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New possibilities for pharmaceutical excipients analysis: Combustion combined with pyrohydrolysis system for further total chlorine determination by ICP-OES

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ABSTRACT

An alternative method for the determination of total chlorine content in hydroxypropyl cellulose (HPC) was applied, combining a recently developed system based on a combustion step followed by pyrohydrolysis reaction. Using this approach it the determination of total chlorine by inductively coupled plasma optical emission spectrometry (ICP-OES) without interferences was feasible. It overcame the limitations of European Pharmacopoeia (EP) method for HPC analysis regarding to the inability to determine total chlorine in HPC, once some chlorine compounds (e.g., chloroform) that can not be identified by the official method (EP). The following parameters of combustion and pyrohydrolysis were evaluated: absorbing solution, sample mass, the use of powdered silica as retardant of combustion, oxygen flow rate and reaction time. Reference values for total chlorine were obtained after digestion using microwave-induced combustion and determination by ion chromatography (IC). Microwave-assisted extraction (MAE) was also investigated for Cl extraction. The accuracy of the proposed method was also evaluated by analyte recovery tests (agreement of 95–103%), as well as by the analysis of certified reference materials (CRMs). The agreement with the certified values was higher than 95% and the limit of quantification (LOQ) was 50 $\mu g\,g^{-1}$. Up to 500 mg of sample were efficiently digested by the proposed method in 5 min (dissolved carbon in digests was below 50 $mg\,L^{-1}$). Total chlorine content in samples of modified cellulose ranged from 284 to 576 $\mu g\,g^{-1}$. Despite the relatively high chlorine content in all samples, the concentration was lower than the maximum limit allowed by the EP for HPC (0.5%).

1. Introduction

Pharmaceutical excipients are important constituents for pharmaceutical industry [1–3]. These compounds have been used to enhance the product performance, enabling formulations, oral bioavailability, patient acceptability and compliance, providing a more efficient and safe treatment [3–6]. Among the several types of excipients available for the pharmaceutical industry, modified cellulose as hydroxypropyl cellulose (HPC) has been extensively used in several pharmaceutical products as oral tablets and suspensions [2,4,7].

Despite the widespread use of pharmaceutical excipients, these compounds can present elemental impurities. The most common source of these impurities in pharmaceutical products is due to the reagents or catalysts used during the synthesis. However, these impurities could be harmful to human health and their content should be monitored. Hydroxypropylmethyl cellulose, for example, could be synthesized by the reaction between cellulose, dichloromethane and other reagents as propylene oxide [8,9]. In this way, residual chlorine can be found in the final products generally from organochlorine reagents. These reagents present adverse health effects, affecting the central nervous system and reproductive system, with high toxicity for liver and kidney, besides being considered carcinogenic [10,11].

The European Pharmacopoeia (EP) has established the limits of chlorine content in modified cellulose in order to monitor the chlorine concentration in excipients to guarantee the quality of the final product. The official method recommends a limit test that consists of a reaction between chlorine and $AgNO_3$ after previous sample dispersion in a 0.85% HNO_3 solution (chlorine should be lower than 0.5%) [12]. The turbidity of the test sample solution should be lower than a standard (0.5% Cl), prepared by dilution of a chlorinated inorganic salt (e.g., NaCl). However, this method is prone to some errors, providing inaccurate results due to the low ability of the analyst to visually compare

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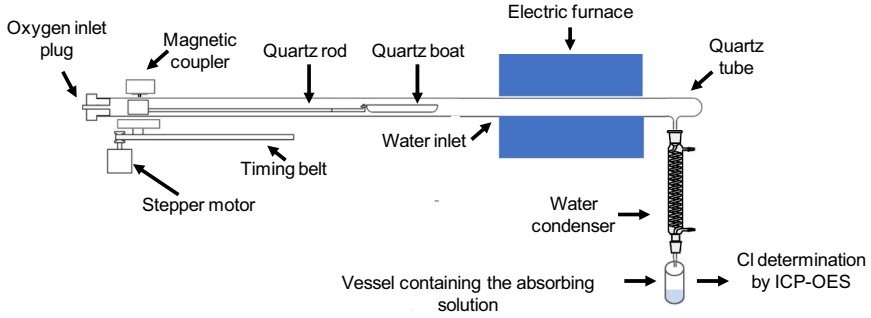

Fig. 1. Combustion followed by pyrohydrolysis system.

the turbidity between the sample and standard solutions, mainly at low concentration. In addition, this limit test does not take into account the presence of organochlorine compounds remaining from the synthesis. In this case, there is no reaction between the organochlorine compounds and $AgNO_3$, indicating a false and underestimated results.

Nowadays, several analytical techniques allow the direct solid analysis of chlorine, such as neutron activation analysis (NAA) [13], elemental analyzers [14], energy-dispersive X-ray (EDX) [15] and high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS GF MAS) [16,17]. Furthermore, in some cases, techniques allowing the direct analysis in solid samples without a sample preparation step are generally susceptible to problems related to heterogeneity, usually requiring a previous step to increase the sample homogeneity [18]. In addition, by using direct solid sampling approach, a calibration step with certified reference materials (CRMs) can be required, making unfeasible for routine analysis due to the high cost of CRMs [15,19]. In this way, other techniques such as ion selective electrode (ISE) [20], ion chromatography (IC) [21,22], inductively coupled plasma mass spectrometry (ICP-MS) [23,24] and inductively coupled plasma optical emission spectrometry (ICP-OES) [25,26] have also been used for halogens determination, since combined with a suitable sample preparation method. Furthermore, chlorine determination in low concentration is not a simple task, mainly due to the problems related to sample preparation step and risk of contamination (in general relatively high when compared to other elements) [27].

In case of organic matrices, they must be digested using concentrated HNO_3 or a mixture of acids [28,29]. However, a special attention should be given when concentrated HNO_3 is used during digestion, because losses of chlorine can be observed [30,31]. It is also important to mention that the dissolved residual carbon (in case of extraction or partial digestion) and the high residual acidity can also interfere during the determination by IC, ISE and ICP-MS, highlighting the requirements for an efficient digestion method [18,32].

To overcome some of the drawbacks of conventional sample digestion methods, combustion reactions have been used for further halogens determination [26,33]. These methods allow the use of relatively high sample mass and diluted solutions for analytes absorption, reducing the generation of residues [34,35]. Pyrohydrolysis has also been successfully applied showing as the main advantage the separation of halogens from the matrix, reducing interferences in the determination step [36,37]. Pyrohydrolysis has been applied for the digestion of several matrices such as soil [38], airborne [39], coal [40] and ~~heavy~~ crude oil and their derivates [41]. However, the conventional pyrohydrolysis system generally requires the use of a reagent (e.g., V_2O_5) to achieve quantitative recoveries, being susceptible to contamination and interferences during the determination step [41,42].

Recently, a new sample preparation method was developed based on the combination of combustion followed by pyrohydrolysis reaction using an automated system [43]. This system can be applied using simple and inexpensive materials, reducing the cost in comparison with other systems, being more attractive for routine analysis and minimizing the limitations of conventional pyrohydrolysis.

In this sense, considering the potential of combustion step followed by pyrohydrolysis approach, the present work aims to investigate this method for the digestion of modified cellulose pharmaceutical excipient for the subsequent chlorine determination by ICP-OES. The absorbing solution, maximum sample mass, the use of powdered silica as retardant of combustion, oxygen flow rate and reaction time were investigated. The reference values for chlorine were obtained by IC analysis after microwave-induced combustion (MIC). The accuracy was evaluated by analyte recovery tests and by analysis of CRMs. Additionally, for results comparison microwave-assisted extraction (MAE) was also investigated.

2. Experimental

2.1. Instrumentations

A homemade pyrohydrolysis system was used for HPC digestion (Fig. 1). The system was composed by a quartz tube heated by an electrothermal furnace (Sanchis, Brazil) containing an automatic temperature control (maximum temperature of 1200 °C) and a quartz condenser. The quartz tube (450 mm length, 18 mm i.d.) was positioned inside the furnace, with a lateral inlet for water, pumped by a peristaltic pump (IPC8, Ismatec, Switzerland) for *in situ* water vapor generation. An automatic homemade sample introduction system was used to introduce a quartz platform (80 mm length) containing the sample into the pyrohydrolysis system. More details about this system can be found in reference [43]. The operational parameters, such as the speed of platform introduction, were controlled by using Arduino Pro Mini (Italy). The program and control of the sample introduction into the system were monitored using a 3 inch display and a rotary encoder placed inside a plastic housing with others electrical components.

The output of the quartz tube was connected to a quartz condenser, cooled with water at 4 °C using a chiller (Q-214U2, Quimis, Brazil) and the end of condenser was connected to a polypropylene vessel containing the absorbing solution. During the heating program, the oxygen flow rate was controlled by using a flowmeter (2A13, Key Instruments, USA).

A microwave oven (Multiwave 3000, Anton Paar, Austria) equipped with eight high-pressure quartz vessels (internal volume of 80 mL, maximum temperature of 280 °C and maximum pressure of 80 bar) was used for sample preparation by MIC and by MAE. A commercial quartz holder (Anton Paar, Austria) was used to insert the samples inside the quartz vessels. An analytical balance (AY 220, resolution of 0.1 mg, Shimadzu, Japan) was used for sample weighing. For MIC, HPC samples were pressed as pellets (diameter of 13 mm) using a hydraulic press at 3 t (Specac, England).

Chlorine determination was carried out using an ICP-OES instrument with axial view configuration (Spectro Ciros CCD, Spectro Analytical Instruments, Germany). The spectrometer was operated with a cross-flow nebulizer (TQ-30-A3, Meinhard, USA) and a Scott-type

double pass spray chamber (Glass Expansion, Australia). The spectrometer is equipped with a monochromator in a Paschen-Rouge configuration. Besides the visible spectral range, the optical system of the ICP-OES equipment is sealed with high purity argon, allowing the wavelength monitoring in the vacuum UV spectral range (up to 125 nm). The argon flow rates (99.998%, White Martins-Praxair, Brazil) used for plasma, auxiliary and nebulization gas were set at 14.0, 1.0 and 1.00 L min^{-1}, respectively. Radiofrequency power was set at 1550 W. The monitored wavelength for chlorine was 134.724 nm. The same spectrometer was used for the determination of dissolved carbon in digests after combustion followed by pyrohydrolysis. The monitored wavelengths for C and Y (used as internal standard only for C determination) were 193.091 and 371.029 nm, respectively.

For the reference method and for results comparison obtained by ICP-OES, an ion chromatography system (Metrohm, Switzerland) consisting of a pump (IC liquid handling unit) and a conductivity detector (model 819, Metrohm) was used for the determination of Cl after MIC. The analytical column was an anion-exchange type (Metrosep A Supp 5, 4 mm i.d.) with a guard column (Metrosep A Supp 5). A chemical suppressor module was used to reduce the conductivity of the mobile phase. A sample loop of 100 μL was used and the mobile phase was 3.2 mmol L^{-1} Na_2CO_3 and 1.0 mmol L^{-1} $NaHCO_3$, at a flow rate of 0.7 mL min^{-1}.

2.2. Samples, reagents and standards

Four commercial HPC samples were purchased from local drugstores (Santa Maria city, Brazil) and named as HPC "1" to "4". Hydroxypropyl cellulose "1" sample was used for the development of the proposed method. The accuracy was evaluated by spike recovery tests, comparison of results with reference values obtained from digests by MIC and also by the analysis of CRMs of apple leaves (NIST 1515) and peach leaves (NIST 1547). No CRM was available for Cl certified values for HPC.

All the reagents were of analytical grade. Water was purified (18.2 MΩ cm) using a Milli-Q system (Millipore Corp., USA). A chlorine stock standard solution (1000 mg L^{-1}) was prepared by dissolution of NaCl (Merck, Germany) in water. Calibration solutions for chlorine determination by ICP-OES (1–10 mg L^{-1}) were prepared by the dilution of the stock solution in 10 mmol L^{-1} NH_4OH. In order to assure a controlled and reproducible combustion, powdered silica (63–210 μm, SysCroma, Brazil) was evaluated as retardant of combustion in the proposed method. The powered silica was previously cleaned with ethanol for 40 min in an ultrasound bath, rinsed with water and dried for 2 h in a class 100 laminar flow bench (CSLH-12, Veco, Brazil). This procedure was required to assure the removal of eventual chlorinated compounds.

Water and NH_4OH solutions (10–100 mmol L^{-1}) were evaluated as absorbing solutions for the proposed method (50 mmol L^{-1} of NH_4OH was used as absorbing solution in MIC method). The NH_4OH solutions were prepared by sequential dilution of a 25% NH_4OH (Merck) solution in water. Water also was used in MAE method as extraction solution.

Ammonium nitrate solution (6 mol L^{-1}) was used as ignition aid for MIC and was prepared by the dissolution of NH_4NO_3 (Merck) in water. A small disc of filter paper (15 mm diameter, 15.3 ± 0.3 mg) with low ash content (Black Ribbon Ashless, Schleicher & Schuell, Germany) was used as combustion aid for the MIC method. The filter paper was cleaned using the same procedure for powered silica.

Carbon standard solutions (5–500 mg L^{-1}) were prepared by sequential dilution of 1000 mg L^{-1} citric acid solution (P.A grade, Dinâmica, Brazil) in 5% HNO_3. Yttrium (1000 mg L^{-1}, Spex CertiPrep, USA) was used as internal standard for C determination at final concentration of 1 mg L^{-1} in blanks, standards and samples. Previously to the C measurements, the volatile carbon species in digests (e.g., CO_2) were removed by applying a flow rate of 0.1 L min^{-1} of argon for 2 min. The limit test based on the EP [12] was performed using NaCl and $AgNO_3$ salts (both from Merck) dissolved in water. Furthermore, chloroform (Merck) was used as organochlorine standard.

2.3. European Pharmacopoeia limit test

The limit test for chlorine was performed as recommended by EP using an inorganic chlorine standard (from NaCl). In this way, in a test tube containing 1 mL of 17 g L^{-1} $AgNO_3$ and 1 mL of 20% HNO_3 solution, a mixture, previously prepared, of 10 mL of 5 mg L^{-1} chlorine and 5 mL of water were added [12].

In order to compare the performance of the EP limit test for inorganic and organic chlorine standards, the same test was carried out using chloroform as organic chlorine standard (3.75 μL of chloroform 99% and volume completed with water up to 15 mL). The volume of chloroform was calculated to achieve the same chlorine maximum concentration allowed by EP for chlorine (3.33 mg L^{-1}).

The limit test was performed by weighing about 1 g of sample 1 and adding 50 mL of hot water (90 °C) and subsequently completing the volume to 100 mL. After, 1 mL of the formed gel was diluted to 15 mL with water and transferred to the test tube containing 1 mL of 17 g L^{-1} $AgNO_3$ and 1 mL of 20% HNO_3. The turbidity was compared with that of standard solution (inorganic chlorine standard).

2.4. Combustion followed by pyrohydrolysis method

For the combustion followed by pyrohydrolysis proposed method, sample masses ranging from 100 to 500 mg were directly weighed on the quartz platform and covered with powdered silica (500 mg). After, samples were introduced into the quartz tube (previously heated at 1000 °C) by using the automatic homemade system. The heating time ranged from 1 to 15 min. Water vapor required for hydrolysis reaction was generated by water pumped through the system (1 mL min^{-1}). Oxygen (99.6%, White Martins, Brazil) and air used as combustion and carrier gas were evaluated at flow rates from 0.1 to 0.5 L min^{-1}. The gaseous products of pyrohydrolysis reaction were cooled and condensed in a quartz condenser and collected in a volumetric flask containing the absorbing solution. The obtained solution was diluted with water up to 25 mL. The concentration of NH_4OH evaluated as absorbing solution ranged from 10 to 100 mmol L^{-1} (10 mL). After each run, the pyrohydrolysis system was cleaned by heating for 30 min at 1000 °C, with introduction of water (1 mL min^{-1}) and air 0.4 L min^{-1} through the system.

2.5. Microwave-induced combustion and Cl determination by IC

For the reference method, the digestion of samples was performed by MIC using the conditions described in previous works [35,44]. In this sense, about 100 mg of HPC were pressed as pellets and placed on the quartz holder containing a filter paper with 50 μL of 6 mol L^{-1} NH_4NO_3. Then, the quartz holder was inserted into the quartz vessel containing 6 mL of absorbing solution After closing the vessels and capping the rotor, vessels were pressurized with 20 bar O_2 and microwave irradiation was applied (1400 W for 5 min, and 0 W for 20 min for cooling) [23]. The digests were diluted with water up to 25 mL for further analysis by IC. After each run, vessels were cleaned using 6 mL of concentrated HNO_3 under microwave irradiation at 1400 W for 10 min and 0 W for 20 min (cooling). After, vessels were rinsed with ultrapure water.

2.6. Microwave-assisted extraction

Microwave-assisted extraction was also performed in order to evaluate the possible quantitative extraction of chlorine of HPC. Thus, 200 mg of sample were transferred to quartz vessels and water (6 mL) was used as extraction solution. After placing the vessels inside the oven, the microwave irradiation program was started by applying *i*)

1400 W with a ramp of 10 min; *ii*) 1400 for 50 min and *iii*) 0 W for 20 min. The digests were diluted with water up to 25 mL for further analysis by ICP-OES. The cleaning conditions of vessels used after MAE procedure were the same applied after MIC digestion [45].

2.7. Method validation

The validation of the proposed method was carried out according to International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) [46]. The accuracy was evaluated by recovery tests in three levels of concentration (250, 500 and 750 $\mu g\, g^{-1}$), results comparison with reference values and also by the analysis of two CRMs (NIST 1515 and NIST 1547). The limit of quantification (LOQ) was calculated based on the standard deviation of 10 measurements of blanks, taking into account the sample mass and the final dilution of digests. Linearity was expressed as related coefficients (R^2) of analytical curves and the acceptable value of R^2 was 0.9995. The robustness was evaluated by the reliability of analysis taking into account variations of the following parameters: absorbing solution (water or NH_4OH from 10 to 100 mmol L^{-1}), sample mass, the use of powdered silica as retardant of combustion, oxygen flow rate and the reaction time. Repeatability (intra-day precision) and intermediate precision (inter day precision) were estimated based on the relative standard deviation (RSD) after analysis in three days and with three replicated analysis per day. All statistical calculations were performed using the GraphPad InStat (GraphPad InStat Software Inc, Version 3.00, 1997) software and a confidence level of 95% was used.

3. Results and discussion

3.1. European Pharmacopoeia limit test

According to EP, modified celluloses used as pharmaceutical excipients must be submitted to the chorine limit test. This test consists in a visual comparison of the turbidity generated after precipitation of AgCl in sample and standard solutions. The visual aspect of these solutions are shown in Fig. 2.

For the chlorine standard (tube A) it can be observed a turbid solution due to the presence of insoluble $AgCl_{(s)}$ formed in the reaction between NaCl and $AgNO_3$. On the other hand, for the standard chlorine solution of tube B ($CHCl_3$), no turbidity can be observed while for HPC 1 solution (tube C) the turbidity seems to be less pronounced than in standard chlorine solution from NaCl (tube A). Despite the presence of the same amount (3.33 mg L^{-1}) of chloride in the standards solutions from NaCl and $CHCl_3$ (tubes A and B, respectively), the aspect of the resulting solutions is different. These results were expected, once the limit test allows only the identification of inorganic chloride ions, being unsuitable for the identification/determination of organochlorine compounds.

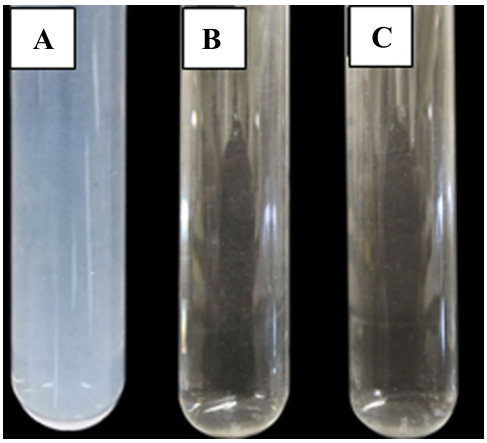

Fig. 2. Visual aspect for the limit test for chlorine: A) standard chlorine (3.33 mg L^{-1}) solution prepared from NaCl, B) standard chlorine solution prepared from $CHCl_3$ (3.33 mg L^{-1}) and C) HPC 1 extract.

3.2. Reference method: MIC and MAE

Sample digestion based on MIC was carried out and a comparison with the proposed combustion followed by pyrohydrolysis method was performed. After digestion by MIC, Cl determination was performed by IC. Results obtained for HPC "1" to "4" were 274 ± 12, 597 ± 36, 401 ± 29 and 430 ± 24 $\mu g\, g^{-1}$, respectively. On the other hand, chlorine extraction based on MAE was not suitable as a method for Cl extraction. The aqueous extracts presented a black solid residue on the bottom of quartz vessels and a brown solution was obtained. For this reason, the Cl determination by IC was not performed. However, the Cl concentration by ICP-OES after MAE was lower than the LOQ (125 $\mu g\, g^{-1}$). Moreover, the carbon concentration in aqueous extracts was in the range of 600 mg L^{-1}. In this sense, it is important the development of a new sample preparation method to obtain digests with low carbon content minimizing possible interferences during total chlorine determination step by ICP-OES.

3.3. Development of the proposed combustion followed by pyrohydrolysis method

Initial experiments were performed to evaluate the type of gas (air or oxygen) used for sample combustion and transportation of generated volatile compounds up to the tube outlet. Both gases were investigated in order to achieve an efficient combustion without remaining of matrix residues on the platform, tube or even able to be transported to the absorption solution. Due to the high kinetics of sample combustion (100 mg) for both gases for different selected flow rates, an incomplete combustion was observed with black solid residues remaining in the quartz tube.

In order to reduce the combustion rate to ensure a complete sample combustion, some strategies can be used as, e.g., by adding some compounds that can act as flame retardants. In this sense, powdered silica has been recently proposed as an useful alternative for this purpose [47]. In the present work, 100 mg of high purity silica were mixed with 100 mg of sample (the same amount of HPC mass), before pyrohydrolysis reaction. When oxygen flow rates of 0.1–0.3 L min^{-1} were evaluated, black solid residues in quartz tube were observed due to incomplete combustion. However, when 0.4 L min^{-1} or higher O_2 flow rate was employed, a complete combustion without solid residues was achieved. Thus, for the subsequent experiments, oxygen flow rate was kept at 0.4 L min^{-1}, while the other parameters (temperature and water flow rate) were not changed. After each test, the residual silica was removed from the quartz platform with a polypropylene spatula and the quartz tube and platform were cleaned by applying an additional full heating program.

3.4. Evaluation of sample mass

Initially, the amount of sample that could be introduced on the platform was evaluated from 100 to 500 mg, using powdered silica as flame retardant and 10 mL of 50 mmol L^{-1} NH_4OH were used as absorbing solution and the digest were diluted up to 25 mL. However, it was necessary to increase the mass of silica due to black solid residues presence after sample preparation. In this way, the amount was fixed in 600 mg.

Due to the limited capacity of the platform, as well as the internal diameter of the reactor, 500 mg was the maximum of sample mass that could be efficiently digested by the proposed system, remaining only the silica in the quartz tube after the combustion. It is important to mention that black residues from HPC were not observed in the quartz

tube, platform or even in the absorbing solution using these conditions.

Despite the efficiency of powdered silica as flame retardant its amount was investigated from 100 to 600 mg, maintaining the sample mass constant (500 mg). When 100–400 mg of powdered silica were used, the combustion was incomplete (similarly when no powdered silica was used), resulting in black residues inside the quartz platform. On the other hand, when 500 or 600 mg of powdered silica were used, samples were digested and no black residues were observed. Thus, further experiments were performed using 500 mg of sample and 500 mg of powdered silica, taking into account that suitable digests were achieved with carbon content lower than 10 mg L^{-1}.

3.5. Evaluation of reaction time

The reaction time is an important parameter that must be evaluated for pyrohydrolysis reaction in order to obtain a suitable matrix digestion and quantitative analyte recoveries. In this sense, the time required for combustion followed by pyrohydrolysis reaction is dependent on the sample matrix and analyte species [39,40]. The pyrohydrolysis reaction time was evaluated from 1 to 15 min using 500 mg of sample 1 and 500 mg of powdered silica at 1000 °C, with oxygen and water flow rates of 0.4 L min^{-1} and 1 mL min^{-1}, respectively, and 10 mL of 50 mmol L^{-1} NH_4OH was used as absorbing solution. Results are shown in Fig. 3.

As can be seen in Fig. 3 and 1 min of reaction time of combustion followed by pyrohydrolysis was not enough for a complete chlorine volatilization (agreement about 78%). However, applying a relatively low reaction time of 5 min, the agreement was considered suitable (higher than 95%) with RSD lower than 5%. It is also important to mention that no memory effects were observed evidencing that the analyte was completely released from the sample matrix. Another important aspect is that blank values obtained after combustion followed by pyrohydrolysis were always negligible.

3.6. Optimization of absorbing solution

For chlorine determination, the absorption solution must be carefully chosen to assure a quantitative recovery and to stabilize the analyte without precipitation or even losses due to the formation of volatile species [39]. It is well known that alkaline solutions are suitable for the absorption of chlorine after pyrohydrolysis [41]. In the present work, experiments were carried out using 10 mL of water or 10, 25, 50 or 100 mmol L^{-1} NH_4OH. Additionally, an experiment was performed without absorbing solution to investigate its necessity for chlorine absorption. Digests were analyzed by ICP-OES and the results

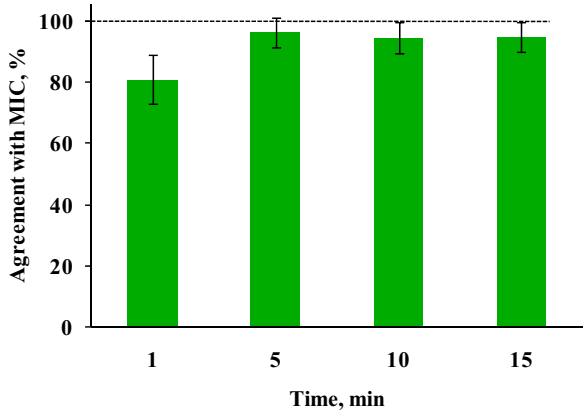

Fig. 3. Evaluation of combustion followed by pyrohydrolysis reaction time for sample 1. The error bars represent the standard deviation, n = 3. Conditions: 500 mg of sample, 500 mg of silica, 1000 °C, oxygen flow rate of 0.4 L min^{-1}, water flow rate of 1.0 mL min^{-1} and 10 mL of 50 mmol L^{-1} NH_4OH as absorbing solution.

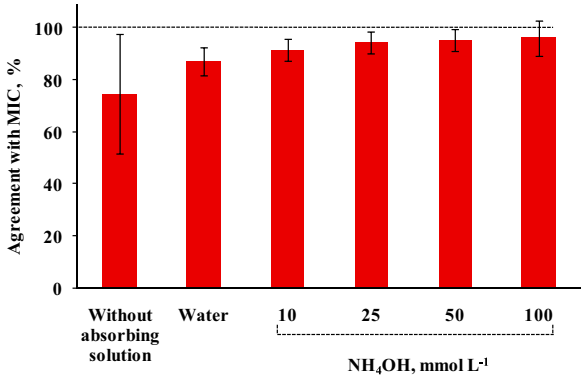

Fig. 4. Influence of the absorbing solution for sample 1 during the combustion followed by pyrohydrolysis reaction. The error bars represent the standard deviation, n = 3. Conditions: 500 mg of sample, 500 mg of silica, 1000 °C, 5 min, oxygen flow rate of 0.4 L min^{-1} and water flow rate of 1.0 mL min^{-1}.

are shown in Fig. 4.

As can be seen in Fig. 4, when no absorbing solution was used, the agreement with results by MIC was below 80%. This unsuitable agreement could be attributed to the analyte losses during absorption. Contrary to previous work [48], the agreement was slightly better when water was used as absorbing solution, but not higher than 90%. On the other hand, when 25, 50 and 100 mmol L^{-1} NH_4OH were used, the agreement ranged from 95% to 102% with RSD values below 6%. Thus, 50 mmol L^{-1} NH_4OH was selected as absorbing solution for subsequent experiments as a compromise condition [39,41].

3.7. Method validation

The accuracy of combustion followed by pyrohydrolysis method was evaluated using two botanical CRMs, NIST 1515 (apple leaves) and NIST 1547 (peach leaves). The experiments were performed using the optimized conditions: 10 mL of 50 mmol L^{-1} NH_4OH as absorbing solution, 500 mg of sample mixed with 500 mg of powdered silica, 5 min of reaction time; reaction temperature of 1000 °C, oxygen flow rate of 0.4 L min^{-1} and water flow rate of 1 mL min^{-1}. The agreement for chlorine in NIST 1515 was 95% while for NIST 1547 was 97%. The accuracy also was evaluated by analyte spike. Thus, chlorine (from NaCl) was added to HPC sample in order to obtain chlorine concentrations of 250, 500 and 750 μg g^{-1}. Suitable recoveries (higher than 95%) and RSD values (around 5%) were obtained for the spike experiments in the three evaluated concentration ranges.

The LOQ was calculated based on the standard deviation of 10 measurements of blanks, taking into account the sample mass and the final volume of digests (500 mg of sample and 25 mL of final volume). The LOQ achieved by chlorine determination by ICP-OES after combustion followed by pyrohydrolysis was 50 μg g^{-1}. The LOQ achieved was considered as suitable being much lower than the limit established by EP (0.5%). It is important to mention that the blanks were always negligible, and the use of powdered silica did not increase the blank values. The correlation coefficient (R^2) of calibration curve was 0.9995, indicating a suitable linear response for the evaluated concentration range (1–10 mg L^{-1}). The proposed method was considered robust since results changed less than 2% even changing the sample mass from 450 to 550 mg, the absorbing solutions from 47 to 53 mmol L^{-1} NH_4OH and the reaction time from 4.5 to 5.5 min. The results for both repeatability and intermediate precision were considered suitable, with RSD around 5%. Based on these results and the quantitative recovery applying the selected conditions, combustion followed by pyrohydrolysis can be considered as suitable for the determination of total chlorine in modified cellulose pharmaceutical excipients.

Table 1
Chlorine content after combustion followed by pyrohydrolysis and determination by ICP-OES. Mean and standard deviation ($\mu g\, g^{-1}$, n = 5).

Sample	Combustion followed by pyrohydrolysis	Reference value[a]	EP limit test
HPC 1	284 ± 8	274 ± 12	Pass the test
HPC 2	576 ± 15	597 ± 36	Pass the test
HPC 3	375 ± 25	401 ± 29	Pass the test
HPC 4	451 ± 20	430 ± 24	Pass the test
NIST 1515	550 ± 37	579 ± 23[b]	–
NIST 1547	349 ± 12	360 ± 19[b]	–

Conditions: 500 mg of sample and 500 mg of powdered silica, oxygen flow rate of 0.4 L min^{-1}, water flow rate of 1.0 mL min^{-1}, 5 min of reaction time and 50 mmol L^{-1} of NH_4OH solution.
[a] microwave-induced combustion and ion chromatography determination.
[b] Certified value.

3.8. Determination of chlorine in modified cellulose pharmaceutical excipients using the proposed method

After optimization of the proposed method it was applied for other HPC samples and the results are shown in Table 1.

Samples used for the development of this work were provided by different manufacturers (synthesis and purification processes used by each one were not informed). According to the results showed in Table 1, chlorine concentration in HPC samples ranged from 284 to 576 $\mu g\, g^{-1}$ (0.0284–0.0576%). These results evidenced that for all HPC evaluated, the chlorine content was lower than limit established by EP (0.5% m/m). It is important to emphasize that the results for chlorine in all HPC obtained by ICP-OES were in agreement (better than 94%) with the results obtained by IC.

The proposed method can be considered a suitable alternative for HPC digestion for further Cl determination by ICP-OES. On the other hand, MIC can also be used, but requires the use of a pressurized system and with more expensive instrumentation.

4. Conclusions

The results showed the applicability of combustion followed by pyrohydrolysis for further total chlorine determination in modified cellulose pharmaceutical excipients by ICP-OES and IC. The combustion followed by pyrohydrolysis system was easily built and feasible for routine analysis. The method showed good performance for modified celluloses even using a high sample mass (500 mg). Using the optimized pyrohydrolysis conditions, quantitative recoveries for chlorine were achieved after 5 min of reaction at 1000 °C and using diluted absorbing solution (50 mmol L^{-1} NH_4OH). Taking into account the advantages of the proposed method, it can be considered as a suitable alternative for the quality control of HPC for further determination of chlorine by ICP-OES with suitable LOQ (50 $\mu g\, g^{-1}$) and high precision (around 5%). The results obtained by the proposed method were also in agreement with those obtained by MIC and IC. It is important to highlight that the use of diluted absorbing solution is an important aspect, once it helps to reduce the reagents consumption and laboratory residues generation. Moreover, blanks were always negligible and only diluted solutions were used as absorbing solution, being in agreement with the green chemistry principles.

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