Physicochemical characterization and microbiological analysis for quality control of olanzapine active substance

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Abstract

Background

Olanzapine is an atypical neuroleptic of the class of thienobenzodiazepines, characterized by its clinical efficacy on the positive and negative symptoms of schizophrenia by preventing the occurrence of extrapyramidal symptoms.
The objective of this work was to identify and to characterize the active drug Olanzapine and its related compounds by HPLC and to evaluate its microbiological quality.

Materials and methods

Olanzapine was identified through its organoleptic characteristics, its melting point and by an infrared absorption using Spectrum One FTIR spectrometer. The determination of its purity, the identification and the dosage of related substances of olanzapine were carried out using a SHIMADZU® LC-2010 CHT HPLC, equipped with a UV detector at 260 nm and Phenomenex C8 column (150 mm X 4.6 mm X 5 **Q**m) which is maintained at room temperature. The flow rate was about 1.5 ml per min for Assay and about 1 ml per minute for related substances. A mixture of acetonitrile and 6.9 g/l sodium monohydrate phosphate, pH= 2.5 (1: 1) was used as mobile phase for the assay and a gradient mobile phase elution consisting of (A): acetonitrile: purified water (20:80, V/V) and (B): acetonitrile: purified water (60:40, V/V) for related substances.
The microbiological cleanliness of Olanzapine was controlled by enumeration of microorganisms.

Results and discussion

The identification of olanzapine active substance and the evaluation of its chemical quality showed conformity with Eur. Ph. 8.0 norms.
The percentage content of Olanzapine calculated was about 99.93%. The analysis of this substance by HPLC showed one non specified impurity, its relative retention time was 0.81 and its percentage content was 0.03%.
The enumeration of aerobic germs, yeasts and total molds and the search for Escherichia Coli showed the microbiological cleanliness of Olanzapine.

Conclusion

Through these results we can conclude that the Olanzapine active substance tested is of good physicochemical and microbiological quality.

KEYWORDS: Olanzapine, characterization, HPLC, related substances, microbiological quality.

Introduction

Olanzapine is a psychoactive substance of the atypical antipsychotic class approved by the Food and Drug Administration (FDA) in September 1996 for the treatment of schizophrenia and moderate to severe manic episodes associated with bipolar disorder. [1, 2]
Olanzapine has the empirical formula C17H20N4S, its molecular weight is 312.4325 gmol-1.[3]
Our present work deals with the identification and characterization of the active pharmaceutical ingredient Olanzapine and its related compounds by HPLC and evaluation of its microbiological quality.

Methods

Olanzapine was identified by an infrared absorption using spectrum one FTIR spectrometer. The organoleptic characteristics, determination of water content by the Karl Fischer method and sulphuric ashes were also studied.
The determination of its purity, identification and the dosage of related compounds of Olanzapine were carried out using a SHIMADZU® LC-2010 CHT HPLC. [4]
The microbiological cleanliness of Olanzapine was controlled by enumeration of microorganisms. [4]

A- Organoleptic characteristics

Through a visual appreciation, we examined the appearanceand color of our raw material.

B- Solubility

We tested the solubility in distilled water, methylene chloride andethanol 96 °. [4]

C- Identification of Olanzapine by Infrared Absorption using Spectrum one FTIR

In order to obtain the IR spectrum of Olanzapine, we mixedand grounded a very small amount of the substance (approximately 5 mg) with potassium bromide (approximately 100 mg) and then compressed the powder into a special moldunder a pressure of 1.5 to 3 MP.cm-2, toobtain a pellet ofpotassium bromide. [4]

D- Determination of purity of Olanzapine and dosage of its related substances by HPLC

Mobile phase	Assay Buffer: 6.9 g/l monobasic sodium phosphate, pH 2.5. Mobile Phase: Acetonitrile: Buffer (1:1)	Related substances Mobile PhaseA:20 V acetonitrile with 80 V water, add 2ml of perchoric acid to each liter. Mobile Phase B:60 V acetonitrile with 40 V water, add 2ml of perchoric acid to each liter.(elution gradient) (Table 2)
Flow rate	1.5ml/min	1 ml/min
Column	C8 (4.6 mm X 15 cm; 5 µm), room temperature.	C8 (4.6 mm X 25 cm; 5 µm)
Injection volumes	20 µl	20 µl
Detection wavelength	260 nm	230 nm

table 1: chromatographic conditions [4].

Standards and Samples solutions:
• For Assay : [4]
• Standard solution: containing 0.1mg/ml of Olanzapine CRS prepared in mobile phase (Table 1).
• Test solution: containing 0.1mg/ml of Olanzapine prepared in mobile phase (Table 1).

• For Related substances : [3]
• Standard solution: containing 1.5µg/ml of Olanzapine CRS prepared in mobile phase A.
• Test solution: containing 0.3mg/ml of Olanzapine prepared in mobile phase A (Table 2).

TABLE 2: Gradient mobile phase system:[3]

Time (min)	Mobile phase A	Mobile 7 phase B
0	93	7
13	93	7
28	0	100
33	0	100
33.1	93	7
44	93	7

E-Microbiological Analysis [4]

** Enumeration of microorganisms
- Preparation of dilution at 10-1 (Stock solution):Dissolve 10 g of olanzapine in 100 ml of peptonewater pH 7.0.
- Enumeration of total aerobic germs:
** In-depth seeding of TSA Agar:
Take 1 ml of the stock suspension and place the volume taken from the bottom of a Petri dish, distributing it in drops. Then aseptically flow about 15ml TSA agar maintained supercooled but slightly cooled.
In parallel, fill another Petri dish with TSA Agar alone as a negative control[4].
- Enumeration of yeasts and total bolds :
** In-depth seeding of SDA Agar:
Take 1 ml of the stock suspension and place the volume taken from the bottom of a Petri dish, distributing it in drops. Then aseptically pour about 15ml of the SDA agar maintained supercooled but slightly cooled.
In parallel, fill another Petri dish with SDA agar alone as a negative control.[4]
** Incubation of culture media:
Incubate the plates of TSA and SDA agar plates in an incubator at 20 ° C to 25 ° C for
5 to 7 days [4].

Results and discussion

A- Organoleptic characteristics

Olanzapine is yellow, crystalline powder (Fig 1).
This aspect is in accordance with the Eur. Ph. 8.0 norms.
The melting point of Olanzapine is about 194.2°C.
Norms: 192.8°C-195°C

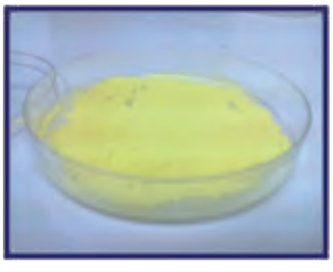


Figure 1: Appearance of Olanzapine.

B- Solubility

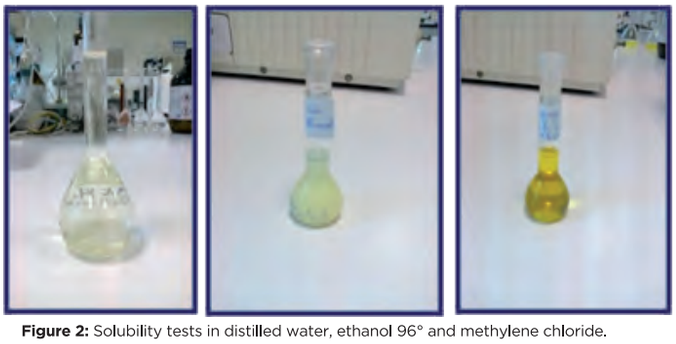
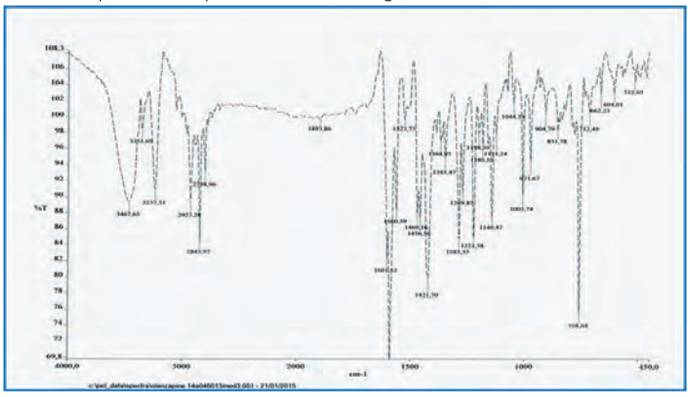


Figure 2: Solubility tests in distilled water, ethanol 96° and methylene chloride.

Olanzapine is insoluble in water, slightly soluble in 96% ethanol and freely soluble in methylene chloride (Fig 2).
- The water content determined using a Karl-Fischer apparatus was about 0.268 %. Eur. Ph. 8.0
(≤ 1.0 %).
- The average rate of sulphuric ashes is equal to 0.024%. Eur. Ph. 8.0(≤ 1.0 %).

C- Infrared spectra

The Olanzapine infrared spectra was Showed in fig 3.



D- Identification and dosage of Olanzapine active substance

The standard deviation (RSD) for a series of standardinjection is 0.012% so is not more than 2%, according to the Eur. Ph 8.0.

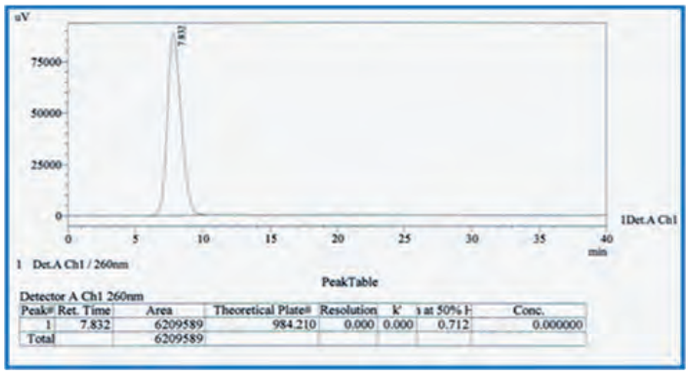


Figure 4: Standard solution chromatogram 0.1 mg/ml.

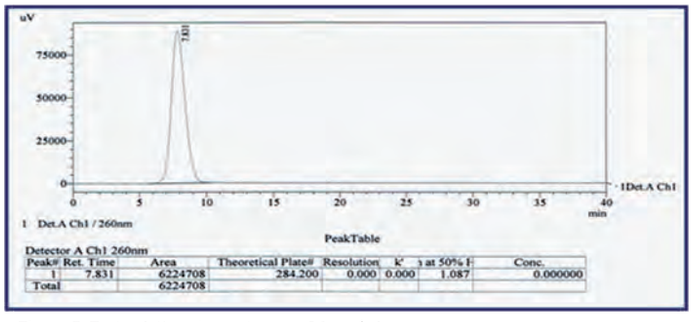


Figure 5: Test solution chromatogram 0.1 mg/ml.

The percentage content of Olanzapine was calculated using the formula (1).

$$T\% = \left(\frac{AUC\ Ech}{AUC\ Std}\right) x \left(\frac{CStd}{CEch}\right) x\ 100$$

P (%) of Olanzapine = 99.93 %. Norms of Eur. Ph 8.0 [98%-102%].

Dosage of Related substances by HPLC:

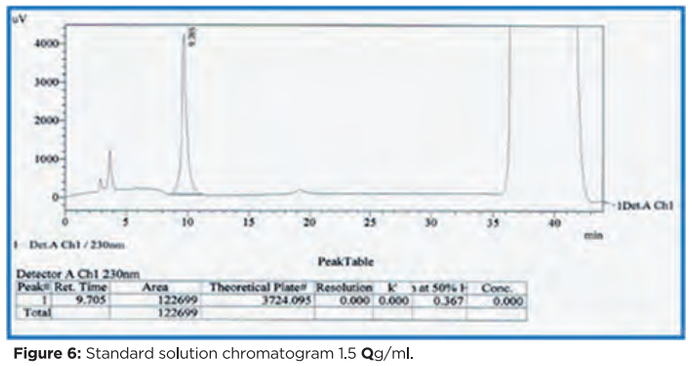


Figure 6: Standard solution chromatogram 1.5 Qg/ml.

The chromatogram of the test solution shows two peaks that correspond to an unspecified impurity and Olanzapine (Fig 7).

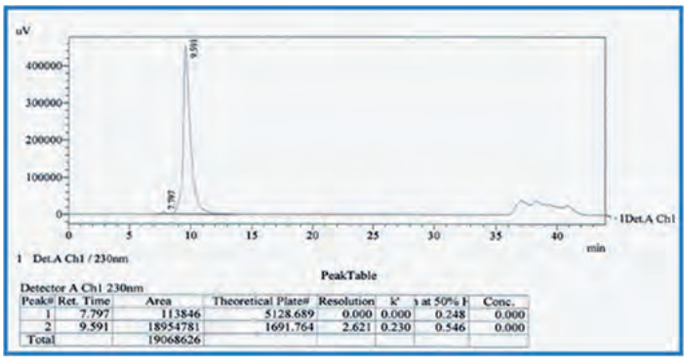


Figure 7: Test solution chromatogram 0.3 µg/ml.

The relative retention time of the unspecified impurity relative to Olanzapine is 0.81.
The percentage content of unspecified impurity was calculated using the formula (2).

$$T\% = \left(\frac{AUC\ Ech}{AUC\ Std}\right) x \left(\frac{CStd}{CEch}\right) x \frac{1}{F} x\ 100, \quad F = 1$$

The content of this unspecified impurity meetsthe standards of the US Pharmacopoeia 37thedition.
T = 0,03 %, ≤ 0.20% (USP 37th)

E- Microbiological Analysis
** Enumeration of microorganisms
After an incubation of 5 days, we made a macroscopic examination of the culture media:

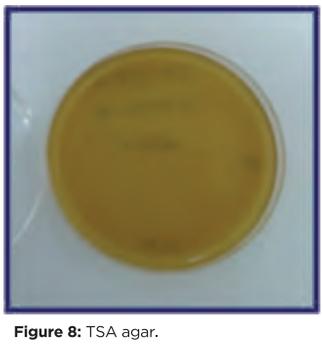


Figure 8: TSA agar.

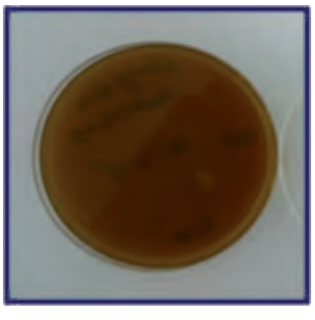


Figure 9:SDA agar.

- **TSA Agar:** No macroscopic signs of aerobic germ proliferation (GAT) were detected.(Figure 8).

The number of total aerobic germs is calculated by the formula(3):

$$N = \frac{NBoite\ 1+NBoite2}{2} x \frac{1}{D} x \frac{1}{V}$$

The number of GAT is equal to 0 CFU / g. Standards: ≤ 103.

- **SDA agar:** no macroscopic signs of yeast and mold proliferation (LMT) were detected.(Figure 9).
The number of total aerobic germs is calculated by the formula (4):

$$N = \frac{NBoite\ 1+NBoite2}{2} x \frac{1}{D} x \frac{1}{V}$$

The number of LMT is equal to 0 CFU / g. Standards: ≤ 102.

The microorganism count of olanzapine indicates that olanzapine is free of aerobic germs, yeasts and molds, in accordance with the standards described by the European Pharmacopoeia 8thedition.

**** Search for Escherichia Coli**
After an incubation of 5 days, we made a macroscopic examination of the culture media:
- TSB Broth: TSB broth is clear in appearance.
- Macconkey Broth:the color did not fire.
- Gelose Macconkey: absence of bacterial colonies.

From these results, we confirm the absence of Escherichia Coli in our raw material.

Conclusion

Olanzapine was identified through organoleptic characteristics, by its melting point and by spectroscopic methods such as infrared spectroscopy (FT-IR).
The identification, determination of purity of Olanzapine active substance and dosage of its organic impurities found results consistent with standards.
The enumeration of aerobic germs, yeasts and total molds and the search for Escherichia Coli showed the microbiological cleanliness of Olanzapine.
Through these results we can conclude that the Olanzapine active substance tested is of good physicochemical and microbiological quality.

References

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[3] United States Pharmacopoeia Convention: United States Pharmacopoeia 37, 2012.
[4] European Pharmacopoeia 8th edition.