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Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 833–836

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

A new HPLC method for azithromycin quantitation

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Received 20 May 1999; received in revised form 1 March 2001; accepted 5 March 2001

Abstract

A simple liquid chromatographic method was developed for the estimation of azithromycin raw material and in pharmaceutical forms. The sample was chromatographed on a reverse phase C18 column and eluants monitored at a wavelength of 215 nm. The method was accurate, precise and sufficiently selective. It is applicable for its quantitation, stability and dissolution tests.

Keywords: Azithromycin; New method; HPLC; UV detection

1. Introduction

Azithromycin is a macrolide antibiotic with a broad spectrum [1]. It plays a leading role in the treatment or prophylaxis of several diseases such as opportunistic infections in AIDS [2], toxoplasmosis [3], pediatric infections [4] and respiratory tract infections [5]. The most innovative feature is the efficacy and safety of a 3-day course of oral suspension or tablets [6]. Several quantification methods have been described but all require detection devices not generally available in routine pharmaceutical analysis laboratories (a liquid chromatograph equipped with an amperometric electrochemical detector with dual glass carbon electrodes) [7]. This led us to develop a quantitative HPLC method using conventional materials, reagents and equipment.

2. Experimental

2.1. Materials

Azithromycin USP Standard from USA, azithromycin raw material 99.4%, from ICN Argentina, tetrabutylammonium phosphate, acetonitrile and methanol, all HPLC grade from J.T. Baker USA and phosphoric acid and ammonium phosphate monoacid from Merck Química Argentina were used.

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00554-4

2.2. Formulation

A commercial Argentine tablet formulation, composed of Azithromycin dihydrated 524 mg, corn starch, lactose, povidone, microcrystalline cellulose, sodium croscarmelose, sodium laurylsulfate, magnesium stearate and opadry II, was studied.

2.3. Instrumentation

The HPLC system consisted of a dual piston reciprocating pump (Model KNK-500 G), an UV-Vis detector (model KNK-029-757), an integrator (Model SP 4600) (all from KoniK, Spain) and a Rheodyne injector (Model 7125).

2.4. Chromatographic conditions

LiChroCART® 125 × 4.6 mm HPLC Cartridge LiChrospher® 100 RPS select B (5 μm) Merck Darmstadt, Germany, was used. Mobile phase was chosen in order to obtain a good peak in a reasonable resolution time and the best selectivity for the drug among its degradation products and excipients. It was a suitable mixture of buffer, acetonitrile and methanol (60:20:20) adjusted to pH 8.0 ± 0.1 with phosphoric acid, Buffer was prepared by dissolving 2.88 g of ammonium phosphate monoacid in 500 ml of water, adding 45.6 ml of a 10% solution of tetrabutylammonium phosphate in water and diluting with water to 1000 ml. Mobile phase was filtered through 0.45 μm nylon membrane and degassed with helium. Flow rate was 1.0 ml/min, at room temperature. Wavelength was set at 215 nm and the volume of each injection was 20 μl. In these conditions azithromycin retention time was roughly 5 min.

2.5. Standard preparation

Approximately 100 mg azithromycin, USP standard, were accurately weighed, sonicated 2 min in mobile phase in order to dissolve and obtain 2 mg/ml, then filtered through a 0.45 μm membrane filter.

2.6. Sample preparation

Twenty tablets were weighed and finely powdered and a accurately weighed powder sample was suspended in mobile phase in order to obtain 2 mg/ml of azithromycin, sonicated 2 min and filtered through a 0.45 μm membrane filter.

2.7. Procedure

The column was equilibrated for at least 1 h with mobile phase flowing through the chromatographic system before starting the assay. System suitability (theoretical plates, tailing factor, resolution) was calculated using USP 23 specifications. Twenty μl of the standard or sample solution were injected into the chromatograph using conditions described above (each solution was injected in triplicate).

2.8. Validation

Considering the possibility of use this analytical procedure in stability studies, five solutions from 0.6 to 3.0 mg/ml (25–150% of theoretical value) were prepared by dissolving azithromycin in mobile phase, in order to study system linearity response. Each solution was injected three times. Six samples of standard preparation (100% of assay solution titer) were prepared to evaluate accuracy by performing three consecutive injections of each sample with the same equipment, on the same day and by the same operator.

Precision was evaluated by studying repeatability and intermediate precision. One of the standard preparations was injected ten times in the same equipment, by the same operator, on the same day in order to study repeatability. Intermediate precision was evaluated by performing two accuracy assays, working 1 day apart and 1 week apart, by two different operators with different equipment, with different chromatographic columns, in the same laboratory. Different aliquots of standard preparation and solutions of all excipients were treated with acid, alkali and hydrogen peroxide and exposed to natural light and UV lamp during 24 h, neutralized and suspended in mobile phase were then analyzed by the

procedure described above in order to study method specificity. Assay preparation stability was studied by running the standard solution kept at room temperature at different times.

2.9. Method application

This method was applied to assay commercial tablets and to evaluate their dissolution test (described in USP XXIII Edition, using an electrochemical detector) [7].

3. Results and discussion

3.1. Linearity

The regression curve of peak areas versus concentrations proved linear with a coefficient of correlation $r = 0.9994$ and with confidence intervals at $P = 0.05$.

$$Y = -1.17 \times 10^3 + 1.13 \times 10^4 \; X$$

3.2. Accuracy

Recovery data obtained was within the 98.7–100.0% range, with a mean value of 99.4% and a CV equal to 0.53% (Table 1). The mean 't' value versus the true value with 95% confidence shows that the experimental mean was not significantly different from true value (t_{n-1}, $\alpha/2$ from tables = 2.571, for five freedom degrees).

3.3. Precision

3.3.1. Repeatability

The following results were expressed as the relative standard deviation of ten replicate analyses of a 100% assay solution titer. The mean area was 18 465 with a CV of 1.49%.

3.3.2. Intermediate precision

For each accuracy assay the results were as follows: mean values 99.46 and 99.85%, S.D. 0.59 and 1.86, and CV 0.59 and 1.86%. Test 't' comparing two sample means with 95% confidence for 10 freedom degrees disclosed that both samples were not significantly different from each other (t_f; $\alpha/2 = 2.571$).

3.4. Selectivity

Neither formulation ingredients nor degradation products interfered with quantitation of azithromycin. All samples were analyzed using the assay chromatographic condition described. No evidence of interactive degradation products was seen during evaluation. However, azithromycin showed degradation products following alkaline and acid hydrolysis, reduction, oxidation and photolysis. Selectivity was demonstrated showing that azithromycin peak was free of interference of degradation products indicating that the proposed method can also be used in a stability assay (Fig. 1).

Table 1
Assay accuracy, n: 6, result of the recovery anlisys of azithromycin

Azithromycin (mg/ml)	Amount recovered (mg/ml)	Recovery (%)	RSD n: 3
1.76	1.75	99.6	0.4
1.85	1.82	98.7	0.3
1.93	1.93	100.0	0.3
1.95	1.95	100.0	0.3
2.10	2.08	99.0	0.4
2.25	2.23	99.3	0.4

RSD: relative standard deviation.

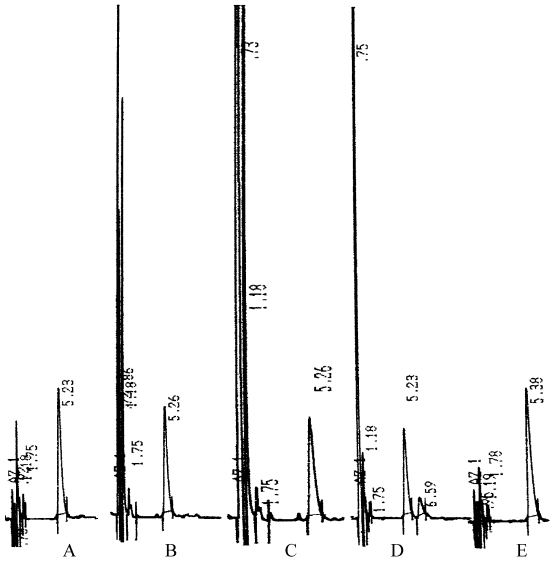

Fig. 1. Chromatogram corresponding to azithromycin as standard (A) and as raw material treated with acid (B), alkali (C) and hydrogen peroxide (D), and exposed to natural light and UV lamp (E).

3.5. Stability of the assay preparation

The assay preparation failed to show any significant degradation over a span of 6 h during which the difference between two determinations remained below 2%.

4. Conclusion

The proposed method for quantitation of azithromycin raw material or pharmaceutical forms, was validated following ICH specifications [8] and proved accurate, precise and selectivity. It is applicable for its quantification, stability and dissolution tests.

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