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Short Communication

On-line photoreaction and fluorimetric determination of diazepam*

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Introduction

Diazepam is 7-chloro-1,3 dihydro-1-methyl-5-phenyl,1-2H-1,4-benzodiazepin-2-one, an off-white to yellow, practically odourless crystalline powder. It is a pharmaceutical compound classified as a minor tranquilizer used in the treatment of psychoneuroses to reduce pathological anxiety, agitation and tension. The side-effects of diazepam are mild and infrequent [1, 2].

Different analytical procedures for diazepam determination have been published, like the non-aqueous titration in acetic media; a single reduction wave proportional to concentration in the range 2×10^{-4}–7×10^{-4} M is a suitable polarographic assay. The direct spectrophotometric analysis of diazepam is applicable provided significant quantities of the hydrolytic contaminants are not present. The separation of diazepam from its metabolites has been reported by means of a HPLC procedure [3]. The acid hydrolysis of blood extracts has been used to prepare volatile derivatives (benzophenones) for GC separation [4].

On the other hand, some recently published work has dealt with different FIA–photodegradation procedures for the determination of drugs [5–8]. The implementation of an unsegmented-flow manifold with the irradiation source demonstrated the possibility of translating the classical photoreaction methods into FIA procedures [9].

This paper deals with a FIA–fluorimetric procedure for diazepam determination on the basis of the formation of fluorescent product by photoreaction.

Experimental

Reagents, apparatus and procedures

All used reagents were of analytical grade unless indicated. The following reagents were used: diazepam (Guinama, Valencia), ethanol (Panreac, Barcelona) sodium hydroxide (Probus, Madrid), copper sulphate (Panreac), piridoxyne (Guinama), glucose (Panreac), lactose (Panreac), sorbitol (Acofarma, Valencia), sucrose (Acofarma).

Flow injection manifold

The analytical procedure was carried out in a mono-channel FIA assembly in which the sample is irradiated through PTEF tubing coiled around a lamp; the lamp was placed in the sample loop of the injecting valve. The manifold (shown in Fig. 1) contained a peristaltic pump Gilson Minipuls, Model 2, and a six-port Rheodyne valve, model 5051. The irradiation was made by a Vilber–Lurmat T-60 mercury lamp and then the sample was transferred to a Perkin–Elmer LS 50 fluorimetric detector furnished with a Hellma flow cell (20 μl inner volume). The PTEF tubing was of 0.5 mm i.d.

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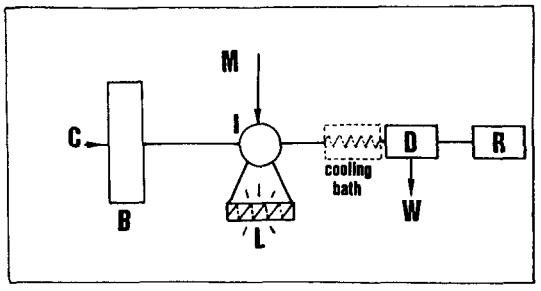

Figure 1
Flow assembly for the determination of diazepam. C, Carrier (pure distilled water); B, peristaltic pump; M, sample solution (in Cu(II) and NaOH media); I, injection valve; L, lamp; D, detector; R, recorder; and W, waste.

Determination of diazepam

For the evaluation of diazepam, the sample containing about 20 μg ml^{-1} is prepared in an aqueous solution of 0.04 M sodium hydroxide containing 6.0 μg ml^{-1} of cupric ions. The sample solution is irradiated inside the sample loop of the injecting valve in the flow assembly and then is inserted into a carrier stream of pure distilled water which carries the sample solution to the fluorimeter flow cell.

Results and Discussion

Preliminary experiments aimed to determine the formation of a fluorescent product from the pharmaceutical in aqueous solutions by direct UV irradiation. The observed effect of irradiation was a spectrum of fluorescence with the maxima at 328 and 382 nm, for excitation and emission, respectively.

These preliminary experiments revealed the formation of a fluorescent product requiring a strong and long exposure to the action of irradiation. The effect of light was noticeable in continuous-flow, by coiling PTEF tubing around the lamp, and it was negligible when the solution was transferred to a beaker with the lamp standing over it.

The small aqueous solubility of the drug imposed the use of the hydro-alcoholic mixture as solvent; the concentration of ethanol did not influence the height of the outputs, but did affect the reproducibility of the results significantly. A concentration of 4% ethanol was selected for further work.

The suitability of different media was tested. Acidic media was found to be unsuitable even for long exposures (up to 5 min). The basic media were more suitable: sodium hydroxide, ammonium, phosphoric/phosphate buffers, etc., were all tested at different temperatures. Sodium hydroxide at low concentrations (about 0.01 M) and room temperature was selected for further experiments.

Various catalysts were also tested in order to speed up the reaction rate; metal ions (Co(II), Mn(II), Fe(III), Cu(II) and Ag(I) with persulphate present) were assayed in different media. The presence of cupric ions in weak alkaline media resulted in a significant increase of the emitted light; the fluorescence intensity was about seven times that obtained without the cupric ions present. Other significant increases in emitted light were obtained with the cupric ions in ammonium medium and with Ag(I); the presence of other oxidative reagents, such as persulphate, also resulted in an increased emission.

Flow injection manifold

The most suitable configuration for a FIA manifold was studied bearing in mind results obtained in the previous experiments. Special attention was paid to the following: (a) the stopped-flow technique should be considered as a consequence of the required irradiation time and, at the same time, sample dispersion should be minimized; (b) the media must be inert with respect to the drug (hydrolytic changes should be avoided). The suitable media was a mixture of cupric ions in sodium hydroxide solution; the ammonia and phosphoric/phosphate buffers being discarded. The selection configuration is shown in Fig. 1, in which the sample loop is coiled around the lamp. Due to the stability of the aqueous solutions of the drug it is possible to prepare and store the drug in an aqueous solution of sodium hydroxide and with cupric ions present. On the other hand, this configuration allows the sample to be in full contact with the irradiation source (as in the stopped-flow mode) without requiring the continuous stop–start sequence of the pump.

Once the continuous-flow assembly had been selected, the optimization of chemical and manifold parameters was carried out by means of the univariate method. The parameters to be optimized included lamp power, irradiation wavelength and time, temperature, influence of other solvents, and concentration of cupric ions or sodium hydroxide. The aim of the optimization was to reach the best compromise peak height/baseline width/repro-

Table 1
Optimization of FIA parameters

Parameter	Tested range	Selected value
Irradiation time (min)	1–15	5
Length of irradiated coil (m)	1–4	3
Cu(II) conc. (ppm)	0.05–10	3.0
NaOH conc. (M)	0.02–0.08	0.02
Carrier flow rate (ml min^{-1})	1.8–6.2	3.0
Length valve-detector (m)	1–4	1.0
Temperature	room temp.–80	room temp.
Wavelength ex. (nm)	317.0–321.0	319.0
Window width ex. (nm)	6.0–10.0	8.0
Wavelength em. (nm)	362.0–366.0	362.0
Window width em. (nm)	6.0–10.0	10.0

ducibility. The tested ranges and the optimum selected value are given in Table 1.

The irradiation time was the most critical parameter; experiments were carried out for different analyte concentrations (Fig. 2), the maximum emission was reached after 5 min. The sample volume (length of tubing coiled around the lamp) had a significant effect on the peak-height and on the width of the base-peak (Fig. 3). The influence of the sample flow rate on the outputs was irrelevant and the carrier flow rate only influenced the sample passage (width of the base-peak).

The influence of temperature was tested by introducing into a water-bath the pre-valve coils for the sample and the carrier. The influence of the temperature was studied up to 80°C. Higher temperatures resulted in a decreased emission intensity and an increase in regular bubbling (which results in changing flow rate and, as a consequence, the reproducibility). Room temperature was selected for further work. The bubbling was also present at room temperature; possibly due to presence of ethanol and the heat from the lamp. The problem was solved by cooling the sample after the irradiation as shown in Fig. 1. Different coil lengths were tested over the range 1–4; 2.0 m was selected as the length producing the highest outputs without bubbling.

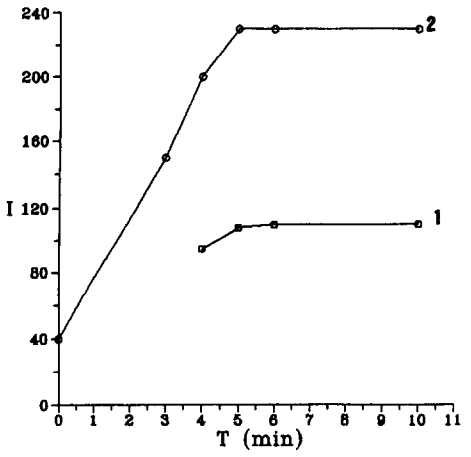

Figure 2
Influence of irradiation time. 1, 2.5 μg ml^{-1} diazepam; 2, 5.0 μg ml^{-1} diazepam.

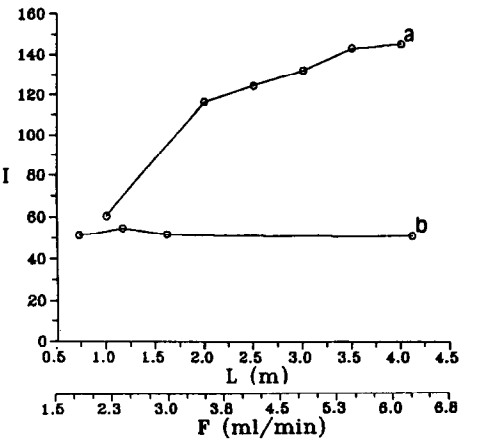

Figure 3
Influence of FIA parameters. (a) Length of irradiated coiled tubing; (b) flow rate of the carrier (pure distilled water).

The re-optimization of chemical variables (concentration of cupric ions and sodium hydroxide) was studied over the ranges 3.0–9.0 μg ml^{-1} and 0.02–0.06 M, respectively. The selected values are given in Table 1.

Analytical applications

The calibration graph was linear from 0.5 to 50 μg ml^{-1} and gave the equation

$$I = 21.344\,x - 2.510,$$

where I is the intensity of fluorescence and x the concentration of the drug ($\mu g\ ml^{-1}$). The correlation coefficient was 0.9998.

A set of 10 different samples containing 7.0 $\mu g\ ml^{-1}$ of diazepam were injected in order to determine the reproducibility (RSD) and sample passage; the obtained results were 2.1% and 10 h^{-1}, respectively.

The tolerance of the method to foreign compounds which can be found in typical pharmaceutical samples containing diazepam was investigated by using solutions containing 20 $\mu g\ ml^{-1}$ of the drug and adding various concentrations of the interferents up to 200 $\mu g\ ml^{-1}$ or when the realtive error (by comparing with pure diazepam solutions, 20 $\mu g\ ml^{-1}$) was about 3%. The results obtained were (conc. in $\mu g\ ml^{-1}$ and relative error in %): pyrydoxyne 1, 6.2; glucose 150, 3.4; lactose 200, 3.0; sorbitol 25, 0.01; and sucrose 200, 4.1. The determination of the drug content was carried out in two different sample solutions: (a) propylene glycol containing 1001.0 $\mu g\ ml^{-1}$ of diazepam; and (b) ethanol containing 5098 $\mu g\ ml^{-1}$ of the drug; both prepared in our laboratory according to ref. 2. Aliquots (5 ml) were taken from the (a) solution and mixed with 2 ml of 2 M NaOH and 6 ml of 100 $\mu g\ ml^{-1}$ Cu(II); the resulting mixture was diluted to 200 ml: found = 25.6 $\mu g\ ml^{-1}$, relative error = 2.1%. Aliquots (1 ml) from the stock solution (b) were mixed with 2 ml of NaOH 2 M and 6 ml of 100 $\mu g\ ml^{-1}$ Cu(II) and then made up to volume: found =25.9 $\mu g\ ml^{-1}$ relative error 1.8%.

Conclusions

An FIA method has been developed for the measurement of diazepam by fluorescence detection following on-line UV irradiation of the sample contained in a PTFE injection loop. The method is quantitative over the range 0.5–50 $\mu g\ ml^{-1}$ with RSD ± 2.1% (7 $\mu g\ ml^{-1}$). The throughput achieved was 10 samples per hour. Although currently less sensitive than chromatographic methods, this approach provides a simple and rapid procedure for typical pharmaceutical samples.

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