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# DETERMINATION OF OXALATE AND SOME INORGANIC ANIONS IN GREEN AND BLACK TEA

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*Oxalate concentration differs in various daily consumed food products. The role of oxalic acid in the human body is very significant, as its compounds are responsible for the stability of biological membranes. However, insoluble calcium and magnesium oxalates can be accumulated in the body in the form of kidney stones. Oxalate concentration has been measured by high performance liquid, gas after derivatization and ion chromatography (IC). The most effective method for the simultaneous determination of oxalate and inorganic anions is ion chromatography with conductometric detection. Here, we report the results of the measurement of oxalic acid in bleak and green tea samples. Separation was performed by IC on an anion-exchange column Shodex IC SI-90 with surface-layer sorbent and conductimetric detection. The main analytical features of the method were: limit of detection of oxalic acid 0.03 mg/l, linear range 0.1–20 mg/l, correlation 0.9998, relative standard deviation 1%. The method did not need specific sample treatment and was successfully applied to the analysis of black and green tea samples. Oxalic acid was determined in the ranges 16.7–84 mg/l for green tea and 63–116 mg/l for black tea. Green tea contained lower oxalate ions concentration than black tea. The IC method has a lower detection limit for oxalate ions than HPLS and GC, ten and two times less, respectively.*

**Key words:** *tea, oxalic acid, ion chromatography, kidney stones.*

## INTRODUCTION

Oxalate can be found in food products such as sorrel, chocolate, cacao and tea (Schroder *et al*., 2011). After water, tea is the second most popular beverage, but it contains high levels of oxalic acid (Sang *et al*., 2011). In the human body, oxalic acid is formed by the metabolism of glycine and ascorbic acid (Rassam *et al*., 2005). The role of oxalic acid in the human body is very significant, because its compounds are responsible for the stability of biological membranes (Melnik, 1999). However, insoluble calcium and magnesium oxalates may be accumulated in the body in the form of kidney stones. Therefore, it is necessary to control the concentration of oxalic acid in food and the human body (Hodgkinson *et al*., 1968). Nowadays, oxalate ions can be identified in complex objects using titration, colorimetry, capillary electrophoresis (CE) (Fu *et al*., 1999), chemiluminescence (Rubinsteln, 1983), high performance liquid chromatography (HPLC), gas (GC) (Kawamura *et al*., 2010) and ion chromatography (IC) (Lachenmeir *et al*., 2005). However, these methods have disadvantages. CE has low reproducibility due to migration times of ions. GC involves difficult sample preparation. Since oxalic acid is a strong organic acid ($pK_{a_1}$ = 1.27; $pK_{a_2}$ = 3.80), the most effective method for the determination of oxalate ions is IC with conductometric detection (Geng *et al*., 2008). In this work, an approach for the simultaneous determination of oxalate, chloride, nitrate, phosphate, sulphate ions is proposed and applied to the analysis of these compounds in tea samples of different brands and manufacturers. We aimed to identify the optimal conditions for determination of oxalate ions in drinks, to determine interference of inorganic anions, describe the comparative characteristics of the method and to apply the method for determination of oxalate ions in tea samples.

## MATERIALS AND METHODS

The chromatographic system HPLC LC-20 Prominence (Shimadzu, Japan) and conductivity detector were used. An anion-exchange column Shodex IC SI-90, 250 mm × 4 mm, 9 μm of particle size and column was filled with KanK-ASt, 120 mm × 5 mm, 14 μm of particle size. The column oven was maintained at 33 $^{o}$C. Loop volume was 20 μl.

An isocratic elution was performed, using a mobile phase of 1.9 $Na_2CO_3$+2.4 mM $NaHCO_3$ at a flow rate of 1.5 ml/min,

and a column was filled with KanK-ASt and 1.8 mM $Na_2CO_3$+1.7 mM $NaHCO_3$ at a flow rate of 1.0 ml/min for column Shodex.

Oxalic acid dihydrate, sodium bicarbonate, sodium digidrokarbonat were HPLC grade. Sodium chloride, sodium nitrate, sodium phosphate, sodium fluoride were analytical grade. Water was purified with an aquaMAXTM Ultra 370 Series – Ultra Water Purification System (Young Lin Instrument Co, Korea). Standard solutions were prepared and diluted in deionised water. Oxalic acid solution was daily diluted.

Samples of black and green tea were purchased from Krasnoyarsk outlets, produced by Russia, Ceylon, China. Assay solutions were obtained by refluxing 2.0 g of tea with hot water (100 $^{o}$C) for 6 min. The obtained extract was filtered through a disposable 0.45 μm filter, diluted 10 times and injected into the chromatographic system.

## RESULTS

In preliminary experiments, the conditions for the separation of the most common inorganic anions (fluoride, chloride, phosphate, nitrate, sulfate) in biological and food products were optimised. It was found out that the best separation conditions for the chromatographic system using a column filled with KanK-ASt was obtained with eluent consisting of 1.9 mM $Na_2CO_3$ +2.4 mM $NaHCO_3$ at a flow rate of elution of 1.5 ml/min. The retention time of oxalate ions in these conditions was $t_R$ = 13.73 ± 0.01 min. Previous reports (Rassam *et al*., 2005) and the experimental data suggested interference from sulfate ions on oxalate ion determination. A chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions is shown in Figure 1. Resolution of the peaks was $R_s$ = 1.16.

In these conditions quantitative identification of oxalate is unreasonable. Therefore, the effects of different factors on the degree of separation of the peaks were studied. The influence of various factors (flow rate and composition of the eluent) on the degree of separation of the peaks were studied by a full factorial experimental design. The data showed that a column was filled with KanK-ASt gave the best results, but was not sufficient to separate of sulfate and oxalate ions. Sorbent KanK-ASt is a bipolar central-localised sorbent, in which the act of sorption and desorption runs inside the grain. The diffusion inside the grain when anions are strongly retained anions, multiply charged and when hydrated ions have large size, is very difficult (Dolgonosov, 1984; Dolgonosov *et al*., 1988). This can explain the observed eroding peaks. Sulfate and oxalate ions have similar constants of ion exchange and retention times, and probably for this reason a satisfactory separation of sulfate and oxalate ions in this system is impossible.

The anion-exchange column Shodex IC SI-90 with surface-layer sorbent was also applied for the separation of sulfate and oxalate ions. Under the selection experimental conditions, the retention time for the oxalic ions was 15.83 ± 0.01 min. Figure 2 shows a chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. The selectivity of the column Shodex IC SI-90 is correct, as because the peaks showed resolution 1.5 for all of the determined compounds. In further work, we used a column Shodex IC SI-90.

Table 1 gives the resolutions of sulphate and oxalate ions at different concentrations. The column Shodex allowed to separate the peaks at all sulphate:oxalate ratios used.

The accuracy of the method was evaluated from recovery assays, preparing spiked samples of tea in triplicate at three levels of concentrations higher than the limit of quantitation (LOQ). The obtained values ranged between 98% and 102% (Table 2). The linearity of the analytical response, calibration curve, limit of detection (LOD) and LOQ were examined (Table 3).

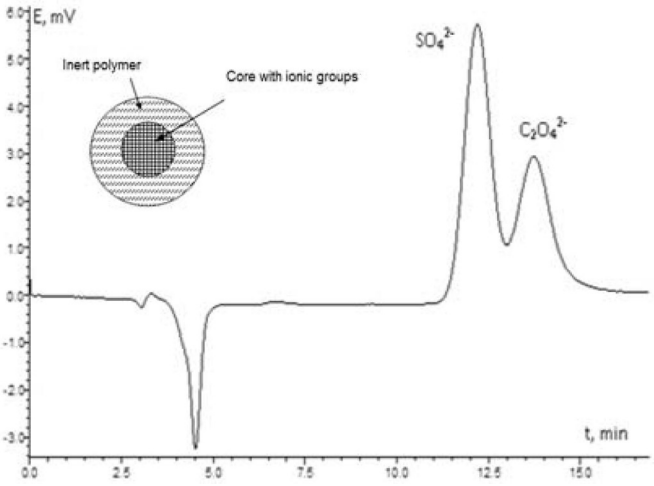


*Fig. 1.* Chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. Conditions: column was filled with KanK-ASt bipolar central-localised sorbent; eluent 1.9 mM $Na_2CO_3$ + 2.4 mM $NaHCO_3$; flow rate 1.5 ml/min.

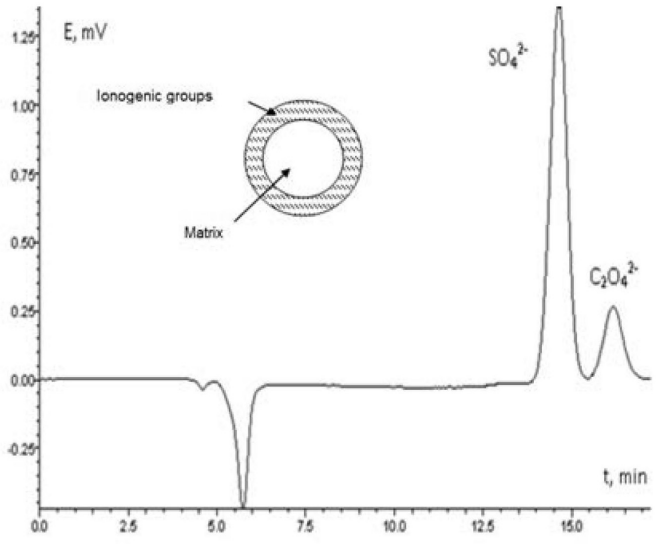


*Fig. 2.* Chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. Conditions: column Shodex IC SI-90 with surface-layer sorbent; eluent 1.8 mM $Na_2CO_3$ + 1.7 mM $NaHCO_3$; flow rate 1.0 ml/min; $R_s$ = 1.64.

Table 1

RESOLUTIONS OF SULPHATE AND OXALATE IONS AT DIFFERENT RATIOS OF CONCENTRATIONS

| Relation of concentrations $SO_4^{2-}/C_2O_4^{2-}$ | Resolution, $R_s$ | Concentration ratio $SO_4^{2-}/C_2O_4^{2-}$ | Resolution, $R_s$ |
|---|---|---|---|
| 1 | 1.64 | 20 | 1.58 |
| 2 | 1.61 | 30 | 1.13 |
| 5 | 1.59 | 50 | 1.01 |

Table 2

RESULTS OF RECOVERY OF OXALIC ACID IN TEA SAMPLES

| Added, mg/l | Found, mg/l | Recovery, % |
|---|---|---|
| 5.0 | 4.9 | 98.0 |
| 10.0 | 10.2 | 102.0 |
| 20.0 | 19.7 | 98.5 |

Table 3

RESULTS OF SENSITIVITY AND LINEAR RANGE OF OXALATE ANIONS

| Migration time, min | Linearity range, mg/l | Calibration curve | r | LOD, mg/l | LOQ, mg/l |
|---|---|---|---|---|---|
| 15.83 ± 0.01 | 0.1–20.0 | C = 0.0002·S + 0.04 | 0.9998 | 0.03 | 0.1 |

To check the precision of the method, seven replicate analysis of a standard solution were performed on different days. The precision expressed as R.S.D. remained 1%.

Chloride, nitrate, phosphate, sulphate and oxalate ions were determined in tea samples using the method proposed. The presence of these compounds was confirmed by comparing their retention times with those of standard solutions. The results are summarised in Table 4 and the chromatograms of the green and black tea samples are shown in Figure 3.

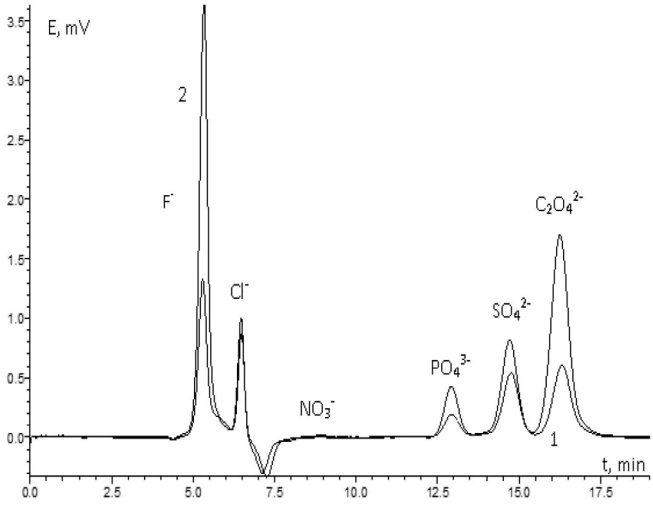


*Fig. 3.* Chromatograms of green (1) and black (2) tea samples brand "Tess". Conditions: column Shodex IC SI-90; eluent 1.8 mM $Na_2CO_3$ + 1.7 mM $NaHCO_3$; flow rate 1.0 ml/min.

## DISCUSSION

As can be observed in chromatograms of samples the fluoride ion peak has an asymmetric shape, indicating the presence of several anions having similar retention times with fluorine; therefore, the separation between these peaks could not be obtained. According to the literature it is

Table 4

CONTENTS OF ANIONS IN TEA SAMPLES

| Sample | Class tea | Concentration of ions (average ± standard error)*, mg/l | | | | |
|---|---|---|---|---|---|---|
| | | $Cl^-$ | $NO_3^-$ | $PO_4^{3-}$ | $SO_4^{2-}$ | $C_2O_4^{2-}$ |
| Rowford | Black | 45 ± 2 | 1,48 ± 0,04 | 38 ± 2 | 42 ± 2 | 89 ± 4 |
| | Green | 28 ± 1 | 0,95 ± 0,03 | 25 ± 1 | 46 ± 2 | 53 ± 2 |
| Riston | Black | 31 ± 1 | Nd | 42 ± 2 | 42 ± 2 | 99 ± 5 |
| | Green | 20 ± 1 | 1,11 ± 0,03 | 35 ± 1 | 39 ± 2 | 84 ± 4 |
| Ahmad | Black | 23 ± 1 | Nd | 24 ± 1 | 21 ± 1 | 63 ± 3 |
| | Green | 14,5 ± 0,7 | 0,24 ± 0,01 | 6,7 ± 0,3 | 22 ± 1 | 34 ± 2 |
| Princess Nuri | Black | 30 ± 1 | 0,85 ± 0,02 | 45 ± 2 | 46 ± 2 | 98 ± 5 |
| Princess Yava | Green | 28 ± 1 | 1,37 ± 0,04 | 28 ± 1 | 60 ± 3 | 66 ± 3 |
| Tess | Black | 35 ± 1 | 0,34 ± 0,01 | 41 ± 2 | 43 ± 2 | 113 ± 5 |
| | Green | 26 ± 1 | 0,44 ± 0,01 | 18,9 ± 0,9 | 44 ± 2 | 43 ± 2 |
| Indu | Black | 29 ± 1 | 0,35 ± 0,01 | 38 ± 2 | 48 ± 2 | 114 ± 5 |
| | Green | 21 ± 1 | 0,72 ± 0,02 | 14,8 ± 0,7 | 37 ± 2 | 50 ± 2 |
| Curtis | Black | 26 ± 1 | Nd | 50 ± 2 | 55 ± 2 | 116 ± 5 |
| | Green | 15,6 ± 0,8 | 0,56 ± 0,02 | 17,9 ± 0,9 | 469 ± 23 | 16,7 ± 0,8 |
| Da Hong Pao | Black | 35 ± 1 | 0,51 ± 0,02 | 25 ± 1 | 37 ± 2 | 64 ± 3 |
| Tie Guan Yin | Green | 16,5 ± 0,8 | 0,25 ± 0,01 | 9,5 ± 0,5 | 28 ± 1 | 21 ± 1 |

*Average of five analysis

Nd, not detected

known that these ions can be acetate and formate. Development of a method for quantification of fluoride ions requires further research. The presence of nitrate, phosphate, sulfate ions in tea samples can be explained by the use of various fertilisers and pesticides in cultivation. The most common class of pesticides is organochlorine and organophosphorus compounds. They easily dissolve in rain water and enter the soil. They are the most dangerous substances, causing of cancers, allergies and other diseases (Flaten, 2002; Tatarchenko *et al*., 2003).

Oxalic acid was present in the ranges 16.7–84 mg/l for green tea and 63–116 mg/l for black tea. Green tea contained lower oxalate ions concentration than black tea. It may be because green tea lacks oxidative processes. The lowest amount of oxalate ions was found in black tea brand Ahmad tea (black sheet tea "English breakfast", Ceylon) (63±3 mg/l) and green tea brand Curtis "Original green tea" (Chinese green long leaf tea, Russia) (16.7 ± 0.8 mg/l). A high concentration of phosphate ions in all samples was indicated.

Finally, the approach developed was compared with other existing methods (Table 5). Table 5 shows that the IC method has a lower detection limit for oxalate ions than HPLS and GC, ten and two times less, respectively.

Table 5

COMPARISON OF METHODS FOR DETERMINATION OF OXALIC ACID

| Method of determination | Detection time, min | Linearity range, mg/l | LOD, mg/l | R.S.D., % | References |
|---|---|---|---|---|---|
| HPLC | 3 | - | 0.3 | - | (Judprasong, 2006) |
| CE | 6 | 0.05–90.00 | 0.01 | 4 | (Fu, 1999) |
| GC | 15 | 0.2–5.0 | 0.06 | 1.3 | (March, 2003) |
| IC | 20 | 0.1–20.0 | 0.03 | 1 | Approach developed |

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## OKSALĀTU UN DAŽU NEORGANISKO ANJONU NOTEIKŠANA ZAĻAJĀ UN MELNAJĀ TĒJĀ


Skābeņskābei ir nozīmīga loma cilvēka organismā, jo tās atvasinājumi ietekmē bioloģisko membrānu stabilitāti. Tomēr nešķīstoši kalcija un magnija oksalāti var uzkrāties organismā nierakmeņu formā. Oksalātu koncentrācijas mērījumiem izmantoja augstefektīvo šķidruma hromatogrāfiju, gāzhromatogrāfiju un jonu hromatogrāfiju. Jonu hromatogrāfija ar konduktometrisku detektoru bija visefektīvākā metode oksalātu un neorganisko anjonu noteikšanai. Pētījums veltīts skābeņskābes mērījumiem melnās un zaļās tējas paraugos. Sadalīšanai izmantoja jonu hromatogrāfu ar anjonu apmaiņas kolonnu *Shodex IC SI*-90 ar virsmas slāņa sorbentu un konduktometrisko detektoru. Skābeņskābes noteikšanas robeža bija pie 0,03 mg/l, lineārais apgabals no 0,1–20 g/l, korelācija 0,9998, relatīvā standartnovirze 1%. Metodei nebija vajadzīga specifiska paraugu apstrāde, un to labi varēja izmantot melnās un zaļās tējas paraugu analīzei.