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RESEARCH PAPER

Single analysis of human hair for determining halogens and sulfur after sample preparation based on combustion reaction

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Abstract

A single analysis of hair for determining halogens (chlorine, bromine, fluorine, and iodine) and sulfur by ion chromatography with suppressed conductivity and mass spectrometry detection (IC-MS) was proposed. Inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) were also used to compare the results. For this purpose, 300 mg of human hair were digested by microwave-induced combustion (MIC) using 20 bar of oxygen pressure. The analytes were absorbed in 100 mmol L^{-1} NH_4OH. Trueness of the proposed method was evaluated by analysis of a CRM of human hair; by recovery tests, using standard solution at two levels (50% and 100%), and by comparison of results with those obtained by ICP OES (Cl and S) and ICP-MS (Br and I). Suitable recoveries (ranging from 92 to 105%) were obtained, and the results from CRM analysis did not differ significantly from those described in the certificate. Moreover, results obtained by IC-MS did not present significant differences ($p > 0.05$) from those obtained by ICP OES and by ICP-MS. Precision was evaluated in terms of repeatability and intermediate precision, and the relative standard deviations were always lower than 8%. The proposed method presented good accuracy and it is a reliable strategy for human hair analysis. Final digests obtained using the MIC method were fully compatible with all proposed determination techniques. Compared to others reported in the literature, the proposed method presents several advantages, especially given that it is possible to determine halogens and sulfur in a single analysis.

Keywords Ion chromatography · Mass spectrometry · Inductively coupled plasma optical emission spectrometry · Inductively coupled plasma mass spectrometry · Microwave-induced combustion · Biological matrices analysis

Introduction

Halogens and sulfur content in human hair are commonly associated with diet, environmental exposure, health problems, medicines, or supplements taken [1–4]. These elements have important functions to the human organism; however, in unsuitable concentrations, they can be related to several disorders [4–6]. Halogens and sulfur concentration in biological samples is also used as an indicator of anthropogenic sources, global contamination, and toxic effects of persistent organic pollutants [2–5, 7].

Some techniques, such as instrumental neutron activation analysis (INAA), X-ray fluorescence, high-resolution continuum source graphite furnace molecular absorption spectrometry, and laser ablation inductively coupled plasma mass spectrometry have been used to determine halogens and sulfur in human hair [2, 5, 8–12]. Other versatile analytical tools, such as combustion ion chromatography or Cl/S multi-analyzer, have been proposed for the direct determination of halogens and sulfur in solid samples. However, although these techniques enable the direct analysis of solid samples, they have some disadvantages related to problems in the calibration step, a lack of sample homogeneity, matrix effects, and, specifically in the case of INAA, requirement for a nuclear reactor as a neutron source [13]. Moreover, some of them may not have

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sufficient sensitivity for the determination of Br and I in the most varied types of human hair.

In contrast to direct solid-sampling methods, analytical tools that require the solid sample conversion in a solution have also been used for human hair analysis. Potentiometric techniques such as ion-selective electrode are still used as a simple way to determine F in human hair [14]. Otherwise, this technique presents some disadvantages, such as monoelemental detection capability, low sensitivity, and low selectivity. Argon plasma-based spectrometric techniques have also been used for halogens and sulfur determination in human hair [15–18]. However, high ionization potentials, low wavelength emission lines, and spectral and non-spectral interferences make the determination of non-metals by argon plasma-based spectrometric techniques a difficult task, especially when compared to the determination of metals [4, 7]. Ion chromatography with suppressed conductivity detection (IC) is a well-established technique that is used in routine analysis for anion determination in a variety of samples [19]. In addition to sequential multielemental determination capability and good sensibility and selectivity, it is an excellent alternative for halogens and sulfur determination as bromide, chloride, fluoride, iodide, and sulfate forms, especially when an anion electrolytically regenerated suppressor is used [19]. Chromatographic separation can also be coupled with mass spectrometry as an additional detector system. Ion chromatography with suppressed conductivity and mass spectrometry detection (IC-MS) is an interesting and promising alternative for routine analysis to ensure analytical confidence and to dramatically improve the method's selectivity, sensibility, and detection capability. Although IC-MS requires a relatively long time for the analysis, it is easy to automate and presents more affordable maintenance costs when compared to plasma-based spectrometry techniques [19–21]. However, an appropriate chromatographic optimization associated with a suitable sample preparation method is critical to the analysis's success.

Microwave-assisted digestion in closed vessels has been considered a state-of-the-art method to convert a solid sample into an aqueous solution [22]. Nevertheless, concentrated nitric acid, which is usually required to assure high digestion efficiency, can cause some problems or even make impossible the analysis by IC [22]. Moreover, non-quantitative halogen recoveries in an acid medium can also occur due to element losses, resulting in volatile compounds [4, 7]. Some studies have employed water or alkaline solutions to extract halogens from different matrices [23]. Despite this, non-quantitative extraction or some interferences related to the matrix compound during the determination step can also occur [4, 7]. To solve these drawbacks, our research group recently proposed the use of microwave-induced combustion (MIC) as an excellent alternative for preparing a human hair sample while aiming to determine Br and I by ICP-MS [24]. The organic matrix was converted to the respective oxidation products, and the Br and I were absorbed in a suitable solution. However, there are other elements such as F, Cl, and S that should also be monitored in human hair. Their determination by ICP-MS is impaired by high ionization potentials (F and Cl) and by several polyatomic interferences (Cl and S) [4, 7]. Thus, suitable analytical tools that allow the determination of Br, Cl, F, I, and S in a single analysis are extremely interesting and they should be proposed.

Thus, this study proposes the feasibility of MIC for human hair sample preparation and subsequent determination of halogens and sulfur by IC-MS. Recovery tests using a standard solution; the analysis of a certified reference material (CRM) of human hair and the comparison between the results obtained with proposed method (MIC + IC-MS); and those using inductively coupled plasma optical emission spectrometry (ICP OES) and using inductively coupled plasma mass spectrometry (ICP-MS) were carried out to demonstrate the trueness. Precision was evaluated in terms of repeatability and intermediate precision. Additionally, microwave-assisted digestion and microwave-assisted extraction using alkaline solutions were evaluated for sample preparation of human hair and subsequent analyte determination by IC-MS. Finally, the proposed method was applied to various hair types to demonstrate its applicability. To the best of our knowledge, combining the MIC method and subsequent halogens and sulfur determination of human hair by IC-MS is being proposed for the first time and should be a powerful strategy for analysis, especially for biological samples.

Experimental

Instrumentation

An analytical mill (226/5, Lucadema Científica, Brazil) and a conventional oven (400/2ND, DeLeo, Brazil) were used to grind and to dry the samples, respectively. Hair masses were measured on an analytical balance (AY220, Shimadzu, Philippines), with a maximum load of 220 g and a resolution of 0.0001 g. A microwave oven (Multiwave 3000, Anton Paar, Austria) was used in the sample preparation. In the MIC, the microwave system was equipped with eight high-pressure quartz vessels (internal volume of 80 mL) and home-made quartz sample holders were used. The temperature and pressure were limited to 280 °C and 80 bar as recommended by the manufacturer (Software Version v2.50, Anton Paar). In the extraction and digestion methods, the system was equipped with eight chemically modified polytetrafluoroethylene (PTFE-TFM) vessels (internal volume of 100 mL). The temperature and pressure were limited to 260 °C and 60 bar as recommended by the manufacturer (Software Version v2.50, Anton Paar).

Halogen determination and sulfur determination were carried out using an ion chromatograph (ICS-5000, Dionex/Thermo Fisher Scientific, USA) with an IonPac AS11-HC analytical anion exchange column (250 mm × 2 mm i.d., particle size 4 μm) and an IonPac AG11-HC guard column (50 mm × 2 mm i.d., particle size 4 μm), at a controlled temperature of 30 °C. A Dionex ERS 500 anion electrolytically regenerated suppressor (2 mm, using the autosuppression external water mode at 0.20 mL min^{-1} and 56 mA), an eluent source EGC 500 KOH generator cartridge with continuously regenerated anion-trap column (CR-ATC) and an AS-AP autosampler were also used. A gradient of KOH from 5 mmol L^{-1} to 90 mmol L^{-1} over 35 min at a flow rate of 0.28 mL min^{-1} was used to separate sample anions and the injection volume was 50 μL. A conductivity cell, at a controlled temperature of 35 °C, was used for Cl^-, F^-, and SO_4^{2-} detection. The conductivity cell was connected to a mass spectrometer with electrospray ionization operated in the negative mode (MSQ Plus Single Quadrupole Mass Spectrometer, Thermo Fisher Scientific) using PEEK tubing (300 mm × 0.25 mm i.d.) for Br^- and I^- detection. The parameters such as the cone voltage, needle voltage, and temperature probe were optimized to give greater sensitivity for these species. Analytes determination was performed in a single chromatography run by peak area integration. Instrument control and data acquisition were performed using Chromeleon 7.0 (Thermo Fisher Scientific).

For comparison of results, Cl and S were determined using an inductively coupled plasma optical emission spectrometer (Spectro Ciros CCD, Spectro Analytical Instruments, Germany), while Br and I were determined using an inductively coupled plasma mass spectrometer (NexION 300X, Perkin-Elmer, Canada). The parameters and details about instrumentation for the determination of Cl and S by ICP OES and of Br and I by ICP-MS are presented in previous studies [24, 25].

Reagents and solutions

All solutions were prepared using ultrapure water (18 MΩ cm) obtained from a purification system (Mega Up, Megapurity, South Korea), and all reagents used in this study were of analytical grade or of higher purity. Ammonium hydroxide and tetramethylammonium hydroxide (TMAH) solutions, used as absorbing and/or extracting solutions for halogens and sulfur, were prepared from 25% ammonia solution (Merck, Germany) and 25% TMAH in water (Sigma-Aldrich, USA), respectively. Nitric acid (65%, Merck) was previously distillated below its boiling temperature using a sub-boiling system (Duopor, Milestone, Italy). Ammonium nitrate solution (6 mol L^{-1}) was used as the igniter of combustion and it was prepared by the dissolution of solid reagent (Merck) in water. Oxygen 99.5% (Linde, Brazil) was used for the pressurization of quartz vessels in the MIC procedure. Acetone 99% (Proquimios, Brazil) was used for hair washing. Low-ash filter paper discs (0.09 mg, Unifil, Germany), used as a combustion aid (15 mm of diameter, 12 mg) and polyethylene films (Congelito, Brazil), used to wrap the samples (8 cm × 8 cm), as well as the vessels and holders used for the digestion by MIC were previously cleaned based on studies presented in the literature [24].

Stock standard solutions containing 1000 mg L^{-1} of Br^-, Cl^-, I^-, F^-, and SO_4^{2-} were prepared by dissolving KBr (Merck, Germany), NaCl (Dinâmica, Brazil), KI (Merck), NaF (Merck), and K_2SO_4 (Synth, Brazil) salts in ultrapure water. These stock standard solutions were diluted and used to prepare the external calibration curves for analyte determination by IC-MS (5.0 μg L^{-1} to 50 μg L^{-1} for Br^-; 0.1 mg L^{-1} to 1.0 mg L^{-1} for Cl^-; 0.01 mg L^{-1} to 0.1 mg L^{-1} for F^-; 1.0 μg L^{-1} to 10 μg L^{-1} for I^- and 0.1 mg L^{-1} to 1.0 mg L^{-1} for SO_4^{2-}). Sodium chloride and Na_2SO_4 (Merck) were used to prepare the calibration standard solutions for Cl and S determination by ICP OES (1.0 mg L^{-1} to 10 mg L^{-1} for Cl and 0.25 mg L^{-1} to 10 mg L^{-1} for S). Potassium bromide and KI were also used to prepare the calibration standard solutions for Br and I determination by ICP-MS (1.0 μg L^{-1} to 10 μg L^{-1} for Br and 0.1 μg L^{-1} to 1.0 μg L^{-1} for I). High-purity argon (99.998%, White Martins, Brazil) was used for plasma generation and nebulization.

Experimental design for optimization of Br and I detection by IC-MS

The manufacturer recommends instrumental parameters for the determination of some elements, such as Br. However, instrumental parameters for I determination by IC-MS were not found in the manufacturer's application. Thus, taking into account that cone voltage, needle voltage, and probe temperature were identified as significant parameters for elemental detection by IC-MS, they were optimized to achieve the higher signal/noise ratio and to enhance Br and I detection. These parameters were optimized for enhancing Br and I detection using a central composite rotatable design (CCRD) 2^3 with six axial points and three replicates of the central point that were randomly injected. The CCRD was carried out with five levels of cone voltage, needle voltage, and probe temperature. The conditions varied from 300 °C to 600 °C (probe temperature), from 20 V to 160 V (cone voltage), and from 1 kV to 5 kV (needle voltage), respectively. The range of the parameters was extended aiming at a better evaluation of the experimental region. Contributions of individual parameters and interaction effects on signal intensities of the analytes were determined. The goodness of fit of the model was evaluated by the determination coefficient (R^2) and the models were validated by analysis of variance (ANOVA) at the 95%

confidence level using the Statistica program (version 10.0, StatSoft, Inc., Tulsa, USA).

Human hair samples and certified reference material

Human hair samples of volunteers without kind restriction were collected at beauty salons in the city of Pelotas (Rio Grande do Sul, Brazil). Human hair was cut by a salon professional with a scissor and it was stored in polyethylene bottle (around 1 g of each hair). The research objectives and the procedures were presented for the salon professional and for the volunteers according to the project approved by the Research Ethics Committee of the Federal University of Pelotas (opinion number: 2.251.932). In the laboratory, the samples were washed three times with acetone and water (between the repetitions) as recommended by the International Atomic Energy Agency (IAEA) [13, 26]. Then, the human hair samples were ground using a knife mill, and they were homogenized to obtain a representative sample for the method development. Finally, human hair samples were dried in an oven at a temperature of 60 °C for 4 h and they were stored in a polyethylene bottle until analyses. In addition, samples of human hair of ten volunteers were collected and each one was submitted to the same washing and milling procedures, and they were analyzed by the proposed method to demonstrate its applicability. A CRM NCS DC73347a of human hair from China National Analysis Center was also analyzed using the same optimized conditions of the proposed method for trueness evaluation.

Evaluation of sample preparation methods based on combustion reaction, extraction with alkaline solution, and digestion with oxidizing acid

The sample preparation methods evaluated in this work were based on a previous study [24]. For MIC, 300 mg of human hair was wrapped in polyethylene film and placed on the base of a quartz holder containing a disc of filter paper previously moistened with 50 μL of 6 mol L^{-1} NH_4NO_3 solution. Quartz sample holders were introduced to quartz vessels containing 6 mL of 100 mmol L^{-1} NH_4OH used as the absorbing solution. The vessels were closed, fixed in the rotor, and pressurized with 20 bar of O_2. The microwave heating program was (i) 1400 W for 5 min (combustion and reflux steps) and (ii) 0 W for 20 min (cooling step). The final solutions were diluted with water up to 20 mL for further halogens and sulfur determination by different determination techniques.

For microwave-assisted extraction, 300 mg of sample and 6 mL of alkaline solution (110 mmol L^{-1} TMAH solution, 100 mmol L^{-1} or 200 mmol L^{-1} NH_4OH solutions) were transferred to the PTFE-TFM vessels. In this case, the microwave heating program was (i) 1000 W for 10 min (ramp), (ii) 1000 W for 50 min, and (iii) 0 W for 20 min (cooling step). The maximum temperature was limited to 90 °C using TMAH to avoid its degradation [23]. For microwave-assisted digestion, 300 mg of hair and 6 mL of concentrated HNO_3 were transferred to the PTFE-TFM vessels, and the microwave heating program was (i) 1000 W for 20 min (ramp of 5 min) and (ii) 0 W for 20 min (cooling step). Final solutions were filtered using filter paper discs (C40, Unifil, Germany) and diluted with water up to 20 mL for the determination of halogens and sulfur determination by IC-MS.

Accuracy, limits of quantification, and statistical analysis

The systematic error (trueness) and the random error (repeatability and intermediate precision) were evaluated in order to express the accuracy. Trueness was initially evaluated by recovery tests and a solution containing 5 mg L^{-1} of Br, 2000 mg L^{-1} of Cl, 5 mg L^{-1} of I, 80 mg L^{-1} of F, and 20,000 mg L^{-1} of S was added (40 and 80 μL) to a human hair sample (300 mg) before closing the PE film. Additionally, trueness was evaluated by analysis of CRM NCS DC73347a (human hair) using the same optimized conditions of the proposed method. The analytes were also determined by ICP OES (Cl and S) and by ICP-MS (Br and I). Precision was assessed by repeatability and intermediate precision according to Eurachem guidelines [27]. Limits of quantification (LOQs) were estimated as LOQ = kQ × s'0, where kQ is the multiplication factor (10 for LOQ) and s'0 is the standard deviation of ten blank readings [27]. However, the blank values were included in the formula to consider the factors related to the contaminations ($\bar{x}$ + kQ x s'0, where $\bar{x}$ is the mean of the blank values). The statistical analysis was performed using Student's *t* test and ANOVA (GraphPad InStat Software Inc., Version 3.00, USA) with a confidence level of 95%.

Results and discussion

Feasibility of halogens and sulfur determination by ion chromatography with conductivity detection and mass spectrometry

Total concentrations of Br, Cl, F, I, and S that were present in the sample were determined as Br^-, Cl^-, F^-, I^-, and SO_4^{2-} by IC, respectively. It was carried out according to the previous systematic studies presented in the literature about the stability of halogens and S species after the MIC method [28, 29]. Moreover, the analytes concentration determined by IC was in agreement with the total concentration determined by ICP OES and ICP-MS. Given that analyte concentration in human hair can vary widely, Cl, F, and S were determined using suppressed conductivity detection, whereas Br and I were

determined by mass spectrometry detection. However, this was performed in a single chromatography run.

The eluent initially evaluated was 30 mmol L^{-1} KOH in isocratic mode, which manufacturers recommend for anion determination [30]. However, matrix interferences were observed in the retention time of F^- (2.95 min). Moreover, in this condition, the retention time of I^- was around 45 min. Thus, a gradient elution method was developed to overcome these chromatographic limitations. The gradient program was as follows: (a) 5 mmol L^{-1} KOH from 0 to 4 min (to solve the common interferences that affect the F^- determination by IC as well as to improve the F^- peak resolution); (b) 5 mmol L^{-1} to 30 mmol L^{-1} KOH from 4 to 8 min (to reduce the analysis time); (c) 30 mmol L^{-1} to 10 mmol L^{-1} KOH from 8 to 13 min (to improve the separation of NO_3^-, Br^-, and SO_4^{2-}); (d) 10 mmol L^{-1} KOH from 13 to 19 min; (e) 10 mmol L^{-1} to 90 mmol L^{-1} KOH from 19 to 22 min (to decrease the retention time of I^-); (f) 90 mmol L^{-1} KOH from 22 to 33 min; and (g) 90 mmol L^{-1} to 5 mmol L^{-1} KOH from 33 to 35 min (for baseline stabilization). It is important to mention that step (c) was required due to the relatively high NO_3^- concentration resulting from the sample and from the igniter solution used in the MIC method. The optimized gradient elution is presented in the Electronic Supplementary Material (ESM) Fig. S1, because the slopes of the gradient program were not linear in some steps.

Mass spectrometry detection was evaluated for Br and I determination because they are generally present in very low concentrations (in the order of parts per billion (ppb)) in human hair [24] and the suppressed conductivity detection did not present enough sensitivity for this task. Cone voltage, needle voltage, and probe temperature are critical parameters on the mass spectrometry response [21]. Thus, these parameters were carefully optimized for Br and I determination using a CCRD with 3 central points ($\alpha = 1.68$), totaling 17 experiments. In this optimization, a standard solution containing 5 mg L^{-1} of the analytes was directly introduced to the mass spectrometer using an auxiliary pump (AXP-MS, Dionex/Thermo Fisher Scientific, USA) with a flow rate of 0.28 mL min^{-1} of water and an injection volume of 500 μL. High-purity nitrogen, used as the nebulizer gas in the electrospray ionization (ESI) source, was produced from a nitrogen generator (18LA, Peak Scientific, Scotland). Nebulizer gas pressure was set at 5.5 bar (measured by the MSQPlus's front gas regulator), which is the recommended general condition on the MSQ instrument for ESI applications [21]. The signal intensities of the species with *m*/*z* 79 (bromide) in the range of 12 to 22 min and *m*/*z* 127 (iodide) in the range of 30 to 35 min were monitored during the chromatographic run. Electrospray ionization in negative selected ion monitoring (SIM) using a 0.2 *m*/*z* span and dwell time of 1.0 s $scan^{-1}$ were used for all scans.

According to the signal intensities of Br and I in the experiments generated by CCRD (ESM Table S1) and to the Pareto charts (ESM Fig. S2), all variables were statistically significant ($p < 0.05$) for Br and I. The results showed that all linear parameters presented a positive effect on the responses in the range evaluated, whereas the quadratic parameters presented a negative effect on the responses. The central points showed little variation (relative standard deviation — RSD ≤ 2%) for both responses (Br and I signal intensities), indicating good repeatability. The adequacy of the model was tested via Fisher's statistical test for the ANOVA, which was used to evaluate the significance of the generated models (ESM Table S2). The ANOVA of the model suggests that it is predictive (i.e., the calculated F is higher than the tabulated one) and significant ($p < 0.05$). Coefficients of determination were 0.961 and 0.939 for Br and I, respectively, indicating that 96% and 94% of the mass spectrometry response variation were attributed to the factors and that only 5% were by chance. The results obtained experimentally were compared with those obtained using the model, and the linear correlation coefficient obtained for both analytes was higher than 0.85; thus, the models for the Br and I signal intensities were generated.

The response surfaces obtained for the Br and I signal intensities by mass spectrometry using different cone voltages (20 V to 160 V), needle voltages (1.0 kV to 5.0 kV) and probe temperatures (300 °C to 600 °C) are shown in Fig. S3 in ESM. The maximum signal intensities for Br and I were obtained when the maximum probe temperature was used in the mass spectrometer. Although the use of higher temperatures could provide higher signal intensities, 600 °C was chosen as the probe temperature, per the manufacturer's recommendations, to minimize damage to the spectrometer. Moreover, it was possible to observe that the use of values about 135 V of cone voltage and 4.2 kV of needle voltage enabled the maximum intensities of Br and I for the mass spectrometry. Thus, these conditions were chosen for Br and I determination by IC-MS. Chromatography separation for a standard solution containing 0.05 mg L^{-1} of F^-, 0.5 mg L^{-1} of Cl^- and SO_4^{2-}, and 50.0 μg L^{-1} of Br^- and I^-, and a sample prepared using the MIC method using the optimized condition are presented in Fig. 1.

As shown in Fig. 1, separating all species in a chromatography run of 35 min was possible. Fluorine, Cl, and S were determined as F^-, Cl^-, and SO_4^{2-} using suppressed conductivity detection (Fig. 1 a), and Br and I were determined as Br^- and I^- using mass spectrometry detection (Fig. 1 b). Dilution factors of around 4 and 20 times were performed to minimize the interferences of matrix compounds and reagents during the determination of F and S, respectively, by IC. As can be seen in Fig. 1, the use of mass spectrometry coupled with IC as an additional detector improved the detectability of Br and I, which are generally present in low concentrations in human

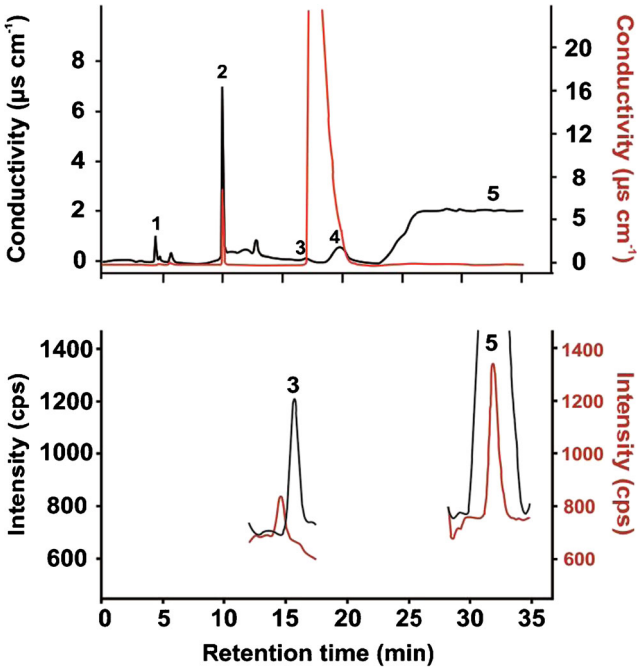

Fig. 1 Chromatograms obtained for a multielemental standard solution (—) and for 300 mg of human hair digested by microwave-induced combustion using 100 mmol L^{-1} NH_4OH as the absorbing solution (▬) — diluted 20 times in suppressed conductivity detection and 4 times in mass spectrometry detection. The analyte concentration of the standard solution is (1) 50 μg L^{-1} for fluoride, (2) 500 μg L^{-1} for chloride, (3) 50 μg L^{-1} for bromide, (4) 500 μg L^{-1} for sulfate, and (5) 50 μg L^{-1} for iodide. **a** Conductivity detection. **b** Mass spectrometry detection

hair. As can be seen in Fig. 1b, it is possible to verify the retention time matching for I^- when a standard solution and a sample were analyzed. However, this behavior was not observed for Br^-. Despite this, the Br^- peak was confirmed on the sample by spiking experiments. Even using a fourfold dilution prior to injection, the Br^- peak on the sample did not exactly correspond to the one in the standard solution. This probably occurred because the hair matrix contains constituents that modify the stationary phase or interact with other anionic species (e.g., NO_3^- and SO_4^{2-}) in higher concentrations, which may result in changes on the Br^- retention behavior. It should be mentioned that this fact did not affect the Br^- determination by IC-MS.

Proposed analytical method for determining halogens and sulfur in human hair

The MIC method was already used for preparation of the human hair sample, aiming subsequent determination of Br and I by ICP-MS [24]. In view of this, the feasibility of the MIC method for further halogens and sulfur determination by IC-MS was evaluated. Trueness was evaluated via recovery tests using a standard solution at different levels (around either 50% or 100% of the Br^-, Cl^-, I, and SO_4^{2-} concentrations previously determined in the homogenized human hair portion, and around 1 time or 3 times the LOQ for F); via CRM NCS DC73347a (human hair) analysis; and via comparison of the results obtained by proposed method with those obtained by ICP OES and ICP-MS. The results are shown in Fig. 2.

As shown in Fig. 2, suitable recoveries (ranging from 92 to 105%) were obtained for all analytes in the two concentration levels evaluated. Moreover, the halogen and sulfur concentrations obtained by the proposed method did not differ significantly ($p > 0.05$) from those described in the certificate of the CRM (agreeing from 91 to 102%). Additionally, as previously mentioned, Cl and S were determined by ICP OES and Br and I were determined by ICP-MS in all samples used in this study. The results did not present significant differences ($p > 0.05$) from those obtained by IC-MS (Table 1). Relative standard deviations using IC-MS for repeatability were always lower than 5% and for intermediate precision, always lower than 8%. Although the RSDs for the optimization of the MIC method were lower than 5%, the RSDs for the applicability of the MIC method to different human hairs were up to 10%. This is probably associated with some of the sample's lack of homogeneity.

The limits of quantification for Cl, F, and S using suppressed conductivity detection were 13 μg g^{-1}, 8 μg g^{-1}, and 0.21 mg g^{-1}, respectively, while Br and I using mass spectrometry detection were 0.7 μg g^{-1} and 0.2 μg g^{-1}, respectively. The limits of quantification using plasma-based spectrometric techniques were 50 μg g^{-1} and 0.02 mg g^{-1} for Cl and S (ICP OES), respectively, and 0.06 μg g^{-1} and 0.02 μg g^{-1} for Br and I (ICP-MS), respectively. In this way, besides the determination of Br and I by ICP-MS [24] and of Cl and S by ICP OES, the proposed method allows the determination of Br, Cl, F, I, and S using IC-MS in a unique chromatography run. This approach presents suitable accuracy, which was demonstrated by the good results for trueness and precision; it has lower maintenance costs than ICP OES and ICP-MS and allows the determination of F, which would not be possible with these techniques.

Comparison of the MIC method with other analytical methods for determining halogens and sulfur in human hair

In microwave-assisted extractions with an alkaline solution, the final solutions presented a dark brown coloration in view of its matrix compounds. To avoid interferences and damage to the equipment, it was necessary to apply a filtration step and successive dilutions (at least 20 times) before the IC-MS determination. Fluorine could not be determined due to a high matrix interference in the retention time of F^-. The concentrations for Cl, Br, and I could not be determined because their

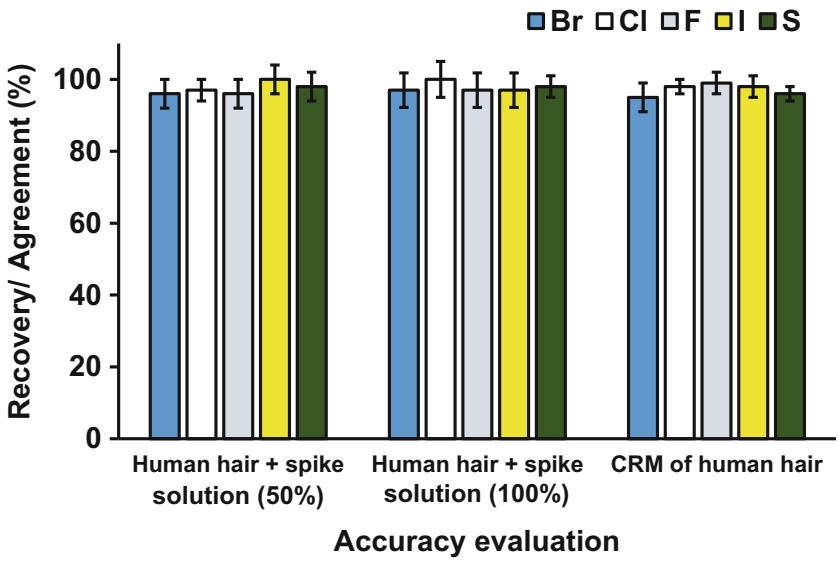

Fig. 2 Analyte recoveries and agreements with the concentration in the certified reference material after microwave-induced combustion method combined with halogen and sulfur determination by ion chromatography with suppressed conductivity and mass spectrometry detection ($n = 5$)

concentrations were below the LOQs (900 μg g^{-1} for Cl using suppressed conductivity detection and 10 μg g^{-1} for Br and 2.0 μg g^{-1} for I using mass spectrometry detection). The obtained S concentrations in human hair (determined as SO_4^{2-}) via IC using suppressed conductivity detection after microwave-assisted extraction were 9.9 ± 3.5 mg g^{-1} using 100 mmol L^{-1} NH_4OH; 10.3 ± 4.5 mg g^{-1} using 200 mmol L^{-1} NH_4OH; and 0.42 ± 0.10 mg g^{-1} using 110 mmol L^{-1} HTMA. These results were excessively lower than the S concentration obtained using the MIC method (47.2 ± 2.6 mg g^{-1}). This indicates that S was not quantitatively extracted from the human hair or it was not extracted in an SO_4^{2-} form.

In microwave-assisted digestion, the human hair was efficiently digested using 6 mL of concentrated HNO_3. However, the final solutions presented high residual acidity and high NO_3^- content in the digests. Even after performing the pH adjustment, the high NO_3^- can saturate the chromatographic column, and extremely high dilution factors would be required prior to sample analysis by IC-MS, which makes the analyte determination difficult. Thus, in view of these drawbacks, the solutions obtained after microwave-assisted digestion were not analyzed to prevent damage to the equipment, and this sample preparation method was not considered suitable for determining halogens and sulfur in human hair by IC-MS.

Table 1 Halogen and sulfur concentrations in human hair after the microwave-induced combustion method using 300 mg of the sample and 100 mmol L^{-1} NH_4OH as the absorbing solution. Determination by different techniques (mean ± standard deviation, μg g^{-1}, $n = 5$)

Samples	IC-MS					ICP OES		ICP-MS	
	Conductivity		Mass spectrometry						
	Cl	F	S[a]	Br	I	Cl	S[a]	Br	I
A	1112 ± 43	< 8	57.6 ± 3.3	2.20 ± 0.20	1.30 ± 0.12	1025 ± 55	53.2 ± 3.1	2.47 ± 0.15	1.27 ± 0.12
B	646 ± 16	< 8	52.5 ± 2.1	4.10 ± 0.32	2.70 ± 0.25	635 ± 25	48.2 ± 2.4	4.17 ± 0.40	2.88 ± 0.27
C	107 ± 8	< 8	50.7 ± 1.5	< 0.7	0.85 ± 0.08	115 ± 8	48.4 ± 3.0	0.41 ± 0.04	0.90 ± 0.09
D	784 ± 49	< 8	51.9 ± 1.2	1.21 ± 0.12	0.71 ± 0.07	720 ± 23	53.6 ± 2.1	1.31 ± 0.07	0.66 ± 0.05
E	120 ± 12	< 8	53.7 ± 0.6	0.98 ± 0.08	1.30 ± 0.11	128 ± 12	52.1 ± 2.4	1.07 ± 0.04	1.19 ± 0.02
F	56.1 ± 2.4	< 8	47.1 ± 0.6	< 0.7	0.61 ± 0.06	53.9 ± 3.8	45.0 ± 2.2	0.24 ± 0.02	0.56 ± 0.02
G	647 ± 51	< 8	51.6 ± 2.4	2.91 ± 0.30	1.90 ± 0.18	645 ± 41	48.8 ± 2.5	2.69 ± 0.10	1.98 ± 0.12
H	1039 ± 60	< 8	56.1 ± 1.5	3.59 ± 0.32	10.1 ± 0.9	1043 ± 68	54.1 ± 2.6	3.50 ± 0.33	9.41 ± 0.61
I	1190 ± 56	< 8	61.2 ± 0.9	3.21 ± 0.30	2.19 ± 0.21	1182 ± 71	62.0 ± 1.5	3.41 ± 0.26	2.29 ± 0.20
J	697 ± 42	< 8	53.4 ± 0.9	3.70 ± 0.25	2.20 ± 0.21	650 ± 50	50.2 ± 3.2	3.82 ± 0.27	2.18 ± 0.16
CRM*	176 ± 4	11 ± 0.3	40.2 ± 0.8	1.04 ± 0.04	0.78 ± 0.02	186 ± 8	39.9 ± 1.2	1.13 ± 0.06	0.75 ± 0.04

*Certified concentrations: Cl 180 μg g^{-1}; F 11 μg g^{-1}; S 41.9 ± 1.1 mg g^{-1}; Br 1.1 μg g^{-1}, and I 0.8 ± 0.2 μg g^{-1}

[a] Values in mg g^{-1}

Given these results, the MIC method overcame the drawbacks currently found in others sample preparation methods for further halogen and sulfur determination in human hair by IC-MS. Using MIC, the human hair was efficiently digested (i.e., residual carbon content was lower than 1%) and a diluted alkaline solution was used for analyte absorption, resulting in low blank values and digests that were fully compatible with different analytical techniques, including IC-MS. The advantages of MIC are highlighted by its high sample throughput (16 samples per hour), low reagent consumption, and, consequently, low generation of laboratory waste.

Determination of halogens and sulfur in human hair by the proposed method

The proposed method was applied to analyze the human hair samples, which were taken from 10 volunteers. The results for halogens and sulfur determined by IC-MS are shown in Table 1. For a comparison of the results, Cl and S concentrations determined by ICP OES and Br and I concentrations determined by ICP-MS were also presented in Table 1.

As can be observed in Table 1, no significant differences ($p > 0.05$) were observed between the results for all analyte concentrations in human hair using different determination techniques after the digestion combustion method. The concentration variations for Br (0.24 μg g^{-1} to 4.17 μg g^{-1}), Cl (56.1 μg g^{-1} to 1190 μg g^{-1}), I (0.61 μg g^{-1} to 10.1 μg g^{-1}), and S (40.2 mg g^{-1} to 61.2 mg g^{-1}) in the analyzed human hair samples can be associated with the habits, environmental exposure, and conditions of each organism. In addition, the observed variations may also be related to the analyzed hair samples' features (e.g., black, blond, red, brown, curly, and straight human hair). Fluorine concentration in all analyzed human hair samples was lower than the LOQ (with the exception of the CRM). The volunteers' clinical status was not evaluated in this study, but the proposed method is a suitable alternative for this purpose, considering that it was possible to determine the analytes with satisfactory accuracy.

Conclusions

In using the MIC method for sample preparation, the obtained solution was suitable for halogen and sulfur determination in human hair by IC-MS, in addition to the determinations by ICP OES and ICP-MS. The additional determination of anions using mass spectrometry coupled with IC is an interesting and promising alternative for routine analysis because this approach provides several advantages mainly related to selectivity, sensibility, detection power, and more affordable maintenance cost when compared to plasma-based spectrometry techniques. The proposed method allows the determination of halogens and sulfur in a single analysis with suitable accuracy and LOQs. The proposed method presented relatively high throughput, low waste generation, and compatible digests with multiple determination technique, which are very important characteristics for routine analysis.

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Compliance with ethical standards

The authors declare that the research objectives and the procedures were presented for the salon professionals and for the volunteers according to the project approved by Research Ethics Committee of the Federal University of Pelotas (opinion number: 2.251.932).

Conflict of interest The authors declare that they have no conflict of interest.

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