hazard[20], simultaneous determination of methanol in the ethanol analysis is recommended; this can be achieved simply by method calibraton for methanol at the time of calibration for ethanol, using the same internal standard for both components. The amended procedure is described in detail in Experimental. The determination of ethanol is thereby not affected.

Due to the sample dilution in specimen preparation necessary for proper ethanol assay by the internal standard method, the sensitivity for methanol detection is inherently lower than it would be in analyzing the sample as is. Nevertheless, methanol at concentrations as low as 50 ng/l ethanol (*i.e.* about 20 ng/l sample, or 20 ppm) was routinely detected, with repeatabilities better than 1% (S.D. between injections).

The other major congeners, propanol, 2-methyl-1-propanol and 3-methyl-1-butanol, common to most alcoholic distilled beverages, all elute well after the internal

standard, 2-propanol, and do not interfere with the ethanol analysis. The retention times under the standard conditions make it prohibitive for the method to be used for simultaneous determination of these components; temperature programming would impair the efficiency, and possibly the accuracy, of the method. Therefore, another procedure is recommended for congener analysis.

The late eluting peaks might, in theory, interfere with an analysis immediately following another. It was found that the ghost peaks do not coincide with the peaks corresponding to ethanol and 2-propanol in the procedure, particularly not when the autosampler is used and properly timed. In case of doubt, a recess of 20 min after a run should alleviate any problems of interference from the previous runs.

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