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Determination of Ethanol In Alcoholic Beverages by Liquid Chromatography Using the UV Detector

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Abstract

Ethanol in certain beverages and in similar solutions may be determined by reversed-phase liquid chromatography (LC) using the UV detector. The mobile phase in this indirect photometric detection technique contains a low concentration of a UV-absorbing compound, such as acetone, that coelutes with the ethanol peak. Several variables such as the choice and concentration of the UV-detection agent are examined regarding their effects on the retention time, magnitude and linearity of peak area, and other aspects of quantitation. Except for filtering to remove particulate matter, samples can be injected without pretreatment. The concentration of ethanol in several types of beverages can be determined with 2% relative standard deviation, calibration is linear to 40% ethanol, and the minumum detectable concentration is 0.1%.

Introduction

A number of papers have appeared in the literature describing the indirect UV photometric detection technique for liquid chromatography (LC), whereby UV-transparent compounds are detectable with the UV-absorbance detector (1–5). The technique involves the addition of a UV-absorbing compound as a component of the mobile phase to act as UV-detection agent. After the distribution of the detection agent between the moving and stationary phases becomes constant, the injection of a sample containing a UV-transparent component (in this case ethanol) produces a number of displacement and vacancy peaks that are either positive or negative relative to the base line and correspond to an excess or deficiency of the chromophore eluting from the column.

Recent papers on indirect photometric detection were summarized in a review article (1). In much of the reported work, however, the mode of separation is by ion-exchange or ion-pairing, wherein the injected analyte either associates with, or displaces, the UV-absorbing ion in the mobile and stationary phases so that the resulting excess or deficiency of the chromophore ion causes the formation of positive or negative peaks (2).

The indirect photometric method was also used with ion-pairing reagents that are in themselves UV-absorbers (3) and was extended to nonelectrolyte compounds as well. Parkin and co-workers have studied some of the lower esters, ethers, and alcohols as analytes using benzene derivatives as the UV-detection compound (4). A similar study was made by Vigh and Leitold (5).

Determination of Ethanol

In the present study, the proposed indirect detection technique was applied to the determination of ethanol in alcoholic beverages and similar aqueous solutions. The specific purposes were to study the effects of several variables on the quantitation of ethanol by this technique and to determine the optimum analytical conditions, precision, linearity, and other characteristics of the proposed procedure. In a typical case, the injection of a UV-transparent compound (in this case, ethanol) produces three peaks, t_o, t_s and t_c, corresponding respectively to the retention time of the organic modifier, the UV-transparent solute (analyte), and the detection agent. These peaks, which may be either positive or negative relative to the base line, can provide much information about the injected sample and the chromatographic system in general. For example, the direction and size of the peaks depend, among other factors, on the relative concentrations and relative polarities of the organic modifier, detection agent, and injected analyte (6).

The technique is illustrated with example A in Figure 1, which shows a chromatogram of an ethanol/water solution injected into a chromatographic system with methyl ethyl ketone (MEK) as the detection agent. The first peak, t_o, is negative and corresponds to a deficiency of MEK in the mobile phase caused by the transfer of some MEK from the mobile phase to the ethanol zone. The second peak t_s, is positive and results from the coelution of MEK with ethanol. The third peak corresponds to the vacancy (MEK deficiency) created by the ethanol on the stationary phase and appears at t_c, the retention time of MEK. This peak indicates that the column has returned to equilibrium.

The three chromatograms in Figure 1, A, B, and C, correspond to water solutions in which the concentration of ethanol is 10%, 5%, and 0%, respectively. The peak height at t_s is proportional to the ethanol content of the sample and may be used as a basis for the determination of ethanol in aqueous solutions.

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Experimental

Instruments

The chromatographic system comprised the following major components from LDC/Milton Roy: variable speed metering pump model VS; variable wavelength detector, Spectromonitor D, equipped with a 10-μL flow cell; and a Model 3402 strip chart recorder. For most of this work, the detector wavelength was set at or near the absorbance maximum of the UV detection compound, usually 280 nm, and the recorder chart speed was 4 mm/min.

Sample volumes of 10 μL (in some cases 50 and 100 μL) were injected with a Valco manual six-port valve (Valco Instruments Co.). The columns were 250 X 4.6 mm Econosil cartridges with 10-μm packings of C_8, CN, or C_{18} from Alltech Associates, Inc. A direct-connect precolumn filter was used between the injector and the column.

Mobile phase

The major components of the mobile phase (water and methanol) were of HPLC-quality and were filtered and degassed using a 0.2-μm filter system. At the start of each day, the mobile phase in the reservoir was sparged with helium for about 5 min.

Other materials

The chemicals used in this study, whether as UV-detection agents or standard solutions, were of analytical quality or HPLC-grade and were filtered through a syringe-fitted 0.2-μm pore Millipore disposable filter unit. The sample analyte solutions (i.e., the sample whiskeys and wines), were used without pretreatment except that they were filtered with the Millipore unit mentioned above.

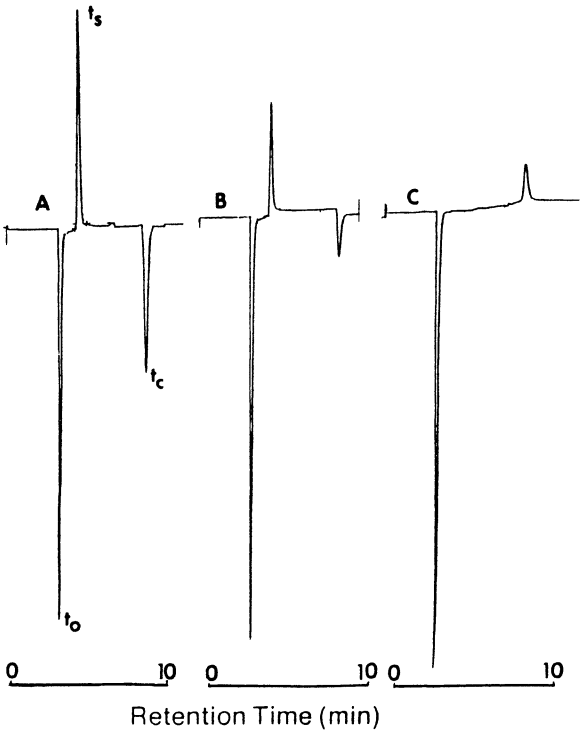

Figure 1. Chromatograms of ethanol/water reference solutions. Column: C_{18}, 250×4.6 mm, 10 μm; mobile phase: 30:70 methanol/water, 0.3% MEK; injection: 10 μL; detector: 280 nm, 0.10 AUFS. A=10% ethanol, B=5% ethanol, C=0% ethanol.

General procedure

The LC column was first equilibrated with the mobile phase without the UV-detection agent. Then, the desired amount of UV detection agent was added to the mobile phase, and the absorbance was monitored until a steady state condition was reached as indicated by the base line. This usually required 40 to 50 mL of eluant. The column effluent was recycled into the mobile phase reservoir to conserve materials.

When the base line was steady, the standard (synthetic) or unknown samples were injected allowing time between runs for the system to return to equilibrium. Standard solutions of ethanol were prepared by pipetting 95% ethanol (corrected to 100% purity) into volumetric flasks. Because of simplicity and convenience, peak heights rather than areas were used in this study.

After the measurements were completed for a particular set of conditions, the column was usually flushed with 70% MeOH/water until the effluent was chomophore-free as indicated by the UV monitor.

Results and Discussion

Chromatographic system and UV-detection agent

The basic approach in selecting the components of the system (LC column, mobile phase, and detection agent) was to keep the system as simple and conventional as possible. Although a number of LC columns (C_8, CN, and C_{18}) were tested with various compositions of mobile phase, it was found that a C_{18} column with 30:70 methanol/water as mobile phase was suitable to resolve ethanol in a reasonable time from the other interfering components found in beverages.

A number of chromophore compounds were screened as UV-detection agents for ethanol based on the following requirements. The compound should have its maximum absorbance near 254 nm, so that the method will have adequate sensitivity in cases where only a fixed wavelength UV detector is available. The compound should be relatively polar (similar to ethanol) in order to provide increased sensitivity. In other words, the retention time of the detection agent should be similar to that of ethanol, because, as reported elsewhere (4) and confirmed in this laboratory (6), the response factor for the analyte increases as the retention times of analyte and detection agent approach each other. Accordingly, it was found that acetone, MEK, and methyl isopropyl ketone (MIPK) were suitable as detection agents for ethanol. But for most of this work, MEK was used as the detection agent because its retention is close to that of ethanol, and at the same time it is sufficiently separated from the peaks of other UV-absorbing compounds encountered in some beverage types.

Concentration of detection agent

In much of the literature of indirect photometric detection in LC, the optimum concentration of the detection agent is set as a compromise between detector response and noise level of the base line. In some instances, the concentration was determined by the maximum absorbance that can still be compensated by the zero adjustment of the instrument being used (5).

It was found that the sensitivity of the method as measured by the size of the coeluted chromophore peak increased with

increasing concentration of the detection agent in the mobile phase. This is shown in Figure 2. But the increase was nonlinear, approached diminishing returns beyound 0.3% MEK, and in any event, above 0.3% concentration the absorbance of MEK exceeds the capacity of the instrument zero adjustment as mentioned above. (Total suppression available with the instrument in hand was ±0.6 AU). Thus, the optimum concentration of MEK in this work was 0.25% to 0.30%.

As previously reported (4), it was observed that the capacity factor of the ethanol peak (marked by the coeluted chromophore peak) remained unchanged as the concentration of the chromophore was increased stepwise 10-fold. This is shown by the retention data in Table I, indicating that the MEK concentration has little or no effect on the retention characteristics of the system, not a suprising observation considering that the concentration of the detection agent is less than 1% of the organic modifier concentration in the mobile phase. It should be noted however, that the concentration of MEK in the mobile phase does affect the retention of the MEK peak, as also shown by the retention data in Table I. As the concentration of MEK is increased 10-fold, the capacity factor of MEK decreased from 1.77 to 1.38. This may be explained by the fact that MEK, being a stronger solvent than the organic modifier (methanol), is more strongly absorbed in the stationary phase.

Calibration

The proposed method was calibrated against reference standard solutions of ethanol/water prepared in the range 0 to 50% ethanol, and the resulting curve is shown in Figure 3. The height of the ethanol (coeluted chromophore) peak is linear with ethanol concentration to about 40%. For higher ethanol concentrations, the relationship between peak height and concentration is not linear. This means that the determination must be done graphically, or alternatively, the analytical sample may be diluted volumetrically with mobile phase, thereby bringing the concentration of ethanol into the linear range, below 40%.

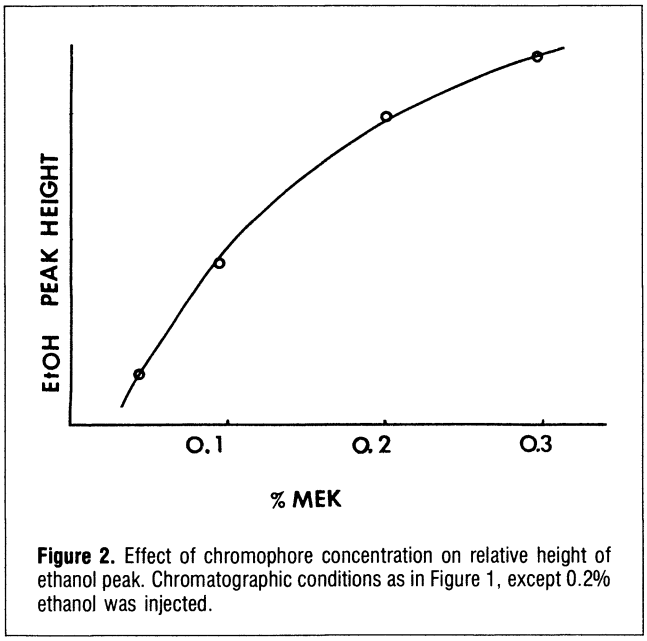

Figure 2. Effect of chromophore concentration on relative height of ethanol peak. Chromatographic conditions as in Figure 1, except 0.2% ethanol was injected.

Table I. Effect of MEK Concentration on Capacity Factors of Ethanol and MEK

	Capacity factor	
% MEK	Ethanol	MEK
0.05	0.30	1.77
0.10	0.30	1.65
0.20	0.32	1.55
0.30	0.31	1.51
0.55	0.30	1.38

Accuracy, precision, and sensitivity

The apparent accuracy of the proposed procedure was estimated by comparing the percent ethanol found with the percent indicated on the label of commercial products. The results shown in Table II indicate that the apparent mean accuracy is better than 2%.

The estimated precision of the proposed method shown in Table III, RSD ≈ 2%, was based on replicate determinations made over several days using the same sample and same mobile phase solution. Peak heights were measured manually, and all the measurements were made by the same person. The sensitivity of this method may depend on a number of variables, including the injection volume, the choice of the specific detection compound, and its concentration in the mobile phase. Under the conditions used in this work (see below), the minimum detectable concentration of ethanol was found to be 0.1%, corresponding to twice the base line noise level at a detector sensitivity 0.005 AUFS and a recorder sensitivity 10 mV FS.

Recommended analytical conditions

Ethanol in certain types of alcoholic beverages and in similar solutions may be determined by the proposed method using the following (or similar) conditions.

LC column: C_{18}, 250×4.6 mm, 10 μm
Mobile phase: 30:70 methanol/water
Detection agent: 0.2 to 0.3% MEK
Flow rate: 1 mL/min
Injection volume: 10 μL
Detector: 280 nm, 0.1 AUFS for
10 to 30% ethanol content
Calibration method: by comparison with
ethanol/water standard solutions

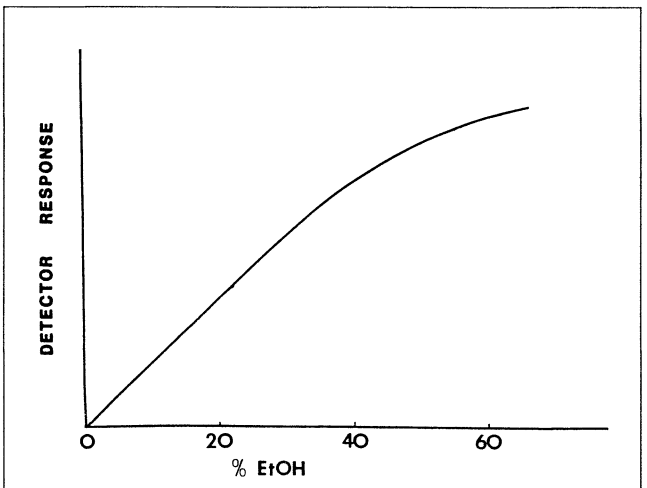

Figure 3. Relative detector response as a function of ethanol concentration. Chromatographic conditions as in Figure 1.

Frequency of calibration: each time a mobile phase solution is used

Typical chromatograms

Chromatograms of samples of gin, vodka, and whiskeys are shown in Figures 4 and 5. These products are apparently free of components that absorb at 280 nm with retention times close to that of ethanol; consequently, the chromatograms are relatively simple. Other beverages such as wines, which contain relatively high concentrations of UV-absorbing components (e.g., fatty acids), may not be suitable for analysis by the present method.

Table II. Ethanol Content of Selected Beverages

Product	Ethanol content (%) Label	Found[a]
Gin	43	42.7
Scotch, blended	40	40.1
Vermouth, very dry	18	17.8
Rye	50	48.9
Sherry	17.1	17.3
Whiskey, sour mash	45	45.0
Vodka	40	40.0

[a] means of three chromatographic runs.

Table III. Estimated Precision*

Run	Ethanol found (%)
1	42.1
2	43.9
3	43.0
4	42.6
5	42.9
Mean 42.9	
RSD ≈ 2	

*Sample: gin (43% ethanol); conditions are the same as those for Figure 1.

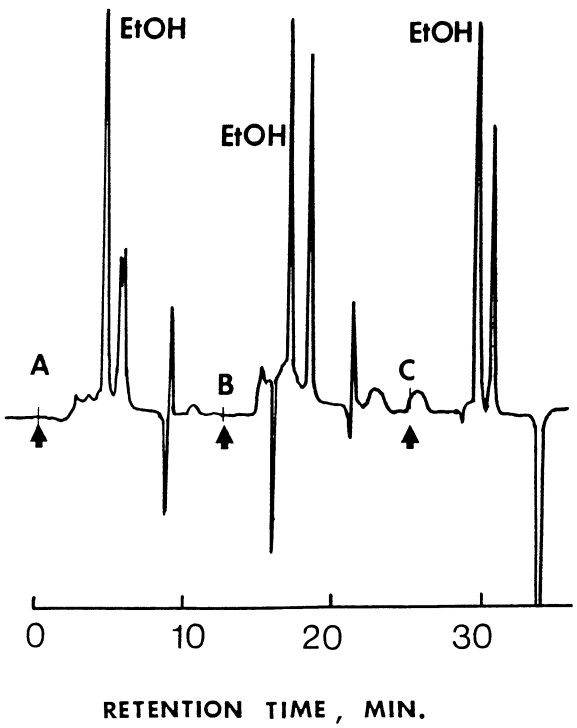

Figure 4. Chromatograms of beverages. Chromatographic conditions as in Figure 1. A=sour mash whiskey, B=scotch, blended, C=vodka.

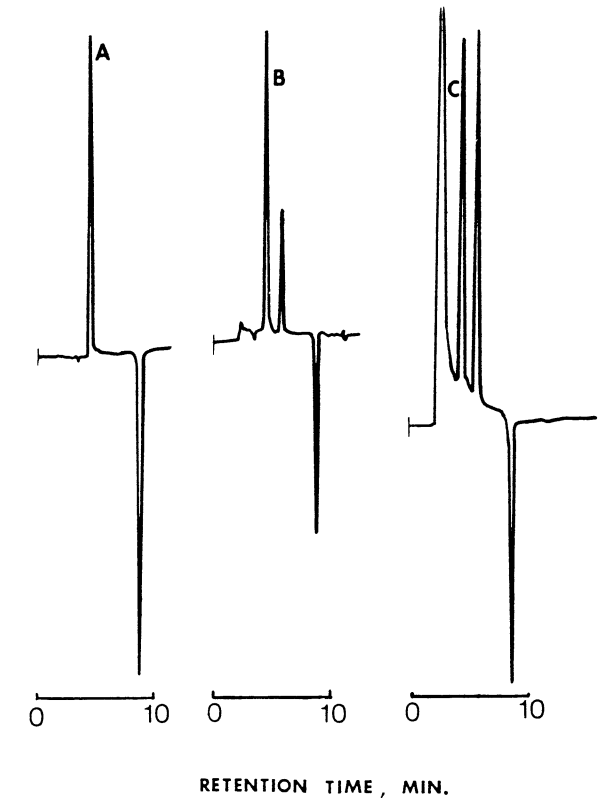

Figure 5. Chromatograms of beverages. Chromatographic conditions same as in Figure 1. A=gin, B=scotch, straight, C=rye.

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