FOOD COMPOSITION AND ADDITIVES

Confirmatory Method for the Determination of Volatile Congeners and Methanol in Turkish Raki According to European Union Regulation (EEC) No. 2000R2870: Single-Laboratory Validation

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A method described by European Union Regulation (EEC) No. 2000R2870 was validated and supported by GC/MS analysis for the determination of volatile congeners and methanol in Turkish raki. The method was validated in terms of specificity, accuracy, precision, LOD, LOQ, linearity, and robustness. The specificity of the method was demonstrated, and the method showed excellent accuracy (97.5–100.1%). Linearity was checked in the ranges of 0.200–26.390 mg/100 mL for more volatile compounds and 1.155–48.00 mg/100 mL for less volatile compounds, after concentrations found in Turkish raki were taken into account. The calibration curves of all analytes showed good linearity ($R^2 > 0.998$). The within- and between-day precision (RSD) values of 11 analytes were in the range of 0.18–4.50%. The LOD and LOQ values were in the range of 0.014–0.362 and 0.045–1.085 mg/100 mL, respectively. The method can be used as an absolute quantification method for the determination of volatile congeners and methanol in Turkish raki and for QC.

Turkish raki is the most popular traditional distilled alcoholic drink in Turkey, with a history going back 300 years (1). According to the Tobacco, Tobacco Products and Alcoholic Beverages Market Regulatory Authority (TAPDK) of Turkey, raki consumption was 42.7 million liters in 2007 (2). In recent years, because alcoholic beverages are subject to a high rate of taxation in Turkey, there is a great difference between the cost and sales price of alcoholic beverages; this difference makes the production of counterfeit beverages attractive. In 2005, 23 people died in Turkey and dozens were hospitalized after drinking counterfeit raki that contained lethal levels of methanol. Therefore, it has become very important to control the quality and safety of the alcoholic beverages produced in Turkey.

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Turkish raki is produced by a second distillation of suma only (the distillate originating from grapes distilled in a continuous distillation column to approximately 94.5% alcohol, with the purpose of keeping the flavor and odor of grapes), or suma mixed with ethyl alcohol having an agricultural origin with aniseed (*Pimpinella anisum*) in traditional copper distillers having a volume of 5000 L. During the second distillation, "head," "medium," and "tail" (last product) are separated, and the medium part is used for raki production. The alcohol concentration of the medium part is diluted by water to 45% (v/v). At the end of production, sugar is added and the raki is then stored immediately in oak barrels for a ripening process (3, 4).

Turkish Food Codex-Distilled Alcoholic Beverages Notification (5) defines some special characteristics of Turkish raki: 65% of the total alcohol in the product should be suma; refined white sugar (saccharose) should be used in the preparation; the maximum concentration of sugar in the product should be 10 g/L; the amount of total volatile compounds should be 100 g/hL in absolute ethanol in the product; the amount of methanol should be 150 g/hL in absolute ethanol in the product; the amount of anethole in the etheric oil originating from the anise seed should be 800 mg/L in the product; the alcoholic strength of the product should be 40%; and the product should be aged 1 month before bottling.

Congeners are volatile substances formed along with ethanol during fermentation and maturation of spirit drinks, and they can be used to provide both qualitative and quantitative information for labeling purposes (6). European Union (EU) Council Regulation (EEC) No. 1989R1576 of 29 May 1989 (7) defines the description and composition of spirit drinks. It also established the general production procedures for some distillates and fixed common analytical composition limits. Recently, the EU has issued a specific regulation decision EEC No. 2000R2870 of 29 December 2000 (8), which sets the reference methods for the analysis of spirit drinks. The method that is used for the determination of volatile substances and methanol is recommended for use in monitoring compliance with EEC No. 1989R1576 and for official purposes in general.

The aim of our study was to test the method that has been successfully used for the determination of volatile congeners and methanol in spirit drinks, and that is defined by EEC No. 2000R2870, by applying it to the analysis of Turkish raki. The method was carefully validated for the analysis of Turkish raki by evaluating specificity, accuracy, within-day repeatability, between-day repeatability, linearity, range, LOD, LOQ, and robustness, according to the guidelines of the International Conference on Harmonization (ICH; 9).

Experimental

All analyses were performed according to EEC No. 2000R2870 (8), with some modification to the GC conditions. Compounds were quantified and identified by GC with flame ionization detection (GC/FID) and a GC/MS, respectively.

GC/FID

GC analyses were performed using an Agilent Technologies 7980A gas chromatograph with an autosampler and a flame ionization detector (Agilent Technologies, Palo Alto, CA). All separations were carried out with a CP-WAX57CB capillary column, 60 m 0.32 mm id 0.4 m film thickness (stabilized polyethylene glycol; Chrompack, Middelburg, The Netherlands). A retention gap of 1 m 0.32 mm was connected to the front of the column to improve the peak shape and GC conditions. Injections were made in the split mode (50:1), and the injection volume was 1 L. The injector temperature was 160 C, and the oven was programmed for 4 min at 40 C, increased at 1.8 C/min to 94 C, then increased at 30 C/min to 180 C, followed by 4 min at the final temperature. The carrier gas was helium at a flow rate of 1.3 mL/min. Detector: 260 C. H_2: 35 mL/min. Air: 350 mL/min. Auxiliary gas (He): 20 mL/min.

GC/MS

To identify the congeners in the raki sample, an Agilent Model 6890 Series II gas chromatograph equipped with an Agilent Model 5975B VL mass selective detector was used. The fused-silica column was a DB-WAX from J&W Scientific (Agilent Technologies, Santa Clara, CA), 30 m 0.25 mm id 0.25 m film thickness. The injector was used in the splitless mode. The temperature program of the gas chromatograph was the same as that for the GC/FID system. The splitless inlet temperature was 220 C, and the carrier gas was He at a flow rate of 3.3 mL/min. Electron ionization (EI) mass spectra were recorded at an ionization energy of 70 eV. The complete scanning mode over the *m*/*z* 29–350 range was used. The identification of compounds was based both on comparison of the linear retention index (RI) values with those reported in the literature and on the matching of mass spectra of the compounds with reference mass spectra of two libraries, Wiley and the National Institute of Standards and Technology, coupled with the GC/MS software. The compounds were identified by comparison of mass spectra and retention time data with those of reference compounds; the identifications were supported by the Wiley GC/MS library. Identification from the analysis of a raki sample was based on RI and comparison of EI mass spectra with those of reference compounds.

Chemicals and Reagents

All chemicals and standards [acetaldehyde, CAS No. 75-07-0; acetal (1,1-diethoxyethane) CAS No. 105-57-7; ethyl acetate, CAS No. 141-78-6; methyl acetate, CAS No. 79-20-9; methanol, CAS No. 67-56-1; 1-propanol (*n*-propanol), CAS No. 71-23-8; 2-butanol, CAS No. 78-92-2; 2-methyl-1-propanol (isobutanol), CAS No. 78-83-1; 1-butanol (*n*-butanol), CAS No. 71-36-3; 2-methyl-1-butanol (active amyl alcohol), CAS No. 137-32-6; 3-methyl-1-butanol (isoamyl alcohol), CAS No. 123-51-3] were analytical grade and purchased from Merck (Darmstadt, Germany). Purities of chemicals (all >98%) were confirmed by injection of congener standards, and all chemicals were free from other congeners. Acetal and acetaldehyde were stored in the dark at <5 C; all other reagents were kept at room temperature. Deionized (18.2 M) water of at least grade 3, as defined in ISO 3696, and purified using a Milli-Q system (Millipore, Billerica, MA) was used for the preparation of standard solutions.

Preparation of Standard Solutions

All standard solutions of congeners were prepared in a 40% (v/v) ethanol solution according to EEC No. 2000R2870 (8) by using the concentration ranges expected in Turkish rakies. 3-Pentanol (CAS No. 584-02-1) was used as an internal standard.

(**a**) *Standard solution A.*—A 3 mL portion of each reagent (acetaldehyde, acetal, ethyl acetate, methyl acetate, methanol, 1-propanol, 2-butanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, and 3-methyl-1-butanol) was pipetted into a 100 mL volumetric flask containing approximately 60 mL ethanol to minimize component evaporation. The contents of the flask were diluted to volume with ethanol and mixed thoroughly.

(**b**) *Standard solution B (internal standard stock solution).*—A 3 mL portion of 3-pentanol was pipetted into a 100 mL volumetric flask containing approximately 80 mL ethanol, and the contents of the flask were diluted to volume with ethanol and mixed thoroughly. The final concentration of the 3-pentanol stock solution was 2332 mg/100 mL.

(**c**) *Standard solution C (internal standard solution).*—A 10 mL aliquot of solution B was pipetted into a 100 mL volumetric flask containing approximately 80 mL ethanol, and the contents of the flask were diluted to volume with ethanol and mixed thoroughly. This solution was used in sample preparation, and the final concentration was 233.2 mg/100 mL.

(**d**) *Standard solutions used to check the linearity of the FID response.*—Aliquots of 0, 0.01, 0.05, 0.1, 0.5, 1, and 2 mL standard solution A and 1 mL standard solution B were pipetted into separate 100 mL volumetric flasks containing approximately 80 mL ethanol, and the contents of the flasks were diluted to volume with ethanol and mixed thoroughly.

Table 1. Retention times, peak symmetries, and resolutions

Compound	Mean t_{Rs}, min[a]	Mean t_{Rs}, min[b]	SD, min	RSD, %	I[c]	Peak symmetry	Peak resolution
Acetaldehyde	5.7628	5.7630	0.0001	0.0025	500	0.87	16.23
Methyl acetate	7.9066	7.9044	0.0016	0.0197	860	0.90	26.69
Ethyl acetate	9.6654	9.6632	0.0016	0.0161	895	0.89	17.21
Acetal	9.8558	9.8544	0.0001	0.0101	900	0.88	1.54
Methanol	10.6352	10.6432	0.0057	0.0532	905	0.84	5.16
2-Butanol	16.8628	16.8644	0.0011	0.0066	1011	0.86	42.19
1-Propanol	17.9998	18.0036	0.0027	0.0149	1042	0.90	5.78
2-Methyl-1-propanol	21.5698	21.6130	0.0306	0.1415	1108	0.95	24.82
1-Butanol	25.2722	25.2978	0.0181	0.0716	1138	0.91	26.64
2-Methyl-1-butanol	29.5604	29.5652	0.0034	0.0115	1206	0.87	30.55
3-Methyl-1-butanol	30.2350	30.2408	0.0041	0.0138	1206	0.89	1.30

[a] Retention times of standard solutions; n = 9.
[b] Retention times of raki samples; n = 9.
[c] Relative retention indexes on DB-WAX column, calculated versus *n*-alkanes (C10-C16).

Procedure

(**a**) *Alcoholic strength.*—A traditional commercial raki sample was obtained from a local market. The alcoholic strength of the raki sample was determined according to EEC No. 2000R2870 (8).

(**b**) *Sample preparation.*—A 0.9 mL aliquot of the raki sample or standard solution A was pipetted into a 1.5 mL vial, and 0.1 mL standard solution C (internal standard solution) was added. The sample was shaken vigorously and stored at <5 C before analysis.

(**c**) *Calculation.*—The concentration of each congener was determined with respect to the internal standard from relative response factors (RRFs), which were obtained during calibration under the same chromatographic conditions as those of the raki analysis. The RRF for each congener was calculated by using Equation 1.

$$RRF = (A_{is}/A_c) \quad (C_c/C_{is}) \qquad (1)$$

where C_c = concentration of congener (mg/100 mL), C_{is} = concentration of internal standard (mg/100 mL), A_c = peak area or height of congener, and A_{is} = peak area or height of internal standard.

The results were calculated by using Equation 2 and were expressed as g/hL in absolute ethanol.

$$C_c = (A_c \quad A_{is}) \quad C_{is} \quad RRF \quad (100/H) \qquad (2)$$

where H = alcoholic strength of sample.

Method Validation

The method was validated according to the guidelines established by the ICH for analytical method validation (9). All results were expressed as percentages, where n represents the number of values or measurements.

Specificity

For chromatographic methods, specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. An example of specificity for an assay method is that the analyte peak will have a baseline chromatographic resolution of 1.5 from all other sample components (10). The ICH documents define specificity as the ability to access the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components (9). The specificity of the method was determined by analyzing standard mixtures and evaluating peaks for purity and resolution from the peak of the nearest eluting component. Specificity was also documented by comparing retention times obtained for three samples of the standard compounds mixture (standard solution A) with those obtained for three raki samples.

Accuracy (Recovery)

Accuracy is the closeness of the test results obtained by the analytical method to the true value. Accuracy can be assessed by analyzing a sample of known concentration (reference materials) and comparing the measured value to the true value (10). Accuracy is often calculated as percent recovery by the assay of known, added amounts of analyte to the sample (11). The ICH documents recommend that accuracy should be assessed by using a minimum of nine determinations over a minimum of three concentration levels (9). The accuracy of the method was assessed at three concentration levels.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation (10). According to the ICH (9), precision should be evaluated at three

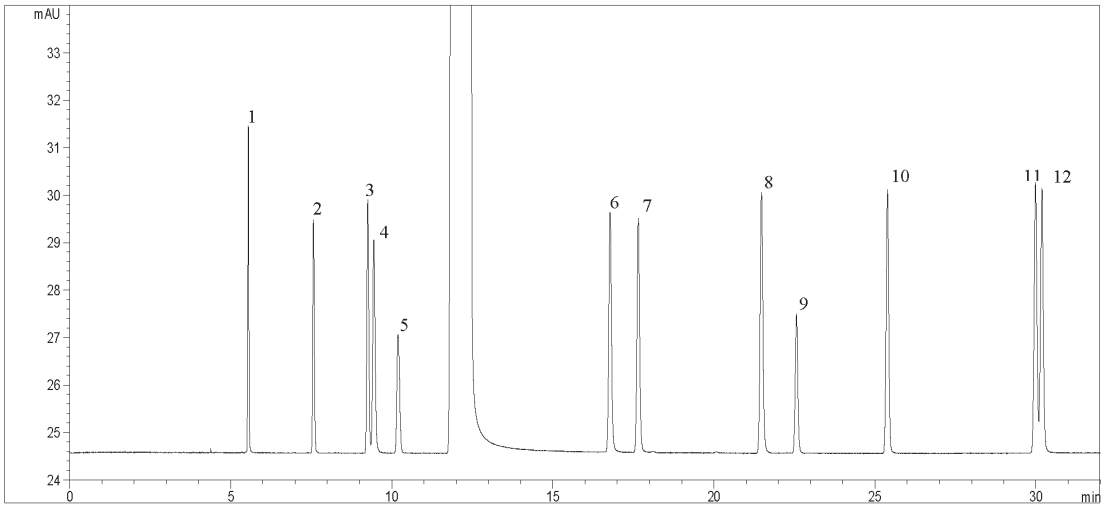

Figure 1. Chromatogram of a standard solution of volatile compounds. Peak identification: (1) acetaldehyde; (2) methyl acetate; (3) ethyl acetate; (4) acetal; (5) methanol; (6) 2-butanol; (7) 1-propanol; (8) 2-methyl-1-propanol; (9) 3-pentanol (internal standard); (10) 1-butanol; (11) 2-methyl-1-butanol; and (12) 3-methyl-1-butanol.

different levels: repeatability, intermediate precision, and reproducibility. Repeatability reflects the results of the method operating over a short time interval under the same conditions. Intermediate precision reflects the results from within-laboratory variations due to random events, such as different days, analysts, equipment, etc. Reproducibility, which is determined by testing homogeneous samples in multiple laboratories, is often a part of interlaboratory crossover studies (10). Repeatability was evaluated by three injections of three levels of standard solutions within one day. Intermediate precision was evaluated by performing the whole method on 3 different days with three levels of standard concentrations. Reproducibility, which refers to the use of an analytical procedure in different laboratories, was beyond the scope of the present study.

LOD and LOQ

The LOD is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. It is expressed as a concentration at a specified S/N (10). The S/N is determined by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. An S/N of 3:1 is generally considered acceptable for estimating the LOD (9). The LOQ is defined as the lowest concentration of an analyte in a sample

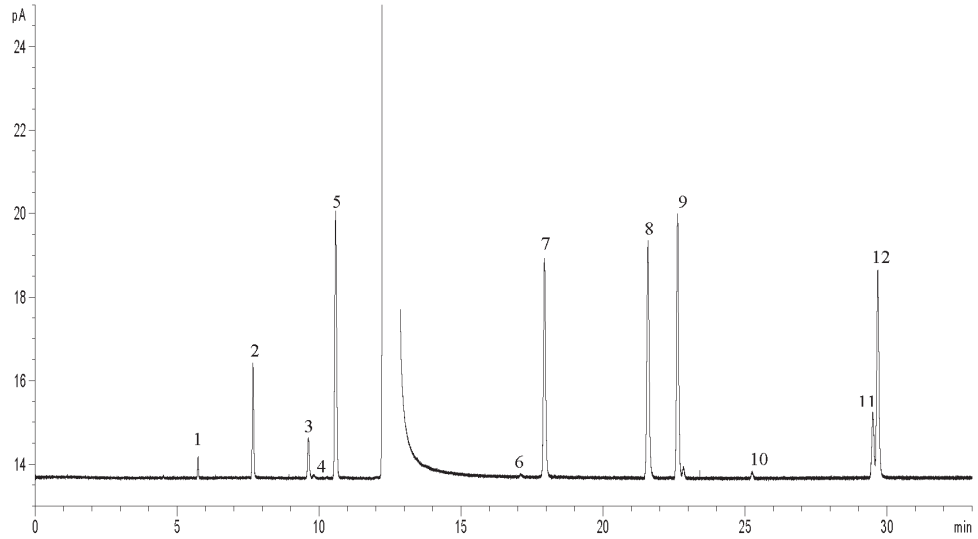

Figure 2. Chromatogram of a raki sample. Peak identification: (1) acetaldehyde; (2) methyl acetate; (3) ethyl acetate; (4) acetal; (5) methanol; (6) 2-butanol; (7) 1-propanol; (8) 2-methyl-1-propanol; (9) 3-pentanol (internal standard); (10) 1-butanol; (11) 2-methyl-1-butanol; and (12) 3-methyl-1-butanol.

Table 2. Linearity parameters and LOD and LOQ values for the compounds in standard solutions

Compound[a]	Range tested, mg/100 mL	Slope (*a*)	Intercept (*b*)	R^{2b}	LOD (3.3 /S), mg/100 mL[c]	LOQ (10 /S), mg/100 mL[c]	LOD (S/N, 3:1), mg/100 mL[d]	LOQ (S/N, 10:1), mg/100 mL[d]
Acetaldehyde	0.200–20.020	0.4973	–0.0008	0.9998	0.054	0.180	0.100	0.300
Methyl acetate	0.261–26.060	0.3842	–0.0017	0.9999	0.113	0.377	0.261	0.782
Ethyl acetate	0.264–26.390	0.5182	–0.0008	0.9999	0.065	0.218	0.176	0.528
Acetal	0.241–24.410	0.4207	–0.0045	0.9981	0.054	0.181	0.362	1.085
Methanol	1.155–46.184	0.4548	–0.0034	0.9999	0.064	0.213	0.277	0.831
2-Butanol	1.186–47.440	0.8478	–0.0092	0.9999	0.051	0.171	0.109	0.328
1-Propanol	1.153–46.100	0.8257	–0.0098	0.9999	0.080	0.265	0.173	0.519
2-Methyl-1-propanol	1.191–47.620	0.9703	–0.0109	0.9999	0.014	0.045	0.159	0.476
1-Butanol	1.200–48.000	0.9191	–0.0107	0.9999	0.137	0.456	0.144	0.432
2-Methyl-1-butanol	1.171–46.820	0.9912	–0.0098	0.9999	0.068	0.228	0.140	0.421
3-Methyl-1-butanol	1.162–46.460	1.0273	–0.0125	0.9999	0.083	0.276	0.139	0.418

[a] Regression equation: $y = ax + b$, where y is the peak area and x is the concentration mg/100 mL.
[b] Correlation coefficient.
[c] Calculated from the SD of the intercept and the slope.
[d] Calculated from the S/N.

that can be determined with acceptable precision and accuracy under the stated operational conditions of method (10). The S/N is determined by comparing the measured signals from samples with known low concentrations of analyte with those of blank samples, and establishing the minimum concentration at which the analyte can be reliably quantified. A typical S/N is 10:1 (9). The injection concentration, which could be detected at the S/N of 3, was considered to be the LOD for all analytes. The LOQ was the injection concentration corresponding to the peak heights with an S/N of 10. The LOD and LOQ were also calculated on the basis of the SD and the slope obtained from the linearity plot of each compound in the standard mixture (9).

Linearity and Range

Linearity is the ability of the method to elicit test results that are directly proportional to the analyte concentration within a given range. Range is the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy and linearity using the method as written (10). The ICH guidelines specify a minimum of five concentration levels, along with certain minimum specified ranges (9). All analytes were evaluated for linearity using five concentration levels, and triplicate injections were made for each concentration level. Acetaldehyde, methyl acetate, ethyl acetate, and acetal were assayed at ranges from 0.20 to 26.40 mg/100 mL; the ranges for the other compounds in the standard mixture were greater because of their greater volatility.

Robustness

The robustness of a method is its ability to remain unaffected by small deliberate variations in method parameters (10). As documented in the ICH guidelines (9), robustness should be considered early in the development of a method. Robustness of the method was evaluated during the development phase by 31 laboratories from eight countries with 10 test materials consisting of rum, whisky, brandy, kirsch, and grappa spirit drinks with and without fortified levels of volatile congeners (6). The method was found to be robust and was recommended for official regulatory purposes.

Results and Discussion

The specificity of the method was assessed by comparing retention times obtained for the standard compounds mixture with those obtained for the raki samples. For acceptable results specificity was also documented by analyzing the standard mixture and a raki sample, and evaluating peaks for purity and resolution from the nearest peak (Table 1). Chromatograms of the standard compounds and a raki sample are shown in Figures 1 and 2, respectively. Retention times, peak shapes, and peak resolutions showed that no interferences were present in the chromatographic region of interest where the peaks of the analytes were found. Peak symmetries were between 0.5 and 1.5, which is the ideal value for purity. All peaks had a baseline resolution of >1.5, except 3-methyl-1-butanol (1.30). However, pretrial studies had suggested that there was little difference in the total congener concentration whether 2-methyl-1-butanol and 3-methyl-1-butanol were quantitated individually or as one peak (6). The difference between the retention times of compounds present in the standard mixture and in the raki samples allows confident and highly specific peak identification.

The linearity was checked by means of the internal standard method. Table 2 shows the linearity parameters and the LOD

Table 3. Accuracy of compounds in standard solutions

Compound	Theoretical concn, mg/100 mL	Concn found, mg/100 mL	RSD, %[a]	Recovery, %	Avg. recovery, %[b]
Acetaldehyde	2.002	1.925	1.394	96.17	97.94
	10.010	10.140	1.104	101.30	
	20.020	19.287	1.464	96.34	
Methyl acetate	2.606	2.494	2.456	95.69	97.51
	13.030	12.860	0.961	98.70	
	26.060	25.574	1.094	98.14	
Ethyl acetate	2.639	2.690	1.814	101.95	100.10
	13.195	13.012	1.091	98.61	
	26.390	26.319	1.351	99.73	
Acetal	2.410	2.674	2.287	110.95	101.13
	12.205	10.570	0.360	86.60	
	24.410	25.833	1.754	105.83	
Methanol	2.309	2.403	1.079	104.06	100.63
	11.546	11.499	0.554	99.59	
	23.092	22.686	1.306	98.24	
2-Butanol	2.372	2.350	1.265	99.09	98.48
	11.860	11.663	0.871	98.34	
	23.720	23.249	1.205	98.01	
1-Propanol	2.305	2.321	1.209	100.67	99.10
	11.525	11.384	0.337	98.78	
	23.050	22.554	1.347	97.85	
2-Methyl-1-propanol	2.381	2.363	0.514	99.24	98.67
	11.905	11.730	0.465	98.53	
	23.810	23.393	1.023	98.25	
1-Butanol	2.400	2.385	0.306	99.35	98.87
	12.000	11.863	0.427	98.86	
	24.000	23.619	1.286	98.41	
2-Methyl-1-butanol	2.341	2.336	1.067	99.78	99.55
	11.705	11.688	0.387	99.86	
	23.410	23.182	0.950	99.03	
3-Methyl-1-butanol	2.323	2.368	1.052	101.92	99.45
	11.615	11.469	0.176	98.75	
	23.230	22.694	0.845	97.69	

[a] $n = 3$.
[b] $n = 9$.

and LOQ values for all compounds. Standard solutions containing an internal standard were assayed in the range of 0.20–48.00 mg/100 mL. A linear fit was obtained from five different concentrations of standard solutions using three replicate injections. The regression line was calculated as:

$$y = ax + b$$

where x is the concentration (mg/100 mL) and y is the response (peak area expressed as mAU). The calibration curve was obtained by using the linear least-squares regression procedure. The coefficient of correlation (R^2) values were >0.999; thus, there was a linear relationship between the analyte concentration and the detector response. The LOD was measured as the lowest amount of analyte that may be detected to produce a response that is significantly different from that of a blank. The LOD and LOQ values were first calculated as those corresponding to S/N values of 3:1 and 10:1, and then as 3.3 /S and 10 /S, respectively, where is the SD of the intercept and S is the slope of the regression line. The calculated values of LOD and LOQ for each compound are reported in Table 2.

Table 4. Interday and intraday precision values for compounds in standard solutions

	Interday[a]		Intraday[b]	
Compound	Mean ± SD, mg/100 mL	RSD, %	Mean ± SD, mg/100 mL	RSD, %
Acetaldehyde	1.93 ± 0.03	1.39	1.95 ± 0.02	1.27
	10.14 ± 0.11	1.10	10.23 ± 0.08	0.81
	19.29 ± 0.28	1.46	19.36 ± 0.09	0.45
Methyl acetate	2.49 ± 0.06	2.46	2.30 ± 0.17	3.25
	12.86 ± 0.12	0.96	12.66 ± 0.19	1.53
	25.57 ± 0.28	1.09	25.20 ± 0.50	1.97
Ethyl acetate	2.69 ± 0.05	1.81	2.79 ± 0.09	3.12
	13.01 ± 0.14	1.09	12.96 ± 0.11	0.87
	26.32 ± 0.36	1.35	25.87 ± 0.58	2.24
Acetal	3.09 ± 0.02	0.71	3.13 ± 0.05	1.69
	10.57 ± 0.04	0.36	10.61 ± 0.04	0.36
	25.83 ± 0.45	1.75	25.46 ± 0.59	2.30
Methanol	2.40 ± 0.03	1.08	2.54 ± 0.11	4.51
	11.50 ± 0.06	0.55	11.68 ± 0.16	1.38
	22.69 ± 0.30	1.31	23.33 ± 0.56	2.40
2-Butanol	2.35 ± 0.03	1.26	2.40 ± 0.05	1.97
	11.66 ± 0.10	0.87	11.82 ± 0.13	1.13
	23.25 ± 0.28	1.20	23.61 ± 0.31	1.33
1-Propanol	2.32 ± 0.03	1.21	2.35 ± 0.06	2.75
	11.38 ± 0.04	0.34	11.60 ± 0.20	1.69
	22.55 ± 0.30	1.35	22.99 ± 0.39	1.71
2-Methyl-1-propanol	2.36 ± 0.01	0.51	2.41 ± 0.05	2.15
	11.73 ± 0.05	0.46	11.90 ± 0.16	1.35
	23.39 ± 0.24	1.02	23.76 ± 0.32	1.33
1-Butanol	2.38 ± 0.01	0.31	2.42 ± 0.04	1.51
	11.86 ± 0.05	0.43	12.03 ± 0.15	1.21
	23.62 ± 0.30	1.29	24.05 ± 0.38	1.57
2-Methyl-1-butanol	2.34 ± 0.02	1.07	2.35 ± 0.01	0.62
	11.69 ± 0.05	0.39	11.82 ± 0.11	0.97
	23.18 ± 0.22	0.95	23.56 ± 0.33	1.40
3-Methyl-1-butanol	2.37 ± 0.02	1.05	2.38 ± 0.01	0.43
	11.47 ± 0.02	0.18	11.63 ± 0.14	1.20
	22.69 ± 0.19	0.85	23.09 ± 0.35	1.52

[a] $n = 9$.
[b] $n = 27$.

Accuracy was calculated from triple injection of standard solutions containing known amounts of compounds at three levels. Table 3 shows the recovery values for all compounds. The range of the recovery values obtained was 97.5–101% with RSD values <2.5% for all analytes. The method showed excellent recoveries and acceptable variation.

Repeatability was determined subsequently from triple injections of the standard compound mixture at three levels within a single day. Intermediate precision was calculated from triple injections of the standard mixture at three concentration levels on three different days. Table 4 shows the precision data for all compounds determined by within- and between-day analysis. For all compounds, satisfactory RSD values (<2.46% for intraday and <4.51% for interday) were found. The RSD values vary with the concentration levels, ranging from 0.3 to 4.5% and indicate that the proposed method shows acceptable repeatability and intermediate precision.

Conclusions

The proposed method exhibits excellent precision and detection limits, as well as excellent recoveries (97.5–101.1%) for all volatile congeners present in Turkish raki. The robustness of the method and its selectivity allowed its application to the measurement of volatile compounds of Turkish raki. All of these results show that this method is suitable for routine determination of volatile congeners for the quality control of raki samples by both manufacturers and control laboratories.

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