SIMULTANEOUS DETERMINATION OF FLUORIDE, ACETATE, FORMATE, CHLORIDE, NITRATE, AND SULFATE IN DISTILLED ALCOHOLIC BEVERAGES WITH ION CHROMATOGRAPHY/CONDUCTIVITY DETECTOR

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Abstract: A novel method of ion chromatography with suppressed conductivity detection was developed for simultaneous determination of fluoride, acetate, formate, chloride, nitrate, and sulfate in distilled alcoholic beverages. In this study, bromide was used as an internal standard. The separation of the anions was accomplished by utilizing an anion exchange column with a gradient eluent program. The chromatographic conditions were as follows: the suppressor current was 31 mA; the flow rate of the mobile phase was 0.25 mL min^{-1}; the column and detector compartment temperatures were 35 °C and 40 °C, respectively; and the sample loop volume was 10 µL. All the calibration curves showed excellent linearity ($r^2 \geq 0.999$). The limits of detection (LOD) values were between 0.56 and 13.2 µg L$^{-1,}$ while the limits of quantification (LOQ) values were between 1.80 and 43.9 µg L^{-1}. A raki sample was spiked with standard solutions at three different concentration levels to evaluate the accuracy, and the average recoveries were found in the range of 94.90% - 101.71%. Intra-day and inter-day precision studies were also investigated, and the relative standard deviations (RSDs %) were less than 5.99%. The validated method was applied to the three kinds of commercial samples: Turkish raki, vodka, and gin.

Keywords: Ion chromatography, anion analysis, distilled alcoholic beverages.

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INTRODUCTION

The chemical composition of the beverages is an essential issue in terms of nutritional, toxicologic, and monitoring of the product quality. Distilled beverages are a mixture of all compounds produced from raw materials such as fruits, cereals, and spices. The process of distillation defines its sensory identity and quality of the drink. Every spirit has its unique aroma and flavoring components which reflect raw materials, distillation, and maturation process. The analysis of lower and higher alcohols is essential in terms of the quality of the product (1). Added to the lower and higher alcohols, the anions in the beverages should be controlled as well for quality purposes.

Determination of organic acids in food and beverages is a critical topic because these compounds define organoleptic quality and may also influence the stability of the product. Moreover, the dose of some organic acids, especially formic acid in food and beverages, is toxicologically significant. Additionally, the measurement of formate may identify methanol contamination (2-4).

Formic acid is an inhibitor leading hypoxia by disrupting cytochrome oxidase activity in the body. Cellular death arising from inhibition of cytochrome oxidase is thought to be based on the discharge of ATP, decreasing level of energy fundamental cell functions cannot be kept on (5). The retinal and optical nerves are vulnerable tissues and easily affected by formate toxicity (6). According to U.S. Environmental Protection Agency (US EPA), the threshold oral dose for formic acid-induced acute mortality ranged from nearly 30 to 45 g (429 to 643 mg/kg, assuming a bodyweight of 70 kg)(7).

Ethyl acetate has an essential effect on the organoleptic characteristics of alcoholic beverages. It is found mainly in the heading fraction. Passing to its following fraction influence the sensory quality of the spirit in an adverse manner (8). Under unsuitable storage conditions, acetate concentration can be increased in the product utilizing bacterial degradation. Besides, some operations such as fermentation process and distillation techniques affect the level of acetate (9). Acetate is a significant source of acetyl-CoA in hypoxia. Prevention of acetate metabolism may weaken tumoral growth (10). According to US EPA 900 mg/ kg/ day considered as No Observed Adverse Effect Level (NOAEL) for ethyl acetate and 3600 mg/ kg/ day was shown a significant toxic effect, and 23 % of rats have died (11).

Fluoride is the smallest anion. It reduces bacterial enzymic activity. Although fluoride is an essential nutrient for the skeletal bone and dental health, high levels of fluoride give rise to skeletal and dental fluorosis (12, 13). According to US EPA, the current maximum contaminant level goal for fluoride in water is set at 4.0 mg/L. The minimum dose that could lead to toxic signs and symptoms, including death for fluoride intoxication, has been set at 5 mg/kg body weight (14).

Chloride is one of the most common anions found in foods. It plays a vital role in the metabolic acid-base equilibrium and responsible for muscular irritability. Excess chloride content leads to severe adverse effects not only on human health but also production and storage process (15, 16). As a result of an extraordinary degree of chloride concentration in biological fluids, cystic fibrosis, myotonia, Bartter syndrome, and startle disease emerge (17). According to the World Health Organization (WHO), a dietary intake for adults of 9 mg of chloride per kg of body weight has been recommended (18).

Because of the widespread use of nitrogenous fertilizers, nitrite and nitrate level increase in foods, nitrites, and nitrates cause methemoglobinemia. Nitrites react with the secondary and tertiary amines in foods, so forming highly carcinogenic nitrosamines (19). The current acceptable daily intakes (ADIs) for nitrite and nitrate, set by the Joint Food and Agriculture Organization /WHO (FAO/WHO) Expert Committee on Food Additives (JECFA) in 2002, is 0.07 mg/kg bw/day and 3.7 mg/kg bw/day, respectively (20).

Sulfate, a basic anion for the human body, plays an essential role in the detoxification and catabolism of various endogenous and exogenous compounds. Sulfate exerts a laxative effect following exposures to high concentrations (21). Even though there is no health-based guideline value for sulfate in drinking water, above 500 mg/liter, a noticeable taste will arise (22).

In the literature, several analytical methods have been utilized for the analysis of small organic acids. The Gas Chromatography (GC) method for direct determination of 13 short-chain volatile organic acids including acetic acid in liquid foods was established, and a method based on continuous solid-phase extraction and GC method was reported for the direct determination of 29 organic acids including acetic acid in food and beverages (23, 24). Similarly, High-Performance Liquid Chromatography (HPLC) methods (25, 26) been reported for the analysis of carboxylic acids. Moreover, short-chain carboxylic acids, including formic and acetic acid in vegetable oils and fats, have been determined using Ion exclusion chromatography (IEC) electrospray ionization mass spectrometry (27). The systems, coupled with mass spectrometry (MS), eliminate the resolution problem and provides a good separation for the small organic acids. Even though MS coupled instruments are advanced technology products, they have some handicaps such as being expensive and time-consuming, requiring clean-up and derivatization procedures, coupled instrumentation, and use/waste of toxic organic solvents. Additionally, in the literature, there are capillary electrophoretic (CE) (28) and ion chromatographic (IC) (29, 30) methods allowing simultaneous quantification of common inorganic anions and small organic acids. However, less sensitivity (31) and precision problems of CE methods make suppressed IC the most potent method for the simultaneous determination of anions in various beverages.

Today, ion chromatography is a well-established method for the analysis of routine inorganic anions for many samples, especially for water analyses. Reagent-free ion chromatography (RFIC) has been utilized for about two decades. The ability to produce high purity eluents from the eluent generator system gives extra accuracy and reproducibility. Besides, suppressor systems decrease the baseline noise. There are few studies in which ion chromatography is employed for the analysis of distilled spirits.

Lachenmeier et al. (32) analyzed 107 different vodka samples with a conductivity meter. They found out that the conductivity of vodka purely derived from inorganic ions. The total ion content had an excellent linear relation with the conductivity. Arbuzov and Savchuk (33) analyzed the vodka samples by IC in combination with GC for the identification of ions. The ionic composition depends on water used for the production of vodka. They indicated that the conductivity of vodka could be affected by additives that were used for water treatment and adjustment of alkalinity. Also, Lachenmeier et al. (34) found that there were no significant differences in anion concentrations between the same samples, which were bottled at different dates. Balcerzak and Kapica (29) analyzed formate by using the IC system. In their study, they eliminated the volatile compounds from the matrices by utilizing an infrared (IR) source. They found LOD and LOQ values to be 0.014 mg L^{-1} and 0.042 mg L^{-1} for formate ion, respectively.

As being distinct from the previous study, to evaporate the volatile compounds from the raki samples, we used a water bath for more effective insulation. So, the time of vaporization was shortened under the nitrogen stream. It was observed that the broad peak disappeared on the IC chromatograms after the water bath vaporization process. Moreover, in our study, LOD and LOQ values were as smaller as 13 times than the previously published results.

This paper presents a simple, sensitive, reliable, and greener IC-CD method for the simultaneous determination of formate, acetate, fluoride, chloride, nitrate, and sulfate in alcoholic beverages. To the best of our knowledge, the proposed method is the first study to describe an IC-CD analysis of the anions in distilled alcoholic beverages utilizing bromide as an internal standard (IS), which minimizes the margin error arising from the loss of analyte in sample preparation procedure and during storage. Moreover, no study on anion analysis in Turkish raki samples has been published in the literature so far. This paper presents and discusses the anion concentration results of the Turkish raki samples. Besides, neither organic solvents nor reagents were used thanks to the RFIC system, which also provides repeatable results and sensitive measurements. In conclusion, it can be claimed that the proposed method is suitable for the determination of the anions in distilled alcoholic beverages at the routine laboratories where an IC system is used.

EXPERIMENTAL

Chemicals and Materials

All reagents were of analytical reagent grade. Sodium acetate (≥ 99.5%) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and ammonium formate (≥ 99.5%) were taken from Honeywell-Fluka (Morris Plains, NJ, USA). Stock solutions (1000 mg L^{-1}) of sodium acetate and ammonium formate were prepared by weighing and dissolving accurate amounts in ultrapure water.

Ultrapure water (min. 18.2 MΩ/cm), which was used for the preparation of standard solutions and dilutions, was supplied by the water purification system of New Human Power I Scholar UV (Human Corporation, Seoul, Korea).

1000 mg L^{-1} of pure standard solutions of fluoride, chloride, nitrate, sulfate, and bromide was purchased from Merck (Darmstadt, Germany). All these stock solutions were stored at 4 °C in tightly closed polypropylene (PP) bottles.

Polyether Sulfone (PES) filter (pore size 0.2 µm, 17 mm) was purchased from Analytical Columns (New Addington, Croydon, CR0 9UG, England).

Instrumentation

Chromatographic separations and analyses were conducted by using Dionex ICS-3000 (Sunnyvale, CA, USA) IC system. A suppressed conductivity detector (ASRS 300 suppressor and conductivity cell) was employed to collect signals after separation in the system. The separation of fluoride, acetate, formate, chloride, nitrate, and sulfate in the samples was accomplished by utilizing Dionex IonPac®AS20 analytical column (2 × 250 mm) with a Dionex IonPac® AG20 guard column (2 × 50 mm) as stationary phases. NaOH was used as a mobile phase automatically generated by the Dionex Eluent Generating Cartridge (EGC)-NaOH EluGen II cartridge using solely ultrapure water. The sample loop volume was 10 µL. Possible contaminants in the IC system were eliminated using Continuously Regenerating Trap Columns (CR-ATC). It is well known that the RFIC systems are superior compared to systems with manually prepared eluents in terms of stability, reproducibility, and minimum contamination risk.

Analysis of volatile compounds present in a raki sample was carried out by performing a Perkin Elmer Clarus 500 Headspace Gas Chromatography/Mass Spectrometry (HS-GC/MS) system.

Calibration and Calculations

0.0354 g of ammonium formate salt was dissolved with some UP water, and then an appropriate volume of UP water was added to bring the volume to the mark on the 25 mL PP of the volumetric flask to prepare the formate stock solution at 1000 mg L^{-1} concentration.

Acetate stock solutions at 100 mg L^{-1} concentration were prepared by dissolving 0.014 g of sodium acetate with 100 mL of UP water in a PP volumetric flask.

A range of working standard solutions containing 2 mg L^{-1} bromide (IS) was prepared by diluting the stock solutions with ultrapure water.

Peaks from the sample were identified by comparing the retention time with those obtained from the individual standard samples. The concentrations of fluoride, acetate, formate, chloride, nitrate, and sulfate were separately calculated by utilizing the IS method. The ratio of the analyte peak area to the IS peak area was measured, and standard calibration curves constructed by plotting concentration versus peak area ratios. All the data acquisition and instrument control were performed via the Dionex Chromeleon® Client (Ver. 6.80) software.

Sample Collection

Three brands of Turkish raki samples, three brands of vodka samples, and three brands of gin samples were purchased from local supermarkets (Istanbul, Turkey). An ouzo sample was also bought from a local supermarket in Greece.

Sample Preparation

Firstly, 5 mL of sample solutions were prepared by mixing 4,990 µL of sample and 10 µL of 1000 mg L^{-1} bromide (IS) solution. Samples containing 2.0 mg L^{-1} bromide (IS) were evaporated approximately until 0.5 mL remains in a water bath at 40 – 50 °C under a nitrogen stream. When the temperature of the sample solutions has reached to room temperature after the evaporation process, samples were diluted to 2.0 mL with ultrapure water. Consequently, final solutions were filtered through a PES filter before IC analysis.

RESULTS AND DISCUSSION

Optimal Chromatography Conditions

The optimized chromatographic conditions were as follows: the suppressor current was 31 mA; the flow rate of the mobile phase was 0.25 mL min^{-1}; the column and detector compartment temperatures were 35 °C and 40 °C, respectively; sample loop volume was 10 µL.

The gradient elution of the mobile phase program provided by EGC was as follows: 0-7 min 5 mM NaOH isocratic, 7-17 min gradient from 5 mM to 23 mM NaOH, 17-22.5 min 23 mM NaOH isocratic, 22.5-23 min gradient from 23 mM to 50 mM NaOH, 23-28 min gradient from 50 mM to 5 mM NaOH, and 28-33 min 5mM NaOH isocratic.

Matrix Elimination

The significant compounds of raki, vodka, and gin samples are alcohols, higher alcohols, and white sugar. When compared to vodka and gin samples, raki has a more complex nature by including trans-anethol and extra white sugar, and it was chosen as a representative sample for validation studies and optimization of pretreatment processes.

Primarily, to eliminate the volatile compounds from the sample solution, a raki sample was exposed to an IR source obeying the method mentioned in the literature (29). However, even though all apparatus were covered with aluminum foil, only max half of the sample could be evaporated within 3 hours. This solution was filtered and directly injected into the IC system. IC chromatogram of the brand, raki sample "a" exposed to IR radiation was shown in **Figure 1**. A broad peak interfered with the other anions in the IC chromatogram. Therefore, we decided to carry out an alternative vaporization method. Then, a pretreatment method employing a water bath (40 – 50 °C) and nitrogen stream was applied. By utilizing this process, sample volume was reached to 0.5 mL within only 1 hour. This solution was diluted to 2 mL, and it was loaded to IC for analysis. As it seen in **Figure 2**, no broad peak on IC chromatogram was observed after applying the water bath accompanied nitrogen stream vaporization process.

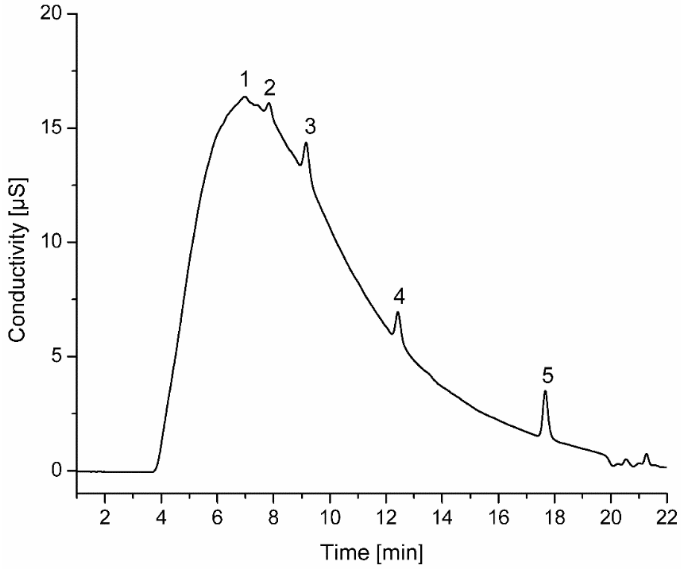

Figure 1. An IC chromatogram of the brand "*a*" raki sample after IR vaporization pretreatment.

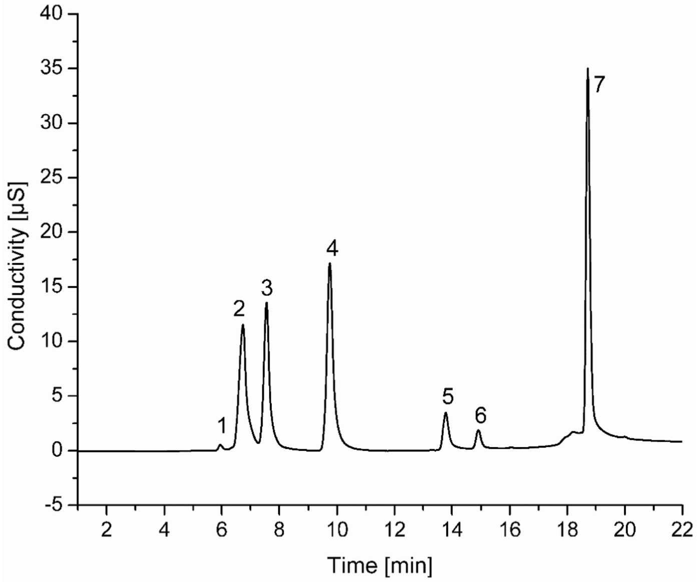

Figure 2. An IC chromatogram of the brand "*a*" raki sample after water bath vaporization. *1*: Fluoride; *2*: Acetate; *3*: Formate; *4*: Chloride; *5*: Nitrate; *6*: Bromide; *7*: Sulfate.

An aliquot 200 µL of the raki sample solutions were transferred into the HS vials and analyzed qualitatively by HS-GC/MS before and after the vaporization process to evaluate whether the pretreatment procedure was effective in terms of elimination of volatile compounds. HS-GC/MS total ion chromatograms were given in **Figure 3**. All volatile compounds of the brand "*a*" raki sample were eliminated by carrying out the latter process.

On the other hand, according to the Turkish Food Codex Distilled Alcoholic Beverage Regulation (35), the permitted maximum concentration of white sugar is 10 g L^{-1}, which is used for taste harmonization during the production of raki. The added sugar did not lead to any damage or interference problem to the IC-CD system since sugars eluted from the anion exchange column (AEC) (36), and they have no response to the conductivity detector.

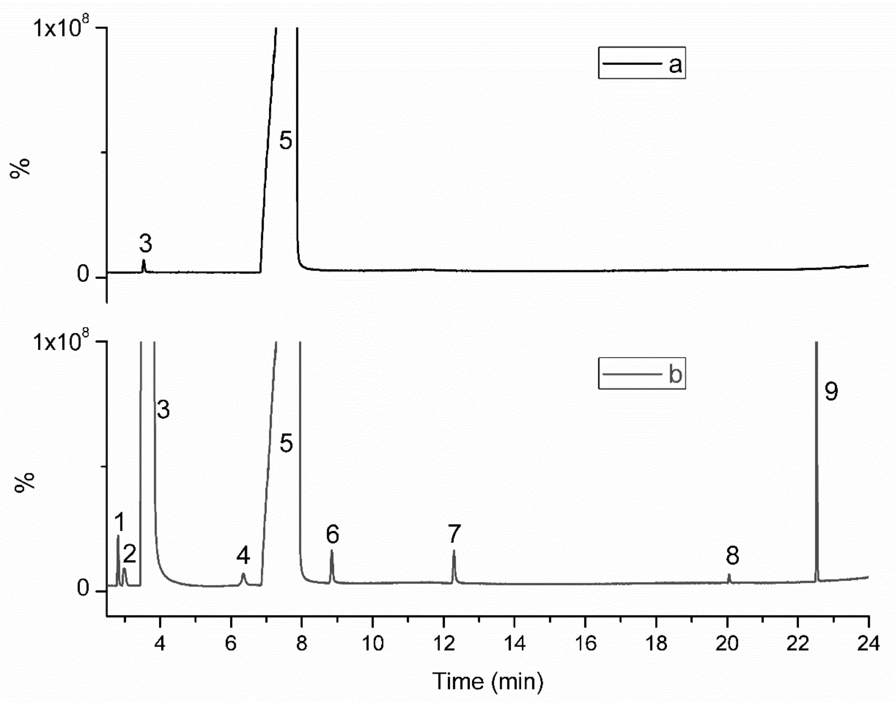

Figure 3. HS-GC/MS total ion chromatogram of the brand "a" raki sample. A) after water bath vaporization; B) before water bath vaporization. *1*: Ethyl acetate; *2*: Methanol; *3*: Ethanol; *4*: 1-Propanol; *5*: Water; *6*: 2-methyl-1-propanol; *7*: 3-methyl-1-butanol; *8*: Estragole; *9*: Anethole.

Method Validation

Some performance characteristics were investigated to evaluate linearity, selectivity, and repeatability.

Linearity

In this study, bromide was used as an IS at 2.0 mg L^{-1}. Thus, the stability and accuracy of the method could be improved. Bromide peak did not interfere with the peaks of fluoride, acetate, formate, chloride, nitrate, and sulfate on the chromatogram of the brand *a* raki sample (See **Figure 2**). Similarly, all analyte peaks of brand "g" vodka sample and brand "h" gin samples could be sufficiently separated from each other on AEC under optimized chromatographic conditions (See **Figure 4** and **Figure 5**, respectively).

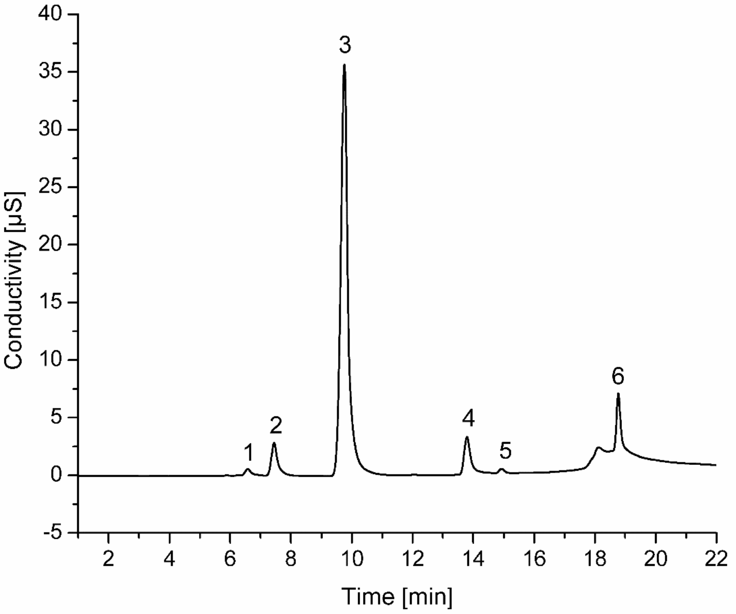

Figure 4. An IC chromatogram of the brand "g" vodka sample. *1*: Acetate; *2*: Formate; *3*: Chloride; *4*: Nitrate; *5*: Bromide; *6*: Sulfate.

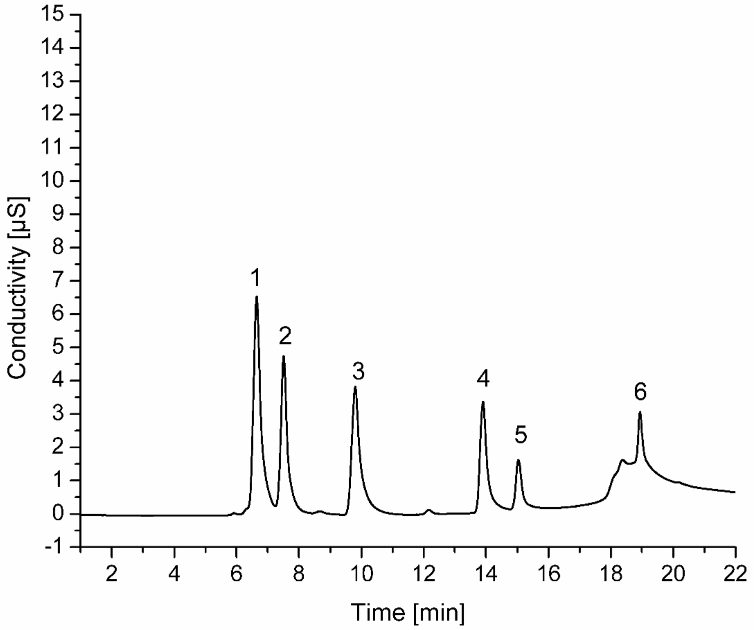

Figure 5. An IC chromatogram of the brand "h" gin sample. *1*: Acetate; *2*: Formate; *3*: Chloride; *4*: Nitrate; *5*: Bromide; *6*: Sulfate.

The linear calibration ranges and correlation coefficients of fluoride, acetate, formate, chloride, nitrate, and sulfate were summarized in **Table 1**. It was clear that a good linear relationship and correlation coefficients ($r^2 \geq 0.999$) were achieved.

Table 1. Linear calibration curve parameters, t_R, LOD, and LOQ.

Analyte	Linear Range (mg L^{-1})	Regression equation	t_R (minute)	LOD (µg L^{-1})	LOQ (µg L^{-1})
Fluoride	0.004-10	$y = 0.5912x - 0.0023$	5.98	1.40	4.64
Acetate	0.010-20	$y = 0.0510x - 0.0002$	6.64	3.20	10.40
Formate	0.003-10	$y = 0.2566x - 0.001$	7.54	0.97	3.25
Chloride	0.002-30	$y = 0.4927x - 0.0019$	9.84	0.56	1.80
Nitrate	0.006-20	$y = 0.4927x - 0.0019$	15.05	1.70	5.85
Sulfate	0.044-20	$y = 0.4927x - 0.0019$	18.94	13.20	43.9

r^2 for all analytes were 0.999.

LOD and LOQ

According to the Eurachem Guide*,* LOD and LOQ were calculated by the standard deviation (s'_0) obtained from ten replicate measurements of low concentration of analytes in the samples, multiplied by k_Q factors, which were 3 and 10 (the IUPAC default values), respectively (37). Linear range, regression equation, the limit of detection (LOD), limit of quantification (LOQ), and retention time (t_R) values were given in **Table 1**.

The values given in **Table 1** confirm that the developed method was highly sensitive even at low ppb levels.

Selectivity

The proposed study offers enhanced selectivity. Quaternary ammonium functional group of the analytical AEC has (+) charge and anionic forms of small organic acids and inorganic anions separated on AEC. Then, each anion is converted into H-form in the suppressor part before arriving at CD. Therefore, the H-form of target analytes must be dissociated for responding to the CD. Sugar molecules, thus, can not be detected by CD since their H-forms can not be dissociated in CD.

In addition to separation on the column, some possibly interfering compounds in the samples were eliminated by suppressed CD. Thanks to the water bath vaporization process, the matrix effect was significantly decreased. Any shouldered or interfered peak was not observed in IC chromatograms.

By considering all these properties, the samples were successfully separated within 20 min without any interference, and it can be confidently asserted that a highly selective method was developed.

Trueness

Trueness is a criterion for the accuracy of a method. One of the statistical methods for accepting accuracy is the significance test (t-test). To evaluate the trueness of the proposed method, brand "a" raki sample was spiked with three concentration levels, about half, equal, and two-fold of the first measured concentration values of the analytes. **Table 2** summarizes the average values of the recovery percentage results. Measured concentration values of the analytes in the solutions were given directly without calculating with a correction factor, which was 2.5. The recovery percentages for the analytes were found between 94.90 and 101.71 %, and they were acceptable according to the AOAC guideline (38). Besides, one of the statistical methods for accepting accuracy is the significance test (t-test). According to the results, the calculated t values were smaller than the theoretical, critical t value for 5 degrees of freedom equal to 2.57 at a level of significance $\alpha = 0.05$ (see **Table 2**).

Precision

The precision of the proposed method was evaluated by carrying out inter-day and intra-day repeatability studies. Repeatability was expressed with RSD% values. Precision was evaluated by continuously performing six replicates each day within 3 days for the determination of the brand *a* raki sample. RSD % values of concentration, area, height, and t_R of the anions were given in **Table 3,** and the results were acceptable according to the AOAC guideline (38). The proposed method was successfully validated.

Analysis of the Samples

Three brands of Turkish raki, an ouzo sample, three brands of vodka, and three brands of gin samples were analyzed. **Table 4** shows the results of the analysis for different brands of distilled alcoholic beverages.

In raki samples, fluoride was the anion with the lowest concentration levels among all analytes, whereas acetate concentrations were the highest compared to the other anions except for sample "c". Although Ouzo is also an anise-flavored drink, acetate was not detected in this sample. Additionally, chloride, nitrate, and sulfate concentrations of the Ouzo sample were found about 75, 135, and 30 times higher than their average concentrations in Turkish raki samples, respectively. These differences between the anise-flavored beverages might arise from the water qualities and distillation procedures.

Nitrate and sulfate concentrations were found below 1.75 mg L^{-1} in all vodka and gin samples. Additionally, chloride concentrations were similarly found low, except brand "g" vodka had 7.03 mg L^{-1}. Formate concentrations were ≤ 2.61 mg $L^{-1,}$ whereas acetate concentrations were measured at a wide range, which was between 0.41 – 15.02 mg L^{-1}.

For instance, according to the average results, when a person weighing 70 kg drinks three double shots (3 × 80 mL) of any legally produced raki sample, he/she approximately takes to his body about 0.42 mg of formate, 0.003 mg of fluoride, 1.39 mg of acetate, 0.46 mg of chloride, 0.03 mg of nitrate and 0.19 mg of sulfate. Similarly, drinking three glasses of vodka (3× 100 mL), he/she takes about 0.29 mg of formate, 0.003 mg of fluoride, 1.39 mg of acetate, 0.46 mg of chloride, 0.03 mg of nitrate and 0.19 mg of sulfate and when he/she drinks three glasses of gin (3× 100 mL) 0.44 mg of formate, 3.01 mg of acetate, 0.29 mg of chloride, 0.06 mg of nitrate and 0.20 mg of sulfate enter to his body. Besides, brand *c* sample has the maximum content of formate brand "a" sample has the maximum anion contents except for formate ion among the branded raki samples. On the one hand, brand ″g" sample has the maximum contents of chloride and sulfate, brand "*e*″ sample has the maximum anion contents except for chloride and sulfate ions among the branded vodka samples. On the other hand, sample "h" has the maximum content of nitrate, brand "i" sample has the maximum anion content except for nitrate ion among the gin samples. According to the results, a similar trend among the types of distilled beverages was not observed. When the legally produced beverage sample possessing maximum examined anion is consumed, taken amounts of all the anions are considerably lower than their toxic doses (7, 11, 14, 18, 20, 22). It was found in this study that no analyte found in the samples exceeded toxic levels.

In comparison, according to the previous studies and this study, one can say that the anionic composition of the distilled alcoholic beverage samples varies from brand to brand. It is well known that the anionic composition of the beverages depends on water used for production. However, among the analyzed brands, some of the anion content was relatively bigger than the other anions, these amounts are not out of the limit values set by the authorities based on average consumption habits (7, 11, 14, 18, 20, 22). Ionic composition of the water allows the differentiation of spirits, which are obtained from different manufacturers (34). If excellently demineralized soft water is used, the stability and sensory properties of drink will be improved.

On the other hand, the incremental amount of minerals may result in precipitation and instability problems (32). Moreover, the conductivity of the beverages is tightly related to the ionic composition. On the other hand, we put forward an argument that the proposed method can be used for authentication of illicit or inadequate quality alcoholic beverages by analyzing their anionic composition in addition to common higher alcohols analyses.

CONCLUSIONS

A reliable method of analysis for the determination of formate, acetate, and the other inorganic anions in alcoholic beverages is crucial for assessing product quality. In this paper, a new ion chromatographic method was developed for the simultaneous determination of fluoride, acetate, formate, chloride, nitrate, and sulfate in distilled alcoholic beverages using bromide as IS. The anion content analysis of the Turkish raki samples utilizing the developed method was performed for the first time in the literature. Experimental results indicated that the method has many advantages, which are smooth to operate, excellent repeatability, and sensitivity. It should be noted that the proposed method was environmentally friendly since no organic solvent or compound was not used throughout analyses. The total time for obtaining the results, including sample preparation, was about 1.5 h. In conclusion, the proposed method can be applied to various distilled alcoholic beverages to analyze the anions at food control laboratories and forensic laboratories.

Table 2. Recovery results of the brand a raki sample for evaluating the trueness of the method.

Analyte	**Concentration (mg L^{-1})**							**Average Recovery (%)**		
	Initial	SD	RSD % (n=6)	Added	Found	SD	RSD % (n=6)	Average	SD (n=6)	t_{cal}
Fluoride	$2.20 \text{ x } 10^{-2}$	7.48×10^{-5}	0.34	0.01	3.17×10^{-2}	1.68×10^{-4}	0.53	97.00	3.83	1.92
				0.02	4.13×10^{-2}	2.56×10^{-4}	0.62	96.50	4.53	1.89
				0.04	6.14×10^{-2}	1.53×10^{-4}	0.25	98.50	3.36	1.09
Acetate	8.15	2.45×10^{-3}	0.03	4.21	12.23	8.56×10^{-3}	0.07	96.91	4.23	1.79
				8.42	16.31	2.60×10^{-2}	0.16	96.91	4.50	1.68
				16.84	24.50	1.47×10^{-2}	0.06	97.09	3.49	2.04
Formate	1.76	2.99×10^{-3}	0.17	0.88	2.62	6.03×10^{-3}	0.23	97.73	4.71	1.18
				1.76	3.55	6.03×10^{-3}	0.17	101.71	1.88	2.23
				3.52	5.33	3.73×10^{-3}	0.07	101.42	2.11	1.65
Chloride	1.32	3.43×10^{-3}	0.26	0.66	1.95	4.87×10^{-3}	0.25	95.45	5.83	1.91
				1.32	2.62	4.72×10^{-3}	0.18	98.48	3.52	1.06
				2.64	3.90	3.90×10^{-3}	0.10	97.72	3.19	1.75
Nitrate	1.94×10^{-1}	9.12×10^{-4}	0.47	$9.65 \text{ x } 10^{-2}$	2.87×10^{-1}	1.00×10^{-3}	0.35	96.37	4.83	1.84
				$1.93 \text{ x } 10^{-1}$	$3.51 \text{ x } 10^{-1}$	2.00×10^{-3}	0.57	95.33	4.99	2.29
				$3.86 \text{ x } 10^{-1}$	5.08×10^{-1}	1.88×10^{-3}	0.37	95.34	5.42	2.11
Sulfate	1.97	4.33×10^{-3}	0.22	0.98	2.91	2.91×10^{-3}	0.10	95.92	4.39	2.28
				1.96	3.89	5.45×10^{-3}	0.14	97.96	3.24	1.54
				3.92	5.69	1.71×10^{-3}	0.03	94.90	5.50	2.27

Table 3. Inter-day and intra-day RSD % (n=6) values of concentration, peak area, peak height, and t_R.

Analyte	Concentration		Area		Height		t_R	
	intra-day	inter-day	intra-day	inter-day	intra-day	inter-day	intra-day	inter-day
Fluoride	0.34	0.84	0.61	2.84	1.08	1.95	0.38	0.63
Acetate	0.04	0.20	0.31	2.43	0.78	1.41	0.44	0.70
Formate	0.17	0.99	0.43	3.28	2.10	5.99	0.46	0.75
Chloride	0.26	1.35	0.49	2.17	1.74	3.58	0.54	0.85
Nitrate	0.47	0.78	0.61	2.90	2.54	3.21	0.28	0.45
Sulfate	0.22	0.76	0.46	2.08	1.92	1.49	0.16	0.26

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COMPLIANCE WITH ETHICS REQUIREMENTS

Conflict of Interest: No conflict of interest exists in the submission of this manuscript, and all authors approve the manuscript for publication. This work described was an original research that has not been published previously and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is submitted. Melike Güler Şimşek, Orhan Destanoğlu, and Gülçin Gümüş have no conflict of interest.

Ethical Approval: This article does not contain any studies with human or animal subjects.

Table 4. IC results of anion concentrations of the raki, vodka and gin samples.

Analyte	**Raki Brands**						**Ouzo Brand**		**Vodka Brands**						**Gin Brands**					
	a		**b**		**c**		**d**		**e**		**f**		**g**		**h**		**i**		**j**	
	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)	C (mgL^{-1})	RSD (%)
Fluoride	2.20 × 10^{-2}	0.34	ND	-	ND	-	ND	-	0.11× 10^{-1}	0.23	ND	-	ND	-	ND	-	ND	-	ND	-
Acetate	8.15	0.03	7.54	0.04	1.70	0.05	ND	-	9.39	0.03	0.41	0.65	0.52	0.57	10.05	0.02	15.02	0.02	5.02	0.14
Formate	1.76	0.17	1.41	0.17	2.05	0.05	0.57	0.01	2.61	0.24	0.11	0.64	0.13	0.03	1.26	0.15	1.53	0.12	1.64	0.99
Chloride	1.32	0.26	0.39	0.13	0.56	0.47	60.43	0.24	2.10	0.31	0.73	0.12	7.03	0.01	0.75	0.85	1.06	0.13	1.03	0.14
Nitrate	0.19	0.47	0.03	0.96	0.10	0.95	13.50	0.02	0.50	0.76	0.12	0.42	0.06	0.39	0.34	0.65	0.15	0.69	0.12	0.89
Sulfate	1.97	0.22	0.29	0.21	0.13	0.78	22.32	0.02	0.22	0.64	0.25	0.15	0.63	0.40	0.18	0.78	1.75	0.07	0.09	0.85

**ND: Not Detected*

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