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Simultaneous Determination of Different Anions in Milk Samples Using Ion Chromatography with Conductivity Detection

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Abstract: The description of a simple method for simultaneous determination of chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotic acid in milk samples was outlined. The method involves the use of dialysis cassettes for matrix elimination, followed by ion chromatography on a high capacity anion exchange column with suppressed conductivity detection. The novelty of dialysis process was that it did not need any chemical and organic solvent for elimination of macromolecules such as fat, carbohydrates and proteins from milk samples. External standard calibration curves for these analytes were linear with great correlation coefficients. The relative standard deviations of analyte concentrations were acceptable both inter-day and intra-day evaluations. Under optimized conditions, the limit of detection (Signal-to-Noise ratio = 3) for chloride, phosphate, thiocyanate, perchlorate, iodide, nitrate, sulfate, and orotate was found to be 0.012, 0.112, 0.140, 0.280, 0.312, 0.516, 0.520, and 0.840 mg L^{-1}, respectively. Significant results were obtained for various spiked milk samples with % recovery in the range of 93.88 - 109.75 %. The proposed method was successfully applied to milk samples collected from Istanbul markets. The advantages of the method described herein are reagent-free, simple, and reliable.

Keywords: Anions; dialysis; milk; ion chromatography; orotic acid.

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Table 1. List of notations/symbols and units.

Notations/Abbreviations		Units	
IC	Ion chromatography	mM	Millimolar
NIS	Sodium iodide symporter	L	Liter
N or n	Number of samples	mL	Milliliter
K_a	Dissociation constant of an acid	g	Gram
pK_a	Is the negative logarithm of K_a (-Log K_a)	mg	Milligram
LOD	Limit of detection	min	Minutes
LOQ	Limit of quantification	°C	Degrees celcius
SD	Standard deviation	h	Hours
RSD	Relative standard deviation	kDa	Kilodalton
t_R	Retention time	MWCO	Molecular weight cut-off

INTRODUCTION

Thyroid hormones are necessary for brain and neural development in fetuses and infants [1, 2]. In the cellular membrane of the cells from thyroid follicles, the protein responsible for iodide capture from the bloodstream was identified as sodium iodide symporter (NIS), which is present in the thyroid gland and also in the mammary glands. Iodide is an important trace element used by the thyroid gland to make thyroid functioning a human beings [3]. Deficiency or excess intake of iodine gives rise to disorders commonly referred to as iodine disorders. In the pregnant and lactating women, iodine deficiency leads to increased early and late loss of gestation, intellectual disability, cretinism and growth retardation [4, 5]. A number of inorganic anions can block iodide (I^-) uptake at the thyroidal sodium iodide symporter (NIS) in a competitive manner. Among these, nitrate (NO_3^-), thiocyanate (SCN^-), and perchlorate (ClO_4^-) are of particular dietary and/or environmental importance [6]. Sufficient inhibition of iodide uptake can lead to decreased thyroid hormone production and, ultimately, result in adverse health effects secondary to hypothyroxinemia. In particular, infants under six months of age are very susceptible to nitrate poisoning. The solubility of $NaClO_4$ in water is 2 kg L^{-1}, and the compound is almost inert in surface waters. For this reason, perchlorate pollution can persist for long periods. Perchlorate is found as a common contaminant of food, water, milk, human milk [7-11]. Chloride (Cl^-) is one of the typical anions which its determination in milk has a great interest. The chloride determination in milk is a useful parameter to understand the degree of subclinical mastitis that may occur in cattle [12]. It is also important to determine phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) concentrations in milk because they are naturally present in the crude matter and may also be introduced during the industrial manipulations [13]. Orotic acid (1,3,5-trihydro-2,6-dioxo-pyrimidine-4-carboxylic acid) is an intermediate product in pyrimidine biosynthesis. The molecular structure of orotic acid is

given in Figure 1. Recent experimental studies have demonstrated that it is involved in carcinogenesis, hepatic lipid storage and renal toxicity [14]. Several studies have shown that repeated dosing of orotic acid promotes the formation of tumors initiated by various known carcinogenic substances [15].

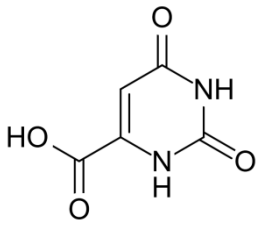

Figure 1. Molecular structure of orotic acid.

Sample preparation is an important part of the analysis of anions in a complex matrix especially in milk to manage a good analytical procedure. Because milk has high molecular mass compounds such as fats, proteins, and carbohydrates that can interfere with the analysis, some initial cleanup procedures are required before the analysis of anions. There are a number of matrix elimination methods, for example, dry ashing, wet digestion in closed system with microwave assistance are currently used [16, 17]. They involve generally laborious sample pre-treatments, too many steps, and time consuming nature, and the sample can lose its volatile species during the process. A micro dialysis sampling technique is gentle and non-degrading procedure because of the fact that it can be used in multi-component analysis. Dialysis process depends on molecular diffusion occurring as a result of the concentration gradient of analyte of interest between donor and acceptor solutions. Dialysis procedure is mostly used in biological fields. In a representive study, cytochrome c, insulin, lysozyme and ribonuclease A were separated from human serum albumin by using dialysis procedure [18]. In another study, the determination of inorganic anions in olive oil mill wastewater can be achieved by using dialysis procedure [19]. However, no previous research had focused on the analysis of anions and orotic acid in milk samples by using dialysis technique for sample preparation. This is the first paper on simultaneously determination of chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate and orotic acid in milk by using dialysis as a sample preparation technique.

A wide range of techniques have been employed for the determination of anions and organic acids in milk, including voltammetry, flow injection methods, X- ray fluorescence spectroscopy, and neutron activation analysis [20,21]. In a representative report, anions, including iodide, nitrate and nitrite in biological samples were analyzed by micellar electrokinetic capillary chromatography [22]. In another study, determination of iodine species in milk was done with using epithermal neutron activation analysis. In this method, pre-concentration of iodide anion was achieved by using polymer inclusion sorbent [4].

Another study showed that benzoic acid and sorbic acid in food products were analyzed by using electrokinetic flow injection analysis [23].

Ion chromatography has been used for determination of anions and organic acids with pK_a value below *ca*. 6, drawing analyst's attention to its capabilities of high sensitivity, rapidness, and the ease of operation, coupled with the advantage of simultaneous determinations [24]. However, up to now chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotic acid in milk are not analyzed using one method. In the present study, we demonstrated an ion chromatographic method for quantification of one organic acid and seven anions at the same time under a suppressed conductivity detector. The aim of this work is to present a simple and accurate procedure for milk pre-treatment. The sample's matrix elimination is done by using dialysis method with dialysis cassette. The proposed method of analysis has the following advantages: (a) addition of chemicals are not required; (b) proteins and lipids can be removed easily.

EXPERIMENTAL

1) Materials and Chemicals

Four milk samples from different brands were purchased from local markets in İstanbul. In addition, a raw milk sample was purchased from dairy farm. Additionally, a bottled daily milk sample was taken from a market. All solutions and reagents were prepared from highly pure analytical grade chemicals using ultra pure water. All chemicals used is in extra pure state (purity is up to > 99%). Sodium perchlorate monohydrate ($NaClO_4.H_2O$), potassium nitrate (KNO_3), potassium iodide (KI) and sodium chloride (NaCl) were obtained from Merck, Darmstadt, Germany. Sodium hydrogen phosphate dihydrate (dibasic) ($Na_2HPO_4.2H_2O$) and ammonium thiocyanate (NH_4SCN) were purchased from Riedel de Haën, Seelze, Germany. Anhydrous orotic acid ($C_5H_4O_4N_2$) and anhydrous sodium sulfate (Na_2SO_4) were provided by Sigma-Aldrich, Steinheim, Germany.

2) Instrumentation

Dionex ICS-3000 (Sunnyvale, CA, USA) ion chromatograph equipped with a suppressed conductivity detector (ASRS ULTRA II-2mm suppressor and conductivity cell) was used for analyses of anions in milk samples. Chromatographic separations were performed at 25 ºC with a Dionex IonPac® AS20 analytical column (2 x 250 mm) equipped with a Dionex IonPac® AG20 guard column (2 x 50 mm). Analytical column resin composition is super macroporous polyvinyl benzyl ammonium polymer cross-linked with divinylbenzene. Guard column's resin composition is microporous polyvinylbenzyl. Eluents' gradients were generated on-line from ultra pure water using the Dionex Eluent Generator, EGC II NaOH

cartridge and then polished from the contaminants using Continuously Regenerating Trap Columns CR-ATCs. The eluent concentrations was controlled by Dionex Chromeleon® Client (6.80) software. The instrument was also equipped with a Pump and attached to an AS autosampler. 10 µL sample loop was used in all analyses unless otherwise stated.

Ultrapure water of 18.2 MΩ cm resistivity or better was obtained from a New Human Power I Scholar UV system (Human Corporation, Seoul, Korea). All solutions were prepared from highly pure analytical reagent grade compounds by using ultra pure water.
3.0 – 12 mL capacity of Slide-A-Lyzer® Dialysis cassette allowing passage of molecules (≤ 2 kDa) was supplied by Thermo Scientific (Rockford, IL 61101, U.S.A.). This cassette (molecular weight cut-off: 2K) facilitate simple and effective removal of ions from macromolecules that are larger than 2000 daltons. Membrane structure the cassette is cellulose.

3) Preparation of Stock and Standard Solutions

Stock solutions of the anions ($Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, I^-, ClO_4^-, SCN^-$) (1000 mg L^{-1}) were prepared by dissolving 0.165 g of NaCl, 0.141 of $NaClO_4.H_2O$, 0.163 g of KNO_3, 0.131 g of KI, 0.187 g of $Na_2HPO_4.2H_2O$, 0.131 g of NH_4SCN and 0.148 g of Na_2SO_4 in different 100 mL volumetric flasks and diluting to the mark with water. A stock solution of 100 mg L^{-1} of orotic acid was prepared by dissolving an appropriate amount of orotic acid in a 500 mL volumetric flask and diluting to the mark with water. The aqueous standard stock solutions of anions was prepared to obtain the calibration curves and to spike the milk samples.

4) Optimization of Dialysis Time

In order to optimize the dialysis time for chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotate, a 5.0 mL mixed standard aqueous solution containing 1.0 mg L^{-1} of each anion was loaded into the dialysis cassette. Subsequently, the cassette was submerged and fitted vertically into a plastic beaker containing 400 mL of ultrapure water. A magnetic stirrer was also utilized to accelerate the dialysis. 0.5 mL portions have been taken from the solution diffused to water in the beaker at different times and were immediately injected into the IC system under optimum ion chromatographic conditions.

5) Dialysis of Milk Samples

In proposed method, the newest sample preparation method including dialysis technique was investigated for matrix elimination of milk samples. The dialysis cassette was utilized for removing macromolecules such as milk fat, carbohydrates, and proteins from milk sample, because the macromolecules can affect ion chromatographic analysis of anions.

Good recoveries of the anions were obtained by using flat cassette chamber with two membranes provides high surface-area to volume ratio. The cassette does not require knots or clips that may result in leaking and sample loss. Rectangular cassette design (see Figure 2) maximizes recovery of entire sample volume with any one of the four corner injection ports.

Dialysis procedure was as follows: a) A syringe was filled with milk sample, b) the syringe needle was inserted through the gasket via one of the corner ports, c) 5 mL of spiked and unspiked milk sample were injected into the cassette, d) the excess air was withdrawn and the syringe was removed, e) a float buoy was attached onto the cassette f) The sample was dialyzed to 400 mL ultra pure water in a plastic beaker in the presence of a magnetic stirrer, f) After a steady state time ~ 45 hours, which is described bottom paragraph, dialysis filtrate was analyzed by ion chromatography with conductivity detector using a computerized gradient elution program.

To clean the used cassette, an empty syringe was inserted to second corner port. Air was injected for expanding cassette chamber. Finally, the dialyzed sample was withdrawn.

Figure 2. Dialysis cassette; 2000 MWCO and 3 – 12 mL capacity.

6) Chromatographic conditions and Measurements

Many experiments targeting the optimal ion chromatographic separation for all anions in samples have been performed. The conditions of the suitable program is given in Table 2. Column temperature was 25 °C, cell temperature was 30 °C; current of the suppressor was 31 mA and injection volume was 10 µL.

Table 2. Optimum ion chromatographic conditions.

Time (min.)	Concentration (mM)
0-1	3 isocratic
1.1-13	3-20 gradient
13.1-20	20 isocratic
20.1-21	20-50 gradient
21.1-24	50 isocratic
24.1-25	50-3 gradient
25.1-30	3 isocratic

External calibration curves drawn by peak areas versus concentrations were used for the quantification of analytes. Calculations were done by utilizing correlation equations ($y = mX + n$). A chromatogram obtained for a standard solution containing chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate and orotic acid of a 3 mg L^{-1} for each is shown in Figure 3.

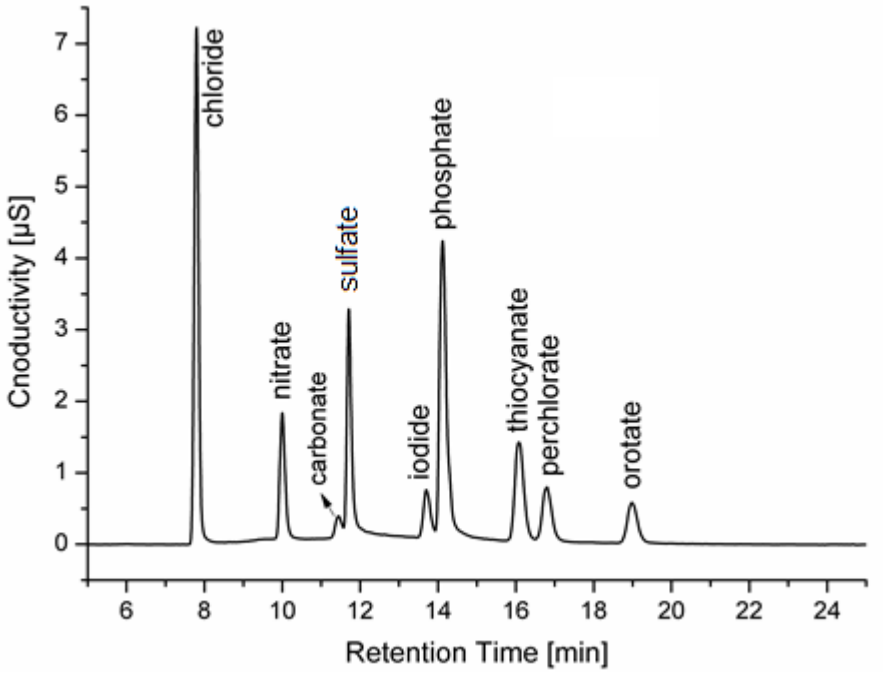

Figure 3. Chromatogram of a standard aqueous solution containing different anions.

7) Theoretical and statistical aspects

6.1) *LOD:* $\frac{signal}{noise} = 3$

LOQ: $\frac{signal}{noise} = 10$ [25]

6.2) *Standard Deviation:* $SD = \sqrt{\frac{(x-\bar{x})^2}{(n-1)}}$

(x: each measured value; $\bar{x}$: the mean value of population; n: number of measurement; $n-1$: degree of freedom)

Relative Standard Deviation: RSD $= \frac{SD}{\bar{x}}$

$RSD\ \% = \frac{SD}{\bar{x}} \times 100$

6.3) $Recovery\ \% = \frac{(\text{Found concentration} - \text{initial concentration})}{\text{added concentration}} \times 100$ [25]

6.4) *t value calculation:* $\text{tcal} = \frac{((\text{Recovery } \% - 100) \times \sqrt{n})}{SD}$ [26]

RESULTS AND DISCUSSION

Optimum dialysis time

After the filtrated 0.5 mL aqueous solutions had been collected at different times from plastic beakers during dialysis, these 0.5 mL portions were injected to the IC system and the recoveries were calculated. Figure 3 exhibits that all anions completely transferred into 400 mL of water after 45 h during dialysis process. It was observed that recovery % values ranged from 96.5 % to 106.7 which are acceptable recovery values [26-28]. Thus, all of the other studies were carried out according to 45 h dialysis time.

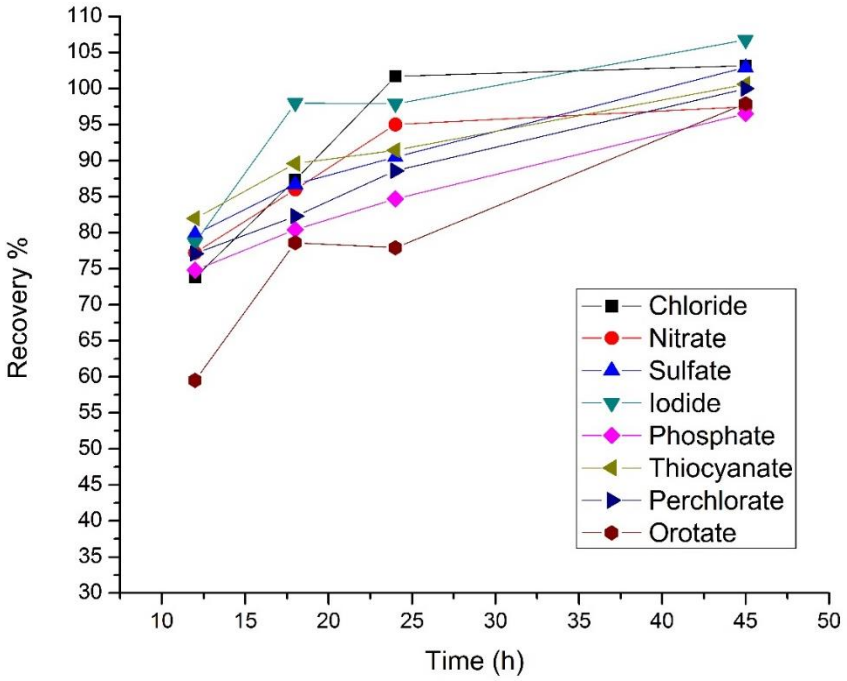

Figure 3. The plot of recovery % values for determination of optimum dialysis time for chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotate.

Validation of the method

To validate the proposed method, numerous analytical parameters such as linearity, limits of detection (LOD) and quantitation (LOQ), with-in run precision, intermediate precision, and accuracy have been evaluated. All of these parameters should be evaluated for validation of the method [25]. Microsoft Excel office software was used for all calculations.

Linearity, limit of detection and quantitation

External calibration method was used for determine the concentration of analytes in the samples. Linear concentration range, linear regression equations, correlation coefficients (r^2), LOD values, LOQ values and retention times are given in Table 3. Under the optimum ion chromatographic *(vice versa)* conditions, aqueous calibration standards have been injected five times into the IC system. The linear relationships could be established with the linear regression equations, $y = ax + b$, wherein y is the peak area (μS min^{-1}) and x is concentration (mg L^{-1}). The experimental coefficients of the linear regression indicated a first-order correlation in all cases. LOD and LOQ values have been calculated from the peak height as the average concentration corresponding to the signal-to-noise ratio equal to 3 and 10, respectively. LOD and LOQ values for samples were again corrected for real samples by multiplying with dilution factor.

Table 3. Linear concentration range, regression equation, retention time, r^2, LOD and LOQ values.

Analyte	Linear Range (mg L^{-1})	Regression Equation	t_R (min)	r^2	LOD* (mg L^{-1})	LOQ* (mg L^{-1})
Chloride	0.01 - 9.0	y = 0.2131x – 0.017	7.76	0.9989	0.012	0.040
Nitrate	0.05 – 9.0	y = 0.0791x – 0.0067	10.01	0.9985	0.516	1.720
Sulfate	0.05 – 9.0	y = 0.0989x – 0.009	11.71	0.9999	0.520	1.733
Iodide	0.03 – 9.0	y = 0.0437x + 0.003	13.76	0.9989	0.312	1.040
Phosphate	0.01 – 9.0	y = 0.154x – 0.0219	14.29	0.9968	0.112	0.373
Thiocyanate	0.01 – 9.0	y = 0.123x – 0.0058	16.13	0.9968	0.140	0.467
Perchlorate	0.03 – 9.0	y = 0.0716x – 0.0114	16.87	0.9975	0.280	0.933
Orotate	0.07 – 9.0	y = 0.0661x – 0.0026	19.08	0.9953	0.840	2.800

*****LOD and LOQ values were of the proposed method.

Precision

Repeatability is a criterion of precision. In addition, SD and RSD values are used for evaluation of repeatability. Therefore, a standard aqueous solution in which the concentration was 3.0 mg L^{-1} for each anion was injected five times to IC system and analyzed. High RSD values are acceptable for very low μg L-1 whereas accepted RSD values decrease as the concentrations increase. To illustrated, the acceptable RSD % is 5.3 for

the analyte concentration of 100 mg L-1 while the acceptable RSD % is 30 for 1 µg L-1 concentration [28]. It is obvious from Table 4 that the repeatability of the IC system is excellent owing to very low RSD values of both peak areas and retention times. Moreover, milk sample 2 treated with dialysis has been injected five times into the IC system at three different days under optimized conditions and intermediate precision was evaluated. RSD values pointed that intermediate precision of the method was very well (see Table 5).

Table 4. Repeatabilities of the analytical signal and retention time for the 3 mg L^{-1} chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotate (n = 5).

Analyte	Area of the signal (µS cm^{-1} min)	RSD (%)	t_R (min)	RSD (%)
Chloride	0.97	0.48	7.76	0.15
Nitrate	0.28	0.58	10.01	0.07
Sulfate	0.43	3.55	11.71	0.01
Iodide	0.14	1.19	13.76	0.10
Phosphate	0.91	0.98	14.29	0.12
Thiocyanate	0.37	0.75	16.13	0.06
Perchlorate	0.21	1.49	16.87	0.05
Orotate	0.17	0.69	19.08	0.09

Table 5. RSD (%) values of within run and intermediate precisions of milk sample 2 for chloride, nitrate, sulfate, iodide, phosphate, and orotate.

	Chloride	Nitrate	Sulfate	Iodide	Phosphate	Orotate
$RSD_{with\text{-}in\ run}$(%) (n=5)	0.80	3.96	4.29	2.53	0.67	4.28
$RSD_{intermediate}$(%) (n=3)	2.96	12.74	3.31	6.46	2.40	3.41

Accuracy

The recovery values were taken into consideration to evaluate the accuracy of the method. Three different concentration levels (see Table 6) were added to milk sample #1, as described below. The significance test (t-test) at 95 % confidence level was applied to recovery values. If the calculated t value is less than the tabulated value for 2 (n - 1) degree of freedom at 95 % significance level, the null hypothesis is accepted and the method is accurate [26]. We feel confident to note that the calculated t values were absolutely adequate for good accuracy as they were smaller than theoritical critical t value equal to 4.30 (see Table 5). At this point, we should emphasize that acceptable recovery percentage range is 70 – 120 % with a precision about 20 % in analytical studies [27,28].

Recovery studies were conducted by spiking a milk sample. Milk sample #1 was spiked with standard analyte solutions at three different concentrations. The added amounts for spike works are given in Table 6. Two mixed standard solutions were primarily prepared because the chloride and phosphate concentrations were very high comparing to the other anions. The first mixed solution contained chloride and phosphate whereas the other mixed solution included nitrate, sulfate, iodide, thiocyanate, perchlorate, and orotate anions. When dialysis time were finished, a 10-mL sample from 400 mL solution was filtered with PES (polyethersulfone) syringe filter and loaded into autosampler vials for IC-CD analysis. Another 10 mL from chloride and phosphate recovery assay was additionally diluted 10 times prior to injection since the concentrations of chloride and phosphate were extremely high (especially in spiked solutions). Increasing concentrations of chloride caused interference with nitrate. On the other hand, additional ten-fold dilution of chloride and phosphate were convenient in terms of performing the analysis within the linear ranges. The concentration values in Table 6 are given after corrected by multiplying dilution factors.

Analysis of milk samples

Figure 4 presents the chromatogram for the milk sample 3. The ion chromatographic results of the anions in milk samples are given in Table 7. The milk samples between number 1 – 4 in Table 7 were different brands purchased from local markets in İstanbul. Sample number 5 was a raw milk sample. The raw milk sample was also boiled and it was analyzed in same conditions as sample 6. It has been observed that the concentrations of anions in raw milk were dramatically changed after boiling. Concentrations of chloride, nitrate, sulfate and orotic acid was increased whereas phosphate was decreased and thiocyanate peak disappeared in boiled milk. Moreover, a bottled daily milk sample (number 7) was analyzed. Chloride, sulfate, phosphate, and orotate were observed in all of the samples. Chloride and phosphate concentrations were at the highest concentrations. Iodide was detected in two samples while thiocyanate were detected only in one sample. Perchlorate was not observed in the samples according to the present method. Nitrate was not detected in only one sample. Figure 5 also more apparently exhibits the concentration distribution of anions by samples. In brief, the found concentrations of anions in milk samples were also ordered as follows:

Sample1: $[PO_4^{3-}] > [Cl^-] >> [SO_4^{2-}] > [Orotate] > [NO_3^-] > (I^-,\ ClO_4^-, SCN^-\ Not\ detected)$

Sample2: $[Cl^-] > [PO_4^{3-}] >> [SO_4^{2-}] > [Orotate] > [NO_3^-] > (I^-, ClO_4^-, SCN^-\ Not\ detected)$

Sample3: $[Cl^-] > [PO_4^{3-}] >> [SO_4^{2-}] > [Orotate] > [NO_3^-] > [I^-] > (ClO_4^-, SCN^-\ Not\ detected)$

Sample4: $[Cl^-] > [PO_4^{3-}] >> [SO_4^{2-}] > [Orotate] > [NO_3^-] > [I^-] > (ClO_4^-, SCN^-\ Not\ detected)$

Sample5: $[PO_4^{3-}] > [Cl^-] >> [Orotate] > [SO_4^{2-}] > [NO_3^-] > [SCN^-] > (I^-, ClO_4^-\ Not\ detected)$

Sample6: $[Cl^-] > [PO_4^{3-}] >> [SO_4^{2-}] > [Orotate] > [NO_3^-] > (I^-, ClO_4^-, SCN^-\ Not\ detected)$

Sample1: $[PO_4^{3-}] > [Cl^-] >> [SO_4^{2-}] > [Orotate] > (NO_3^-,\ I^-, ClO_4^-, SCN^-\ Not\ detected)$

Table 6. Recovery (%) and calculated t values for chloride, phosphate, nitrate, sulfate, iodide, thiocyanate, perchlorate, and orotate in milk sample 1.

Anion	**Concentration (mg L^{-1})**			**Recovery (%)**	**SD**	**RSD (%)**	t_{cal}
	Initial± SD	**Added**	**Found**	**(n=3)**			
Chloride	913±10	296	1208	99.83	4.13	4.14	0.07
		631	1598	108.56	4.45	4.10	3.33
		1215	2246	109.75	3.98	3.63	4.24
Phosphate	1678±19	496	2182	101.55	3.93	3.87	0.68
		664	2301	93.88	2.88	3.07	3.68
		1420	3133	102.44	2.38	2.32	1.78
Nitrate	4.72±0.16	2.0	6.79	103.31	11.3	10.94	0.51
		4.0	9.00	106.88	4.19	3.92	2.84
		13.2	18.67	105.67	4.20	3.97	2.34
		26.8	31.26	99.04	2.58	2.60	0.64
		53.2	59.23	102.46	1.50	1.46	2.84
Sulfate	97.60± 0.40	30.4	127.92	99.75	6.85	6.87	0.06
		200.0	329.72	116.06	7.83	6.75	3.55
		400.0	530.00	108.10	3.32	3.07	4.23
Iodide	N.D.[a]	44.36	44.48	100.27	0.81	0.81	0.58
		55.16	55.68	100.94	1.31	1.30	1.24
		115.16	116.43	101.11	0.97	0.96	1.98
Thiocyanate	N.D.	37.40	37.96	101.50	0.85	0.84	3.06
		50.0	49.56	99.12	1.24	1.25	1.23
		122.0	120.72	98.95	0.66	0.67	2.76
Perchlorate	N.D.	38.24	39.20	102.51	1.26	1.23	3.45
		41.44	42.38	102.27	0.94	0.92	4.18
		122.0	116.32	95.34	2.14	2.24	3.77
OA	74.60 ± 0.60	19.40	96.55	113.14	7.14	6.31	3.19
		37.48	112.07	99.97	5.73	5.73	0.01
		81.60	161.89	106.63	3.74	3.51	3.07

[a] N.D. : Not Detected

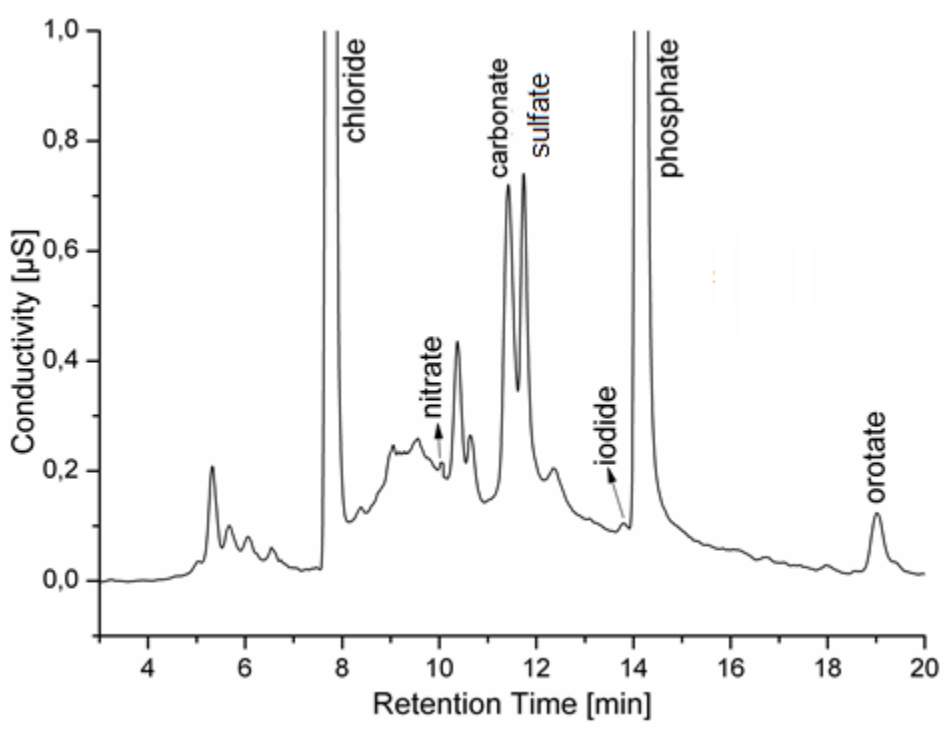

Figure 4. Chromatogram of milk sample 3.

Table 7. Concentration results of chloride, nitrate, sulphate, iodide, phosphate, thiocyanate, perchlorate, and orotate in real milk samples (n = 5).

Analyte anions		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6 (Boiled)	Sample 7
Cl^-	C (mg L^{-1})	913	999	896	939	1177	1474	1099
	RSD %	1.1	0.8	0.1	0.7	0.5	0.1	0.7
NO_3^-	C (mg L^{-1})	4.72	6.37	8.06	9.30	3.86	4.98	N.D.
	RSD %	3.4	12.4	1.9	2.2	2.9	2.4	-
SO_4^{2-}	C (mg L^{-1})	97.6	156.5	79.9	80.1	43.4	85.7	56.0
	RSD %	0.4	4.3	0.8	1.5	2.2	1.8	3.0
I^-	C (mg L^{-1})	N.D.	N.D.	7.87	7.45	N.D.	N.D.	N.D.
	RSD %	-	-	2.29	3.76	-	-	-
PO_4^{3-}	C (mg L^{-1})	1680	730	799	673	1278	839	1107
	RSD %	1.2	0.7	0.6	0.1	0.4	0.4	0.4
SCN^-	C (mg L^{-1})	N.D.	N.D.	N.D.	N.D.	2.8	N.D.	N.D.
	RSD %	-	-	-	-	0.7	-	-
ClO_4^-	C (mg L^{-1})	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	RSD %	-	-	-	-	-	-	-
orotate	C (mg L^{-1})	74.6	52.6	62.7	58.1	52.1	67.4	49.1
	RSD %	0.8	4.3	3.4	1.0	1.5	1.8	3.6

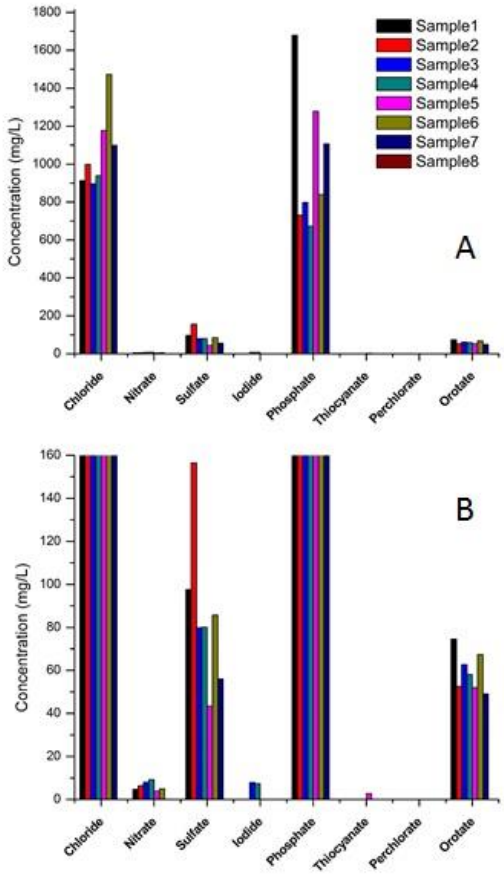

Figure 5. Distribition of results of anion concentrations in milk samples. A: Full scale distribition, B: zoomed image of A.

CONCLUSION

The most important advantage of this study is that this is the first study in the literature for the simultaneous determination of chloride, nitrate, sulfate, iodide, phosphate, thiocyanate, perchlorate, and orotate in milk by using dialysis technique for matrix elimination. This study may be a good alternative for studies related to needed difficult matrix elimination before analysis. The proposed method possesses many advantages which included easy preparation, the absence of chemicals, simple sample pretreatment procedure even if the dialysis time is long. The method was validated in terms of linearity, limits of detection and quantitation, intra-/inter-day accuracy, precision, and recovery. After hundreds of injections, no significant changes were observed on retention times. Very stable retention time values indicated that not only our method had good precision, but the life time of analytical column has also been prolonged as well. LOQ values were adequately small even though the analytes were diluted to 400 mL of water.

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Türkçe Öz ve Anahtar Kelimeler

Simultaneous Determination of Different Anions in Milk Samples Using Ion Chromatography with Conductivity Detection

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Öz: Bu çalışmada aynı anda klorür, nitrat, sülfat, iyodür, fosfat, tiyosiyanat, perklorat ve orotik asidin süt örneklerinde belirtilmesi incelenmiştir. Kullanılan yöntem matrisin ayrılması icin diyaliz kasetleri kullanmaktadır, ardından iyon kromatografisi ile bastırılmış iletkenlik ölçümü ile yüksek kapasiteli anyon değiştirme kolonu üzerinden ayırma sağlanmaktadır. Diyaliz prosesinin yeniliği yağ, karbohidrat ve protein gibi makromoleküllerin süt örneklerinden giderilmesi icin hiç bir kimyasal veya organik çözücü ihtiyacı olmamasıdır. Bu analitlerin dış standart kalibrasyon eğrileri büyük korelasyon katsayıları ile doğrusal olarak elde edilmiştir. Analit derişimlerinin relatif standart sapmaları gün arası ve gün içi değerlendirmelerde kabul edilir seviyede bulunmuştur. En uygun koşullar altında, belirtme sınırı (sinyal-gürültü oranı 3) klorür, fosfat, tiyosiyanat, perklorat, iyodür, nitrat, sülfat ve orotat icin sırası ile 0,012, 0,112, 0,140, 0,280, 0,312, 0,516, 0,520 ve 0,840 mg L^{-1} olarak tespit edilmiştir. Çeşitli katkılanmış süt örneklerinde % geri kazanım değerleri %93,88 - %109,75 olarak belirgindir. Önerilen yöntem İstanbul'dan satın alınan süt örneklerine başarı ile uygulanmıştır. Yöntemin avantajları reaktifsiz, basit ve güvenilir olmasıdır.

Anahtar kelimeler: Anyonlar; diyaliz; süt; iyon kromatografisi; orotik asit.

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