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Automatic miniaturized fluorometric flow system for chemical and toxicological control of glibenclamide[☆]

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ABSTRACT

In this work, and for the first time, it was developed an automatic and fast screening miniaturized flow system for the toxicological control of glibenclamide in beverages, with application in forensic laboratory investigations, and also, for the chemical control of commercially available pharmaceutical formulations. The automatic system exploited the multipumping flow (MPFS) concept and allowed the implementation of a new glibenclamide determination method based on the fluorometric monitoring of the drug in acidic medium ($\lambda_{ex} = 301$ nm; $\lambda_{em} = 404$ nm), in the presence of an anionic surfactant (SDS), promoting an organized micellar medium to enhance the fluorometric measurements.

The developed approach assured good recoveries in the analysis of five spiked alcoholic beverages. Additionally, a good agreement was verified when comparing the results obtained in the determination of glibenclamide in five commercial pharmaceutical formulations by the proposed method and by the pharmacopoeia reference procedure.

1. Introduction

Glibenclamide, is a potent, second generation antidiabetic drug in a class of medications known as sulfonylureas widely used to lower glucose levels in patients with type II non-insulin-dependent diabetes mellitus.

Alongside its therapeutic use, a literature survey reveals that glibenclamide has been subject to an increasing attention among the drugs that are used to incapacitate victims, mainly youngsters or elderly, with a criminal purpose that include sexual abuse or robbery. On the other hand, some reports have emphasized the danger of hypoglycemia with glibenclamide, even at low dose, especially among the elderly due to classic overdosage, leading to the use of this drug to commit suicides or even homicides [1,2]. Sedation induced by glibenclamide can be observed as overdosage presents lethargy. This can be a cause of incapacitation of a victim. Diagnosis can be difficult to achieve when aiming to the discrimination between insulinoma and antidiabetic drug-induced hypoglycemia. In order to differentiate between these two causes of hypoglycemia, antidiabetics must be screened and their concentrations evaluated.

Being glibenclamide one of the most commonly used sulfonylureas for type II diabetes treatment, related health problems are a major public concern not only for its potential toxicity (an overdose of glibenclamide can be fatal) or as a consequence of abuse practices (suicides or homicides), but also due to inappropriate dose administrations (very common among old people), putting in evidence the need for simple and expeditious methods for its chemical control. If one analyses the broad application of glibenclamide in our days, as stated before, it is emphasized the need to develop straightforward and versatile automatic procedures to carry out its screening in drug formulations that can be easily manipulated nowadays, as well as, in beverages that might be the "vehicles" for its surreptitious administration.

Several methodologies were already proposed for the determination of glibenclamide in pharmaceutical formulations involving chromatographic [3–12], potentiometric [13] and fluorometric [14] procedures. These methods are highly time consuming, expensive and often require the use of environmental hazard reagents. Besides, it could be considered somehow excessive applying chromatographic techniques for the analysis of samples that exhibit usually an uncomplicated composition, and not requiring low detection limits, as is the case of pharmaceutical formulations. Therefore, automatic flow analysis can play a prominent role in the chemical control of pharmaceutical formulations since it enables the development of miniaturized flow systems allowing a higher degree of simplicity and improved operational versatility.

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More important, when considering the toxicological screening of beverages for the presence of glibenclamide, not a single scientific work can be found in the literature. Normally, in criminal situations where exists the suspicion of drug abuse with glibenclamide, only biological testing is performed (e.g. hair through LC–MS/MS [15]), which involves laborious assays in a laboratory environment, even considering the risk of not obtaining any validated result due to the short half-lives of most drugs in biological fluids. Glibenclamide has been reported to be cleared within 5 h in human plasma.

This work was the first attempt to develop an automatic and fast screening miniaturized flow system for the chemical control of commercially available pharmaceutical formulations containing glibenclamide, and also, for the toxicological control of some beverages possibly used in hypothetic criminal practices, and thus, it may contributes to build strong legal evidences in a drug-facilitated crime. The implementation in a flow system of an originally developed glibenclamide detection procedure capable of rapidly quantify the drug in some alcoholic beverages and pharmaceutical formulations, documents the importance of versatile and miniaturized detection methods in forensic situations requiring the analysis of medications and food samples collected in crime scenes.

The exploitation of the multipumping flow concept constitutes a major contribution in the development of compact and portable analytical flow systems allowing a higher degree of automation, operational simplicity and improved versatility, bringing together all the advantages associated to miniaturization of flow analysis procedures.

In this work, the originally developed quantification method was based on the fluorometric monitoring of glibenclamide in acidic medium (λ_{ex} = 301 nm; λ_{em} = 404 nm), in the presence of an anionic surfactant (SDS), promoting an organized micellar medium to enhance the fluorometric measurements. The analytical potential of this novel chemical approach for glibenclamide determination was further enhanced through its implementation in a fully automated multipumping flow system [16].

2. Experimental

2.1. Apparatus

The developed flow manifold comprised three 120SP solenoid actuated micro-pumps (Bio-Chem Valve Inc., Boonton, NJ, USA), of fixed displacement diaphragm type, dispensing 10 μL per stroke. All flow lines were made of 0.8 mm i.d. PTFE tubing. Homemade end-fittings, connectors and confluences were also used.

The detector used to monitor the fluorescence intensity (λ_{ex} = 301 nm, λ_{em} = 404 nm) was a fluorometer Jasco (Easton, MD, USA), model FP-2020/2025, equipped with a 16 μL internal volume flow cell.

Automatic control of the analytical system was accomplished by means of a Pentium based microcomputer and software developed using Microsoft Quick-Basic 4.5. A CoolDrive™ power drive board (NResearch Inc., West Caldwell, NJ, USA) was used to activate the solenoid of micro-pumps through the LPT1 computer port.

2.2. Samples, standards and reagents

All solutions were prepared with doubly deionised water and analytical grade chemicals were used.

A 200 mg L^{-1} glibenclamide stock solution was prepared by dissolving 50 mg of the bulk drug (Sigma®, ≥99%) in 250 mL of absolute ethanol (Panreac®, 99.5%). This stock solution was protected from the light and stored under refrigeration.

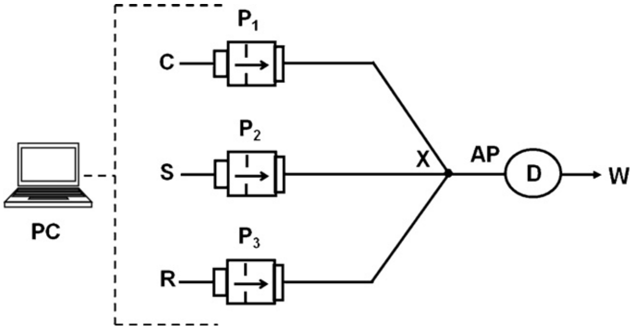

Fig. 1. Multipumping flow system (MPFS). PC, microcomputer; P_1–P_3, solenoid micro-pumps (internal volume 10 μL); X, confluence point; D, fluorometer detector (λ_{ex} = 301 nm and λ_{em} = 404 nm); AP, 10 cm analytical path; S, sample: glibenclamide in 50% ethanol/0.2 mol L^{-1} H_2SO_4; C, carrier: 0.2 mol L^{-1} H_2SO_4; R, 0.03 mol L^{-1} SDS; W, waste.

A solution containing 0.1 mol L^{-1} sodium dodecyl sulphate (SDS) was prepared by dissolving 7.21 g of SDS (Fluka®) in a 250 mL volumetric flask, using deionised water as solvent.

The working glibenclamide standards (5–75 mg L^{-1}) were prepared, on a daily basis, by appropriate dilution of the stock solution: aliquots of glibenclamide stock solution (0.5–7.5 mL) were transferred into a series of 20 mL volumetric flasks and 2 mL of 2 mol L^{-1} H_2SO_4 solution was added. In order to obtain the same ethanol concentration (50%, v/v) for all working glibenclamide standards, appropriate aliquots of absolute ethanol (Panreac®) were also added. The volume was subsequently made up to the mark with deionised water.

Five commercially available pharmaceutical formulations containing glibenclamide were used to prepare sample solutions, by weighing and powdering a representative number of tablets. Afterwards, an appropriate amount of sample, corresponding to 3.0 g of glibenclamide, was dissolved in absolute ethanol (Panreac®, 99.5%) by stirring for 25 min. The resulting solutions were filtered, transferred to 50 mL volumetric flasks and diluted with deionised water. Finally, appropriate volumes of the obtained solutions, with glibenclamide concentrations of approximately 60 mg L^{-1}, were transferred to 20 mL volumetric flasks and 2 mL of a 2 mol L^{-1} H_2SO_4 solution was added. The final volume was subsequently made up with deionised water. At last, the obtained solution was filtered with a syringe filter i.d. 0.20 μm (Corning®) before its insertion in the flow system.

Five commercial alcoholic beverages were selected for analysis. The drinks were spiked aiming at a glibenclamide concentration of 50 mg L^{-1}, by adding 6.25 mL of the 200 mg L^{-1} glibenclamide stock solution into a 25 mL volumetric flask and the final volume completed with the respective alcoholic beverage under analysis. Then, 2 mL of 2 mol L^{-1} H_2SO_4 solution were transferred into a series of 20 mL volumetric flasks, and the volume was made up to the mark with the respective alcoholic beverage previously spiked.

2.3. Flow manifold

The developed flow system exploiting the MPFS approach for the fluorometric determination of glibenclamide is depicted in Fig. 1. The manifold employed three solenoid micro-pumps (P_1, P_2, P_3), which were responsible for the individually handling of three different solutions.

The micro-pump P_1 was responsible for insertion and propelling of the 0.2 mol L^{-1} sulphuric acid solution, which was used as carrier; P_2 and P_3 were used for inserting the sample and 0.03 mol L^{-1} SDS solutions, respectively.

Preceding the analytical cycle, all flow tubing had to be filled with the corresponding solution. Then by actuating P_1, the sul-

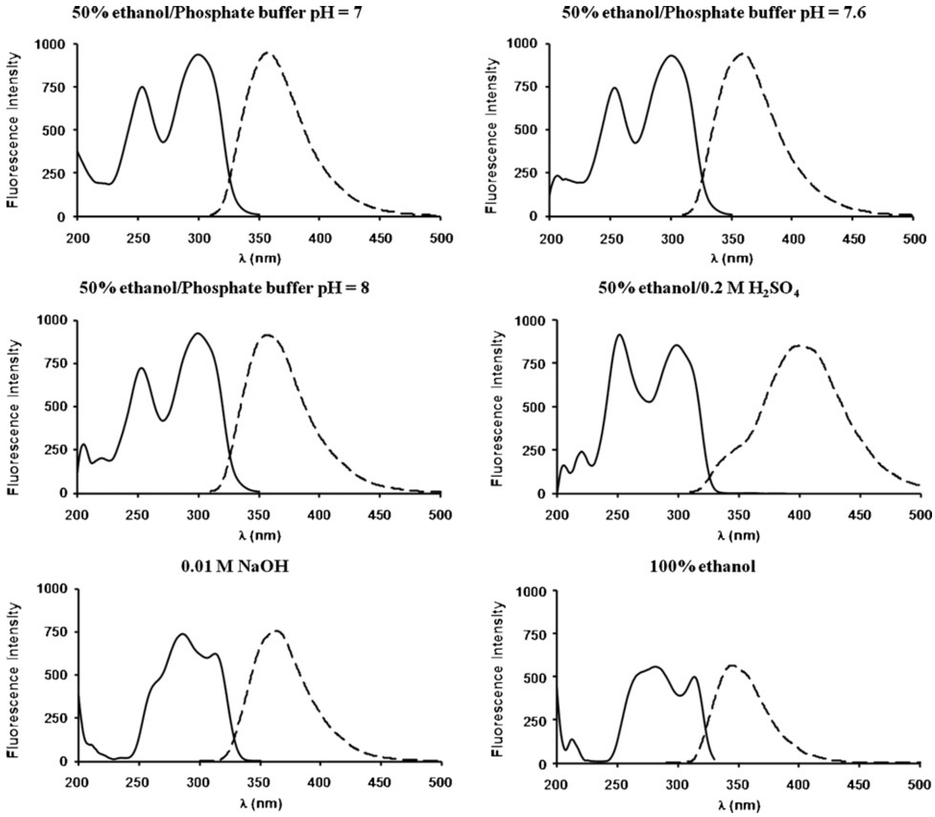

Fig. 2. Fluorescence excitation and emission spectra of glibenclamide solutions prepared in different reaction media.

phuric acid solution was inserted and carried out towards detection in order to establish baseline.

The analytical cycle started by intercalating a unique volume of sample solution between two identical plugs of the SDS solution. The sampling stage consisted in the insertion of the SDS solution for a pre-set number of pulses, through the actuation of micro-pumps P_3 (total volume of 10 μL per stroke), at a fixed pulse time of 0.2 s, corresponding to a pulse frequency of 171 min^{-1}, which defined the flow rate at 1.71 mL min^{-1}. Then, by inserting a pre-set number of pulses of micro-pump P_2, at a fixed pulse time of 0.2 s, a unique volume of sample solution was intercalated with another identical small plug of the SDS solution.

Thereafter, the established reaction zone was carried out towards the detector through the repeated actuation of P_1 (10 μL per stroke), at a fixed pulse time of 0.2 s, corresponding to a pulse frequency of 171 min^{-1}, which defined the flow rate at 1.71 mL min^{-1}. The fluorescence emission of the compound was monitored at 404 nm (λ_{ex} = 301 nm).

2.4. Reference procedure

For validation of the results furnished by the developed methodology, the pharmaceutical formulations containing glibenclamide (tablets) were also analyzed by reversed-phase liquid chromatography, according with the reference methodology [17].

3. Results and discussion

3.1. Preliminary assays

Some preliminary batch assays, aiming at evaluating the fluorescence properties of glibenclamide in different reaction media, were performed by carrying out the fluorescence excitation and emission spectra of six different glibenclamide standard solutions containing 50 mg L^{-1} of the drug. From this set of solutions, three were prepared in 50% ethanol and phosphate buffer with different pH values of 7, 7.6 and 8, whilst of the three remaining solutions one was prepared in 50% ethanol and 0.2 mol L^{-1} of H_2SO_4, another in alkaline medium, containing 0.01 mol L^{-1} NaOH and the last one in 100% ethanol. The selection of the composition of these solutions used to evaluate the glibenclamide fluorescence properties was based and dependent on the characteristic dissolution profile of the drug [18].

The analysis of the recorded glibenclamide excitation and emission spectra of the previous solutions (Fig. 2) demonstrated that the solutions prepared in 50% ethanol/phosphate buffer with a pH of 7, 7.6 and 8 exhibited maximums λ_{ex} = 254 and λ_{ex} = 300 nm and a maximum λ_{em} = 357 nm. Additionally, the results revealed no significant differences on the fluorescence excitation and emission spectra between the different fixed values of pH with phosphate buffer. A glibenclamide solution prepared in 50% ethanol/0.2 mol L^{-1} of H_2SO_4 exhibited λ_{ex} = 252 nm and λ_{ex} = 301 nm and λ_{em} = 404 nm. When the glibenclamide was dissolved in an alkaline solution of 0.01 mol L^{-1} NaOH it revealed fluorescence properties with maximum λ_{ex} = 284 nm and λ_{em} = 363 nm. For the solution of glibenclamide in 100% ethanol, the recorded spectra demonstrated that when the drug was excited at a wavelength 282 nm and/or 315 nm emitted a weak fluorescence at λ_{em} = 345 nm. Other compositions for glibenclamide solutions were assayed in order to evaluate fluorescence properties involving lanthanides, namely, europium and terbium, but they revealed that no detectable increment in fluorescence was measured.

Subsequently, after obtaining the maximum λ_{ex} and λ_{em} for each different solution of glibenclamide previously prepared, the fluorometric detection procedure was implemented in a miniaturized and automated analytical flow system based in the

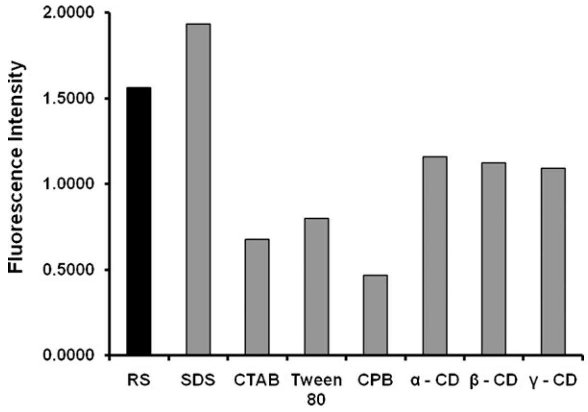

Fig. 3. Influence of surfactants and cyclodextrins on analytical signal. RS – reference sample; SDS – sodium dodecyl sulphate; CTAB – cetyl trimethylammonium bromide; CPB – cetylpyridinium bromide; α-CD – α-cyclodextrin; β-CD – β-cyclodextrin; γ-CD – γ-cyclodextrin.

multipumping concept, with the aim of confirming which one of the solutions media under evaluation allowed achieving the maximization of the analytical signal. The solutions containing 50 mg L^{-1} of glibenclamide dissolved in the different studied reaction media were inserted into the developed miniaturized multipumping flow system and the intensity of fluorescence for each of the solutions was recorded. For this, a MPFS with only two micro-pumps was used, in which, one micro-pump was responsible for the sample insertion and the other was used to propel the carrier solution (solution media under evaluation). The results showed that the glibenclamide solution prepared in a mixture of 50% ethanol and 0.2 mol L^{-1} sulphuric acid exhibited a higher fluorescence intensity (FI), approximately 165% when compared with the ones obtained with the others studied reactions media. Thus, a solution of glibenclamide prepared in 50% ethanol/0.2 mol L^{-1} sulphuric acid (λ_{ex} = 301 nm and λ_{em} = 404 nm) was selected to perform the optimization of the flow system.

3.2. Optimization of chemical parameters

It is documented in the scientific literature that the presence of reagents such as surfactants and sensitizers can have a strong effect on the fluorescence signal. In fact, some surfactants are known to originate an organized micellar system in the surrounding medium for concentrations higher than its CMC, increasing the analyte's concentration on the micellar surface, causing changes in the photophysical properties of analytes by improving its susceptibility to photoradiation and enhancing fluorescence emission.

Therefore, some experiments, with the goal of increasing the fluorescence intensity, were performed by studying the influence on analytical signal of the presence of different substances. Surfactants such as sodium dodecyl sulphate (SDS), cetyl trimethylammonium bromide (CTAB), Tween 80 and cetylpyridinium bromide (CPB) were used at concentrations above their critical micelle concentration (cmc). With the aim of assuring that all the surfactants used in the assay were at concentrations higher than their CMC, a concentration of 0.02 mol L^{-1} was selected for the surfactants SDS, CTAB and CPB, whilst for Tween 80 was 0.01%. Also, some cyclodextrins, namely, α, β and γ-cyclodextrin were used at a concentration of 5×10^{-3} mol L^{-1}. The assays were performed in the MPFS previously used, but comprising a third micro-pump that was responsible for the insertion of the studied solutions. The latter was merged with the stream of the glibenclamide sample solution by using the merging zones approach. A carrier solution of sulphuric acid 0.2 mol L^{-1} was used. Fig. 3 represents a compilation of the obtained results and a comparison with the FI originated by a 50 mg L^{-1} glibenclamide standard solution (reference sample). The analysis of the results demonstrated a decrease in FI for the glibenclamide solutions merged with α, β and γ-cyclodextrins, CTAB, Tween 80 and CPB surfactant solutions. However, by merging a solution of an anionic surfactant such as SDS to the glibenclamide solution, an increase of approximately 24% on the fluorescence signal was observed relatively to the reference sample. These results indicate that the use of SDS improves the sensitivity for the glibenclamide determination, most probably because the organized microenvironment promoted by this surfactant improved energy transfer and fluorescence efficiencies [19].

Subsequently, the influence of sulphuric acid (used as carrier) and SDS concentration on the analytical signal was assessed over a concentration range from 0.005 to 2 mol L^{-1} and 0.001 to 0.075 mol L^{-1}, respectively. The study of the influence of sulphuric acid concentration on the analytical signal was performed using several sample solutions all containing 50 mg L^{-1} of glibenclamide, 50% ethanol and different H_2SO_4 concentrations under evaluation. For these assays, a volume of 100 μL (10 pulses) of sample solution was inserted in the developed MPFS (Fig. 1) and merged with the same volume of 0.02 mol L^{-1} SDS solution. Next, the reaction zone was carried towards the detection with the sulphuric acid solution under evaluation. In this study, a significant increase of FI was observed for sulphuric acid concentrations up to 0.20 mol L^{-1}, whilst for higher concentrations stabilization of FI occurred. Thus, a concentration of 0.20 mol L^{-1} sulphuric acid was selected for further optimization assays.

In the assessment of the influence of SDS concentration on the analytical signal, a volume of 100 μL (10 pulses) of sample solution (50 mg L^{-1} of glibenclamide in 50% ethanol and 0.20 mol L^{-1} H_2SO_4) was merged with the same volume of solution containing different SDS concentrations under evaluation (0.001–0.075 mol L^{-1}), and subsequently the reaction zone was carried towards the detector with the 0.20 mol L^{-1} H_2SO_4 carrier solution. The obtained results showed an increase in FI for a SDS concentration of up to 0.01 mol L^{-1}, which was the value selected for the subsequent assays.

3.3. Optimization of physical parameters

Since the analytical signal could be influenced by physical parameters such as the flow rate, analytical path length (Fig. 1, AP), sample volume and sampling strategy that could affect the extension of the reagents mixture and also its dispersion inside the flow system, some optimization studies involving these parameters were carried out aiming at the maximization of the analytical signal.

The optimization of the analytical path length and the sample volume inserted in the flow system were assessed simultaneously. In this study, for each AP length evaluated, namely 10, 20, 40, 65 and 110 cm, was varied the number of pulses between 2 and 16 (corresponding to sample volumes between 20 and 160 μL) of sample solution (50 mg L^{-1} of glibenclamide in 50% ethanol/0.20 mol L^{-1} H_2SO_4) merged with the same number of pulses of a 0.01 mol L^{-1} SDS solution. The carrier was a solution 0.20 mol L^{-1} in H_2SO_4. The reaction zone was carried towards detection by actuating the micro-pump responsible for carrier insertion with a pulse time of 0.2 s, which corresponded to a flow rate of about 1.71 mL min^{-1}. The obtained results (Fig. 4) showed that for an AP length of 10 cm the highest analytical signal was achieved in all the experimental conditions, and that FI increased until a sample volume of approximately 10 pulses. The smallest AP length ensures the minimization of dispersion phenomena of the reaction zone and, consequently, enabled the maximization of FI.

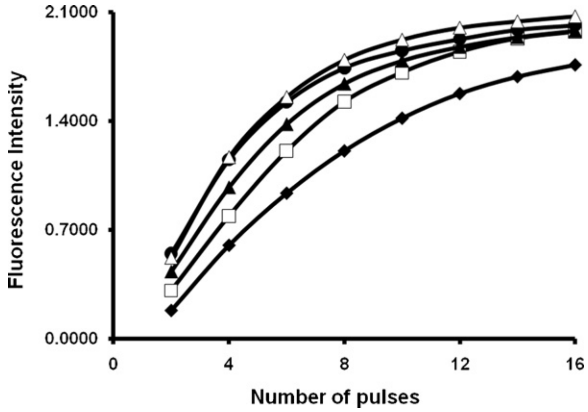

Fig. 4. Influence on the analytical signal of different path lengths. (♦) 110 cm; (□) 65 cm; (▲) 40 cm; (●) 20 cm; (△) 10 cm.

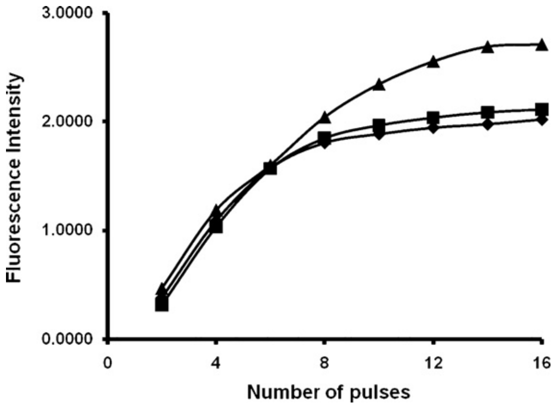

Fig. 6. Influence on the analytical signal of sampling strategies. (▲) Unique volume; (■) merging zones; (♦) binary sampling.

The study of the influence on the analytical signal of flow rate was also performed simultaneously with the inserted sample volume. In these assays, for each different pulse time of 0.1, 0.15, 0.2, 0.3 and 0.4 s (corresponding to flow rates of 2.40, 2.00, 1.71, 1.33, 1.09 mL min^{-1}), the number of inserted sample pulses was also varied between 2 and 16 (corresponding to volumes between 20 and 160 μL) of a solution containing 50 mg L^{-1} glibenclamide in 50% ethanol/0.20 mol L^{-1} H_2SO_4. In this study, the parameter that demonstrated a more pronounced influence on analytical signal was sample volume, which revealed an accentuated increase on the FI up to approximately 10 pulses (100 μL), tending towards stabilization (Fig. 5, graphic A) for higher values. On the other hand, it was verified that the pulse time (flow rate) had a very small influence in analytical signal, which could be explained by the small length of the analytical path (10 cm). However, by fixing the sample volume to 10 pulses and by analysing the variation of the FI with the pulse time, it was verified an increase of the analytical signal up to 0.2 s and a subsequent decrease in fluorescence intensity for higher values (Fig. 5, graphic B). Therefore, as a compromise between determination rate and sensitivity, a pulse time of 0.2 s (corresponding to a flow rate of 1.71 mL min^{-1}) and 10 pulses of sample (corresponding to a sample volume of 100 μL) was chosen.

Another flow parameter of great importance was the sampling strategy, since it could influence the degree of reagents mixture, and hence, the supramolecular organization in the reaction zone, being this fact determinant in the FI measured. Thus, some assays were carried out by exploiting different sampling approaches, such as merging zones, binary sampling and unique sample volume, and at the same time varying the number of pulses of the inserted sample solution within 2 and 16. The study of sampling strategy with number of sample pulses was important because, this way, a fine optimization of the number of pulses could be performed, taking into account that, the two mentioned parameters are very dependent one on another. For the accomplishment of these assays, the pulse time was modified and adjusted for each evaluated sampling strategy, in order to achieve a similar flow rate throughout the system during sample insertion and transport stages. This was important because when micro-pumps are activated simultaneously, theoretically the overall flow rate corresponds to the sum of the flow rates of all propelled solutions.

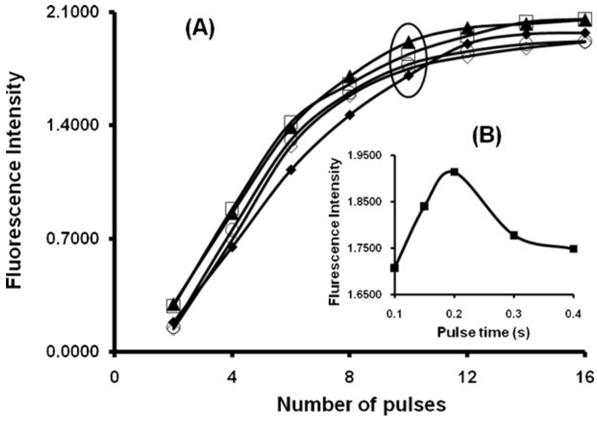

Fig. 5. Influence on the analytical signal of the pulse time (flow rate) and number of sample pulses (sample volume). (A) and (B) explained in the text. (♦) 0.1 s; (□) 0.15 s; (▲) 0.2 s; (○) 0.3 s; (◊) 0.4 s.

The obtained results (Fig. 6) demonstrated that there were no significant differences in the analytical signal for the three sampling strategies when inserting up to 6 pulses of sample solution in the flow system. However, for values higher than 6 sample pulses, a pronounced increase in analytical signal was observed when using the unique volume strategy comparatively with the others sampling strategies.

Accordingly with the principle of sampling strategy associated with binary sampling or merging zones, it should be expected from the results a higher efficiency in the mixture of sample and SDS solutions relatively to the unique volumes strategy. Nevertheless, the obtained results showed a higher FI when using the unique volumes strategy, indicating that a higher degree of mixture actually caused an increase in the dilution of the reaction zone, impairing the analytical signal. In this case, the influence on analytical signal of the degree of dilution markedly compromised the supramolecular organization created by SDS, in the enhancement of the fluorescence signal. Taking into account this data, and in order to obtain a better comprehension on the influence of SDS surfactant in combination with a unique volumes strategy (low degree of mixture), it was performed an assay involving again the study of the influence of the concentration of SDS on analytical signal when using the unique volumes strategy. As expected, and with the aim of assuring a micellar organization of the reaction zone to enhance the FI, a 0.03 mol L^{-1} SDS concentration was chosen for the determination of glibenclamide in samples.

3.4. *Analysis of pharmaceutical formulations*

In order to apply the developed methodology to the determination of glibenclamide in pharmaceutical formulations, the influence of some compounds commonly used as excipients was assessed. Different sample solutions containing a fixed amount of glibenclamide (30 mg L^{-1}) and different quantities of the excipients under evaluation were analyzed by the developed methodology. A com-

Table 1
Comparison of analytical results obtained in the determination of glibenclamide in pharmaceutical formulations by the proposed and the reference method.

Sample	Declared dosage (mg/formulation)	Amount found (mg/formulation)[a]		R. D. (%)[b]
		MPFS methodology	Reference method	
Pharmaceutical sample 1	2.5	2.52 ± 0.02	2.54 ± 0.08	0.82
Pharmaceutical sample 2	5	4.97 ± 0.05	4.976 ± 0.004	0.09
Pharmaceutical sample 3	2.5	2.47 ± 0.01	2.39 ± 0.02	−3.53
Pharmaceutical sample 4	5	4.86 ± 0.02	4.829 ± 0.001	−0.73
Pharmaceutical sample 5	2.5	2.43 ± 0.04	2.45 ± 0.07	0.74

[a] Mean ± $t0.05$ (Student's t-test) × $(S/\sqrt{n})$.
[b] Relative deviation of the developed method regarding the reference procedure.

Table 2
Results obtained in the toxicological control of glibenclamide in spiked alcoholic beverages.

Spiked sample	Concentration added (mg L^{-1})	Concentration found (mg L^{-1})[a]	Recovery (%)
Sample brand 1	50.0	49.3 ± 0.9	98.6
Sample brand 2	50.0	50 ± 2	99.2
Sample brand 3	50.0	49.8 ± 0.8	99.6
Sample brand 4	50.0	49 ± 1	98.7
Sample brand 5	50.0	49 ± 3	98.7

[a] Mean ± $t0.05$ (Student's t-test) × $(S/\sqrt{n})$.

pound was considered as non-interfering if the analytical signal variation was ±4% when compared to the analytical signal obtained in the absence of the referred compound. The results revealed that the excipients hypromellose, lactose, cellulose starch, povidone, magnesium stearate and colloidal silica up to a 100-fold mass ratio, did not interfere.

The linear working response range was assessed by inserting in the MPFS, several standard solutions containing different concentrations of glibenclamide and by using the previously referred optimized physical and chemical parameters. A linear response range for glibenclamide concentrations of up to 75 mg L^{-1} was obtained and the calibration curve was represented by FI = 0.0512 (±0.0007) × C + 0.09 (±0.03), R = 0.9992, in which FI was the fluorescence intensity and C was glibenclamide concentration, in mg L^{-1}. The detection limit calculated from the equation of the calibration curve according to Miller and Miller [20] was about 2.75 mg L^{-1}.

The validation of the proposed automatic methodology, based in a miniaturized multipumping flow system, was accomplished by comparison of the results obtained in the determination of glibenclamide in 5 commercial pharmaceutical formulations with those furnished by the reference procedure of the British Pharmacopoeia [17]. The results, summarized in Table 1, showed a good agreement between both methods, with relative deviations between −3.53 and 0.82%. Additionally, a paired Student's t-test [20] confirmed that there were no statistical differences ($t_{\text{estimated}} = 0.763$, $t_{\text{tabulated}} = 2.776$) between the results obtained by both procedures, for a confidence level of 95% ($n = 5$).

An evaluation of the precision of the proposed MPFS for glibenclamide determination in pharmaceutical formulations, through the repeated analysis of each commercial pharmaceutical formulation (5 consecutive determinations for each sample) revealed a very good repeatability (Table 1), at a confidence level of 95%.

The developed methodology allowed a determination rate of 39 h^{-1}.

3.5. Toxicological control in beverages

The configuration simplicity, versatility in controlling analytical parameters, portability, low power consumption of the proposed automatic flow system and the proved analytical performance of the novel chemical approach for the quantification of glibenclamide implemented in the MPFS, makes the analytical methodology described in this work a promising analytical tool with relevant applicability in the forensic or toxicological analysis of samples collected in crime scenes. Besides that, glibenclamide has been reported to be cleared quickly within 5 h in human plasma, emphasizing the need for new fast screening methodologies for glibenclamide detection in the possible sources for drug ingestion. Taking into account the history of glibenclamide abuse found in literature [15], the developed flow system was applied to the determination of glibenclamide in five alcoholic beverages previously spiked with the drug. However, additional tests have shown that some components of soft drinks and red wine interfered in the analytical response and make in general glibenclamide detection uncertain in these samples.

The samples were prepared accordingly with the procedure mentioned in Section 2.2. Only recovery studies were performed since in the scientific literature it cannot be found an alternative method for glibenclamide (or similar) determination in beverages. Hence, this work scientific novelty.

The obtained results (Table 2) revealed good recoveries comprised between 98.6% and 99.6% of glibenclamide from the spiked samples.

4. Conclusions

This work describes the development of the first automatic miniaturized flow system for the fast screening of glibenclamide in pharmaceutical formulations and alcoholic beverages, involving the implementation of a novel reactional scheme. The drug glibenclamide was directly monitored by fluorescence without any sample pre-treatment, like for example, pre-concentration or photodegradation of the analyte, and the analytical signal was enhanced by performing the determinations in an organized microsystem obtained with sodium dodecyl sulphate and sulphuric acid.

The developed analytical approach was characterized by high simplicity and versatility. The results obtained in the fluorometric determination of glibenclamide in commercial pharmaceutical formulations proved that the developed methodology can be a valuable and promising tool for pharmaceutical chemical control, being this way an advantageous alternative to the reference procedure, only requiring low quantities of reagents and producing reduced volumes of residues. The standard procedures for glibenclamide monitoring involve chromatographic techniques that require high volumes of reagents often dangerous for environment, expensive equipment and are time-consuming in sample and solutions preparation.

Additionally, the developed automatic system demonstrated its potential for carrying out routine laboratory procedures in forensic and toxicological analysis, being an important analytical tool for the detection and quantification of glibenclamide in alcoholic beverages that can be used to perpetrate suicides, homicides and/or robberies. Rapid glibenclamide screening in beverages through this automatic flow system should be used to complement conventional blood and urine analysis as it helps to rapidly identify drug-induced hypoglycemia and permits to take proper clinical and legal actions, all in the good health and safety of the victim. Considering the high portability and the easy control of these systems, one could expect its application for *in situ* analysis performed by police technicians, being one advantage of the proposed work over standard chromatographic techniques. Nevertheless, the proposed system lacks of sensitivity and selectivity for samples with higher matrix complexity. However, the real potentiality of the developed system is still to be achieved through its application for the analysis of samples with different matrices after implementation in-line of pre-concentration procedures, relatively easily to perform due to the modular structure of MPFS.

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