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Artigo

Versatility of a Multicommuted Flow System in the Spectrometric Determination of Three Analytes

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Versatilidade de um Sistema em Fluxo Multicomutado na Determinação Espectrofotométrica de Três Analitos

Resumo: Neste trabalho foi desenvolvido um sistema de multicomutação em fluxo para a determinação de diclofenaco de sódio, ácido acetilsalicílico e fluoretos em injetáveis, comprimidos e águas de torneira, respectivamente. Para o diclofenaco de sódio, o método usado foi baseado em sua reação com permanganato de potássio (20 - 80 mg L^{-1}).Para o ácido acetilsalicílico, o método usado foi baseado na reação de Trinder após hidrólise alcalina do analito (25 - 100 mg L^{-1}). Para os íons fluoreto, o método usado foi o bem conhecido SPADNS (0,4 – 1,6 mg L^{-1}).Os resultados foram comparados com seus respectivos métodos de referência e, aplicando o teste-*t* emparelhado, nenhuma diferença estatisticamente significativa foi verificada entre eles ao nível de confiança de 95%. Após a otimização das variáveis do sistema analítico, ele foi capaz de analisar 80, 72 e 68 amostras h^{-1} contendo diclofenaco de sódio, ácido acetilsalicílico e fluoretos, respectivamente com geração de resíduos reduzida de acordo com os princípios básicos da química verde.

Palavras-chave: Sistema de injeção em fluxo; multicomutação; injetáveis; comprimidos; águas de torneira.

Abstract

In this work was developed a multicommuted flow system for determination of sodium diclofenac, acetylsalicylic acid and fluoride ions in ampoules, tablets and tap waters, respectively. For sodium diclofenac the method used was based on its reaction with potassium permanganate (20 - 80 mg L^{-1}). For acetylsalicylic acid the method used was based on Trinder's reaction after alkaline hydrolysis of the analyte (25 - 100 mg L^{-1}). For fluoride ions the method used was the well-known SPADNS (0.4 - 1.6 mg L^{-1}). Results were compared with their respective reference methods and, by applying the paired *t*-test, no statistic difference between them was verified at the 95% confidence level. After optimization of the variables of the analytical system, it was able to analyze 80, 72 and 68 sample h^{-1} containing sodium diclofenac, acetylsalicylic acid and fluoride, respectively with reduced waste generation according to basic principles of green chemistry.

Keywords: Flow-injection system; multicommutation; ampoules; tablets; tap waters.

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Versatility of a Multicommuted Flow System in the Spectrometric Determination of Three Analytes

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1. Introduction

As consequence of strict regulations, the pharmaceutical industry is now seeking development of fast, less expensive and accurate analytical methodologies.[1] These new methodologies aim to: guarantee drug content, identify adulterants in pharmaceutical formulations which represent health risks,[2] and reduce (or replace)

materials harmful to human health and environment.[3] In this sense, flow systems are excellent tools for dealing with solutions in wet chemical analysis.[4] Among them, Multicommuted Flow Analysis (MCFA) can be considered an evolution of the flow injection concept towards Green Analytical Chemistry (GAC),[5] since the addition of the reagent to the sample zone occurs only in a strict amount when required in the analytical procedure.[4,6] MCFA design uses solenoid valves on binary sampling operation mode that makes its operation straightforward, very versatile, robust and enables the development of green analytical methodologies with low consumption of reagents and samples.

MCFA is a hot trend in automation and its principles can be applied in all kinds of measurements. Literature repports several techniques using MCFA and among them spectroanalytical (molecular spectrophotometry, chemiluminescence, fluorescence, atomic spectrometry and vibrational spectrometry) are often chosen by researchers as strategies;[8] followed by electroanalytical.[7] In terms of applications, environmental analysis is the main field, followed by food and beverages, pharmaceutical, biochemical and industrial using sample dilution, titrations, separation/concentration, sample stopping, sequential/ simultaneous determinations and miscellaneous .

Sodium diclofenac is the sodium salt of 2-[(2,6-dichlorophenyl)amino] benzene acetic acid and is relatively safe and effective non-steroidal anti-inflammatory drug (NSAID)with pronounced anti-rheumatic, anti-inflammatory, analgesic and antipyretic properties.[9] Sodium diclofenac inhibits reversibly Cyclooxygenases (COX)[10] enzymes and consequently the biosynthesis of prostaglandins. Most of prostaglandins are enzymatically derived from arachidonic acid by the metabolic pathway of the ‘‘arachidonic acid cascade’’ which is associated with inflammatory processes.[11]

2-acetoxy-benzoic acid is the systematic name (IUPAC) for acetylsalicylic acid (ASA), more popularly known as aspirin. This is one of the oldest medicines, and play an important role in modern therapeutics with use for headaches, fever, muscular pain, and inflammations due to arthritis or injury.[12] ASA acts as an acetylating agent of the serine residue in the active site of the prostaglandin-endoperoxide synthase enzyme (PTGS) which inhibits irreversibly the synthesis of prostaglandin and thromboxanes.[13,14]

Fluoride (F^-) ions occur in almost all waters and its presence cause significant effects in human beings drinking it[15-18] especially the control and diminution of dental caries.[19] After ingestion fluoride ions are readily transmitted through the bloodstream and deposited in mineralized tissues such as bones and teeth. The effectiveness of fluoride ions in preventing dental caries is due to three factors: (i) strengthening of the dental enamel by reducing its solubility to the acid attack, inhibiting demineralization, (ii) favoring of the remineralization and (iii) changing and decreasing the number and cariogenic potential of microorganisms.[20]

The goal of the present paper is to propose a unique multicommuted flow analyzer which is able to determine sodium diclofenac in ampoules, ASA in tablets and fluoride (F^-) ions in tap waters by changing only operational parameters for three different reactions. The choice of analytical determinations previously mentioned have been motivated by the importance these analytes share.

2. Material and methods

2.1. Reagents and solutions

All chemicals were of analytical grade and water recently deionized by a Milli-Q (Millipore) system was employed throughout.

For determination of sodium diclofenac: Stock solution of 1000 mg L^{-1} sodium diclofenac (Sigma, St. Louis, MO, USA) was

prepared dissolutioning a suitable amount of the salt in deionized water. The calibration solutions with five levels of concentration of sodium diclofenac (20.0 - 80.0 mg L^{-1}) were prepared by suitable dilution from stock solutions in deionized water. An approximately 0.3 mmol L^{-1} $KMnO_4$ (Merck, Darmstadt, Germany) solution was prepared by dissolution of suitable amount of the salt in approximately 100.0 mmol L^{-1} H_2SO_4 (Merck, Darmstadt, Germany) aqueous solution. Deionized water was used as carrier solution.

For determination of ASA: Stock solution of 1000 mg L^{-1}ASA (Sigma, St. Louis, MO, USA) was prepared by dissolution of suitable amount of the acid in 10.0 mL of 1.0 mol L^{-1} NaOH (Merck, Darmstadt, Germany) solution followed by heating during 10 minutes for complete hydrolysis and the volume was up to 250 mL with deionized water. The calibration solutions with five levels of concentration of ASA (25.0 - 100.0 mg L^{-1}) were prepared by suitable dilution from stock solutions in deionized water. The Trinder's reagent was prepared dissoluting of 1.0 g of $Fe(NO_3)_3 \cdot 9H_2O$ (Merck, Darmstadt, Germany) in deionized water and the volume was up to 100 mL. An approximately 100.0 mmol L^{-1} HNO_3 (Merck, Darmstadt, Germany) solution was used as carrier solution.

For determination of fluoride ions: Stock solution of 100 mg L^{-1} fluoride was prepared by dissolution of suitable amount of the NaF (Sigma, St. Louis, MO, USA) in deionized water. The calibration solutions with five levels of concentration of fluoride (0.4 - 1.6 mg L^{-1}) were prepared by suitable dilution from stock solutions in deionized water. The zirconium-SPADNS reagent was prepared by dissolution of suitable amount of $ZrOCl_2 \cdot 8H_2O$ (Merck, Darmstadt, Germany) and SPADNS (1,8-Dihydroxy-2-(4-sulfophenylazo)naphthalene-3,6-disulfonic acid trisodium salt) (Sigma, St. Louis, MO, USA) in 4.0 mol L^{-1} HCl (Merck, Darmstadt, Germany) aqueous solution. Deionized water was used as carrier solution.

2.2. Samples

Five brands of ampoules drugs with a nominal content of 25 mg mL^{-1} of sodium diclofenac and five brands of tablets with a nominal content of 500 mg of ASA were purchased from local drugstores. Five tap water samples were collected in five points in the city of São Luís, Maranhão, Brazil.

Ampoules samples, before analysis, were only suitably diluted in deionized water in order to read analytical signals in the linear response of the method.

Twenty tablets containing ASA were grinded in a mortar to yield a fine powder and the average mass of them was dissolved in 10 mL of 1.0 mol L^{-1} NaOH solution and boiled for 10 minutes for complete hydrolysis. Afterwards, a filtration to remove the insoluble particles was performed with ash less filter paper (Whatman n° 40). The resulting solution was diluted to a final volume (100 mL) with deionized water. This solution was suitably diluted in deionized water in order to read analytical signals in the linear response of the method.

Tap water samples were collected and preserved according to American Public Health Association recommendations to determine fluoride in water samples.[21] Before analysis were added 0.5% (wv^{-1}) sodium arsenite, $NaAsO_2$ (Merck, Darmstadt, Germany) solution to remove residual chlorine interference, suitably filtered in order to remove eventual insoluble particles with ash less filter paper (Whatman n° 40) and then evaporated up to 1/3 of their initial volume.

2.3. Multicommutation system

Measurements were performed in a 700 Plus FEMTO spectrophotometer with quartz cells with 1 cm of optical path. The multicommutation system depicted in Figure 1 (a)comprises three three-way solenoid

valves 161 T031 (NResearch Inc., West Caldwell, USA) and a IPC-8 peristaltic pump (Ismatec, Zurich, Switzerland) with polyethylene pumping tubes (i.d. = 0.8mm) propulsion system. The different components of the multicommuted flow system were connected with polyethylene tubing (i.d. = 0.8mm) and a reaction coil (50, 60 and 75 cm for sodium diclofenac, ASA and fluoride ions, respectively) was made with the same polyethylene tube. A four-way homemade confluence connector in acrylic was also used. Control of the analytical system, data acquisition and processing were carried out by means of a Pentium II microcomputer running a software written in QuickBasic Version 4.5. A PCL711S (Advantech corp. Ohio, USA) interface was used to generate control signals of the solenoid valves which were sent to power interface based on UNL2803 integrated circuit.

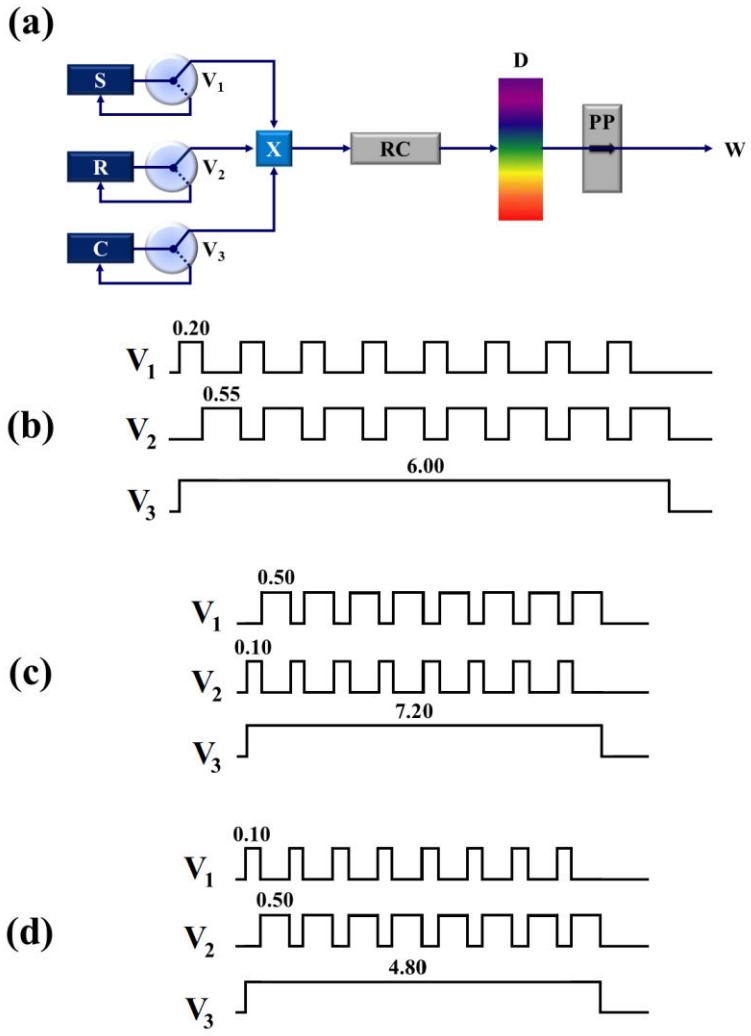

Figure 1. (a) Schematic diagram of the multicommutation system, (b) Sodium diclofenac, (c) ASA and (d) fluoride determination time diagrams for the samples. V_1, V_2, V_3 – Solenoid valves; S – sample; R – reactant; C – carrier fluid; PP – peristaltic pump; M – Mixing zone (reactor); D – detector; X – confluence point; W – waste. For determination of sodium diclofenac: R, potassium permanganate; C, deionized water. For determination of ASA: R, Trinder's reagent; C, 0.1 mol L^{-1} HNO_3 aqueous solution. For determination of fluoride: R, SPANDS + $ZrOCl_2$; C, deionized water. Time intervals (in seconds) T_1, T_2 and T_3 correspond to V_1, V_2 and V_3 valves respectively

2.4. Multicommutation procedure

Three three-way solenoid valves (V_1, V_2, and V_3) assessment to the system was enabled by the sample and reagents: V_1 for the sample (S), while V_2 and V_3 inserted the reactant (R) and carrier fluid (C), respectively.

In the analytical cycles as presented in Figure 1(b), (c) and (d) all valves are switched off and the carrier solution (C) is aspirated through solenoid valve V_3 towards detector in order to obtain the baseline. Afterwards, V_1, V_2 and V_3 are switched on, but V_1 and V_2 are alternately switched on while V_3 remains always switched on up to a defined number of cycles (Figure 1 (b), (c) and (d)) and then all valves are switched off. This sequence of actions alternately inserts in the analytical course defined amounts of sample and reactant which are directed towards the confluence (X) and reaction coil (RC).

2.5. Multicommutation methods

For determination of sodium diclofenac: in aqueous solution, potassium permanganate reacts with sodium diclofenac in acid medium to produce Mn^{2+} ions which exhibits maximum absorbance at 450 nm. The absorbance is proportional to sodium diclofenac concentration on the sample. Semi reactions are showed in Figure 2 (a).

For determination of ASA: a first step of alkaline hydrolysis is carried out in order to produce salicylate ions. The excess of is NaOH is neutralized and the salicylate ions react with Fe (III) ion to produce a 1:3 violet complex with maximum absorption at 525 nm (Trinder's reaction), as showed in Figure 2 (b).

For determination of fluoride: first step zirconium reacts with SPADNS to produce a zirconium-dye lake. Then, fluoride ions react with the zirconium-dye lake, dissociating a portion of it into a colorless complex anion ($[ZrF_6]^{2-}$) and the dye (maximum absorption at 570 nm) showed in Figure 2 (c). As the amount of fluoride increases, the color produced becomes progressively lighter.[21]

2.6. Reference methods

According to Brazilian pharmacopoeia,[22] the reference methods for determinating sodium diclofenac and ASA are based on direct UV spectrophotometric measurements at 258 nm in methanol medium (solvent/blank) and acid-base back titration using phenol red as indicator respectively. For determination of fluoride the reference method is the SPADNS which is based on spectrophotometric measurements at 570 nm in aqueous solution (solvent/blank).[21]

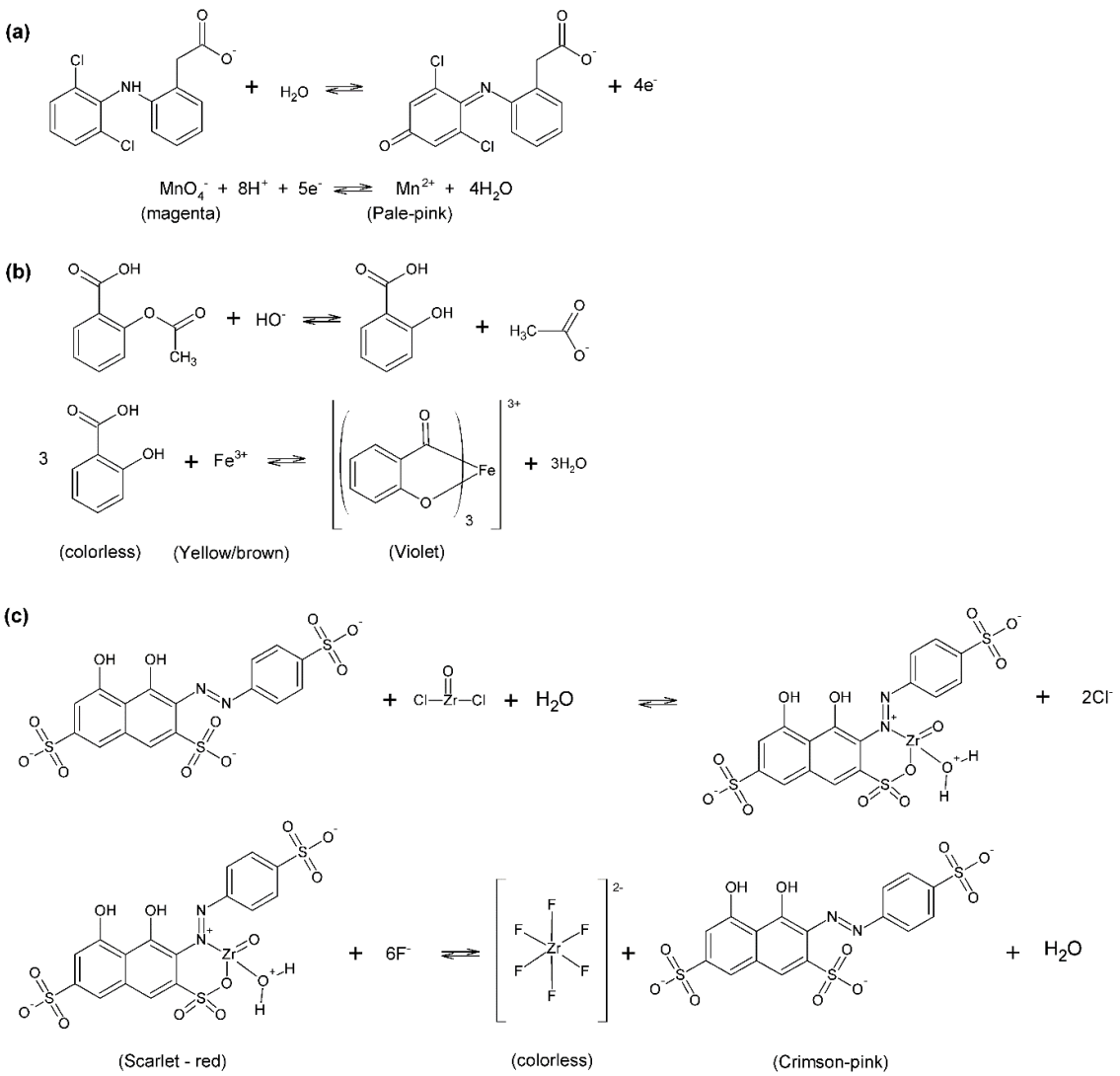

Figure 2. Reactions for determination of: (a) sodium diclofenac, ASA (b) and (c) fluoride ions

3. Results and discussion

3.1. Optimization of multicommutation system

Opening time of V_1 and V_2, flow rate, concentration of reactant and carrier fluid, number of cycles and coil length have been optimized through means of univariate method. The influence of each variable in the absorbance of a defined standard solution on the analysis is observed through a variable variation in a defined range. This procedure was carried out aiming to find the combination of factors which provide the best compromise between sensitivity and reproducibility of the analytical signal, as well reach lower values of limit of detection (LOD) and limit of quantification (LOQ). For each analyte these factors have been evaluated in different ranges and a unique value was chosen as showed in Table 1.

Table 1. Selected parameters for the proposed multicommutation system

Analyte	Parameter	Evaluated range	Selected value
Sodium diclofenac[a]	V_1 time (s)	0.20 - 0.50	0.20
	V_2 time (s)	0.25 - 0.55	0.35
	Flow rate (mL min^{-1})	2.5 - 4.0	3.5
	Reactant (mol L^{-1})	(1.0 - 4.0) x 10^{-4}	3.0 x 10^{-4}
	Carrier fluid (mol L^{-1})	-	-
	Number of cycles	6 - 12	8
	Reaction coil (cm)	0.10 - 0.50	100
ASA[b]	V_1 time (s)	0.10 - 0.70	0.50
	V_2 time (s)	0.10 - 0.50	0.10
	Flow rate (mL min^{-1})	2.5 - 4.0	3.5
	Reactant (mol L^{-1})	0.05 - 0.4	0.1
	Carrier fluid (mol L^{-1})	0.01 - 0.4	0.1
	Number of cycles	8 - 14	12
	Reaction coil (cm)	60 - 120	80
Fluoride[c]	V_1 time (s)	0.30 - 0.50	0.50
	V_2 time (s)	0.10 - 0.30	0.10
	Flow rate (mL min^{-1})	1.0 - 4.0	2.8
	Reactant (mol L^{-1})	(1.68 - 3.36) x 10^{-3}[d] (0.40 - 1.66) x 10^{-3}[e]	3.36 x 10^{-3}[d] 1.66 x 10^{-3}[e]
	Carrier fluid (mol L^{-1})	-	-
	Number of cycles	8 - 14	8
	Reaction coil (cm)	50 - 125	75

[a]Using a 50.0 mg L^{-1} sodium diclofenac solution, [b]Using a 62.5 mg L^{-1} ASA solution, [c]Using a 1.00 mg L^{-1} fluoride solution, [d]SPADNS, [e]$ZrOCl_2 \cdot 8H_2O$.

3.2. Analytical curves and figures of merit

A linear relationship between the absorbance (analytical response) and the concentrations of the analyte in the calibration solutions was observed for the three cases. The confidence intervals of the calibration model parameters ($\hat{y} = \alpha + \beta X$) at the 95% confidence level for the three analytes are shown in Table 2. Since the confidence intervals for estimated parameters of the calibration models contain the ''zero'', they are considered statistically significant.[23]

Table 2. Confidence intervals for the parameters of the linear model and figures of merit for each analyte

Analyte	Confidence intervals for the parameters of the model ($\hat{y} = \alpha + \beta X$)		Values of merit figures (x 10^{-1} mg L^{-1})	
	$\alpha \pm t_{13}\, s(\alpha)$	$\beta \pm t_{13}\, s(\beta)$	LOD	LOQ
Sodium diclofenac	-0.0767 ± 0.0008	0.0108 ± 4.43 x 10^{-6}	0.10	0.33
ASA	0.0090 ±0.0002	0.0061 ± 9.29 x 10^{-7}	0.57	1.90
Fluoride	0.0040 ± 0.0003	0.1367 ± 9.25 x 10^{-5}	0.02	0.07

In order to validate the linear calibration models an analysis of variance (ANOVA) was done. For this purpose, the *F*-test for lack of fit and for significance of regression were applied.[24] The analytical curves were constructed based on three genuine repeated measurements in five levels of concentration. The values of regression, residual, lack of fit and pure error were calculated using the mean squares (MS) presented in Table 3.

In all cases, the values of $MS_{lof}/MS_{pure\ error}$ are smaller than the point of *F*-distribution at a 95% confidence level with equivalent freedom degrees (3 and 10, respectively). This indicates that there is no evidence off it lack for linear models, in other words, a good fit. In all cases, the values of $MS_{regression}/MS_{residual}$ are much larger than the point of *F*-distribution at a 95% confidence level with equivalent degrees of freedom (1 and 13, respectively). This indicates that the results of linear regressions are significant.

Table 3. Analysis of variance for the fit of a linear model ($\hat{y} = \alpha + \beta X$) of the analytical curves

Analyte	Source	Degrees of freedom	Mean square (MS)	$\frac{MS^{c}_{lof}}{MS_{pure\ error}}$	$\frac{MS_{regression}}{MS_{residual}}$
Sodium diclofenac	Regression	1	7.87×10^{-1}	1.97[a]	2.77×10^{7}[b]
	Residual	13	2.84×10^{-8}		
	Lof[c]	3	4.58×10^{-8}		
	Pure error	10	2.32×10^{-8}		
ASA	Regression	1	3.92×10^{-1}	1.35[a]	2.01×10^{8}[b]
	Residual	13	1.95×10^{-9}		
	Lof[c]	3	2.44×10^{-9}		
	Pure error	10	1.80×10^{-9}		
Fluoride	Regression	1	5.05×10^{-2}	1.55[a]	1.02×10^{7}[b]
	Residual	13	4.95×10^{-9}		
	Lof[c]	3	9.56×10^{-9}		
	Pure error	10	6.15×10^{-9}		

[a]$F_{critical}$ = 3.71, [b]$F_{critical}$ = 4.67, Both critical values are at the 95% confidence level, [c]lof = lack of fit.

Since the analytical curves were validated (no lack of fit and significant regression), figures of merit were estimated according to IUPAC recommendation.[25] Twenty measurements of the blank was used to estimate limit of detection (LOD) and limit of quantification (LOQ) for each analyte. Table 2 shows that the proposed method presented low values LOD and LOQ and good performance in terms of linear ranges of response.

3.3. Analytical determinations

After variables of the multicommuted system optimization, validation of the analytical curves and estimation of merit figures the proposed method was applied in the determination of sodium diclofenac, ASA and fluoride in ampoules, tablets and tap water, respectively. As presented inTable 4the proposed method and reference methods have yielded similar results in the determination of the three analytes. In fact, there is no statistic difference between the results through applying the paired *t*-test at the 95% confidence level was verified. The proposed method presented a precision as good as the reference method revealed by the closer values of overall relative standard

deviation (R.S.D.) (n = 5). This satisfactory precision can be ascribed to the optimization of the variables.

Table 5 presents selected analytical features of the proposed multicommutation system which represents a good performance in terms of LOD, overall R.S.D., working rage, sampling rate and consumption of reactants and samples when compared to batch reference methods.

Table 4. Results of the determinations of sodium diclofenac, ASA and fluoride by using proposed and reference methods

Samples	Proposed Method	Reference Method
Sodium diclofenac	25 mg mL^{-1} nominal content	
(1)	24.9 ± 0.1	25.0 ± 0.2
(2)	24.8 ± 0.1	24.7 ± 0.1
(3)	24.9 ± 0.2	25.0 ± 0.1
(4)	24.9 ± 0.1	24.8 ± 0.1
(5)	24.7 ± 0.2	24.9 ± 0.2
Overall R.S.D. (%)	0.60	0.62
ASA	500 mg nominal content	
(1)	497.4 ± 1.9	497.2 ± 2.5
(2)	495.1 ± 1.5	495.3 ± 2.1
(3)	498.6 ± 1.6	498.2 ± 2.4
(4)	496.0 ± 1.7	496.3 ± 2.3
(5)	499.0 ± 1.8	498.2 ± 2.2
Overall R.S.D. (%)	0.34	0.47
Fluoride	mg L^{-1}	
(1)	0.33 ± 0.02	0.35 ± 0.01
(2)	0.34 ± 0.01	0.32 ± 0.02
(3)	0.13 ± 0.02	0.17 ± 0.02
(4)	0.35 ± 0.01	0.32 ± 0.01
(5)	0.21 ± 0.01	0.25 ± 0.02
Overall R.S.D. (%)	5.45	5.76

Table 5. Proposed multicommutation system analytical features for each analyte

Parameter	Sodium diclofenac	ASA	Fluoride
Working range (mg L^{-1})	20 - 80	25 – 100	0.4 - 1.6
Overall R.S.D. (%)	0.60	0.34	5.47
Sampling rate (sample h^{-1})	80	72	68
Sample consumption (μL)	20.4	29.2	187.0
Reagent consumption (μL)	11.6	5.8	37.0
Carrier consumption (μL)	350.0	420.0	224.0
Waste generation (μL)	382.0	455.0	448.0
Method	Permanganate's reaction	Trinder's reaction	SPADNS' reaction
Carrier fluid	Deionized water	100.0 mmol L^{-1} HNO_3 solution	Deionized water

4. Conclusions

This work demonstrated the viability of the use an unique multicommuted system determination of sodium diclofenac, ASA and fluoride in ampoules, tablets and tap waters, respectively. The binary way operation of the solenoid valves, which were inserted discrete volumes of sample and reagent, showed to be effective for determination of the three analytes. Its operation is easy and robust that makes possible to determine others analytes changing two parameters: the reaction coil (physical) and the number of cycles (operational). Emphazing the proposed system is not a "polyvalent system" is very important because variables should be optimized according to reactions used, even using the same manifold.

Variables optimization allowing fast homogenization, low consumption of sample and reagents and low waste generation according to basic principles of GAC. Moreover, provided analytical curves with suitable linear range of response and sensitivity as revealed by low values of LOD and LOQ, for accurate and precise determination of the three analytes. Unfortunately, there are not in literature others flow analyzers that use this paper's reactions to do a fair analytical performance comparison.

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