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Determination of chloride and sulfate in bio-ethanol by ion chromatography

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Bio-ethanol is produced from biomass or biodegradable waste and is used as a blending component in commercial gasoline fuel. Bio-ethanol can be contaminated with chloride and sulfate ions which can cause various unwanted side effects. European norm, EN 15376 defines the maximum permitted level of ions present and by that maximum chloride and sulfate content should not exceed 6.0 $mg \cdot kg^{-1}$and 4.0 $mg \cdot kg^{-1}$, respectively. The optimization and validation of ion chromatography method for the quantitative determination of chloride and sulfate ions in bio-ethanol by direct injection ion chromatography using anion-exchange stationary phase, carbonate/hydrogen carbonate mobile phase and conductivity detection has been described. The method is optimized regarding mobile phase composition and flow rate as well as the length of the chromatographic column. Validation of the method has been performed in order to confirm its applicability. The developed method is applied for the determination of chloride and sulfate ions in real bio-ethanol samples used for inter-laboratory comparative testing. The obtained results are compared with the official tests results and *z*-score has been determined. For chloride determination higher *z*-scores is obtained (from 1.4 to >3) than for the determination of sulfate (from -0.9 to 0.1).

Keywords: Bio-ethanol, Chloride, Ion chromatography, Sulfate

Legislation and environmental awareness introduces an alternative to the increasing use of bio-fuels. Therefore, replacement of regular fuels by bio-ethanol blended fuels is endorsed and favored worldwide. Ethanol is one of the main components for future reformulated fuels and offers great advantages due to its physical–chemical characteristic, raw materials availability, low production costs and beneficial environmental effects[1].

From the environmental standpoint bio-ethanol as a fuel have considerable advantages. Many advantages are achieved by replacing the usual additives with ethanol. In the reformulated gasoline, bio-ethanol replaces additives which increase octane number, such as tetraethyl lead, benzene or methyl tetra-butyl ether (MTBE) and all of them are strong pollutants.

The problem of using ethanol as a component of gasoline is due to their incompatibility with the materials that are used in the engines. Bio-ethanol added to bio-fuels may contain chloride and sulfate ions that can cause corrosion and deposit formation[2, 3]. There are numerous data on the influence of chloride ions on the formation of pitting and stress corrosion[4, 5, 6]. The sulfates can react with sodium in the ethanol distribution system to form insoluble sulfate salts and deposits.

For the above reasons, the European standard EN 15376[7] determined the maximum allowed amount of chloride (6 $mg \cdot kg^{-1}$) and sulfate (4 $mg \cdot kg^{-1}$). If the amount of chloride and sulfate in bio-ethanol exceed a defined maximum allowed amount then it is unacceptable for blending of bio-fuels. A variety of methods have been employed for the analysis of inorganic ions: wet chemical methods such as colorimetry, gravimetry and titrimetry, electrochemical techniques such as use of an ion-selective electrode and amperometric titrations. Method for determination of chloride and sulfate in the bio-ethanol by X-ray fluorescence after a precipitation procedure was also described[8]. Many of these methods suffer from interferences, limited sensitivity and time consumption; they are often difficult to automate. Some of the methods are based on previous reactions of analytes with reagents, sample burning, etc. For multiple ions determination use of several different methods is required. For such determination ion chromatography (IC) is favorable by European norms EN 15376 and EN 15492[9] and defined as the standard method for the determination of chloride and sulfate ions in bio-ethanol.

In this paper the IC method optimization and validation for chloride and sulfate content determination in bio-ethanol samples by anion exchange stationary phase, carbonate/bicarbonate puffer mobile phase and conductivity detection is presented. The possibility of analysis of bio-ethanol by direct injection of the sample onto the chromatographic column without tedious sample preparation step was investigated. EN 15492 describes a procedure for determination of ions in bio-ethanol indirectly with sample preparation procedure that involves the evaporation of bio-ethanol on a water bath, drying, and then dissolving in water. All these steps are possible sources of measurement error. The main advantage of the developed method is that it allows bio-ethanol analysis without previous sample preparation step thus significantly improving sample throughput and reduce sources of possible errors. Since the chloride content in bio-ethanol is usually less then levels set up in EN 15492 (from 4 $mg \cdot L^{-1}$ to 30 $mg \cdot L^{-1}$), the aim of our work was to examine the applicability of the proposed method with respect to the precision of EN 15492 method in the case when the chloride content is less than 4 $mg \cdot L^{-1}$.

The method was optimized regarding the mobile phase composition, flow rate and the length of the chromatographic column. After optimization of the method, validation was performed to verify the proposed method for a specific purpose.

Experimental Section

Materials

As mobile phase in the system, mixture of sodium carbonate, Na_2CO_3 (Kemika, Zagreb, Croatia) and sodium hydrogencarbonate, $NaHCO_3$ (Merck, Darmstadt, Germany) of chromatographic purity in deionised water (18 MΩ) obtained from TKA Pacific/Gen Pure water purification system (TKA Water Purification Systems GmbH, Germany) has been used. For chemical suppressor regeneration sulfuric acid (H_2SO_4), p.a. (Kemika, Zagreb, Croatia) has been used. Organic modifier acetone (CH_3COCH_3) (Gram-mol d.o.o., Zagreb, Croatia) was added for mobile phase optimization. For calibration purpose commercial chloride and sulfate standard solutions were used with concentration of 30 $mg \cdot L^{-1}$ and 150 $mg \cdot L^{-1}$ (Dionex, USA), respectively. Standard solution except chlorides and sulfates also contains fluoride, nitrite, bromide, nitrate and phosphate ions. Standard stock solutions were diluted to appropriate concentrations with deionized water and calibration curves were constructed.

IC procedure

Ion chromatography system (Metrohm IC 761, Switzerland) used for analysis is a complex modular system consisting of the injection system (766 IC Sample Processor, Metrohm, Switzerland), high pressure pump with flow rate ranged from 0.2 $mL \cdot min^{-1}$ to 2.5 $mL \cdot min^{-1}$ and maximum pressure of 15 MPa, peristaltic pump used for suppressor regeneration, tanks with solvents ($Na_2CO_3/NaHCO_3$ mobile phase, H_2SO_4 for suppressor regeneration, deionized water for system purification), conductivity detector, chemical suppressor (100 $mmol \cdot L^{-1}$ H_2SO_4 at flow of 0.6 $mL \cdot min^{-1}$ and deionized water at same flow rate were used for suppressor regeneration) and chromatographic columns Metrosep A Supp 5/150 (150 × 4.0 mm I.D.) and Metrosep A Supp 5/250 (250 × 4.0 mm I.D.). Both columns have same carrier material polyvinyl alcohol with quaternary ammonium groups and particle size of 5 mm. In a system for ion chromatography there is also suppressor module included when the conductivity detectors are used and the mobile phase is intensively conducting, saturating the detector's response. Injection volume was 20 µL.

Chromatographic system is completely controlled by the Methrom IC Net 2.1 programme. The same computer program collects and processes analytical data.

Instrument has been prepared by including all individual modules of the instrument and computer software to the computer. If necessary, the solvent tank was filled with fresh mobile phase and purge for 5 minutes. Run flow rate was set at 0.5 $mL \cdot min^{-1}$ and then after 20 minutes gradually increasing to the working flow rate. After stabilization of the pump pressure and conductivity detector, the instrument is ready for the operation.

Samples

In this investigation bio-ethanol samples from inter-laboratory comparative testing were analyzed as unknown samples. Before analysis, the samples of bio-ethanol were filtered through a regenerated cellulose membrane filter with pore size of 0.45 µm, to remove undissolved particles from the sample. Volume of injected sample was 20 µL.

Measured results ($mg \cdot L^{-1}$) were converted to $mg \cdot kg^{-1}$ by division with the bio-ethanol density ($kg \cdot L^{-1}$).

Results and Discussion

The aim of this work was to develop ion chromatography method suitable for determination of chloride and sulfate ions in bio-ethanol at low concentration levels avoiding laborious sample preparation and with accuracy comparable to official EN 15376 method. For that purpose method was optimized, validated and tested by analysis of real bio-ethanol samples.

Method optimization

Method optimization included the examination of the effect of chromatographic column length, mobile phase composition and flow rate on retention and separation of tested components. Detection of components was performed by suppressed conductivity.

Mobile phase composition

During the separation process, ions present in the sample are separated in the column based on their size and charge. The mobile phase has generally a low to medium conductivity and choice of the mobile phase depends on the compatibility with the detection mode, nature and concentration of the competing ion, buffering capacity of the mobile phase and adding of organic modifiers[10]. A mixture of sodium carbonate and sodium bicarbonate (Na_2CO_3/$NaHCO_3$) was used as the mobile phase. Effect of organic modifier addition to mobile phase was examined over the organic nature of ethanol. Organic modifier was added to reduce the impact of negative peak on the chloride peak due to their close retention times. Retention of chloride and sulfate ions was examined with mobile phase consisted of 0.339 g Na_2CO_3 and 0.084 g $NaHCO_3$ per liter with the addition of organic modifier, CH_3COCH_3, at concentrations of 20% (v/v) and 2.5% (v/v). When the acetone was added to the mobile phase analysis time was increased from 21 to 40 min.

By adding an organic modifier in the system, expected improvements are not achieved. Moreover, significant reduction in resolution was obtained as well as overlapping of the chromatographic peaks (Fig. 1).

Although organic modifier can increase the retention time of inorganic ions at the same time it reduces their mutual separation, i.e. decreases separation power of carbonate solution. Therefore, inorganic ions do not come out as single chromatographic curves but as a group of ions.

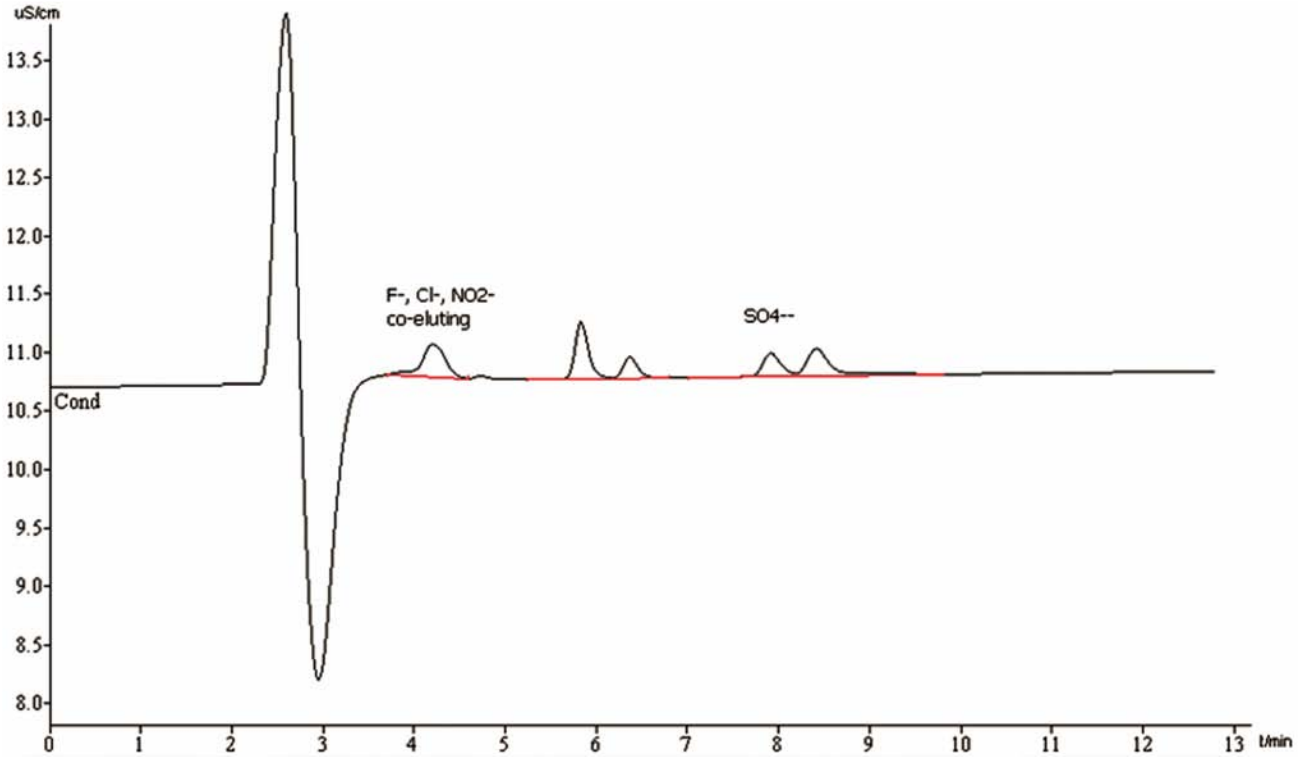

Fig. 1 — Chromatogram of anion standard solution (0.25 mg·L^{-1} both chloride and sulfate concentration), mobile phase Na_2CO_3/$NaHCO_3$ with 20% (v/v) acetone

Table 1 — Retention times of ions at flow rate of 0.6 and 0.7 mL·min^{-1}

t_R/min	Flow rate	
	0.6 mL·min^{-1}	0.7 mL·min^{-1}
Negative peak	4.4	2.5
Cl^-	10.6	6.0
Δ (t_RCl -t_R negative peak)	6.2	3.5
SO_4^{2-}	26.2	16.4

Table 2 — Retention times on 150 mm and 250 mm column length (flow rate, 0.7 mL·min^{-1})

t_R/min	Column length	
	150 mm	250 mm
Negative peak	2.6	4.3
Cl^-	5.9	10.6
Δ (t_RCl -t_R negative peak)	3.3	6.3
SO_4^{2-}	16.6	30.1

Mobile phase flow rate

Tendency in changes of retention times and width of chromatographic peaks were monitored at two mobile phase flow rates: 0.6 and 0.7 mL·min^{-1}. It was noted that the time between negative peak and chloride ion peak is getting longer at flow rate of 0.6 mL·min^{-1}, while the total analysis time was about ten minutes longer. Extended time between negative peak and chloride ion peak is desirable from the analytical point of view because of the potential overlap and overlay of chromatographic peaks that can occur in real samples. Table 1 shows the retention times of chloride and sulfate ions and negative peaks at two investigated mobile phase flow rates.

Column length

Chromatographic retention of chloride and sulfate ions was examined on chromatographic columns of different lengths: 150 mm (Metrosep A Supp 5/150) and 250 mm (Metrosep A Supp 5/250). On the longer chromatographic column retention time of individual ions is higher. The obtained results are expected considering that the number of theoretical plates in a longer column is higher (Table 2). Total analysis time was also increased on the longer column from the same reason.

The resolution between the negative peak and chloride ion peak is higher on the longer column and the possible impact of negative peak on chloride ion determination was reduced by using longer column.

Calibration

Based on results of preliminary tests, the optimal chromatographic conditions were defined as follows: carbonate/bicarbonate mobile phase without organic modifier added, flow rate 0.6 mL·min^{-1} and 250 mm length columns. On these working conditions the calibration is performed by means of external standard method. Calibration curves were prepared by plotting the peak area versus the analyte concentration expressed in mg·L^{-1}. By means of calibration curves, individual ions are quantified as mass concentration. Each standard was measured in triplicate in seven calibration points in concentration range from 0.25 to 20 mg·L^{-1}. For chloride, calibration curve equation was y=13.68x+0.0066 with the correlation coefficient R^2=0.9997, and for sulfate calibration curve equation was y=10.01x+0.00184 with the correlation coefficient R^2=0.9999.

Table 3 — Validation results

Selectivity	Acceptance criteria Information	Cl^- satisfies	SO_4^{2-} satisfies
Linearity	$R^2 \geq 0{,}999$	R^2=0.9997	R^2=0.9997
Accuracy	RSD ≤10 %	RSD = 6.4 %	RSD = 1.7 %
Repeatability	RSD ≤ 5%	RSD = 0.9 %	RSD = 3.2 %
LOD	Information	0.023 mg·L^{-1}	0.033 mg·L^{-1}
LOQ	Information	0.069 mg·L^{-1}	0.100 mg·L^{-1}
Robustness	Information	satisfies	satisfies

Method validation

After optimization, validation of the method has been performed in order to confirm its validity and applicability. Prior to performing validation, validation parameters and criteria of their acceptability are defined (Table 3). Parameters were selected based on laboratory experience and according to good laboratory practice.

Selectivity was tested by comparing the chromatograms of real samples of bio-ethanol with the chromatogram of standard. It was noted that the ions in both sample and standard solution have same retention times, so it was concluded that the method is selective.

Linear calibration curves were obtained with coefficients of correlation higher than 0.999 thus confirming the linearity of the method.

Repeatability is calculated by measuring the ion content in a corresponding sample six times under identical conditions and performed by the same operator in the laboratory. Since the relative standard deviation is below 5% for both investigated anions, the method is considered repeatable.

Limits of detection (LOD) and quantification (LOQ) were experimentally estimated from the injection of standard solutions serially diluted until

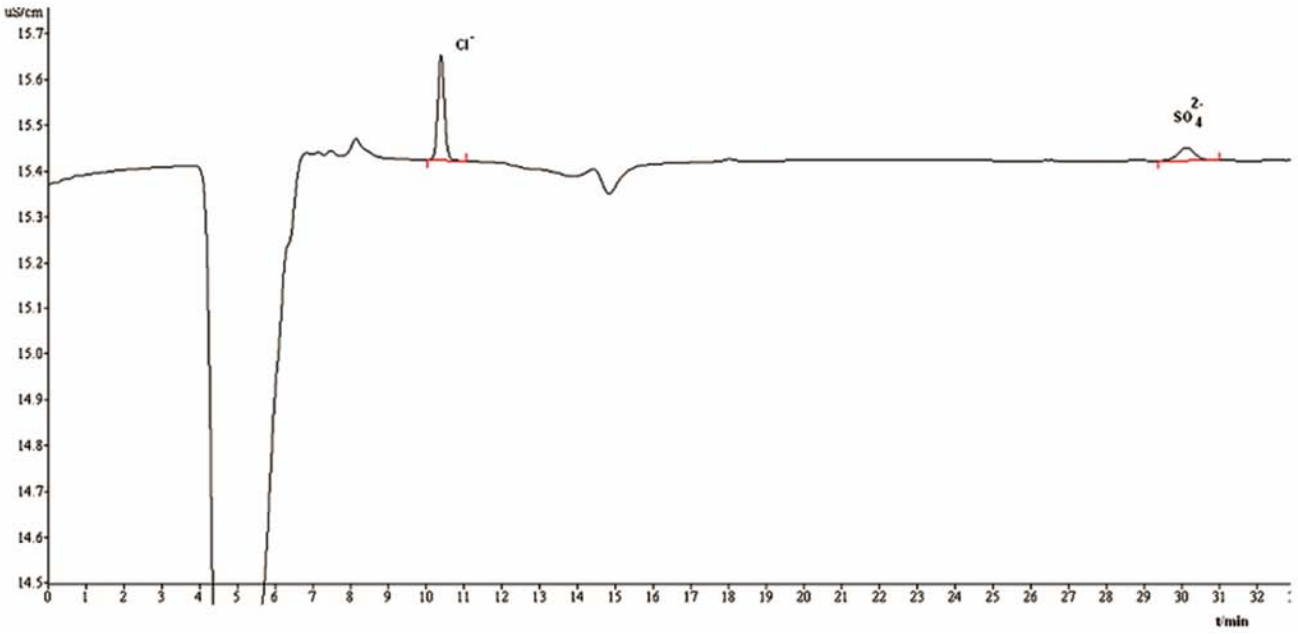

Fig. 2 — Chromatogram of real sample of bio-ethanol (BioEtOH2)

the signal-to-noise ratio for analyte reached a value of ten for LOQ and three for LOD. Under the given conditions, noise of base line has been measured. The limits of detection of 0.023 $mg \cdot L^{-1}$ and 0.033 $mg \cdot L^{-1}$ were obtained for chloride and sulfate, respectively.

The accuracy was checked as yield at three concentrations (1.67 $mg \cdot L^{-1}$, 5.00 $mg \cdot L^{-1}$ and 20.00 $mg \cdot L^{-1}$) by comparing the measured and actual values of concentration. Dividing the measured and actual values, yield is calculated for every concentration measured and expressed as a percentage value. RSD of the yield for chloride ion is significantly larger (6.4%) than for the sulfate ion (1.7%), although both are in the desired range.

The goal of robustness examination was to determine the effect of small changes in operating parameters on the analytical results. Changes in operating parameters are usually appears as a result of human factor or conditions in the laboratory. Since the conditions in the laboratory are usually under control, robustness of the method is the mostly influenced by the analyst work. In the proposed method the greatest impact of analysts is in the preparation of the mobile phase, which involves weighing and dilution. Impact of changes in the strength of mobile phase on the final result of the quantitative analysis was tested by adding small portions of deionised water in mobile phase. By diluting mobile phase, retention time of each ion in the chromatographic column was extended. However, at the quantification there was no significant deviation. Based on the obtained results it can be concluded that a small error during dilution of the mobile phase didn't affect the performance of method.

Analysis of bio-ethanol samples

Given the lack of real samples of bio-ethanol on the market, method was applied to the analysis of the inter-laboratory samples. After method was validated, four real bio-ethanol samples from inter-laboratory comparative testing were analysed: BioEtOH1, BioEtOH2, BioEtOH3 and BioEtOH4. Inter-laboratory testing included the results obtained from ten laboratories. Chromatogram of BioEtOH2 sample as representative chromatogram is presented on Fig. 2.

The content of chloride and sulfate ions in analyzed samples was determined. Measurement results obtained in units of $mg \cdot L^{-1}$ (from calibration curves) were expressed in $mg \cdot kg^{-1}$ taking into account density of each bio-ethanol sample. The obtained results were compared with the official tests results (OTR) and *z*-score was evaluated (Tables 4 and 5).

Considering that baseline has not been stable at the start of the chromatogram as well as a wide negative peak, the results obtained for the determination of chloride have higher *z*-scores than the results of the determination of sulfate.

Both, chloride and sulfate contents in inter-laboratory samples are for a concentration decade

Table 4 — Comparison of chloride concentrations obtained in laboratory and inter-laboratory testing (official tests results, OTR)

Sample	w (mg·kg^{-1})	OTR(mg·kg^{-1})	z-score
BioEtOH1	0.36	0.14	1.4
BioEtOH2	0.29	0.12	1.4
BioEtOH3	0.29	0.07	>3
BioEtOH4	0.44	0.04	>3

Table 5 — Comparison of sulfate concentrations obtained in laboratory and interlaboratory testing (official tests results, OTR)

Sample	w (mg.kg^{-1})	OTR (mg.kg^{-1})	z-score
BioEtOH1	0.14	0.22	-0.3
BioEtOH2	0.85	1.26	-0.9
BioEtOH3	0.89	0.82	0.1
BioEtOH4	0.14	0.21	-0.4

smaller than the allowable limits of 6 mg·kg^{-1} for chloride and 4 mg·kg^{-1} for sulfate.

Requirements of EN 15492 method for precision are defined in concentration range from 4 to 30 mg·L^{-1} while for the lower chloride content there are no data available and therefore can not be applied. For sulfate content precision was not established yet in EN 15492. For both ions precision should be determined in much lower range comparing to their content in real samples. From January 2014. on the Croatian market sale of gasoline which contains bio-component is mandatory and bio-ethanol as a feedstock for their mixing is routinely analyzed in terms of determining the content of chloride and sulfate. The optimized method will be able to use as a quick and adequate method for this analysis.

Conclusion

Recent introduction of bio-ethanol as a component for the mixing into gasoline, requires the development of new fast and reliable analytical methods. The results obtained in this work indicate that the proposed ion chromatography method offers adequate solution for chloride and sulfate content determination in bio-ethanol.

By method optimization, the impact of organic modifiers on improving the resolution has been tested and it is found that organic modifier causes a reduction in the resolution while lower flow rate and longer chromatographic column have a positive impact on method resolution. Results of method validation confirmed the applicability of the method for determination of chloride and sulfate in bio-ethanol since all validation parameters (selectivity, linearity, accuracy, repeatability, LOD, LOQ and robustness) met the established criteria.

The main advantage of the developed method is that it allows bio-ethanol analysis without previous sample preparation step thus significantly improving sample throughput and reduce sources of possible errors. Furthermore, the method is able to determine low chloride and sulfate levels (LOD below 0.1 mg·kg^{-1}) expected in real bio-ethanol samples.

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