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Flow and sequential injection methods for the spectrofluorimetric determination of aluminium in pharmaceutical products using chromotropic acid as chromogenic reagent

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Abstract

This work reports rapid and sensitive FI and SI spectrofluorimetric methods for the determination of aluminium in pharmaceutical formulations. The methods are based on the reaction of aluminium with chromotropic acid (CA) in acidic medium to form a water-soluble complex ($\lambda_{ex.} = 360$ nm, $\lambda_{em.} = 385$ nm). The proposed methods were validated in terms of linearity, repeatability, detection limit, accuracy and selectivity. The calibration curves were linear over the range of 0.03–2.0 and 0.1–4.0 mg/l of aluminium using the FI and SI assays, respectively. The repeatabilities ($s_r = 0.8\%$ and 1.1% at 1 mg/l aluminium using the FI and the SI assay, respectively, $n = 12$) were satisfactory. The FI and SI methods proved to be adequately selective and sensitive with respective 3σ limits of detection equal to $c_L = 0.01$ and 0.03 mg/l Al(III). The sampling rates were 120 and 72 h^{-1} with the FI and SI assay. The methods were applied successfully to the analysis of pharmaceutical formulations (tablets and suspensions). The results were in good agreement with those by an official reference method and the nominal values of the pharmaceutical products.
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1. Introduction

Aluminium is a harmful element for humans. Entering the blood, aluminium is accumulating in tissues such as bone, liver and the central nervous system with toxic consequences. When it reaches a certain concentration in the human body it mostly causes either renal failure in patients undergoing treatment with peritoneal dialysis and hemodialysis due to aluminium accumulation in kidneys [1] or diseases from the nervous system such as dementia and encephalopathy [1], Alzheimer's disease [2] and Parkinson's disease [3].

On the other hand, aluminium is used in pharmaceutical products in the form of aluminium hydroxide as an antacid agent that neutralizes or reduces stomach acid. It helps relieve symptoms of excessive stomach acidity in patients with indigestion, heartburn, gastroesophageal reflux disorder, or stomach or duodenal ulcers. In large doses, it can act as a laxative. It may also be used in certain kidney conditions to reduce phosphate levels. Most common side effects include: confusion, decreased alertness, drowsiness or dizziness, headache, loss of appetite, nausea, vomiting, weakness, chalky taste, diarrhea and constipation [4].

Therefore, the determination of aluminium in biological samples and pharmaceutical products is very important and several methods have been developed for its determination in such matrices. Among the batch methods developed are spectrophotometry in Mocaine® Suspension [5]; spectrofluorimetry in glucose injection [6]; laser ablation inductively coupled plasma atomic emission spectrometry and mass spectrometry in Neusilin tablets [7]; reversed-phase high performance liquid chromatography in synthetic renal dialysate, serum, parenteral solution and pharmaceutical-grade organic acid (fumaric acid) [8]; potentiometry in aluminium magnesium syrup [9]; differential pulse voltammetry in synthetic renal dialysate, sodium chloride injection, sucralfate, hydrothorax, blood and urine [10]; post-column derivatization ion chromatography [11].

An ideal analytical method for routine analysis and quality assurance should be automatic, simple, cost-effective, robust, precise and accurate, and have a high sample analysis frequency. Flow injection (FI) and sequential injection (SI) analysis

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are well-established analytical techniques that fulfil the above mentioned demands. They present extensive applicability in pharmaceutical industry analysis, where the quality control of the pharmaceutical formulations is rather critical. These automatic techniques offer significant advantages for the determination and the monitorship of one analyte (e.g. the active ingredient) and therefore can be applied to routine analysis. These advantages are simplicity, high accuracy, cost-effectiveness, repeatability, high sampling rate and enhanced selectivity as a result of the kinetic nature of the techniques. Furthermore, the advantages of SI over FI are the simpler flow manifold, the reduced consumption of sample and reagents, the easier and more convenient variation of the experimental parameters and the greater potential for fluidic handling.

There are few FI methods for determining aluminium in parenteral solutions [12], hemodialysis solutions [13], parenteral nutrition [14], blood serum [15], dialysis solutions [16,17] and dialysis fluids and concentrates [18]. Regarding the pharmaceutical product aluminium hydroxide as an antacid agent no FI and SI method has been reported so far. The present work reports the first FI and SI methods for determining aluminium hydroxide in pharmaceutical formulations as an antacid agent. The methods are based on the reaction of aluminium with chromotropic acid in acidic medium to form a water-soluble complex ($\lambda_{ex.} = 360$ nm, $\lambda_{em.} = 385$ nm). The proposed methods are simple, rapid, sensitive, cost-effective, adequately selective and were applied successfully to the analysis of pharmaceutical formulations (tablets and suspensions).

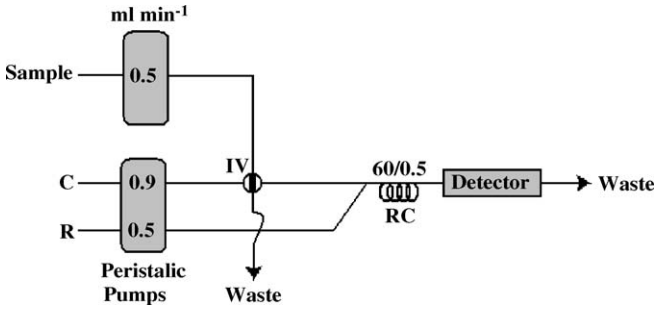

Fig. 1. FI set-up for the determination of Al(III). C: 2×10^{-3} mol/l HCl; R: 7.5×10^{-4} mol/l CA in buffer solution of CH_3COOH/CH_3COONa (pH 4.6); IV: injection valve (sample loop = 200 μl); RC: reaction coil; numbers above coil denote length/i.d. (cm/mm) ratio.

2. Experimental

2.1. Apparatus

The FI system used was a Tecator 5010 analyzer (Tecator, Hoganas, Sweden) with a Tecator chemifold Type III SR manifold, which are shown schematically in Fig. 1. The flow system was 0.5 mm i.d. Teflon tubing throughout, while the aqueous solutions were delivered by Tygon pump tubes.

A schematic diagram of the SI manifold used is shown in Fig. 2. It comprised the following parts: a micro-electrically actuated 10-port valve (Valco, Switzerland) and a peristaltic pump (Gilson Minipuls3, Villiers-le-Bel, France). The hardware

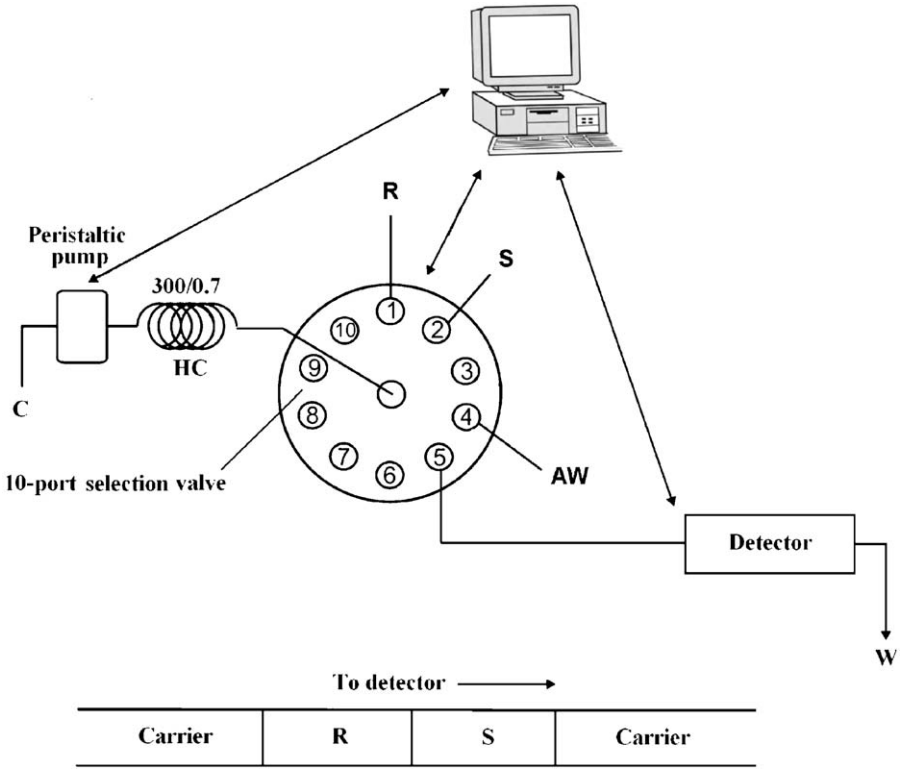

Fig. 2. SI manifold for the determination of Al(III). S: sample; R: 5.0×10^{-5} mol/l CA in buffer solution of CH_3COOH/CH_3COONa (pH 4.6); C: carrier (water); W: waste; AW: auxiliary waste; HC: holding coil; numbers above coils denote length/i.d. (cm/mm) ratio.

was interfaced to the controlling PC through a multi-function I/O card (6025 E, National Instrument, Austin, TX). The control of the system and the data acquisition from the detector were performed through a special program developed in house using the LabVIEW 5.1.1 instrumentation software package (National Instrument, Austin, TX). The response signals from the detector were acquired digitally and the data were saved in ASCII format for further manipulation (peak height measurement, digital filtering, etc.), using a software running in Microsoft Visual Basic® 6.0. The flow system used 0.7 mm i.d. Teflon tubing throughout. Tygon pump tubes of 0.5 mm i.d. were used for aspirating/delivering the solutions.

In both systems spectrofluorimetric detection was performed on a Shimadzu RF-551 flow through detector. The fluorescence intensity was monitored at $\lambda_{em.} = 385$ nm with excitation at $\lambda_{ex.} = 360$ nm.

An Orion (Cambridge, MA, USA) EA940 pH meter was employed for pH measurements with absolute accuracy limits of the pH measurements being defined by NIST buffers.

2.2. Chemicals and reagents

All chemicals were of analytical-reagent grade and were provided by Merck (Darmstadt, Germany), unless stated otherwise, and all the solutions were prepared by using doubly de-ionized water.

The standard solution of chromotropic acid, $c(CA) = 0.001$ mol/l, was prepared daily, by dissolving 0.01 g of chromotropic acid disodium salt (dihydrate) in 25 ml of water.

The standard stock solution of Al(III), $\gamma(Al(III)) = 100$ mg/l, was prepared by dissolving 0.2781 g of $Al(NO_3)_3{\cdot}9H_2O$ in 200 ml of water. The pH of the stock solution (pH 2) was adjusted by adding 0.15 ml concentrated HNO_3 in order to prevent hydrolysis of the ion.

The working buffer solution (pH 4.6) was prepared by mixing 17.3 ml of 1 mol/l CH_3COOH and 5 ml of 2 mol/l NaOH and finally diluted to 100 ml with doubly de-ionized water.

More dilute solutions of Al(III) and CA were prepared daily by dilution with doubly de-ionized water and buffer solution (pH 4.6), respectively, immediately before use.

2.3. FI determination of aluminium in aqueous solutions

The FI setup used is depicted schematically in Fig.1. Two hundred microliters of sample/standard solution Al(III) was injected directly into the 2×10^{-3} mol/l HCl carrier stream C. The carrier/sample stream was merged with the reagent stream R (R: 7.5×10^{-4} mol/l CA, pH 4.6). The flow rates of the C and R streams were 0.9 and 0.5 ml/min, respectively. The water-soluble product was formed on passage of the mixture through a 60 cm reaction coil, 0.5 mm i.d. The cycle time was set to 30 s with 15 s cycle injection time. By using the cycle time of 30 s, 120 injections h^{-1} were made.

The transient signal from the detector was recorded as a peak, the height of which was proportional to the aluminium mass concentration in the sample, and was used for all measurements. The recorded peaks were sharp and the baseline was stable. Five replicate injections per sample were made in all instances.

Table 1
Sequence steps of a complete measurement cycle in the SI system

Time (s)	Pump action	Flow rate (ml/min)	Valve position	Action description
0	Off	–	1	Selection of CA/buffer solution port
10	Aspirate	0.6	1	Aspiration of CA/buffer solution in holding coil
1	Off	–	2	Selection of sample port
15	Aspirate	0.6	2	Aspiration of sample in holding coil
1	Off	–	5	Selection of detector port
25	Deliver	1.4	5	Propulsion of reaction mixture to detector and recording of signal
0	Off	–	5	End of measuring cycle

2.4. SI determination of aluminium in aqueous solutions

The sequence for the determination of Al(III) by the proposed method is shown in Table 1. One hundred microliters of the 5×10^{-4} mol/l CA/buffer solution (pH 4.6) and 150 μl of standards/samples were aspirated in this order in the holding coil (HC), through ports 1 and 2 of the selection valve, respectively. The two zones were propelled to the detector through port 5 at a flow rate of 1.4 ml/min. The water-soluble complex was formed as the reagent/buffer solution and the sample zones were mixed toward the detector. The reaction is instantaneous and therefore no reaction coil (RC) was needed prior to the detector. The cycle time was 50 s (72 injections h^{-1}). When changing between samples or standards, an additional washing step was performed in order to avoid carryover effects; the new sample/standard was aspirated to the HC for 10 s at 2.0 ml/min, and then flushed through port 4 to the auxiliary waste (AW) for 20 s at 2.0 ml/min. Note that in order to avoid overpressure and/or bubble formation in the valve, the pump was stopped for 1 s between changing ports.

The transient signal from the detector was recorded as a peak, the height of which was proportional to the aluminium mass concentration in the samples, and was used for all measurements. Five replicates per sample or standard were made in all instances.

2.5. Determination of aluminium in pharmaceutical formulations

Pharmaceutical formulations (tablets and suspensions) were purchased from a local drug store. Five tablets of the aluminium-containing formulation were weighed, grounded and homogenized in a mortar and pestle. 1.15 g of the powder was accurately weighed and dissolved in 20 ml of de-ionized water. Afterwards 30 ml of 3 mol/l HCl were added and the solution was heated for 30 min in a waterbath at 80 °C. The resultant solution was diluted to 200 ml. The above solution and the suspension solutions were diluted with de-ionized water in order to operate within the linear

range of the method and were filtered through a 0.45 μm membrane (Schleicher & Schuell, Dassel, Germany) before analysis. Appropriate volumes of HCl solution were added in the sample solution in order its final amount concentration to be equal to 2×10^{-3} mol/l before analysis.

Finally, the samples were analyzed using both the above described FI and SI procedures for aqueous solutions. Each sample was injected in five replicates.

3. Results and discussion

3.1. Preliminary studies

A two-line manifold (Fig. 1) was chosen for FI experiments. Since the dilution medium of the samples was HCl, the same acid was used as a carrier in order to minimize any matrix effects. The effect of acidity was examined changing the amount concentration of HCl in the range of 1×10^{-3} to 1×10^{-2} mol/l. The increase in the amount concentration of HCl did not alter the fluorescence intensity of the formed complex until the amount concentration of 2×10^{-3} mol/l. Higher amount concentrations cause the decrease of the fluorescence intensity, probably due to decrease of pH. Therefore, the amount concentration of 2×10^{-3} mol/l HCl was chosen for further experiments. The starting values of the chemical and FI variables were: pH 4.6, $c(\mathrm{CA}) = 1 \times 10^{-3}$ mol/l, $\gamma(\mathrm{Al}^{3+}) = 1$ mg/l, $V = 100$ μl, $l(\mathrm{RC}) = 60$ cm and $q_V(\mathrm{C}) = q_V(\mathrm{R}) = 0.7$ ml/min. The cycle time was set to 30 s with 15 s cycle injection time.

Preliminary experiments using the SI set-up depicted in Fig. 2 show that aluminium reacts with CA instaneously under SI conditions. The formed complex is monitored spectrofluorimetrically ($\lambda_{ex.} = 360$ nm, $\lambda_{em.} = 385$ nm). The initial operating values of the chemical and SI variables were the following: pH 4.6, $c(\mathrm{CA}) = 1 \times 10^{-4}$ mol/l, $V(\mathrm{Al(III)}) = V(\mathrm{CA, buffer}) = 100$ μl, $l(\mathrm{RC}) = 0$ cm, while the reaction mixture was delivered to the detector at a flow rate of 1.2 ml/min. The order of the aspiration of the sample and the buffered reagent solution proved not to be critical. The almost negligible differences in the signals were caused by slightly different dispersion effects on the sample zone according to the order of its aspiration. In addition, stopped-flow experiments showed that the reaction was completed within 5 s.

3.2. Study of chemical and FI variables

The various chemical and instrumental variables of the FI system were studied using the univariate approach at a fixed Al(III) mass concentration of 1.0 mg/l. The starting values of the studied variables were: pH 4.6, $c(\mathrm{CA}) = 10^{-3}$ mol/l, $V(\mathrm{sample}) = 100$ μl, $l(\mathrm{RC}) = 60$ cm and $q_V(\mathrm{C}) = q_V(\mathrm{R}) = 0.7$ ml/min.

The influence of the pH was studied in the range 3.8–5.6. Increase of pH results in better sensitivity until the value of 4.6 and then it is decreased. Therefore, this value was selected for further experiments.

The effect of the amount concentration of CA was studied in the range 10^{-4} to 2×10^{-3} mol/l. Maximum sensitivity, linearity and determination range were achieved at value of 7.5×10^{-4} mol/l and therefore this value was selected. Increasing further the amount concentration of CA the fluorescence intensity decreases as any further production of Al(III)–CA complex was negated by the dispersion effects.

The effect of the sample injection volume was studied in the range 30–230 μl by suitable variation of the loop volume of the injection valve. The peak heights increased non-linearly with increasing sample injection volume in the range 30–200 μl, as the injection volume is inversely proportional to the dispersion of the sample zone, and leveled-off at higher injection volumes. Two hundred microliters was selected for further studies as a compromise between sensitivity and sampling frequency.

The effect of the length of the reaction coil on the determination was examined in the range of 0–300 cm. Maximum sensitivity was achieved at 60 cm. At lower values the sensitivity decreases as the reaction time between Al(III) ion and CA is limited, while at higher values the fluorescence intensity reduces owing to dispersion effects. A length of 60 cm was chosen for further experiments.

The flow rate of the carrier stream is a very important variable in a FI determination, because it influences the dispersion of the sample zone and, thus, the sensitivity of the determination. The flow rate was studied between 0.5 and 1.4 ml/min. The peak heights increased non-linearly with increasing the carrier flow rate, showing a maximum at a flow rate of 0.9 ml/min which was chosen.

The flow rate of the CA stream was studied in the range 0.3–1.4 ml/min. The flow rate of the carrier was kept constant at 0.9 ml/min. As expected, the peak heights increased non-linearly with increasing CA flow rate. The flow rate of 0.5 ml/min was chosen in terms of sensitivity.

3.3. Study of chemical and SI variables

The various chemical and instrumental variables of the SI system were studied using the univariate approach at a fixed Al(III) mass concentration of 1.0 mg/l. The initial experiments were carried out using the following conditions: pH 4.6, $c(\mathrm{CA}) = 10^{-4}$ mol/l, $V(\mathrm{S}) = V(\mathrm{R_1}) = 100$ μl, $l(\mathrm{RC}) = 0$ cm, while the reaction was propelled to the detector at a flow rate of 1.4 ml/min.

The influence of pH on the reaction was studied in the range 3.7–5.6, using a series of standard acetate buffers. When the values of pH vary from 3.7 to 5.4 the fluorescence intensity of the product increases non-linearly and leveled-off at pH 5.4–5.6. Both maximum linearity and determination range were achieved at the range of 4.6–5.0. The fluorescence intensity of the blank solution remains constant until the value of 4.6 and subsequently it increases significantly. Therefore, the value of 4.6 is selected for further experiments as a compromise of sensitivity, linearity and determination range.

The effect of the CA amount concentration was investigated in the range 0.5×10^{-4} to 10^{-3} mol/l where the fluorescence intensity of the product increases non-linearly. Maximum linearity was achieved in the range of 5×10^{-4} to 10^{-3} mol/l, without affecting significantly the sensitivity of the method. The

value of 5×10^{-4} mol/l was selected as at this value the highest ratio of the fluorescence intensities of product/blank solution was noted.

The effect of the sample volume was studied in the range 50–200 μl. The fluorescence intensity increased non-linearly with increasing sample volume and a volume of 150 μl was selected. The effect of the CA solution volume was studied in the range 25–200 μl. The fluorescence intensity increased with increasing CA solution volume and leveled-off at $V(CA) = 100$ μl. At the same time the background signal also increases slightly with the CA solution volume. The value of 100 μl was selected as the ratio of the fluorescence intensity of the product/blank solution was highest at this value.

With the selected sample and buffered reagent volumes and at a propulsion flow rate of 1.4 ml/min, the influence of the reaction coil length was examined in the range 0–75 cm. The length of the reaction coil determined both the degree of overlapping of sample and reagent zones and the period of time that the reaction was allowed to proceed. The results showed that maximum sensitivity was achieved when no reaction coil was used. This means that the reaction is instantaneous and therefore no reaction coil was needed prior to the detector.

3.4. Determination of aluminium with FI assay

Using the FI set-up of Fig. 1 and under the selected chemical and FI variables, a calibration curve was recorded for the determination of aluminium in aqueous solutions. The recorded calibration curve was linear in the range 0.03–2.0 mg/l of Al(III) and was described by the regression equation:

$$F = (446.1 \pm 6.6)\gamma(\mathrm{Al(III)}) + (1.6 \pm 2.5)$$

where F is the fluorescence intensity in arbitrary units and γ(Al(III)) is the mass concentration of aluminium (in mg/l). The relative standard deviation was 0.8% at 1 mg/l. The correlation coefficient was $r = 0.9999$ and the 3σ limit of detection was $c_L = 0.01$ mg/l. All the standards were run in five replicate injections. The measurement frequency was 120 injections h^{-1}.

3.5. Determination of aluminium with SI assay

Using the SI set-up of Fig. 2 and under the selected chemical and SI variables a calibration graph was constructed for aluminium in aqueous solutions. The calibration curve was linear in the range 0.1–4.0 mg/l and is described by the equation:

$$F = (91.4 \pm 1.7)\gamma(\mathrm{Al(III)}) + (20.4 \pm 1.2)$$

where F is the fluorescence intensity in arbitary units and γ(Al(III)) is the mass concentration of aluminium in the aqueous solutions (in mg/l). The correlation coefficient was $r = 0.9998$ and the relative standard deviation was 1.1 % at 1 mg/l Al(III). The 3σ detection limit was calculated to be 0.03 mg/l. The sampling throughput was 72 samples h^{-1}.

3.6. Selectivity study

The selectivity of the proposed methods was evaluated at $\gamma(\mathrm{Al(III)}) = 1$ mg/l, by studying their tolerance against Mg(II) ion which coexists as an active ingredient in the pharmaceutical formulations as well as against implicit interference caused by a placebo mixture of all excipients apart from the active ingredients.

The $\gamma(\mathrm{Mg(II)})/\gamma(\mathrm{Al(III)})$ ratio was 0.40, 1.05 and 0.43 in the pharmaceutical formulations Aludrox® Suspension, Maalox® Suspension and Aludrox® Tablets, respectively. The experimental results showed that the above ratio was 70 and 50 using the FI and SI assay, respectively. Therefore, the proposed methods exhibit selectivity against Mg(II) ion and allow the determination of aluminium in these formulations.

The selectivity was also evaluated by studying the tolerance against placebo mixture. The placebo mixture comprised of methylparaben, propylparaben, saccharin sodium, citric acid, sorbitol liquid, mannitol, starch maize, talc, calcium stearate, benzoic acid and menthol at equal quantities. Three placebo mixtures were prepared at mass concentrations 5000, 10,000 and 25,000 mg/l. These mixtures were added in HCl medium as it was described in Section 2.5 and afterwards diluted in order the final amount concentration of HCl to be 2×10^{-3} mol/l. The resulting solutions were spiked with 1 mg/l Al(III), filtered through 0.45 μm disposable filters and analyzed using the procedures for aqueous solutions. The experimental results showed that a placebo mass concentration of 111.25 mg/l did not cause any interference. It should be noted that the criterion for interference was set at a relative error of less than ±3.0% to the fluorescence intensity of the respective aluminium standard solution ($\gamma = 1$ mg/l).

3.7. Accuracy of the method

In order to validate the accuracy of the methods, placebo solutions ($\gamma = 111.25$ mg/l) were spiked with three different amounts of aluminium covering the mass concentration range of the calibration curve. The solutions were pretreated as it was described in Section 2.5, filtered through 0.45 μm disposable filters and analyzed using the FI and SI assays shown in Figs. 1 and 2. The experimental results shown in Table 2 verified the accuracy of the methods, as the recoveries were in the range 94.0–103.0% using the SI set-up and 98.7–102.0% using the FI set-up.

3.8. Determination of aluminium in pharmaceutical formulations

The applicability of the developed FI and SI methods were checked by analyzing commercially available pharmaceutical formulations containing different amounts of aluminium. The formulations chosen were tablets and suspension Aludrox® (Wyeth Hellas A.E.B.E., Athens, Greece) and suspension Maalox® (Aventis Pharma SpA, Italy). The obtained results are shown in Table 3. The table also includes the comparison of the found values with those stated by the manufactures and those determined by the official reference method of the

Table 2
Determination of aluminium in synthetic samples using the FI and SI set-ups

Synthetic sample	Placebo added (mg/l)	Al(III) added (mg/l)	Al(III) found[a] (mg/l)	100R[b]
(a) FI set-up				
1	111.25	0.50	0.51 ± 0.01[c]	102.0
2	111.25	1.00	0.99 ± 0.02	99.0
3	111.25	1.50	1.48 ± 0.02	98.7
(b) SI set-up				
1	111.25	0.50	0.47 ± 0.01[c]	94.0
2	111.25	1.00	1.03 ± 0.01	103.0
3	111.25	3.00	3.05 ± 0.02	101.7

[a] Mean of five results.
[b] Mean recovery.
[c] Confidence limits ($n = 5$, $P = 0.05$).

Table 3
Determination of aluminium in pharmaceutical formulations using the FI and SI set-ups

Sample	$Al(OH)_3$ found[a]	Ref. method[b]	e_r[c] (%)	Nominal value	e_r[c] (%)
(a) FI assay					
Aludrox® Suspension (mg/5 ml suspension)	304.3 ± 7.8[d]	307.6 ± 4.8[d]	−1.07	307	−0.88
Maalox® Suspension (mg/5 ml suspension)	201.3 ± 4.0	202.8 ± 2.6	−0.74	200	+0.65
Aludrox® Tablets (mg/tablet)	235.2 ± 4.5	232.4 ± 2.7	+1.20	233	+0.94
(b) SI assay					
Aludrox® Suspension (mg/5 ml suspension)	309.4 ± 10.7[d]	307.6 ± 5.5[d]	+0.59	307	+0.78
Maalox® Suspension (mg/5 ml suspension)	198.5 ± 6.1	202.8 ± 3.3	−2.12	200	−0.75
Aludrox® Tablets (mg/tablet)	230.1 ± 5.2	232.4 ± 2.8	−0.99	233	−1.24

[a] Mean of five results.
[b] United States Pharmacopeia 29 reference method.
[c] Relative error.
[d] Confidence limits ($n = 5$, $P = 0.05$).

United States Pharmacopeia 29 [19]. According to this method, 10 ml of the sample is pipetted into a 250-ml beaker. Twenty milliliters of water was added and then in the order named and with continuous stirring 25 ml EDTA sodium salt, 20 ml of CH_3COOH–CH_3COONH_4 buffer (77.1 g CH_3COONH_4 and 57 ml glacial CH_3COOH in 1000 ml de-ionized water) were added and finally heated near the boiling point for 5 min. The resulting solution was cooled and 50 ml of alcohol and 2 ml of dithizone (25.6 mg dithizone in 100 ml alcohol) were added and mixed. Finally, the resulting solution was titrated by 0.05 mol/l $ZnSO_4$ until the color changes from green-violet to rose-pink.

The comparison of the developed FI and SI methods and the reference one verified the accuracy of the proposed method, since the mean relative error, e_r, ranges from −1.07% to +1.20% using the FI set-up and −2.12% to +0.78% using the SI set-up.

3.9. Critical comparison of the FI and SI methods

The critical comparison of the developed FI and SI methods for the determination of aluminium leads to the following advantages/disadvantages:

1. Both methods are sufficiently sensitive and selective for the determination of aluminium in its pharmaceutical formulations.
2. Both methods are automatic. Moreover, the SI method is fully automatic since the instrumental parameters are easily computer-controlled.
3. The FI method offers higher sampling rate than the SI method (120 samples h^{-1} versus 72 samples h^{-1}).
4. The SI method offers wider determination range (100–4000 $\mu g\,l^{-1}$ using SI assay while 30–2000 $\mu g\,l^{-1}$ using FI assay).
5. The FI method is more sensitive than the SI method comparing the slopes of their calibration graphs.
6. The SI method is more cost-effective due to the low reagents consumption.

4. Conclusions

The development and the optimization of a reliable FI and SI assay for the automatic quality control of aluminium-containing formulations is presented. The proposed methods are simple, rapid, accurate and adequately sensitive. The linearity and the selectivity are satisfactory, while the FI method offers higher sampling frequency than the SI method. The results from the analysis of real samples compared favorably to an official reference method suggested by American Pharmacopoeia USP 29 and the nominal value of the formulations. As the proposed methods are adequately sensitive, on-going research is in

progress for application of methods to biological samples which require higher sensitivity.

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