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ORIGINAL PAPER

Diazepam Fluorimetric Monitoring Upon Photo-Degradation in an Automatic Miniaturized Flow System

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Abstract The present work describes the fully integration in line of a photo-degradation unit, comprising a low pressure UV lamp, in a Multipumping Flow System (MPFS), for the fluorimetric chemical control of commercially available pharmaceutical formulations containing diazepam. The utilization of an organized micellar medium provided enhanced fluorescence emission. The results allowed to obtain a linear working range for diazepam concentrations of up to 40 mg L^{-1} (r=0.9998) and the detection limit was about 0.97 mg L^{-1}. The results obtained by the miniaturized and automatic flow system were in agreement with those furnished by the reference procedure, with relative deviations comprised between −2.09% and 2.13%.

Keywords Miniaturized · Flow analysis · Multipumping · Diazepam · Photo-degradation · Spectrofluorimetry

Introduction

Diazepam is a benzodiazepine that is used for the management of anxiety disorders or for the short-term relief of related symptoms. Diazepam may also be used to relieve agitation, shakiness, and hallucinations during alcohol withdrawal and to relieve certain types of muscle spasms. It may also be used to treat seizures, insomnia and other conditions.

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Nowadays, worldwide benzodiazepines consumption has reached very high values, mostly in developed countries, becoming a serious health problem as it frequently reached abuse levels. Either under prescription or by auto-medication, benzodiazepines abuse is frequently a consequence of their widespread availability and of their toxic effects causing dependence and habituation. Although death and serious illness problems resulting from benzodiazepine abuse are relatively rare, their combined administration with either alcohol or other medications could have fatal consequences. Being one of the most commonly used benzodiazepines, diazepam related health problems are a major public concern not only for its potential toxicity (an overdose of diazepam can be fatal) or as a consequence of abuse practices, but also due to inappropriate dose administrations, which requires the development of improved methods for the chemical control of diazepam in pharmaceutical formulations. Several methodologies have already been proposed for the monitoring of diazepam in pharmaceuticals such as thin-layer chromatography—densitometry technique [1], capillary electrophoresis and reversed phase-high performance liquid chromatography (RP-HPLC) [2], potentiometry using solid contact ion-selective electrodes [3], fluorometry [4], extractive-spectrophotometric method [5] and colorimetry [6, 7].

Distinct continuous flow methodologies resorting to a variety of detection techniques were also developed, such as, flow injection analysis combined with fluorimetry [8, 9] and UV spectrophotometry [10] and sequential injection lab-on-valve with potentiometric [11] detection.

Nevertheless, a fast, simple, low cost and miniaturized analytical system for diazepam determination would allow a more expeditious and simplified chemical control of the formulations, and most probably, the encouragement of the use of manipulated formulations with adapted drug dosages

for every clinical situation. Ideally, the use of adapted dosages should always be applied whenever treating a clinical illness.

In pharmaceutical chemical control, the main aim is to develop fast, simple, versatile and reliable methods, which can be readily adapted for routine analysis at relatively low cost. In this context, multipumping flow analysis can play a prominent role since, due to the specific nature of components that comprised the flow system, they enable the development of miniaturized flow systems allowing a higher degree of simplicity and improved operational versatility. Therefore, its application in the chemical control of pharmaceutical formulations represents an attractive perspective, as an advantageous alternative to the reference methodologies that usually involve chromatographic techniques, being these highly time consuming, expensive and often requiring the use of environmental hazard reagents. Besides, applying separation 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, could be considered somehow excessive.

The multipumping flow analysis methodology [12] is based on the use of very small size solenoid actuated micropumps controlled by computer, that make possible a very simplified configuration of the flow system as well as its automatic control, since the basic operations in a chemical flow determination, namely, sample insertion, reagents addition, strategy for solutions mixing and transport of the reaction zone towards the detector unit are carried out by a single type component, being the solenoid micro-pumps the only manifold active elements. The fully automatic control of these devices, under time-based and pulse-counting routines, makes MPFS an attractive methodology for implementation of reliable and versatile analytical alternatives for determination of pharmaceutical compounds, with the additional advantage of permitting a runtime access to important analytical techniques and parameters, such as solutions manipulation (namely, stopped flow and reaction zone formation), flow rate and sample insertion.

The proposed miniaturized flow system enabled the determination of diazepam in commercially available formulations by spectrofluorimetric monitoring after the photo-degradation of the drug, which was accomplished by using of a low pressure UV radiation source coupled in line with the flow system. The proposed methodology was very simple, since it just involved a hydrolysis reaction catalyzed by UV radiation, which was only dependant on the solutions medium used. The use of a low pressure mercury lamp, instead of a high pressure arc lamp, avoided the cumbersome excessive heating of the solutions exposed to the radiation, thus preventing air bubbles formation that impair detection.

Materials and methods

Samples, standards and reagents

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

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. A 2 mol L^{-1} NaOH solution was prepared by dissolving 40 g in 500 mL of deionised water.

The diazepam solutions were prepared from the pure drug supplied by "Laboratórios Bial" (Porto, Portugal). A 200 mg L^{-1} diazepam stock solution was prepared by dissolving 20 mg of diazepam in 40 mL of absolute ethanol (Panreac®, 99.5%) and diluted to 100 mL with deionised water. This stock solution was protected from the light and stored under refrigeration.

The working diazepam standards (5–40 mg L^{-1}) were prepared, on a daily basis, by appropriate dilution of the stock solution: aliquots of diazepam stock solution (0.5–5.0 mL) were transferred into a series of 20 mL volumetric flasks and 2 mL of 2 mol L^{-1} NaOH solution and 6 mL of 0.1 mol L^{-1} SDS solution, were added. In order to obtain the same ethanol concentration (20%, v/v) for all working diazepam standards, appropriate aliquots of absolute ethanol (Panreac®) were also added. The volume was subsequently made up to the mark with deionised water.

Ten commercially available pharmaceutical formulations containing diazepam were used to prepare sample solutions, by weighing and powdering a representative number of tablets. Afterwards, an appropriate amount of sample, corresponding to 2.5 g of diazepam, 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 diazepam concentrations of approximately 50 mg L^{-1}, were transferred to 20 mL volumetric flasks and 2 mL of a 2 mol L^{-1} NaOH solution and 6 mL of a 0.1 mol L^{-1} SDS solution were added. The final volume was subsequently made up with deionised water.

Apparatus

The detector used to monitor the fluorescence intensity (λ_{ex}=272 nm, λ_{em}=450 nm) was a spectrofluorimeter Jasco (Easton, MD, USA), model FP-2020/2025, equipped with a 16 μL internal volume flow cell.

The developed flow manifold comprised two 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 irradiation of the solutions was carried out by using a 15W Philips TUV 15W/G15T8 low pressure mercury lamp, emitting short-wave ultraviolet radiation with a maximum radiation peak at 253.7 nm.

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.

Operation of the flow manifold

The developed analytical flow system, depicted in Fig. 1, employed two solenoid micro-pumps which were responsible for the individual handling of sample and carrier solutions.

The micro-pump P_1 (Fig. 1) was responsible for inserting the diazepam solution, while micro-pump P_2 was used for inserting and propelling the 0.2 mol L^{-1} NaOH solution, which was used as carrier. This flow scheme allowed a higher versatility in the control of the parameters that influenced the extension of diazepam photodegradation, particularly the time of exposure to UV radiation, as the placement of micro-pump P_1 responsible for samples insertion and transport prior to the UV light source, assembled in line with sample insertion tubing, easily enabled halting the flow for the time interval necessary to promote diazepam degradation.

The analytical cycle started by actuating P_1 for inserting the sample solution into the analytical manifold, filling the 2 m reactor tube (R) that was helically coiled around the UV lamp (L). The number of pulses was enough as to ensure that the reactor coil was completely filled with the sample solution, allowing, after sample irradiation, the carrying out of five consecutive measurements. Thereafter, P_1 was switched off and the UV lamp was turned on, starting the sample irradiation stage for a period of 15 min.

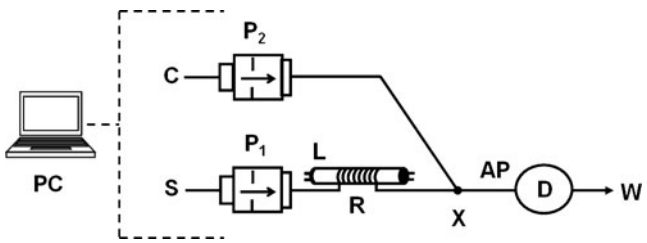

Fig. 1 Multipumping flow system (MPFS). *PC*—computer; *P1*, *P2*—solenoid micro-pumps (internal volume 10 μL); *X*—confluence point; *R*—2 m reactor; *D*—fluorimeter detector (λ_{ex}=272 nm; λ_{em}=450 nm); *L*—Philips UV lamp; *AP*—10 cm analytical path; *S*—sample; *C*—carrier (0.2 mol L^{-1} NaOH); *W*—waste

Following completion of the irradiation time, the lamp was turned off, and P_1 was reactivated, allowing the transport to waste of approximately 180 μL of non-irradiated sample solution. This non-irradiated volume was contained within the terminal 36 cm of the reaction tubing required to connect the UV sealed box (used to prevent any hazardous exposure) to confluence point X. This way, only the solution contained in the tubing coiled around the lamp was subjected to fluorimetric measurements. Subsequently, P_1 was switched off and by actuation of P_2 the NaOH carrier solution was inserted and propelled through confluence point (X) and through the detector (D) for cleanup and baseline establishment.

The analytical cycle proceeded with the activation of P_1, for the insertion of 100 μL of irradiated solution (ten pulses) in the analytical path. Then, by repeated actuation of micro-pump P_2, the reaction zone was carried toward the detector generating an analytical signal whose magnitude was proportional to the diazepam concentration. This process was repeated five times allowing the accomplishment of five consecutive measurements.

In all analytical procedures, the micro-pumps were operated at a pulse time of 0.2 s, establishing a flow rate of 1.71 mL min^{-1}.

Reference procedure

For assessing the accuracy of the results furnished by the developed procedure, commercially available pharmaceutical formulations containing diazepam were analyzed according to the reference procedure recommended by the British Pharmacopoeia [13], which rely on UV spectrophotometry. All pharmaceutical samples were weighed, powdered, and a determined amount was dissolved in a methanolic sulphuric acid solution, followed by dilution and filtration. The absorbance of the final solutions was determined by UV spectrophotometry at a wavelength of 284 nm.

Results and discussion

High pressure mercury lamps capable to emit radiation in the UV range have been used to carry out the photodegradation of several drugs. Nevertheless, it is well know that these lamps produce a significant temperature increase in the solutions that could lead to undesirable chemical processes, parallel to those induced by the UV radiation. Moreover, in flow analysis increased temperatures result in oscillations in flow-rate and in the formation of air bubbles, leading to reduced reproducibility and sometimes even impaired detection. The use of high pressure arc UV lamps usually requires a cooling system, placed after irradiation,

to eliminate air bubbles formed at high temperatures [8]. To prevent this situation, and since preliminary results have shown that diazepam was adequately susceptible to UV irradiation, a low pressure mercury lamp was used to promote the photo-degradation of this compound. In order to evaluate the advantages in implementing the photo-degradation process in a miniaturized flow system by exploiting the multipumping concept, a comparison study of the results obtained upon diazepam exposure to UV radiation in glass beakers and inside a flow tube coiled around the UV lamp, was carried out. Therefore, batch assays were performed involving the exposure to UV radiation of several 200 mg L^{-1} diazepam solutions in 2 mol L^{-1} sulphuric acid contained in glass beakers, for 10 min, 25 min and 45 min. For the proposed flow-based procedure, a multipumping flow system comprising a UV lamp was implemented. A solution containing 50 mg L^{-1} diazepam in 2 mol L^{-1} sulphuric acid was inserted in the flow tubing coiled around the UV lamp, and exposed to the radiation by exploiting the stopped-flow technique, maximizing this way the radiations effects.

The results of the batch assays, involving the analysis of the recorded excitation and emission spectra, revealed that the effect of UV radiation for the assayed exposure intervals was insignificant and the formation of fluorescent compounds was negligible. On the other hand, when observing the results obtained from the flow-based procedure, it was concluded that even by exposing the solutions to UV radiation for a short time period of 10 min, a noteworthy production of fluorescent products (λ_{ex}=262 nm; λ_{em}= 463 nm) was accomplished, even taking into account that the parameters controlling the flow system were not optimized. These results confirmed that the fully integration of a UV light source in a miniaturized flow system, by coiling a flow tube around a UV lamp, exhibits several advantages, namely, the reduction of the distance between the solutions and the light source, the increase of the exposed area of solutions to radiation, the high reduction in the solutions volume used, and finally, the accomplishment of a fully automation of the whole analysis procedure. Therefore, it was concluded that the implementation of the photo-degradation of diazepam in a miniaturized flow system effectively promotes the hydrolysis of diazepam with improved efficiency, by means of increasing the reaction extension and the intensity of fluorescent signal.

In photochemical systems, the source intensity, irradiation time and medium composition, influence the nature and concentration of the degradation products formed in the process, which are frequently obtained via a multitude of possible pathways. Aiming at a high photo-degradation yield, some studies were conducted for optimization of chemical and physical parameters.

Optimization of chemical parameters

Since the pH of sample solutions can influence the extension of the photo-degradation process several studies involving the photo-degradation of diazepam in different acid and alkaline mediums were carried out. Simultaneously, several substances, namely, metal ions and surfactants, were tested for their capacity to increase the extension of the diazepam photo-degradation in acid and alkaline mediums. These studies were aimed at obtaining the higher fluorescence intensity for the degradation products. Several solutions were prepared and irradiated with UV light by making use of the developed flow system. Two groups of sample solutions were prepared: one containing 50 mg L^{-1} of diazepam in 0.050 mol L^{-1} sulphuric acid and a second one containing 50 mg L^{-1} of diazepam in an equimolar concentration of sodium hydroxide. Each of these solution groups was constituted by different solutions containing different substances that one would expect to increase the fluorescence intensities of the analyzed samples. The substances used were (i) Cu(II), Fe(III) and Ce(IV), in a concentration of 5×10^{-4} mol L^{-1}; (ii) non-ionic, cationic and anionic surfactants, respectively, methylcellulose (MC), cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS), in concentrations of 0.01% for MC and 0.01 mol L^{-1} for SDS and CTAB. The selected concentration values for the surfactants were higher than the respective critical micelle concentrations (cmc). For comparison purposes two reference solutions (RS) that contained only diazepam in the acid or alkaline medium (0.050 mol L^{-1} of sulphuric acid and sodium hydroxide, respectively) were used. All solutions were irradiated for 10 min.

A compilation of the assays and the obtained results is represented in Table 1.

The obtained results show a decrease of fluorescence intensity for the acid solutions containing the metal ions, SDS and CTAB surfactants, when comparing with the acid reference solution (Acid-RS). In the case of MC surfactant the differences of analytical signal comparatively to the reference solution were not significant. On the other hand, the assays in alkaline medium revealed a pronounced increase in fluorescence intensity when using Fe (III), MC and SDS, in relation to the alkaline reference solution (Alk-RS). Also, when using Cu (II) and Co (II) in alkaline medium a decrease in fluorescence intensity was observed. The highest increment value (110% regarding the reference solution) was obtained with SDS.

These results clearly demonstrate that enhanced fluorescence intensity is obtained under alkaline conditions in the presence of SDS. The formed micellar medium results in an organized microenvironment that promotes the photo-degradation reaction and/or the fluorescence emission. In

Table 1 Evaluation of the influence of pH and different substances in the photo-degradation process of diazepam

	[Diazepam]	[Acid] or [Base]	[Tested substance]	λ_{ex} λ_{em}	Fluorescence intensity
Acid-RS			–		0.2871
Acid-Cu (II)			Cu (II)		0.2470
Acid-Fe (III)			Fe (III)	λ_{ex}=262 nm	0.0779
Acid-Ce (IV)	50 mg L^{-1}	$[H_2SO_4]$ = 0.050 mol L^{-1}	Ce (IV)	λ_{em}=463 nm	0.1889
Acid-CTAB			CTAB		0.1971
Acid-SDS			SDS		0.2592
Acid-MC			MC		0.2961
Alk-RS			–		0.1758
Alk-Cu (II)			Cu (II)		0.1233
Alk-Fe (III)	50 mg L^{-1}	[NaOH]=0.050 mol L^{-1}	Fe (III)	λ_{ex}=272 nm	0.2123
Alk-Ce (IV)			Ce (IV)	λ_{em}=450 nm	0.1697
Alk-SDS			SDS		0.3690
Alk-MC			MC		0.2420

order to discriminate between these two potential effects of SDS, some studies were performed involving the addition of the SDS reagent either before or after the irradiation stage. These studies were easily implemented in the flow system, due to its modular structure that allowed the inclusion of a third micro-pump, which was responsible for the insertion of SDS after the irradiation stage. Two different solutions of diazepam were prepared: one containing 50 mg L^{-1} of diazepam in 0.05 mol L^{-1} of NaOH, and a second one containing the same concentration of diazepam in 0.05 mol L^{-1} NaOH and 0.01 mol L^{-1} SDS. In the experiments, three different determinations using the flow system with three micro-pumps were conducted: (i) the first procedure, used as reference, involved the irradiation for 10 min of the 50 mg L^{-1} diazepam in 0.05 mol L^{-1} NaOH solution, followed by the insertion, using the merging zones approach, of ten pulses of the irradiated solution and ten pulses of 0.05 mol L^{-1} NaOH solution; (ii) in the second procedure, the 50 mg L^{-1} of diazepam in 0.05 mol L^{-1} NaOH solution was irradiated for 10 min followed by the insertion, using the merging zones approach, of ten pulses of the irradiated solution and ten pulses of 0.01 mol L^{-1} SDS solution; (iii) finally, the third procedure involved the irradiation for 10 min of the 50 mg L^{-1} diazepam in 0.05 mol L^{-1} de NaOH and 0.01 mol L^{-1} SDS solution, followed by insertion of ten pulses of irradiated solution and ten pulses of 0.05 mol L^{-1} NaOH solution by merging zones. For each of the described approaches, the 0.05 mol L^{-1} NaOH solution was used as carrier for the transport of the reaction zones towards the fluorescence detector.

The results revealed fluorescence intensities of 0.280 (±0.007), 0.256 (±0.004) and 0.580 (±0.02) for the (i), (ii) and (iii) procedures, respectively. Accordingly, it could be concluded that the use of SDS surfactant promoted the photoreaction development, since it was verified an increase of 109% in the fluorescence intensity when the diazepam solution was irradiated in the presence of SDS. For the subsequent assays, the solutions of diazepam were always prepared in NaOH and SDS solution.

Aiming at increasing the fluorescence intensities in the proposed methodology, several concentrations of sodium hydroxide (0.01–0.50 mol L^{-1}) were tested in solutions containing 50 mg L^{-1} diazepam and 0.01 mol L^{-1} SDS. After the UV irradiation stage for 10 min, by exploiting the stopped-flow method, 100 μL of sample (ten pulses) were transported towards the detector by using as carrier the corresponding solution of NaOH at the concentration under evaluation. The results showed that the fluorescence intensity increased at approximately up to a concentration of 0.20 mol L^{-1} of NaOH and, that for higher concentration values, the analytical signal tended to stabilization. For the subsequent assays a concentration of 0.20 mol L^{-1} of NaOH was selected.

The influence of the SDS concentration on fluorescence intensities was evaluated by analyzing several diazepam solutions containing different concentrations of SDS (0.001–0.05 mol L^{-1}). Several solutions containing 50 mg L^{-1} of diazepam, 0.20 mol L^{-1} NaOH and different concentrations of SDS, were inserted in the multipumping flow system and subjected to UV radiation for a period of 10 min. Afterwards, ten pulses of sample (100 μL) were transported to the fluorescence detector, using as carrier a 0.20 mol L^{-1} NaOH solution.

The obtained results demonstrated that the fluorescence intensity increased pronouncedly until a SDS concentration value of 0.030 mol L^{-1} and that for higher concentrations it approached stabilization. Thus, for the

remaining assays it was selected a SDS concentration of 0.030 mol L^{-1}.

The ethanol concentration used to prepare the stock solution of diazepam (200 mg L^{-1} in 40% ethanol), from which the diazepam standard solutions were prepared, could also affect the obtained fluorescence intensity. This way, the effect of ethanol in the analytical signal was assessed up to concentration value of 20% (v/v) but no significant differences in the fluorescence intensities were observed for all ethanol concentrations assayed.

Optimization of physical parameters

Since the fluorescence intensity could be influenced by the time of irradiation that determined the extension of diazepam photo-degradation, and by other parameters, namely the analytical path length (AP, Fig. 1), flow rate and sample volume inserted that could affect not only the residence time but also the extension of the reagents mixture and its dispersion inside the flow system, some studies were conducted aiming at the optimization of these physical parameters.

Different periods of irradiation with UV light, comprising 0 min, 1 min, 3 min, 5 min, 8 min, 10 min, 12 min, 15 min, 20 min and 25 min, were applied to solutions containing 50 mg L^{-1} diazepam in 0.20 mol L^{-1} NaOH and 0.030 mol L^{-1} SDS, by the stopped-flow technique. After the irradiation stages, 100 μL of irradiated sample was inserted into the system and transported toward the fluorescence detector for monitoring. The results revealed a small increase in fluorescence intensity when increasing the irradiation time up to approximately 5 min, but a pronounced increase of the fluorescence intensity signals between 5 min and 15 min. For irradiation periods higher than 15 min the fluorescence intensity tended to stabilization. Considering the results, an irradiation time of 15 min was selected for posterior assays, enabling this way the maximization of the photo-degradation process.

The optimization of the analytical path length (AP, Fig. 1) and inserted sample volume was performed simultaneously. This study involved the irradiation of solutions containing 50 mg L^{-1} diazepam in 0.20 mol L^{-1} NaOH and 0.030 mol L^{-1} SDS, for periods of 15 min. Next, by using a fixed pulse time of 0.2 s (1.71 mL min^{-1}), different sample pulses (two to 18 pulses, corresponding to 20–180 μL of sample) were inserted in the flow system and transported towards the detector for fluorescence intensity monitoring. This experiment was repeated for each of the AP lengths tested, namely, 10 cm, 30 cm, 60 cm and 85 cm. The obtained results, represented in Fig. 2, were in agreement with what would be expected, confirming that an AP length of 10 cm allowed obtaining the highest fluorescence intensity in all the experimental conditions. In

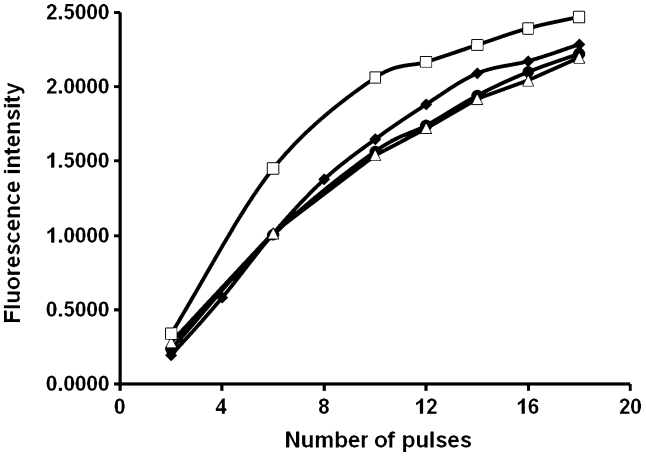

Fig. 2 Influence on analytical signal of the number of pulses using different AP lengths. □—10 cm; ♦—30 cm; ●—60 cm; △—85 cm

fact, a small AP length causes a minimized dispersion of the reaction zone and therefore an analytical signal maximization. Additionally, from Fig. 2 it can be observed an accentuated increase on the analytical signal up to approximately ten pulses (100 μL) and a subsequent stabilization for higher values. As a compromise between determination rate and sensitivity, this sample volume was selected for the analysis.

The study of the influence of flow rate on the fluorescence intensity signal was also performed. In this assay, different pulse times of 0.1 s, 0.2 s, 0.3 s and 0.4 s (corresponding to flow rates of 2.40 mL min^{-1}, 1.71 mL min^{-1}, 1.33 mL min^{-1}, 1.09 mL min^{-1}) were used to transport to the fluorescence detector the reaction zone, constituted by ten pulses (100 μL) of a previously irradiated solution (15 min) containing 50 mg L^{-1} diazepam in 0.20 mol L^{-1} NaOH and 0.030 mol L^{-1} SDS. After analysis of the results (Fig. 3), it was verified that the flow rate had a very small influence in the analytical signal intensity, for pulse times comprised between 0.2 s and 0.4 s, which is clearly explained by the short length of the reaction coil used. For 0.1 s of pulse time, the working frequency of the micro-pumps is high, and therefore the chaotic movement of the solutions inside tubing due to the characteristic pulsed flow nature of the MPFS originates a slightly increase of the sample dispersion, causing thus, a decrease in the fluorescence intensity. As a compromise between sampling rate and precision, a pulse time of 0.2 s (flow rate of 1.71 mL min^{-1}) was chosen.

Interferences

In order to apply the developed methodology to the determination of diazepam in pharmaceutical formulations, the influence of some compounds commonly used as excipients was assessed. Different sample diazepam sol-

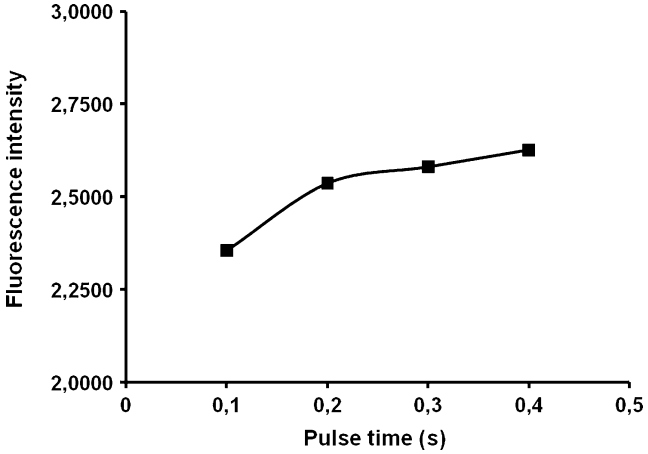

Fig. 3 Influence on analytical signal of different pulse times: 0.1 s, 0.2 s, 0.3 s and 0.4 s (corresponding to flow rates of 2.40 mL min^{-1}, 1.71 mL min^{-1}, 1.33 mL min^{-1}, 1.09 mL min^{-1})

utions containing a fixed amount of diazepam (20 mg L^{-1}) and different quantities of the excipients under evaluation were analyzed by the developed methodology. A compound 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 and cellulose up to a 100-fold mass ratio, starch, povidone and colloidal silica up to a 50-fold mass ratio, and lactose up to a 25-fold mass ratio, did not interfere.

Chemical control of pharmaceutical formulations

By using the previously referred optimized experimental conditions a linear response range for diazepam concentrations of up to 40 mg L^{-1} was obtained. The calibration curve was represented by $FI = 0.0451\ (\pm\ 0.0004) \times C - 0.0083\ (\pm\ 0.0080)$, in which *FI* was the fluorescence intensity and *C* was diazepam concentration, in mg mL^{-1}. A correlation coefficient of 0.9998 was verified. The detection limit calculated from the equation of the calibration curve according to Miller and Miller [14] was about 0.97 mg L^{-1}.

In order to validate the proposed methodology, based in a miniaturized multipumping flow system, the results obtained in the determination of diazepam in ten commercial pharmaceutical formulations were compared with those furnished by a reference procedure of the British Pharmacopoeia [13]. The results, summarized in Table 2, showed a good agreement between both methods, with relative deviations between −2.09% and 2.13%. A paired Student's t-test [14] confirmed that there were no statistical differences ($t_{estimated} = 0.474$, $t_{tabulated} = 2.260$) between the results obtained by both procedures, for a confidence level of 95% ($n = 10$).

The results attained in the evaluation of the developed methodology precision, by repeated analysis (four consecutive determinations) of each commercial pharmaceutical formulation revealed a good repeatability (Table 2), at a confidence level of 95%.

The developed methodology allowed a determination rate of 14 h^{-1}.

Conclusions

The implementation of an automated multipumping flow system with a photo-degradation unit for diazepam determination, based on the susceptibility of this benzodiazepine to UV irradiation and the subsequent generation of fluorescent products further reinforced by a micellar medium, proved to be a valuable alternative for the chemical control of this drug, since the developed flow system was very simple to operate and control and exhibited high versatility and low reagents consumption, when in comparison with traditional methods, like for example, chromatographic procedures.

Table 2 Comparison of analytical results obtained in the determination of diazepam in pharmaceutical formulations by the proposed and the reference method

Pharmaceutical sample	Declared dosage mg/formulation	Amount found (mg/formulation)[a]		R. D. %[b]
		MPFS methodology	Reference method	
Valium roche 10	10	9.75±0.03	9.9±0.4	−1.40
Valium roche 5	5	4.93±0.04	4.89±0.05	0.92
Diazepam labesfal 10	10	10.1±0.1	10.02±0.01	1.16
Diazepam labesfal 5	5	4.87±0.04	4.81±0.09	1.29
Bialzepam 3	3	2.88±0.07	2.86±0.01	0.69
Bialzepam 6	6	5.68±0.01	1.51±0.05	0.67
Diazepam ratiopharm 10	10	10.0±0.2	10.10±0.02	−0.06
Diazepam ratiopharm 5	5	4.90±0.06	4.80±0.02	2.13
Metamidol winthrop 10	10	10.06±0.08	9.95±0.08	1.14
Metamidol winthrop 5	5	4.82±0.05	4.9±0.3	−2.09

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

[b] Relative deviation of the development method regarding the reference procedure

The developed MPFS can be further applied in the monitoring of natural fluorescence substances, or of their degradation compounds, obtained by photo-degradation through exposure to UV radiation, assuming that they exhibit fluorescence properties. The UV lamp coupled to the developed MPFS can be easily turned on or off, emphasizing the versatility of the analytical system. Additionally, by implementing a UV lamp in an automatic flow system the life-time of the lamp is increased, since the system control enables the use of the lamp only when it is strictly necessary. Moreover, it contributes to a lower power consumption and a lower and more stable operational temperature within the flow analytical system that present several advantages such as to avoid the formation of air bubbles and to prevent fluctuations in flow rate and interferences in the analytical signal reproducibility and magnitude.

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