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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 45 (2007) 318–321
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Fluorimetric SIA optosensing in pharmaceutical analysis: Determination of paracetamol

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Received 25 November 2006; received in revised form 2 May 2007; accepted 3 May 2007
Available online 10 May 2007

Abstract

The coupling of sequential injection analysis (SIA) and fluorimetric solid phase transduction is here applied to the determination of paracetamol in pharmaceuticals. The reaction product between the analyte and sodium nitrite in acidic medium is inserted, after alkalinization, in the system. This product is transitorily retained on the active solid sensing phase (the anionic solid support QAE A-25) developing its native fluorescence signal, which is measured at 325/430 nm for the excitation and emission wavelengths respectively. The described system is linear within the range 6.6–80 μg ml^{-1}, with a 2 μg ml^{-1} detection limit and a 2.5% R.S.D ($n = 10$). The proposed fluorimetric SIA optosensor has been applied to the determination of paracetamol in several pharmaceutical preparations, obtaining satisfactory results.

Keywords: Optosensing; Sequential injection analysis; Pharmaceuticals; Nitrosation

1. Introduction

Sequential injection analysis (SIA) is based on the forward and reverse movement of the piston of a syringe pump, as long as a multi-position selection valve automatically controlled by a computer. The use of the computer software enables the precise sampling of chemicals into the system and propelling of the sequenced zones to the reactor and detector in a reproducible way [1,2]. The main advantages of SIA are its versatility, high sample throughput, low sample and reagent consumption and complete automation by means of appropriate software.

The coupling of SIA and solid phase spectroscopy (SPS) has been described [3] as an alternative to conventional optosensors, that is, the coupling of flow injection analysis (FIA) and SPS [4,5]. The main handicaps of conventional FIA, that is, lack of automation (the introduction of reagents into the system is manually controlled with rotary valves) and high reagent consumption (the carrier is continuously flowing through the system) are avoided by using SIA. Hence, the implementation of automatic flow methodologies, such as SIA, in SPS is an interesting research field. Here we propose a SIA–SPS methodology for the fluorimetric determination of paracetamol after appropriate derivatization reaction.

Paracetamol, PCT, (acetaminophen, *N*-acetyl-*p*-aminophenol, 4-acetamidophenol) is an extensively employed analgesic and antipyretic drug, which can be prescribed solely or with other related drugs [4–6]. Due to the importance of this drug, a high number of analytical methods have been previously described for its determination in pharmaceuticals. When the UV spectrophotometry has been used, the direct measurement of PCT is not possible due to the spectral overlapping with other compounds that usually come along in pharmaceuticals and also absorb in the UV spectral region, so different strategies have been used, such as derivatization reactions [7], partial least-squares [8], or the use of flow-through optosensing [4–6]. The determination of PCT by using fluorescence after a derivatization reaction has also been used [9–11], wasting a lot of time in the reaction process [9] or needing an extraction step to avoid interferences [11] as main handicaps. Other separations methods, such as high performance liquid chromatography [12,13], ion chromatography [14] or micellar liquid chromatography [15] with UV detection have been developed too.

The reaction between nitrite and paracetamol in acidic conditions, followed by alkalinization with sodium hydroxide is

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doi:10.1016/j.jpba.2007.05.004

here applied to the determination of paracetamol in pharmaceuticals. This reaction has been previously described and used for determining PCT with UV spectrophotometric detection [7] or nitrite with fluorescence detection [16]. In the case of determining PCT [7], the obtained detection limit was very high and a proper interference study was not carried out. In this paper, we have highly improved the detection limit (in two orders of magnitude) by using fluorescence detection and the solid support in the flow-through cell and a proper interference study has been performed (acetylsalicylic acid interference, normally problematic [11] is here easily eliminated). The fluorimetric SIA optosensor here developed is based on the continuous measurement of the fluorescence signal from the oxidation product of the analyte directly retained on the solid sensing phase. The method has been satisfactorily applied to the determination of PCT in pharmaceuticals, and an additional recovery study has been also performed, obtaining excellent results.

2. Experimental

2.1. Reagents and solutions

PCT, sodium hydroxide, hydrochloride acid and sodium nitrite were purchased from Sigma–Aldrich (Alcobendas, Madrid, Spain). Stock solution of 1000 mg l^{-1} of PCT was prepared in double deionized water and was kept in the dark under refrigeration.

Sephadex QAE A-25, 40-120 μm average particle size (Sigma–Aldrich) and C_{18} bonded phase silica gel beads (Waters, Milford, USA) with 55–105 μm of average particle size were tested as sensing supports.

2.2. Instrumentation

A commercially available instrument FIAlab® 3500 system (FIAlab® Instruments, USA) with a syringe pump (syringe reservoir 5.0 ml) and an 8-port selection Cheminert valve (Valco Instrument Co., USA) was used. The manifold was equipped with fiber-optic fluorimetric detector PMT-FL (Ocean Optics, Inc., USA) with UV light source D-1000-CE. A 340 (300–380) nm primary filter and a 435 (390–510) nm secondary filter were used (Edmund Industry Optic, GmbH, Germany). The whole SIA system was controlled by the latest version of program FIAlab for Windows 5.0. Flow lines were made of 0.8 mm i.d. PTFE tubing.

A Hellma flow cell 176.752-QS (25 μl of inner volume and a light path length of 1.5 mm) was used. The cell was filled with QAE A-25 solid support micro beads, and was blocked at the outlet with glass wool to prevent displacement of the particles.

2.3. Preparation of the samples and reaction conditions

The pharmaceutical samples were chosen in several presentation ways. Two activated tablets were completely dissolved in double-distilled water, filtered and diluted to 200 ml in a volumetric flask. In the case of granular packets, one granular packet was dissolved in double distilled water by using sonication and diluted to 200 ml in a volumetric flask.

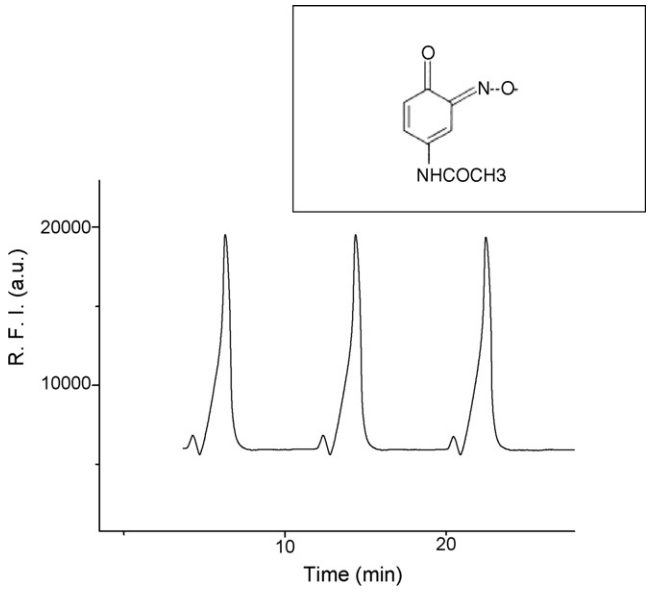

Fig. 1. Flow profile of the transient signal obtained for 10 μg ml^{-1} of PCT. Structure of the final reaction product.

The procedure for the reaction was as follows: a suitable volume of the sample (or PCT stock solution) was placed in a 25 ml volumetric flask. After that, 30 μl HCl 0.2 M and 50 μl $NaNO_2$ 1000 mg l^{-1} were added, and the flask was vigorously stirred. After 5 min, 1 ml NaOH 2 M was added and the flask was completed to volume with double distilled water. After 5 min, the reaction product is stable and the measurement can be carried out.

2.4. General SIA procedure

In the first step, 2.5 ml of the 0.2 M HCl carrier/eluting solution and 1.5 ml of sample (after the derivatization reaction took place) were aspirated by the syringe pump. The sample was pumped towards the flow-though cell at 0.9 ml min^{-1} and the transitory signal (peak height) from the reaction product from PCT was recorded. The analyte was eluted from the solid support by the carrier solution itself. Each sample was analyzed by triplicate. A typical profile of the signal is shown in Fig. 1.

3. Results and discussion

3.1. Reaction product

The reaction of PCT and nitrite has been previously described [7,16]. The first step consists of the reaction of PCT with nitrite under acidic conditions, the nitrosation of PCT occurring under these conditions. The second step is the stabilization of the reaction product, the nitroso compound, by means of using a sodium hydroxide solution. The resulting compound in this alkaline medium, which is a fluorescent stable one, is retained on the

QAE A-25 microbeads and its fluorescence (directly measured on the solid support) is the analytical signal used in the SIA optosensor. The structure of the final compound is depicted in Fig. 1.

3.2. Selection of the solid support

The reaction product in the basic medium is present in anionic form, as can be observed in Fig. 1. Hence, we decided to test the anionic QAE A-25 solid support. In addition, we also tested silica gel C_{18} non-ionic solid support.

Similar analytical signals were obtained with both solid supports, but the compound could not be eluted from silica gel C_{18}; therefore, Sephadex QAE A-25 anionic solid support was selected.

3.3. Instrumental variables

The net fluorescence spectra of the reaction compound were recorded on the solid support. The maximum excitation and emission wavelengths were 325 and 430 nm respectively. Therefore, 340 (300–380) and 435 (390–510) nm were selected for the primary and secondary filters respectively.

When using the Shimadzu spectrofluorimeter for comparative purposes (only when acetylsalicylic acid is present in the samples) the excitation and emission wavelengths were 350 and 535 nm respectively, with 10/10 nm slit widths.

3.4. Chemical variables

3.4.1. Selection of the carrier solution

In order to elute the analyte from ionic Sephadex solid support, changes of pH or high salt concentrations are normally used. It was observed that using water as carrier/eluting solution the elution was not achieved; therefore, different concentrations of HCl were tested. For HCl concentrations lower than 0.2 M the elution was not complete. Hence, 2.5 ml of 0.2 M HCl was selected in order to complete the elution of the reaction product from the solid support, obtaining a good sensitivity and repeatability.

3.4.2. Reaction conditions

Different variables had to be studied: concentrations of HCl, $NaNO_2$ and NaOH, as long as the reaction and stabilization times. Therefore, for the study of each variable, all others were maintained constant as the one under study was changed. The study was carried out for 50 $\mu g\,ml^{-1}$ PCT.

The first step is the reaction of PCT with nitrite in HCl medium. The optimum conditions in this step were 50 μl $NaNO_2$ 1000 $mg\,l^{-1}$ and 30 μl HCl 0.2 M. The optimum reaction time was observed to be 5 min.

The second step is the stabilization of the reaction compound by adding NaOH. A volume of 1 ml NaOH 2 M and 5 min were observed to be needed in order to the stabilization of the fluorescence signal. After this time the measurements could be carried out.

3.5. SIA variables

The sample and carrier volumes, as long as the effect of the flow-rate were the studied SIA variables. The sample volume study was carried out with 50 $\mu g\,ml^{-1}$ PCT; the volume was varied from 100 up to 2000 μl. When increasing the volume of the sample injected, the amount of species of interest sorbed on the solid support also increases, therefore, the analytical response is higher and sensitivity is increased, but the sampling frequency diminishes. In this case, the signal increased linearly up to 1500 μl hence, this volume was chosen for the rest of the experiments.

The volume of the carrier solution was also studied; 2500 μl of carrier solution was enough to regenerate the solid support, restoring the baseline.

The flow-rate was investigated from 0.6 to 1.1 $ml\,min^{-1}$. By increasing it, the sampling frequency increased, but the analytical signal decreased. So a compromise between sensitivity (analytical signal obtained) and the time involved in the analysis had to be taken. The finally selected flow-rate was 0.9 $ml\,min^{-1}$.

3.6. Figures of merit

Taking into account the optimized conditions, the analytical parameters of the system were studied. The linearity range is 6.6–80 $\mu g\,ml^{-1}$, being the detection and quantification limits 2 and 6.6 $\mu g\,ml^{-1}$ respectively; the 3 and 10σ criterion was used to calculate detection and quantification limits respectively. Using 20 $\mu g\,ml^{-1}$ PCT, the intra-day R.S.D. was 2.5% and the inter-day R.S.D. was 7.2%. The solid support used is useful for at least 200 measurements without being replaced.

3.7. Interference study

In order to determine the effect of possible interferences, a tolerance study was carried out with those compounds that are usually found along with PCT in pharmaceuticals. A compound was considered to interfere if a variation of more than ±5% was observed in the analytical signal. If such a variation was observed, the foreign species concentration was diminished until an error less than ±5% was obtained.

The study was carried out with 20 $\mu g\,ml^{-1}$ of PCT. Foreign species were added to the samples at concentrations higher than those usually found in pharmaceuticals.

In the case of acetylsalicylic acid (ASA), the use of the filters was not enough for eliminating the interference. The usual ASA/PCT ratio in pharmaceuticals is 1.25, and even a 0.5 ratio showed high interference when using only filters. Therefore, the use of a spectrofluorimeter was required. By using a spectrofluorimeter and changing the measuring wavelengths to 350/535 nm for excitation and emission respectively, the tolerated ASA/PCT ratio could be increased up to 1.6, which is enough for the required applications.

Satisfactory results were obtained in all cases, as the tolerated ratio was higher that the usually found in pharmaceuticals for all the tested compounds. The results are detailed in Table 1.

Table 1
Effect of foreign species

Foreign species	Tolerated interferent/analyte (w/w) ratio[a]
Fructose, saccharine	>100[b]
Saccharose, sorbitol	80
Lactose	40
Caffeine, benzoic acid	>5[b]
Ascorbic acid, propyphenazone	4
Acetylsalicylic acid	1.6[c]

[a] For a 20 μg ml^{-1} PCT concentration.
[b] Maximum ratio tested.
[c] Using the spectrofluorimeter.

3.8. Analytical applications

Following the previously described general procedure, the system was applied to the determination of PCT in pharmaceutical preparations. Different pharmaceutical preparations were used, such as activated tablets and granular packets. Several active principles were present in the analyzed pharmaceuticals; the maximum active principle/analyte ratios found in these pharmaceuticals were 0.02, 0.8, 0.015, 1.25, 0.25 and 2 for codeine, ascorbic acid, phenylephrine hydrochloride, acetylsalicylic acid, caffeine and propyphenazone respectively. Taking into account the tolerated ratios observed in the interference study, good selectivity was expected. In all cases, the obtained PCT value was in agreement with the provided value by the manufacturers.

In addition, a recovery study was performed by adding three different amounts of PCT to each tested preparation. Excellent recovery results were found, ranging from 96 up to 104%.

3.9. Conclusions

The coupling of SIA and SPS has been here satisfactorily described and applied for the fluorimetric determination of PCT after nitrosation reaction followed by alkaline stabilization. By means of using the solid support in the flow-through cell, the elimination of almost all possible interferences was achieved. In addition, the elimination of the acetylsalicylic acid interference was also achieved by changing the measuring wavelengths. The obtained linear dynamic range is enough for the determination of PCT in pharmaceuticals, as demonstrated in this paper. The coupling of SIA and SPS has demonstrated to be a powerful methodology for the routine analysis of pharmaceuticals as an alternative methodology to FIA–SPS. In the opinion of the authors, it is an interesting approach which can offer an analytic potential in pharmaceutical analysis similar to that of the FIA optosensors [17], by appropriate combination of both the design of the SIA manifold and the use of appropriate carriers/eluents solutions, on-line separations, etc.

Acknowledgements

E.J.L.M. acknowledges a program scholarship from Junta of Andalucia. The financial support of the Czech Ministry of Education project MSM 0021620822 is gratefully acknowledged. The authors also acknowledge the financial support from MCYT, Spain, (project no. BQU 2002-02872), partially co-financed by FEDER funds.

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