Pergamon

0731-7085(94)00108-1

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 12, pp. 1585–1589, 1994

0731-7085/94 $7.00 + 0.00

Short Communication

Determination of ampicillin or amoxycillin in pharmaceutical samples by flow injection analysis

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Keywords: *Ampicillin; amoxycillin; palladium(II); FI; pharmaceuticals.*

Introduction

Ampicillin, (α-aminobenzylpenicillin) and amoxycillin (α-amino-p-hydroxybenzylpenicillin) are two semisynthetic β-lactam antibiotics widely used for the treatment of commonly-occurring bacterial infections [1].

Several analytical procedures have been described for the analysis of these aminobenzylpenicillins including fluorimetric [2–5], spectrophotometric [6–12], electroanalytical [13–16], chromatographic [17–21] and microbiological methods [22, 23]. The spectrophotometric methods for ampicillin and amoxycillin determination are mainly based on the use of equilibrium methods, which usually involve a previous step where an acid [6, 7] or base [9] is used to hydrolyse the β-lactam ring. The treatments take between 20 min and 2 h, so the equilibrium determinations involved are very slow and hinder applications to routine analysis.

Flow injection analysis (FI) is characterized by its simplicity, speed and the use of inexpensive equipment. Its results are accurate and precise, and there are clear advantages in terms of the short time required for each assay. The usefulness of the FI methods for routine analysis has been shown in a large number of determinations developed for clinical, pharmaceutical, food and environmental analysis. However, it has rarely been used for the determination of amoxycillin and ampicillin [24, 25]. The objective of this work was the development of two simple, inexpensive and rapid FI methods to determine ampicillin or amoxycillin in pharmaceuticals.

Experimental

Apparatus

The FI system comprised a Gilson HP4 peristaltic pump (Worthington, OH, USA), an Omnifit injection valve (NY, USA), a Hellma 18-μl flow cell (Jamaica, NY, USA) and a Philips PU 8625 UV/VIS spectrophotometer (Cambridge, UK) as the detector. Connecting tubing (0.5-mm bore) poly (tetrafluoroethylene) (PTFE) tubing and various end-fittings and connectors (Omnifit) were used. A Colora Ultra-Thermostat V5 (Lorch, Würt, Germany) was used.

Reagents

All chemicals were of analytical reagent grade and the solutions were prepared with double-distilled water.

Palladium dichloride standard solution (5×10^{-3} M). The standard solution was prepared by dissolving 0.2216 g of $PdCl_2$ (Merck) in 5 ml of water, to which 0.5 ml of concentrated HCl had been added, and warming the mixture in a water bath. The solution was cooled and diluted with water in a 250-ml calibrated flask.

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More dilute solutions were obtained by appropriate dilution with water.

Hydrochloric acid (6 M). This was prepared by dilution of the concentrated acid.

Stock ampicillin solution (1×10^{-3} M). The stock solution of ampicillin was prepared by dissolving 0.0371 g of sodium ampicillin (The Sigma Chemical Co., St Louis, USA) in 100 ml of water; the stock solution was kept at ≈4°C.

Stock amoxycillin solution (1×10^{-3} M). The stock solution of amoxycillin was prepared by dissolving 0.0419 g of amoxycillin trihydrate (Sigma) in 1 ml of 1 M HCl and diluted with water in a 100-ml calibrated flask. It was stored at ≈4°C.

Samples of sodium ampicillin or amoxycillin trihydrate were previously titrated potentiometrically by the mercurimetric method, which has been adopted in the European Pharmacopoeia [26, 27].

Working solutions were made daily by suitable dilution of the stock solution.

Dosage forms of ampicillin. (1) Britapen injection (Beecham Research, Lab., Spain): 250 mg/vial sodium ampicillin. (2) Bisolvon–ampicillin suspension (Fher Lab., Spain): 125 mg ampicillin trihydrate, 2 mg bromhexine clorhydrate, lactose and other excipients in 5 ml of suspension. (3) Miliken mucolytic retard injection (Liade Lab., Spain): 200 mg/vial sodium ampicillin and 1000 mg/vial ampicillin benzatine.

Dosage forms of amoxycillin. (1) Clamoxyl suspension (Beecham Research, Lab.): 100 mg amoxycillin trihydrate and excipients in 100 ml of suspension. (2) Metifarma capsules (Merck Lab., Spain): 500 mg amoxycillin trihydrate and excipients. (3) Ardine bronchial capsules (Antibióticos, Farma, Lab., Spain): 500 mg amoxycillin trihydrate, 8 mg bromhexine clorhydrate and excipients.

Recommended procedures for calibration

Figure 1 shows the flow-injection system: 70 µl of ampicillin or amoxycillin solution were injected into an inert carrier stream, which then joined the reagent stream (3×10^{-3} M $PdCl_2$ in 0.01 M HCl); the peak height was measured at 400 nm. A calibration graph was prepared by plotting the peak height (h) vs ampicillin or amoxycillin concentration over the range 2.0×10^{-5}–6.0×10^{-4} M.

Procedure for the assay of dosage forms

An amount of the sample powder or solution equivalent to 37 mg of ampicillin or 40 mg of amoxycillin was weighed or measured accurately, dissolved in water or 1 ml 1 M HCl, respectively, and diluted to 100 ml with water in a calibrated flask. A suitable aliquot was analysed by FI procedures.

Results and Discussion

Preliminary studies

Benzylpenicillins interact with metallic ions to form complexes [28–33]. It was found that in 0.01 M HCl and 40°C ampicillin or amoxycillin reacts with Pd (II) to produce yellow compounds that present two maxima of absorption, one at 280–285 nm and the other at 385–400 nm. Individually, neither drugs nor palladium (II) chloride absorb at these wavelengths. The absorbance measurement was made at 400 nm in all subsequent studies.

The method of continuous variations [34] was employed to determinate the stoichio-

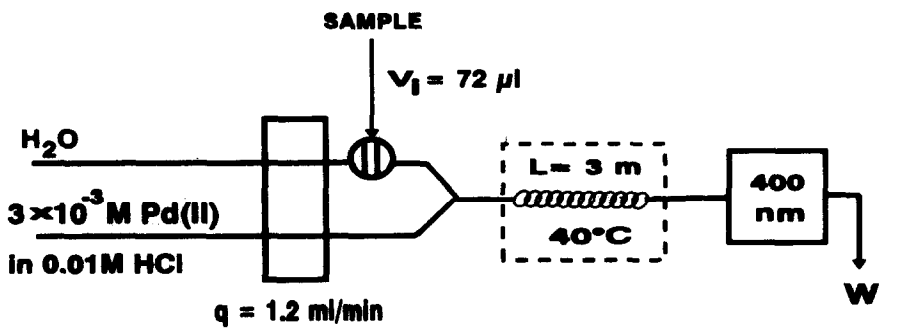

Figure 1
FIA manifold for the determination of ampicillin or amoxycillin.

metry of the two compounds. A maximum molar fraction of 0.5 was found in both cases, which indicates a stoichiometry for the complexes of 1:1.

Longridge *et al.* [35] reported that the penicillins (I) have a tautometric structure with that of penicillenic acids (II) and that these acids are hydrolysed at pH 0.5–2.0 to penamaldic acids (III). These processes are slow but in the presence of Pd (II) the rate of equilbration is rapid. See Fig. 2.

In agreement with the results of our research, the yellow compounds formed between the Pd (II) and the ampicillin or amoxycillin under our experimental conditions, pH ≈2 and 40°C, would be complexes of an aminobenzylpenicillin hydrolysis product, α-aminobenzylpenamaldic acid or α-amino-p-hydroxybenzylpenamaldic acids (III) with Pd (II).

The molar absortivities calculated for these complexes are 9885 and 12 000 l mol^{-1} cm^{-1} (λ = 280 nm) and 3937 and 4250 l mol^{-1} cm^{-1} (λ = 390 nm), respectively.

The compounds obtained in this work between Pd (II) and ampicillin or amoxycillin in the experimental conditions mentioned are used in this work to develop two spectrophotometric–FI methods for determining the antibiotics, both in their pure forms and in pharmaceutical products.

Flow system

The design of the manifold shown in Fig. 1 is simple. After some preliminary studies, a carrier stream of water was adopted because injection of the sample into the reagent stream led to negative peaks. The reagents and the carrier stream of water were pumped at the same flow-rate in order to achieve effective mixing of the sample and reagent solutions. The sample was injected into a water stream, which was then mixed with a stream of palladium (II) chloride dissolved in hydrochloric acid. The palladium (II) reacted at 40°C with ampicillin or amoxycillin and formed the yellow complexes previously described, whilst the absorbance was measured in the detector at 400 nm. In the absence of the antibiotic (blank) a very small noise signal was obtained. The presence of the drug caused an increase in the analytical signal, which was proportional to its concentration.

The use of FI as an alternative to existing methods for the determination of ampicillin or amoxycillin is dependent on the optimization of this system to achieve maximum peak height, with low residence time and minimum dispersion. As a consequence, several experiments were conducted in order to establish the optimum conditions to operate the FI manifold.

A loop size of 72 μl was chosen, a sample volume at which a sufficient sensitivity was obtained with no excessive waste of sample.

A reactor length of 3 m (0.5 mm i.d.) was selected in both cases as this provided a high sampling frequency and reproducibility.

For both ampicillin and amoxycillin a flow rate of 1.2 ml min^{-1} was selected as a compromise between sensitivity and sampling rate.

Influence of reagent concentration and temperature

Based on preliminary studies it is advisable to use an acidic medium for the determination of ampicillin or amoxycillin with palladium (II). Maximum peak height was obtained in both cases at a concentration of 0.01 M HCl.

The influence of palladium (II) concentration was studied in the range 2.0×10^{-4}–5.0×10^{-3} M with a fixed concentration of $4.0 \times$

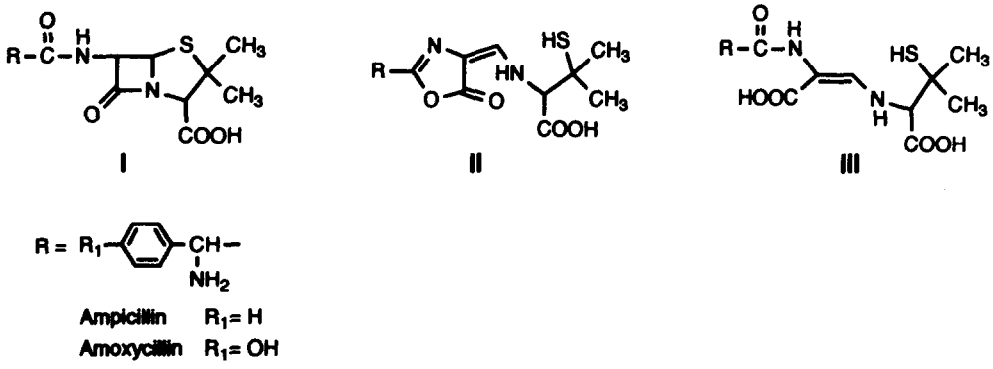

Figure 2

10^{-4} M ampicillin or amoxycillin. A concentration of 3.0×10^{-3} M $PdCl_2$ was selected in both cases.

In the selected conditions the effect of temperature was studied on 4.0×10^{-4} M ampicillin and amoxycillin between 20 and 75°C. A temperature of 40 ± 0.2°C was chosen.

Determination of ampicillin or amoxycillin

With the described manifold and under the selected experimental conditions (3.0×10^{-3} M $PdCl_2$ in 0.01 M HCl and at 40°C) a series of standard solutions of ampicillin or amoxycillin were injected in triplicate to test the linearity. The calibration graphs were found to be linear from 2.0×10^{-5} to 6.0×10^{-4} M in both cases. The regression equations were: $A = 698.9$ [ampicillin] + 0.001; and $A' = 826.2$ [amoxycillin] + 0.001; where a is the peak absorbance and drug concentrations are expressed in M; the correlation coefficients were 0.9998 and 0.9999, respectively. The relative standard deviations for 10 determinations of 1.9×10^{-4} M ampicillin or 2.1×10^{-4} M amoxycillin were ±0.16 and ±0.22%, respectively.

The detection limits (signal-to-noise ratio = 3) were 8.0×10^{-6} and 7.3×10^{-6} M of ampicillin or amoxycillin, respectively. The sampling rate was 45 samples h^{-1}.

Study of possible sources of interference

The influence of commonly used excipients and additives in pharmaceutical dosages of ampicillin or amoxycillin was investigated. Solutions of the drugs and each compound tested were mixed to obtain samples containing 10^{-4} M of the antibiotic and different concentrations of the foreign compound. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error of less than ±3% in the analytical signal. Glucose, sucrose, lactose, galactose, maltose, fructose, saccharine, caffeine, propylene, glycol mannitol were tolerated in large amounts (50-fold excesses were the maximum molar ratio tested). Bromhexine chlorhydrate, did not interfere in the amounts contained in the pharmaceuticals assayed.

Applications

The proposed FI procedures were applied to the determination of ampicillin or amoxycillin in various pharmaceutical preparations. Interference from the sample matrix was not a problem. The data in Tables 1 and 2 show that the ampicillin or amoxycillin contents measured by the proposed FI procedures were in excellent agreement with the labelled contents and with those obtained by the standard method of Pharmacopoeia [26, 27]. For all the formulations examined the results obtained by the reference and FI methods were compared by applying the F-test and the t-test at the 95% confidence level. The calculated F and t values did not exceed the theoretical ($F = 9.60$, $t = 4.30$), which indicates that there is no significant difference between the two methods with respect to precision and accuracy, in the determinations of ampicillin and amoxycillin. Recovery studies were also carried out on samples to which known amounts of ampicillin or amoxycillin had been added. In all cases quantitative recoveries between 99.0 and 102.2 were obtained.

Table 1
Determination of ampicillin in pharmaceutical preparations

	Ampicillin content		
Sample	Labelled	Reference method*	FI method*
Britapen	250†	248.9 ± 0.6	249.4 ± 0.9
Bisolvon	100‡	102.1 ± 0.3	102.3 ± 0.7
Miliken	1200†	1160.8 ± 6.4	1137.6 ± 5.1

* Average of five determinations ±SD.
† mg/vial.
‡ mg ml^{-1} of suspension.

Table 2
Determination of amoxycillin in pharmaceutical preparations

	Amoxycillin content		
Sample	Labelled	Reference method*	FI method*
Metifarma	500†	504.9 ± 3.5	506.5 ± 3.5
Ardine	500†	514.5 ± 2.4	510.0 ± 1.5
Clamoxyl	100‡	104.5 ± 0.9	102.5 ± 0.8

* Average of five determinations ±SD.
† mg/capsule.
‡ mg ml^{-1} of suspension.

Conclusions

The proposed FI methods for the determination of ampicillin or amoxycillin showed good accuracy and reproducibility and were faster and simpler than most of the methods reported for these compounds.

The FI methods proposed are useful for the quality control of ampicillin or amoxycillin in

pharmaceutical dosage forms, since there is no interference from the common excipients that might be found in commercial preparations.

Acknowledgement — The authors are grateful to the DGICYT (Project PB 90-0008) for financial support.

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[Received for review 14 December 1993; revised manuscript received 18 May 1994]