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Article

Determination of Metoprolol, Acebutolol and Propranolol in Pharmaceutical Formulations using the Same SIA System

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Apresenta-se um sistema mecanizado para a determinação de β–bloqueadores em preparações farmacêuticas. Através da utilização da técnica de Análise de Injeção Sequencial (SIA) foi obtida uma metodologia simples, econômica e versátil, adaptável a todo o tipo de controlo farmacêutico envolvendo estas substâncias. As amostras não necessitam de pré-tratamento devendo apenas ser dissolvidas em ácido antes de serem analisadas. Foram obtidas faixas lineares de trabalho para o metoprolol (40,52 - 250 mg L^{-1}), acebutolol (32,85 - 140 mg L^{-1}) e propranolol (16,58 - 120 mg L^{-1}). O desvio padrão relativo foi inferior a 5 % em todas as determinações. A metodologia foi aplicada em comprimidos, injectáveis e cápsulas de libertação prolongada. Os excipientes que, usualmente, são empregues nas preparações farmacêuticas, não interferem. Os resultados obtidos usando a metodologia proposta foram estatisticamente comparáveis com os obtidos pelos métodos de referência (nível de confiança de 95%). O sistema SIA produz apenas 2,5 mL de efluentes por determinação enquanto que os métodos de referência originam 140 mL.

A mechanized system for the determination of β–blockers in pharmaceutical formulations is presented. Using the Sequential Injection Analysis (SIA) technique it was achieved a simple, economical and versatile methodology adaptable to any pharmaceutical control involving these substances. It does not require any pre-treatment for the samples, as they must only be dissolved in acid before analysis. Linear calibration plots were obtained for metoprolol (40.52 - 250 mg L^{-1}), acebutolol (32.85 - 140 mg L^{-1}) and propranolol (16.58 - 120 mg L^{-1}). A R.S.D. lower than 5% was attained. The methodology was used in tablets, injections and prolonged-release capsules. Common excipients used in pharmaceuticals do not interfere. Statistical comparison of the results obtained with the proposed methodology and with the official methods showed good agreement (95% confidence level). SIA system produces only 2.50 mL of effluents per determination whereas the reference methodologies consume around 140 mL.

Keywords: beta-blockers, sequential injection analysis, SIA, spectrophotometry, pharmaceutical formulations

Introduction

Beta-adrenergic blocking agents, commonly known as β-blockers, are very effective in the treatment of high blood pressure (hypertension). Some β-blockers are also used to angina relieve (chest pain), to correct cardiac arrhythmias and in hypertrophic cardiomyopathy. In addition, β-blockers are also used to prevent migraine headaches, reduce symptoms of hyperthyroidism and treat some forms of tremor. Several types of β-blockers exist, which differ in receptor selectivity, lipophilic characteristics and intrinsic sympathomimetic activity.[1] Metoprolol, acebutolol and propranolol belong to subgroups with different pharmacodynamics properties. Metoprolol is a β-blocker with cardioselectivity and without sympathomimetic activity, acebutolol has cardioselectivity and intrinsic sympathomimetic activity while propranolol has neither cardioselectivity nor intrinsic sympathomimetic activity.

The therapeutical relevance of these compounds justifies the development of methods for their determination in pharmaceutical formulations including spectrophotometry,[2-4] chromatography,[5-8] atomic absorption spectrometry,[9]

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angle synchronous fluorimetry,[10] potenciometry[11] and colorimetry.[12] Some of the mentioned techniques are sensitive but costly[9,10] or require a laborious sample clean-up procedure prior to analysis and use organic solvents.[5-8] Others involve rigorous temperature control and prolonged stop periods.[2-4,12]

The appearance of other methodologies with characteristics such as rapidity, efficiency, reliability and economically, both in running cost and in sampling consumption, is of great importance to the field of pharmaceutical analysis in quality control of drug dosage forms, in continuous monitoring of drug production processes, or for dissolution studies. Flow methodologies fulfil these above-mentioned requirements and present advantageous features for the automation of wet chemical assays. They have been widely applied in pharmaceutical analysis,[13,14] including a few dedicated to metoprolol[15] and propranolol.[16-19] Regarding acebutolol, and to our best knowledge, no method based on the use of these techniques has been reported.

The main goal of this work was the development of an automatic system for the determination of metoprolol, propranolol and acebutolol that could constitute an economic and expeditious alternative to the available procedures.

For that it was selected sequential injection analysis[20] (SIA) a well established, powerful, sample handling procedure. Its computer-controlled nature allows modifying the most relevant analytical parameters at run-time assuring a great operational flexibility and the establishment of distinct analytical strategies without physical reconfiguration. Moreover the multiposition selection valve, the core of the system, allows the clustering of all type of devices, such as dissolution apparatus, mixing chambers, dialysis units, in each of its inlets. These, along with the bi-directional nature of fluid handling and stopped-flow periods, can extend the scope of SIA to encompass a variety of on-line sample manipulations. The referred features along with robustness, ease of operation and low reagent consumption inherent to SIA guarantee a noteworthy analytical potential for its application in the pharmaceutical analysis and so in the determination of the three β-blockers herein presented.

Experimental

Reagents and solutions

All solutions were prepared with analytical reagent grade, high purity water (milli Q) with a specific conductivity of < 0.1 μS/cm. All chemicals were of analytical reagent grade. A 1.5 mol L^{-1} sulphuric acid solution was used as carrier and was prepared by dilution, in water, of the required 98% (m/m) sulphuric acid solution volume (Merck). A 7.5×10^{-3} mol L^{-1} potassium permanganate solution was prepared by dilution with 1.5 mol L^{-1} sulphuric acid from the 7.5×10^{-2} mol L^{-1} stock solution prepared from the solid (Riedel-de-Haën) in the same sulphuric acid. Metoprolol (Sigma), acebutolol (Sigma) and propranolol (Sigma) working standard solutions were prepared by dilution with 1.5 mol L^{-1} sulphuric acid of the 1 g L^{-1} stock solution, prepared in 1.5 mol L^{-1} sulphuric acid. The solutions of commercially available pharmaceutical preparations were prepared by dissolving the required amounts of powdered tablets or by diluting the required volume of the liquid formulation in a 1.5 mol L^{-1} sulphuric acid solution. The sample solutions were analysed by the developed SIA procedure without any pre-treatment.

Apparatus

SIA flow system (Figure 1) consisted of a Gilson Minipuls 3 (VilliersleBel, France) peristaltic pump, equipped with a 0.90 mm i.d. Gilson PVC pumping tube and a 10-port selection valve (Valco, Vici C25-3180EMH, Houston, USA).

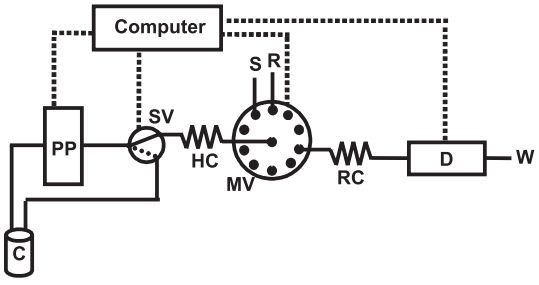

Figure 1. SIA manifold for the determination of β-blockers compounds. C, carrier (H_2SO_4 1.5 mol L^{-1}); PP, peristaltic pump; SV, solenoid valve; HC, holding coil (2 m/0.8 mm); R, $KMnO_4$ 7.5 x 10^{-3} mol L^{-1}; S, sample; MV, multiposition selection valve; RC, reaction coil; D, detector; W, waste.

All connections, including the holding and reaction coils were made with 0.8 mm i.d. PTFE tubing. The holding and reaction coils were 2 m in length and both were figure eight-shaped in configuration. In this type of system the aspiration and propulsion volumes of the diverse solutions are controlled within a time based, according to the flow rate used. In order to guarantee reproducibility in the aspirated and propelled volumes, especially when dealing with reduced volumes,[21] the starting position of the peristaltic pump at the beginning of each cycle was controlled. To this end, it was used a NResearch 161 T031 solenoid valve (W. Caldwell, NJ, USA), a magnet and a reed relay fixed, on the rotative and fixed components of the pump head,

respectively. The two opposed electrical contacts of the reed relay were connected to the digital-in port of the interface card and the ground. With the motion of the rotative head there was an approximation between the magnet and the reed relay and, in the presence of the magnetic field created, the reeds experience a force and move to make contact with one another to complete the circuit. The signal detected by the interface card of the computer set the beginning for each step of the analytical cycle from a fixed position of the peristaltic pump. The solenoid valve, placed between the pump and the holding coil was activated, enabling the solutions to flow through the holding coil. At the end of the cycle, the peristaltic pump returned to the initial position. During this time, the carrier solution flowed in closed circuit by inactivation of the solenoid valve.

This system was controlled by a homemade programme written in QuickBasic language and implemented in a microcomputer equipped with an interface card (Advantech Corp., PCL 711B, San Jose, CA). A Jenway 6100 spectrophotometer, with an 18 μL Helma flow cell (178712 QS, Mullheim/Baden, Germany) was also used as a detection system. Analytical signals were recorded on a Kipp & Zonen BD 111 (Delft, The Netherlands) strip chart recorder or acquired via computer.

Sequential injection procedure

The determination based in the reduction of permanganate by the β-blockers has an analytical cycle (Table 1) that begins with the aspiration of 25 μL of potassium permanganate solution to the holding coil (step 1), followed by 100 μL of sample (step 2). These plugs were aspirated at a flow rate of 1.04 mL min^{-1}. Thereafter, this aspirated sequence was sent by flow reversal, at a flow rate of 2.05 mL min^{-1} to the reaction coil (step 3) where the flow was stopped for 180 seconds (step 4). Finally, the reaction zone was moved directly towards the spectrophotometric detector (λ = 525 nm), at a flow rate of 2.05 mL min^{-1} (step 5).

The decrease of signal, due to the transformation of permanganate in colourless Mn^{2+} is proportional to β-blockers concentration in the sample. Determinations started with the measurement of a blank signal by aspirating a 1.5 mol L^{-1} acid solution (blank solution). The obtained blank signals correspond to the maximum absorbance signal in the absence of drug.

Reference methods

Aiming at the evaluation of the accuracy of the results furnished by the developed procedure, the pharmaceutical formulations were also analysed using the British Pharmacopoeia 2005 methods (BP).[22] Almost of the BP methodologies involve a rigorous pre-treatment of the samples followed by direct measurement of the obtained solutions in the UV region. The samples preparation procedure differed for each of the pharmaceutical preparations. For metoprolol tablets, it consisted of dissolving a given amount of powered tablets in absolute ethanol followed by shaking with the help of ultrasound for 15 minutes before filtering and diluting in absolute ethanol. The acebutolol tablets were dissolved in water. The obtained solution was diluted twice, the latter being in hydrochloric acid. In the case of propranolol preparations, the procedures were different for tablets, injections and prolonged-release capsules. The procedure for propranolol injection was based only on a dilution in methanol while that for propranolol tablets was additionally necessary to do consecutive periods of shacking and filtration. The prolonged-release propranolol capsules procedure was the most laborious one and include after boiling, a prolonged period of shaking, filtrations and dilution steps.

Table 1. SIA analytical cycle used in the determination of metoprolol, acebutolol and propranolol in pharmaceutical formulations

Step	Position	Volume/ (μL)	time/(s)	Flow rate/ (mL min^{-1})	Direction
1	1	25	1.45	1.04	Aspiration
2	2	100	5.8	1.04	Aspiration
3	3	682	20	2.05	Propulsion
4	3	0	180	0	Stopped flow
5	3	1706	50	2.05	Propulsion

Results and Discussion

Flow system optimisation

The optimisation of the general characteristics of the automatic methodology used for the determination of the three distinct β-blockers, was subsequently performed using metoprolol. The concentrations, volumes and order of aspiration of sample and potassium permanganate solutions together with the physical parameters of the SIA manifold were tested. Factors such as precision, sensitivity and linear calibration plots whose lower and upper limits corresponded to at least 80 and 120% of the analyte's tested concentration[23] were preponderant factors in the selection of these parameters.

Thereafter, the performance of the proposed system was evaluated for the determination of the three β-blockers: metoprolol, acebutolol and propranolol regarding analytical range, detection and quantification limits, accuracy and repeatability as well as sampling frequency.

As acidic conditions were necessary for the reaction,[24] it was evaluated the influence of acid concentration in the analytical signals. It was tested different sulphuric acid concentrations ranging from 0.1 to 2 mol L^{-1}, first in the carrier solution and then in the reagent and samples solutions. It was observed an increase in the reaction rate with the increase in carrier acid concentration but beyond 1.5 mol L^{-1}, a loss in linearity was observed. Regarding the effect of acid concentration in sample and reagent solutions results showed that there was a fourfold increase in sensitivity till the 1.5 mol L^{-1} sulphuric acid solution and then the signal approached stabilisation.

Thereafter, the potassium permanganate concentration was studied in the interval 3.75 x 10^{-3} to 1.5 x 10^{-2} mol L^{-1}. The absorbance value of the analytical signal obtained with permanganate solution in the absence of drug (blank signal) should be high in order to get a wider interval in absorbance values between the different analyte concentrations tested. The results revealed that 7.5 x 10^{-3} mol L^{-1} was best since for higher values, a decrease in accuracy was observed and for lower concentrations, despite attaining the same sensitivity, the decrease in amplitude of the blank signal (0.633 AU to 0.384 AU) gave rise to a smaller linear concentration interval. Having selected this concentration, the volume of permanganate solution was tested between 12.5 and 37.5 μL. As expected, the blank signal increased with the volume tested, but it was necessary to obtain a compromise between the absorbance value obtained and the desired linear calibration range, as this changed with the increase in permanganate ion in the reaction zone. This compromise was achieved with 25 μL of reagent solution, which was selected for further experiments. Thereafter, the optimum sample volume was investigated in the range 75 to 125 μL. Between 75 and 100 μL there was a 22% increase in attained sensitivity and an enlarged linear concentration range was obtained. For sample volumes greater than 100 μL the proportion of reagent/sample becomes slower and insufficient to obtain the same linear concentration range, for which it would be necessary to use greater volumes of permanganate solution.

These optimisations were performed with the aspiration of potassium permanganate solution followed by sample solution to the holding coil to get the lower sample dispersion and greater reagent zone penetration,[25] thereby yielding the best efficiency in terms of mixing and sensitivity. The mixture between reagent, sample and carrier began with the aspiration of solutions to the holding coil and the residence time of the reaction zone in the system depended both on aspiration and propulsion flow rates. Therefore, the effect of flow rate used for the aspiration of the solutions to the holding coil was investigated. The sample and reagent volumes aspirated were kept constant by changing the aspiration time in accordance with the flow rates tested (0.51 - 1.55 mL min^{-1}). The propulsion flow rate was also studied between 0.49 and 3.11 mL min^{-1}. An aspiration flow rate of 1.04 mL min^{-1} and propulsion flow rate of 2.05 mL min^{-1} were selected. Higher flow rates of the solutions produced poor repeatability (RSD > 2%) and resulted in analytical signals 10% lower. A 2 meter, figure-eight configured holding coil was sufficient to prevent the stack of zones aspirated from entering the pump conduit which would result in carrier solution contamination.[25] A coil with the same length and shape placed before the detector was selected as a compromise between sampling rate, loss of analytical signal due to the dispersion and the promotion of an adequate interpenetration of the sample and reagent zones.[25] Then, to extend the reaction time without having the unwanted dispersion along the reaction coil it were tested stopped-flow periods in the reaction coil between 120 and 240 seconds, which corresponded to residence times between 172 and 292 seconds. An increase in sensitivity of 30% was noticed up to 180 seconds. Longer periods did not yield improved sensitivities and impaired sampling rate. Consequently, a stopped flow of 180 seconds and a 232 seconds residence time were selected for the optimised system. This allowed a sampling rate of approximately 14 determinations per hour.

Interferences

Considering that the developed methodology was to be applied in the determination of β-blockers, not only as pure substances but also as the active components, in pharmaceutical formulations it was important to assess the potential interfering effect of several compounds commonly used as excipients in the analysed formulations. Solutions with a fixed amount of drug and increasing concentrations of polysorbate 80, talc, magnesium stearate, cellulose, silica and a copolymer based on polyacrylic and metacrylic (Eudragit®) were analysed in the flow system. A species was considered as non-interfering when the analytical signal variation regarding that obtained in its absence was lower than 3%.

It was observed (Table 2) that up to a ratio of 100 (interferent/drug) for talc, magnesium stearate, cellulose and Eudragit®, no interfering effect was noticeable. Regarding silica, no interference was observed for a ratio under 50. Finally, polysorbate 80 was shown to interfere with the analytical signal at a ratio of 5. However this did not affect the determination of β-blockers in the pharmaceutical preparations since this excipient is present at very low concentrations with ratios lower than 1.

Table 2. Interfering effects of excipients on the developed methodology

Excipient	Tolerance weight ratio
Polissorbate 80	5[a]
Silic	50
Talc	100[b]
Magnesium stearate	100[b]
Celulose	100[b]
Eudragit®	100[b]

[a]The lowest value tested. With this value the interference was noted; [b]The highest value tested.

Analysis of pharmaceutical formulations

After system optimisation it was evaluated for each pure drug (metoprolol, acebutolol and propranolol) the linear working range, detection and quantification limits, sampling rate and repeatability (Table 3).

The detection and quantification limits were obtained with the blank solution (sulphuric acid 1.5 mol L^{-1}) and calculated as the concentration corresponding to the absorbance value plus three times or ten-times, respectively, its standard deviation. Sample throughput was calculated by adding the time necessary to perform each step of the protocol sequence, including aspiration of solutions to the holding coil, propulsion to the detector and stopped flow at reaction coil. Repeatability was estimated by calculating the relative standard deviation (RSD%) from ten consecutive sample injections of different concentrations.

The developed analytical methodology was then applied to commercially available pharmaceutical formulations containing metoprolol, acebutolol and propranolol. The accuracy of the results obtained was evaluated by comparison with the concentration values furnished by the reference methods.[22] These values are shown in Table 4.

For the results obtained for propranolol pharmaceutical preparations it was also made the methodologies comparison using the *t*-test, carried out as a bilateral coupled test.[26] The tabulated *t* value (2.45) when compared with the calculated *t* value (0.27) shows the absence of statistical differences for those results obtained by the methodologies at the 95% confidence level. The agreement between both methods was also evaluated by a linear relationship obtained,[26] C_{SIA} (mg L^{-1}) = (0.993 ± 0.025) C_{REF} (mg L^{-1}) − (0.3 ± 2.1) where C_{SIA} and C_{REF} are the sequential injection and reference procedure results for propranolol pharmaceutical preparations respectively, with 95% confidence limits for the intercept and slope. The values obtained show that the intercept is close to zero and the slope close to unit,

Table 3. Figures of merit of SIA system

	Metoprolol	Acebutolol	Propranolol
Regression equation	AU = (1.340 ± 0.076) x 10^{-3} Conc + (0.1 ± 1.2) x 10^{-2}	AU = (1.56 ± 0.22) x 10^{-3} Conc – (1.6 ± 2.3) x 10^{-2}	AU = (2.28 ± 0.14) x 10^{-3} Conc– (1.3 ± 1.2) x 10^{-2}
R^2	0.9983	0.9939	0.9988
Detection limit (mg L^{-1})	12.16	9.86	4.97
Quantification limit (mg L^{-1})	40.52	32.85	16.58
Upper limit (mg L^{-1})	250	140	120
RSD % (sample concentration, mg L^{-1})	4.98 (109.8)	3.75 (78.19)	4.43 (40.57)
RSD % (sample concentration, mg L^{-1})	2.50 (198.2)	3.08 (107.6)	2.59 (76.51)
Determination frequency (determination h^{-1})	14	14	14

Table 4. Results obtained by the proposed flow methodology and the comparison reference methodologies for the determination of metoprolol, acebutolol and propranolol in pharmaceutical formulations

Drug	Pharmaceutical preparation	Amount declared (mg/formulation)	Amount found (mg/formulation) ± SD[a]		Relative error (%)
			Reference methodology	Developed methodology	
Metoprolol	Lopressor 100 (tablets)	100	93.70 ± 0.34	97.54 ± 0.24	+4.10
	Lopressor 200 (tablets)	200	207.9 ± 1.6	207.2 ± 7.9	–0.34
Acebutolol	Prent (tablets)	200	195.16 ± 0.90	207.3 ± 6.4	+6.22
Propranolol	Propranolol Ratiopharm (tablets)	80	82.71 ± 0.34	84.5 ± 2.5	+2.16
	Inderal (injection)	1	1.0210 ± 0.0028	1.070 ± 0.021	+4.80
	Inderal LA 80 (delayed-release capsules)	80	83.2 ± 6.5	81.92 ± 0.68	–1.54
	Inderal LA (delayed-release capsules)	160	164 ± 13	163.2 ± 7.2	–0.49
	Inderal 10 (tablets)	10	10.19 ± 0.19	10.34 ± 0.13	+1.47
	Inderal 40 (tablets)	40	40.20 ± 0.63	41.2 ± 2.3	+2.49
	Inderal 80 (tablets)	80	78.9 ± 1.3	77.1 ± 2.2	–2.28

[a]mean and standard deviation obtained after four-fold sample processing

which confirms yet again, the agreement between the two methodologies.

Conclusions

The developed methodology for the determination of metoprolol, acebutolol and propranolol presents good sensitivity, precision and a wide linear concentration range. The analytical procedure is in good agreement with the current Green Chemistry recommendations as it uses only 0.188 μmol of potassium permanganate (25 μL) and 3.77 mmol of sulphuric acid (2.29 mL) per determination in opposition to the consumed by the reference procedures. Besides, while the analysis of any of the pharmaceutical preparations of the three β-blockers by SIA involves only an acid dissolution of the sample before its insertion in the system, when using Pharmacopoeia reference methods it is necessary to apply five different procedures to obtain similar results.[22] In particular, the procedure for prolonged-release propranolol capsules is extremely complicated and time consuming. Additionally, this new SIA method also allows a drastic reduction in the time necessary for each determination from 10-90 minutes using BP procedures to 5 minutes in the SIA procedure. These differences constitute a significant handicap in pharmaceutical quality control, and advocate the use of the SIA methodology that consume little sample, and present characteristics to be used for on-line sample pre-treatment and an almost real time monitoring of dissolution processes. Furthermore, it is the first flow procedure performing the determination of several β-blockers drugs in pharmaceutical preparations with significant differences in terms of structure, which anticipate the possibility of applying the methodology to the determination of other β-blocker pharmaceutical preparations.

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