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Determination of Fluoride Ions in Urinary Stones by Ion Chromatography

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Abstract—A method of the ion chromatographic determination of fluoride ions in urinary stones has been developed. Sample preparation of solid mineral–organic samples includes dissolution in concentrated hydrochloric acid, dilution with deionized water, and the elimination of excess calcium and magnesium cations by adding a KU-2 sulfo cation exchanger in the H-form to samples and filtration through a membrane filter. Anions were separated on a Shim-pack IC-AIS anion-exchange column (100×4.6 mm) with elution with a mixture of 2.0 mM phthalic acid and 1.2 mM sodium hydroxide (pH 3.5). The linearity range of the fluoride ions in the obtained solutions of urinary stones with conductometric detection was 0.01–300 mg/L, the limit of detection calculated by the $3s$-test was 0.004 mg/L. The quantitative determination of fluoride ions in 20 samples of urinary stones was performed; in 80% of cases the presence of fluorides in the stones at a level from 0.01 to 4 mg/g of the stone was detected. The average concentration of the fluoride ions was 0.3 mg/g of stone. For 20% of the samples an elevated concentration of fluoride ions compared to the average one was found.

INTRODUCTION

Urinary stone disease (**USD**) is one of the most common human diseases [1] and, undoubtedly, one of the leading diseases among the urological disorders. The USD morbidity in different countries of the world varies from 1 to 40% [2], and 34.2% of the population in Russia suffer from this disease [3]. The study of the chemical composition of urinary stones (**USt**) is an important part of patient examination; it provides the necessary information about the type of metabolic disorder and the cause of stone formation, and can serve as a basis for the prescription of a necessary therapeutic diet. Approximately 80% of USt are formed by inorganic compounds of calcium (calcium oxalate, calcium phosphate, and hydroxyapatite) and only 10% are formed by magnesium compounds. Urate and cystine stones formed by uric acid and cystine are much less common.

Causes and processes of the formation of USt in kidney have not been completely understood; therefore, the prevention of USD based on testing various hypotheses and factors affecting stone formation is an important task of present-day urology. It is known that fluoride ions posses exceptive affinity to calcium ions, which gives a certain ground to consider hyperfluoro-

sis (chronic disease developing as a result of a long term excessive ingress of fluorine and its compounds into the body) to be one of possible causes of USD [4].

As a rule, the chemical analysis of USt is focused on the determination of the type or the major components of a stone with the aim to select a proper treatment and diet for a patient. The USt type can be relatively easily determined by IR spectrometry, naturally after the USt leaves the body [5, 6]. The structure and mineral and chemical composition of USt are determined by X-ray powder diffraction [7–9], polarization and scanning electron microscopy [10], and thermal analysis [11]. These methods usually the determination of only the main components and compounds in the USt.

Additional data on the concentrations of the microcomponents of stones, for example, of fluoride ions, can be obtained only after the decomposition and dissolution of the USt. Thus, capillary isotachophoresis was used [12] in one of a few works dealing with the determination of ions in dissolved USt samples. Unfortunately, the authors of the work did not succeed to determine fluoride ions. Presently fluoride ions in USt are determined by a spectrophometric method after the reaction with sodium 2-(*p*-sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonate [13] and by potenti-

ometric titration [14]. Sample preparation of USt in both cases takes more than 10 h, and the determination of the fluoride ions concentration in solution takes more than 2 h. The methods are labor- intensive, require the implementation of special reagents, and possess insufficient sensitivity. The limits of detection for fluoride ions in both methods are approximately 0.02 mg/L.

Ion chromatography (**IC**) in a single-column (without a suppressor column) [15–18] or two-column [19–21] versions is the most selective and precise method of the determination of fluoride ions in various objects. It should be noted that the data on the implementation of IC for the determination of the ionic composition and concentrations of individual ions in USt are lacking in publications. Apparently, this is associated with the complexity and labor-intensity of the preparation of corresponding samples.

The aim of this work was the development of a method of USt sample preparation and the selection of ion-chromatographic conditions for the subsequent quantitative determination of fluoride ions.

EXPERIMENTAL

Reagents. Working solutions of fluoride, chloride, phosphate, nitrate, and sulfate ions were prepared using standard samples (State Standard Samples GSO, Ural Plant of Chemical Reagents, Russia, ion concentrations 1 g/L) by dilution with deionized water (aquaMAX™-Ultra Younglin, Korea) immediately before the analysis. Working solutions of oxalate, acetate, and formate ions (1 g/L) were prepared by the dissolution of accurately weighed portions of oxalic acid, sodium acetate, and sodium formate (Sigma-Aldrich, United States) in deionized water. Sodium carbonate and sodium hydrogen carbonate, phthalic acid (Sigma-Aldrich, United States), and sodium hydroxide of cp grade were used to prepare eluents. Samples of urinary stones were prepared using hydrochloric acid of cp grade and a KU-2-8 sulfo cation exchanger in the acidic form. The cation exchanger was converted into the H-form by washing with 1 M HCl for 24 h until complete equilibration followed by washing with deionized water to neutral pH.

Equipment. Two liquid chromatographs were used in the work: LC-20 Prominence and PIA-1000 (both from Shimadzu, Japan). The LC-20 Prominence chromatographic system was equipped with conductometric and spectrophotometric detectors, an IC SI-90 4E separation column (250×4.0 mm) filled with an adsorbent of 9 μm particles size, a guard column IC SI-9μm suppressor column (200×6 mm) (Akvilon, Russia). The separation of anions was performed using an eluent containing 1.8 mM of sodium carbonate and 1.7 mM of sodium hydrogen carbonate at a flow rate of 1.0 mL /min and column temperature 33°C. The volume of the analyzed sample was 20 μL. System opera-

tion and the processing of chromatograms were performed using an LCsolution software.

A PIA-1000 portable one-column ion chromatographic system consisted of a conductometric detector, a Shim-pack IC-AIS separation column, and a Shim-pack IC-GAIS guard column (both from Shimadzu, Japan). In working with this chromatographic system, a mixture of 2.0 mM of phthalic acid and 1.2 mM of sodium hydroxide (pH 3.5) was used as an eluent; it was pumped through the column at a flow rate of 0.7 mL/min. The temperature of the column was maintained at 33°C, and sample injection volume was 10 μL. System operation and the processing of chromatograms were performed using an onboard software of the PIA-1000 chromatograph.

Sample preparation for ion-chromatographic analysis. The method of sample preparation included the dissolution of 5 mg of USt, preliminary dried and grounded in a porcelain mortar, in 20 mL of conc. HCl to which 5 mL of deionized water was added. Since 80% of the USt are inorganic compounds of calcium and magnesium, prior to the determination by IC tone should eliminate the excess of these metal cations from the solution of the analyzed sample to prevent the formation of precipitates of poorly soluble carbonates and, probably, phosphates in the chromatographic column in using carbonate–hydrogen carbonate and phthalate eluents, respectively. For elimination of metal cations 0.1 mL of a KU-2-8 ion-exchange resin in the H-form was added to the solution. The mixture was stirred on a water bath for 20 min, filtered through a membrane filter (ReZist Syringe Filter 13 mm, PTFE, Whatman, United States) with pore size of 0.2 μm to eliminate mechanical impurities. The obtained filtrate was transferred into a 25.0-mL volumetric flask and diluted to mark with deionized water.

RESULTS AND DISCUSSION

Samples. Twenty urinary stones from USD patients of the age 20 to 70 years living within the territory of Krasnoyarsk Krai, which were under the care in the Urology Department of the Krasnoyarsk Regional Clinical Hospital, were analyzed. The appearance of the stones varied. The color of the stones varied from white to dark brown; most of them were grey and brown with solid rough-spicular surfaces. The stones were 0.3 to 2 cm long.

Sample preparation. The complexity of sample preparation of mineral–organic samples for IC analysis consisted in the fact that the stones can be dissolved only using concentrated inorganic acids. As a result, a sample solution should contain high concentrations of strong acid anions interfering with the ion-chromatographic determination of other anions. Therefore, USt samples should be dissolved in a minimal quantity of acid calculated in accordance with the weighed stone portion. The time necessary for the quantitative transition of fluoride ions from a stone into a solution was
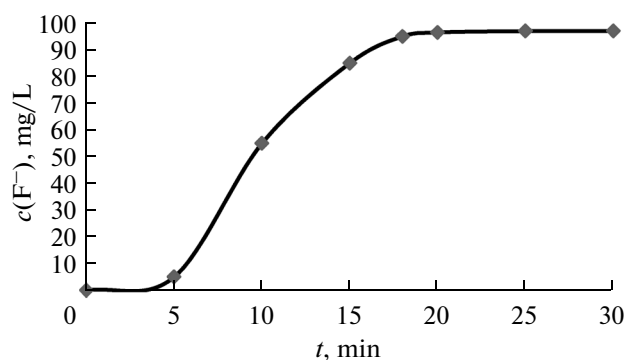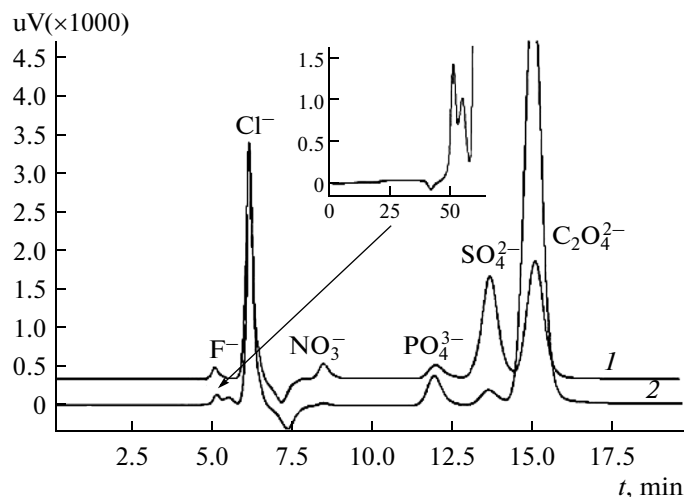
Fig. 1. Dependence of the concentration of fluoride ions in solution on the duration of dissolution of a weighed portion of calcium fluoride in HCl.

Fig. 2. Chromatograms of a model mixture of anions: (*1*), F^- (0.1 mg/L), Cl^- (4.0 mg/L), NO_3^- (3.0 mg/mL), PO_4^{3-} (1.5 mg/L), SO_4^{2-} (4.5 mg/L) and $C_2O_4^{2-}$ (8.0 mg/L); and (*2*), aqueous solution of a sample of urinary stone, on a column Shodex IC SI-90 4E using conductometric detection. The chromatographic conditions are described in the *Experimental* section.

evaluated using a model experiment on the kinetics of the dissolution of solid calcium fluoride. Figure 1 presents a graph of the dependence of the concentration of fluoride ions on the time of dissolution. It can be seen that the time necessary for the quantitative transition of the components into a solution was 20 min. In the dissolution of one of the analyzed USt it was also found that the complete dissolution of the sample fluorides took 20 min.

Previously it was noted that most of the USt consist of insoluble calcium and magnesium salts; the average concentration of calcium in USt is approximately 24.4 wt % [14]. If we assume that USt consists only of calcium oxalate monohydrate, then the solutions obtained after the dissolution of 5-g weighed portions of such USt in 25 mL of acid can contain approximately 48.8 mg/L of Ca^{2+}. For the elimination of excessive concentrations of Ca^{2+} and Mg^{2+} cations from the samples solutions, a KU-2-8 sulfo cation exchanger with an ion exchange capacity of 1.3–1.6 mEq/mL, approximately corresponding to 52–64 mg of Ca^{2+}/g, was added to prevent the formation of precipitates of calcium and magnesium carbonates and phthalates in the chromatography column. It is important that the addition of 0.1 mL of an ion-exchange resin at this stage provided sufficient sample purification and reliable performance of the ion chromatographic system when a phthalate eluent was used, because, after the retreatment of the precipitate remained on the filter with the resin, the fluoride ions were not detected in the solution.
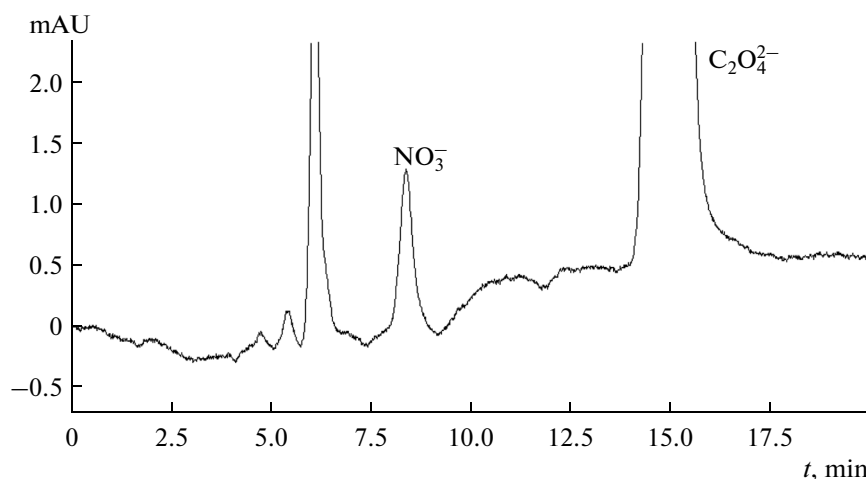
Ion-chromatographic determination. At the first stage the analysis of an USt sample solution was performed on a LC Prominence chromatograph under the conditions described in [22] for the separation and determination of oxalate ions. The components in the chromatogram were identified by retention times for each anion using a comparison of the chromatograms of the sample and the model solution. The separation of a model mixture of anions is presented in Fig. 2. It can be seen that several peaks with close retention

times possess asymmetrical shapes and are poorly resolved ($R_s < 1.0$) in the chromatogram in the region of the retention time of the fluoride ion.

Fluoride, chloride, nitrate, phosphate, sulfate, and oxalate ions were identified in the analyzed USt samples. Considering differences in the absorption spectra of the anions, one can obtain additional information about the composition of the sample using spectrophotometric detection. A chromatogram of an USt sample solution obtained under identical conditions but with spectrophotometric detection at 199 nm is presented in Fig. 3. Several peaks can be clearly seen in the chromatogram in the region of elution of poorly retained anions. Spectrophotometric detection at other wavelengths did not lead to the disappearance of the peaks; consequently, these peaks can be attributed to organic acid anions present in the matrix. It is known [15] that these anions can be acetate and formate ions, which are selectively determined using a system of one-column IC with a more selective anion-exchange column Shim-pack IC-A1S and a phthalate eluent. The obtained chromatogram of an USt sample solution is presented in Fig. 4. The given chromatographic column provides the baseline resolution of the chromatographic peaks of poorly retained fluoride, formate, and acetate ions. It can be seen that actually not only fluoride, but also formate ions are present in an aqueous solution of an USt sample; therefore, the selected conditions of chromatographic separation were used in the next experiments. The components were identified as it was described previously for a two-column chromatographic system.
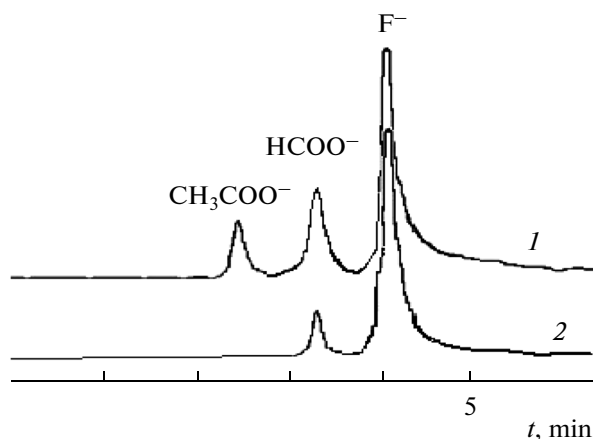
Fig. 3. Chromatogram of a urinary stone sample on a column Shodex IC SI-90 4E using spectrophometric detection at 199 nm. The chromatographic conditions are described in the *Experimental* section.

The results of the determination of fluoride ions in USt solutions are presented in Table 1. Fluoride ions were detected in 80% of the studied USt in the range from 0.01 to 4.0 mg/g of stone. The average concentration of fluoride ions was 0.3 mg/g of stone. In 20% of the samples an elevated concentration of fluoride ions was found at a level of 1.0 mg/g of stone. The accuracy of the determination of fluoride ions was tested using the standard addition method (Table 2).

Figure 5 presents a histogram of sample distribution by the concentration of fluoride ions in the USt. To build the histogram, 20 samples were divided into classes with concentration increments of 0.07 mg/g of stone. The samples were divided into 10 classes according to the fluoride ions concentration. The concentration of fluoride ions below the limit of detection was taken as zero. It follows from the histogram that a maximum is seen for the first class (with the concentration <0.7 mg/g of stone), and distribution near the zero value is close to the normal one (the concentration was always a positive value; therefore, we observed only the right side of the curve). Thus, we can conclude that the concentration of fluoride ions in USt is close to zero and that USt with elevated concentrations of fluorides should be separately classified as fluorine-containing.

The patients with elevated concentrations of fluorides in the stones were 37−39 years old, lived in Krasnoyarsk for 10 years, and led sedentary life. In the medical examination of urological disorders, pyelonephrities (inflammatory kidney disease) was found in all patients. The high concentration of fluoride ions in

Fig. 4. Chromatograms of a model mixture of anions: (*1*) CH_3COO^- (10.0 mg/L), $HCOO^-$ (10.0 mg/L), F^- (5.0 mg/L); and (*2*) solution of a sample of urinary stone on a column Shim-pack IC-A1S.
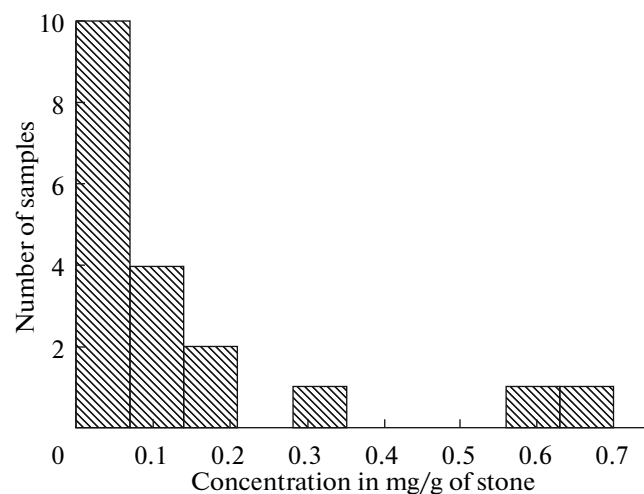
Fig. 5. Histogram of the distribution of the fluoride ions concentration in urinary stones ($n = 20$, $\Delta c = 0.07$ mg/g, division into 10 classes).

Table 1. Results of determination (mg/g of stone) of fluoride ions in urinary stones ($n = 5$, $P = 0.95$)

Sample	Found, mg/g of stone;	RSD, %	Sample	Found, mg/g of stone	RSD, %
1	0.34 ± 0.01	2	11	<0.004	
2	0.025 ± 0.002	1	12	0.16 ± 0.02	5
3	0.041 ± 0.003	2	13	0.111 ± 0.003	1
4	0.016 ± 0.002	4	14	0.122 ± 0.002	2
5	<0.004		15	0.042 ± 0.003	4
6	0.644 ± 0.007	4	16	0.603 ± 0.002	5
7	<0.004		17	0.0844 ± 0.0008	3
8	0.033 ± 0.001	3	18	0.15 ± 0.02	1
9	<0.004		19	0.045 ± 0.004	1
10	0.117 ± 0.003	0.3	20	4.0 ± 0.3	4

USt can be associated with the quality of the used drinking water. In [13] a correlation between the concentration of fluoride ions in drinking water and in USt was presented. Use of dentures and highly fluorinated tooth pastes also can affect the concentration of fluoride in the body.

Thus, the possibility of the highly sensitive quantitative determination of fluorides in urinary stones was demonstrated for the first time. In 1998 up to 80% of medical laboratories all over the world could not perform chemical analysis of USt of the mass less than 10 mg [23]; therefore, an important advantage of the method we developed is the possibility to using small USt samples with the mass less than 5 mg even for the determination of trace amounts of fluorides. The results of this work demonstrated that the USt classification must be updated by adding the concentration of fluorides as a new characteristic and that the composition of samples with the maximum concentration of fluorides must be studied additionally.

Table 2. Results of tests of the accuracy of the determination of fluoride ions in USt sample using the standard addition method ($n = 5$, $P = 0.95$)

Added F⁻, mg/L	Found F⁻, mg/L		Δ, %
	total concentration	standard addition	
0	3.57 ± 0.05		
2.5	6.11 ± 0.09	2.54	1.6
5.0	8.6 ± 0.1	5.05	1.0
10.0	13.5 ± 0.2	9.92	0.8

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