Determination of Olanzapine by UV Spectrophotometry and Non-aqueous Titration

S. FIRDOUS*, T. AMAN AND ALIM-UN.NISA
PCSIR Laboratories Complex, Lahore, Pakistan

(Received 29th March, 2003, revised 7th September, 2004)

Summary: Two new methods for the determination of olanzapine, based on UV spectrophotometry and non-aqueous titration, have been developed. The UV absorbance of the methanolic solution of olanzapine was measured at 226nm. The method obeys Beer's Law from 0.1µg to 50 µg/ml and the inter-day precision of UV procedure is 0.97%. The non-aqueous titration was carried out by titrating olanzapine with 0.1N perchloric acid, using naphthobenzene as an indicator, and the inter-day precision is 0.35%. The developed procedures provide a basis for a new spectrophotometric and a non-aqueous titrimetric determination of olanzapine. The quantitative assessment of tolerable amount of commonly used excepients and other similar acting drugs were also studied for both the procedures. The proposed methods were applied to the analysis of pure and pharmaceutical dosage form of olanzapine. The percentage recovery in both the procedures lies between 99.0 and 100.67%.

Introduction

Olanzapine, 2-methyl-4- (4-methyl) 1-piperazinyl-OH-thieno (2,3-b) (1,5) benzodiazepine (Fig. 1) is an anti-psychotic drug of the diazepine class. It is indicated for the treatment of schizophrenia and other psychotic disorders such as delusion, hallucinations, emotional and social withdrawal and poverty of speech [1]. However side effect includes somnolence, obesity, peripheral odema, dizziness, orthostatics hypo tension and mild or transient anticholinergic effect [2].

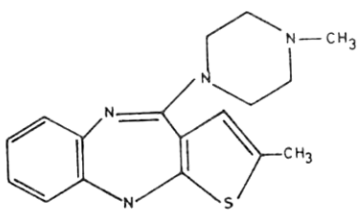

Fig. 1: Structural Formulae of Olanzapine.

Olanzapine is a relatively new drug and only the few chromatographic techniques have been reported for the determination of olanzapine. In the HPLC with amperometric method olanzapine was determined in human plasma with a linear response in the concentration range of 2-100ng/ml and isolation from plasma was accomplished by a solid phase extraction procedure using C_8 cartridge [3]. Olanzapine in human milk was determined by reverse phase HPLC with electrochemical detection. The method involves too many steps of separation, washing and elution prior to the injection of sample into the YMC basic column [4-6], whereas the HPLC determination of olanzapine in rat plasma showing inter assay precision (CV) as high as 15.9% [5]. Other methods includes liquid chromatography-atmospheric pressure chemical ionization mass spectroscopy [7], liquid chromatography/ tandem mass spectrometry [8] and UV detection after chromatographic separation [9] in which long and tedious methods are used involving too many steps and reagents with an average recovery of > 80% [7], while inter-day accuracy was 12.46% [8] and in UV detection the inter-day variation was <8% and risperidone interfered.

The aim of the present study is to develop and compare two procedures viz UV spectrophotometry and non-aqueous titration, so as to monitor olanzapine in pure and in commercial dosage form. Both the procedures have not been reported in literature [10]. The UV detection method is simple, accurate, precise and sensitive. It obeys Beer's Law from 0.1µg to 50 µg/ml with inter-day precision of 0.97%. Whereas the non-aqueous titration of the olanzapine is carried out with 0.1N perchloric acid in a non-aqueous media and the inter-day precision is 0.35%.

*To whom all correspondence should be addressed.

Results and Discussion

Absorption Spectrum

The UV spectrum of olanzapine exhibited three maximas i.e. 205nm, 226nm and 272.5nm (Fig.2). However, the working λ_{max} was 226nm because 205nm is very near to the vacuum region and 226nm shows maximum absorbance than at 272.5nm.

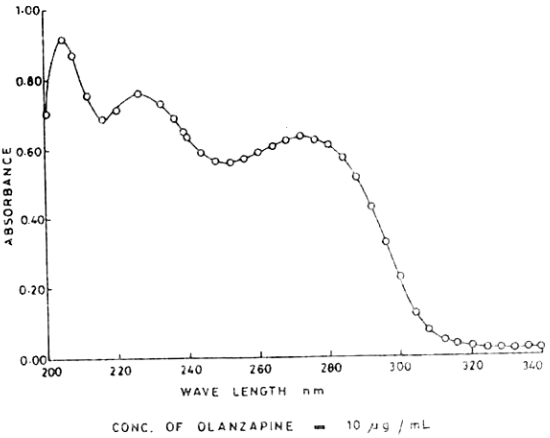

Fig. 2: Absorption Spectra of Olanzapine in Methanol.

Comparison of Results

The result for the determination of olanzapine by UV method and non-aqueous titration procedures are shown in Table 1 and 2 respectively indicating the sensitivity, validity and robustness of the methods. These procedures are reasonably precise and accurate, as the amount taken from the identical sample is known and the amount found by the above procedures do not exceed the inter-day precision (RSD %) of 0.97 and 0.35% for UV and non-aqueous titration, respectively.

Replicate readings of the absorption spectra and determination by non-aqueous titration at medium concentration level 10µg/ml for UV and 50 mg/ml for non-aqueous titration on the same day, allowed calculation of the repeatability (n=3). Inter-day precision was calculated by replicate analysis over a three days period. From the values given in Table 1 and 2 it can be seen that the two procedures gave similar repeatability values. However in term of inter-day precision non-aqueous titration provides better values (c.f. Table.3)

Table 1:Determination of olanzapine in pure solution by UV-spectrophotometry

Olanzapine taken (µg/ml)	Olanzapine found*(µg/ml)	Inter-day precision (RSD) (%)
0.1	0.101	0.53
0.2	0.199	0.40
0.3	0.297	0.42
0.5	4.99	0.37
1.0	1.010	0.23
5.0	0.499	0.15
10.0	9.91	0.10
20.0	20.05	0.09
30.0	30.25	0.27
40.0	40.17	0.21
50.0	49.50	0.97

*Every reading is a replicate of three independent measurements

Table 2:Determination of olanzapine in pure solution by non-aqueous titration

Olanzapine taken (mg)	Olanzapine found*(mg)	Inter-day precision (RSD) (%)
0.1	0.1008	0.35
0.2	0.201	0.33
0.5	0.504	0.23
1.0	1.012	0.25
5.0	4.98	0.19
10.0	10.05	0.21
20.0	19.99	0.15
40.0	40.03	0.07
60.0	60.52	0.10
80.0	80.63	0.12
100.0	99.67	0.13

*Every reading is a replicate of three independent Measurements

Table 3: Optical characteristics, precision and accuracy of the proposed methods

Parameters	Values: UV Spectro-photometry	Values: Non-aqueous titration
λ_{max} (nm)	226	
Beer's Law Limit/ Range	0.1-50µg/ml	0.3-100mg
Limit of detection	0.1µg/ml	0.3mg
Molar absorbitivity ($mol^{-1} cm^{-1}$)	$2.3745X10^4$	
Specific absorbance	760	
Regression equation (Y)*		
Slope (b)	0.074	
Intercept (a)	0.0122	
Correlation coefficient (r)	0.999	
% age range of error (Confidence limit) at 95% Confidence level	100±0.56	100±0.27
Inter-day precision (RSD)** (%)	0.97	0.35

*Y=a+bC where C is the concentration of analyte in µg/ml and Y is the absorbance unit

**Calculated from three independent measurements

Validity and Applicability of the Methods

Validity of both the methods were checked by preparing solution of different known concentrations

Table 4: Determination of olanzapine from available pharmaceutical preparations

Drug	Trade name	Pharmaceutical preparations	UV spectrophotometry			Non-aqueous titration	
			Amount present (mg)	Amount Found (mg)	Recovery (%)	Amount found (mg)	Recovery (%)
Olanzapine	Zyprexa (Eli Lilly & Co Ltd. England)	Tablets	5.0	5.01	99.00	5.04	99.90
		Tablets	7.5	7.45	98.66	7.55	100.67
		Tablets	10.0	9.90	98.75	10.50	99.20

and the quantity was determined by both the procedures and applicability was checked by analyzing pharmaceutical preparations available in market (c.f. Table. 4). The average inter-day accuracy was 99.0 and 100.67% with an average precision of 0.97 and 0.35% for UV spectrophotometry and non-aqueous titremetric methods, respectively.

Effect of Organic Solvents

Different solvents such as benzene, hexane, acetone, chloroform, ethanol and isopropanol were tested for drug solubility and stability. Since it was not soluble in benzene, chloroform and sparingly soluble in isopropanol, that is why no solvent has been employed. However methanol or ethanol could be use as drug solvent but in the present UV method methanol was used as it showed efficient solubility of drug and more stability in the UV region.

Beer's Law, Sensitivity and Precision

The results for the determination of olanzapine are given in Table 1 and 2, which shows the reliability, validity and sensitivity of the method. It is also reasonably precise and accurate, as the amount taken from identical samples are known and the amount found by the above mentioned procedures do not exceed the inter-day precision for UV procedure of 0.97 and that for non-aqueous titration of 0.35%. The calibration graph (Fig. 3) is linear in the range of 0.1µg to 50 µg/ml. The apparent molar absorbitivity calculated was 2.3745×10^4 and regression equation [11] was calculated by the method of least square line from fourteen points, each of which is the replicate of three determinations. The correlation coefficient is 0.999 (c.f. Table. 3). The limit of detection is 0.3mg by non-aqueous titration, so it can be concluded that both the procedures are equally good to carry out the micro and macro determination of olanzapine in pure and pharmaceutical preparations.

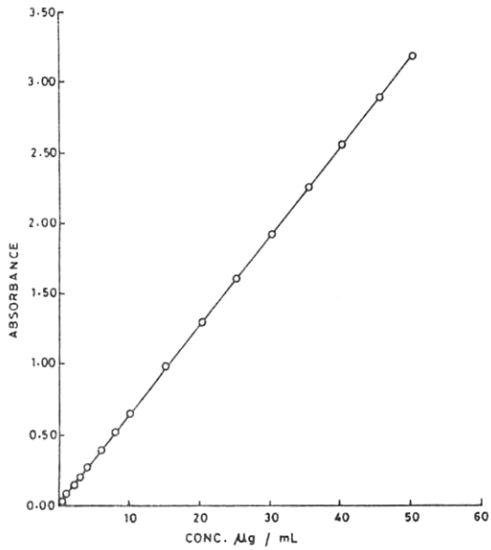

Fig. 3: Calibration Curve of Olanzapine at 226 nm.

Interferences

The quantitative assessment of tolerable amount of different organic compounds and excepients used in pharmaceutical preparations under experimental conditions is given in Table 5. Various amounts of diverse interfering compounds were added to a fixed amount of olanzapine and both the procedures were followed. It was found that commonly used antidepressant and traditionally used anti-psychotic drugs did not interfere.

Applications

The proposed methods are successfully applied for the quality control of pure olanzapine and in the pharmaceutical dosage forms, as shown in Table 4. The two methods provided results in agreement with the labeled contents and the recovery is 99.00 and 100.67% for UV and non-aqueous procedures respectively.

Table 5: Quantitative assessment of tolerable amounts of other drugs not interfering

Drug	Maximum amount not interfering	
	UV spectro-photometry	Non-aqueous titration
Barbituric acid	200	410
Chloral hydrate	150	270
Chlorpromazine	168	328
Diazepam	50	79
Glucose	300	670
Loranzapine	108	58
Methyl parabene	428	298
Naproxen	120	200
Phenobarbitone	200	400
Propyle parabene	410	75
Risperidone	91	189

* The value is the percentage of the drug with respect to 10 µg/ml of olanzapine for UV spectrophotometry that causes ±0.01 change in absorbance and 50 mg for non-aqueous titration that causes ±0.01 variation in factor

Experimental

Apparatus

A Pye Unicam UV-500 spectrophotometer with 1cm silica cells and a slit width of 0.2 nm over a range of 190-1100nm used to measure the absorbance.

Reagents

Olanzapine (Schazoo labs. (Pvt.) Ltd Lahore) standard solution (1mg/ml) (w/v) was prepared in methanol (E.Merck Germany) to get a stock solution, which was further diluted with methanol as required.

For non-aqueous titration glacial acetic acid (99.5%) was used as such.

0.2% naphthobenzene (E.Merck Germany) was prepared in glacial acetic acid and was used as indicator.

0.1N perchloric acid (E.Merck Germany) was prepared by adding 900ml of glacial acetic acid to 8.2ml of perchloric acid (72%) (w/v) and 32ml of acetic anhydride. After mixing and cooling the volume was made up to the 1000ml with glacial acetic acid and was allowed to stand for 24hrs.

Standardization of 0.1N perchloric acid was carried out by titrating 0.5gm of potassium hydrogen phthalate with an aliquot of perchloric acid. Each ml of which is equivalent to 0.02042 gm of potassium hydrogen phthalate [12].

General Procedures

UV Spectrophotometry

An aliquot of olanzapine containing 0.1µg to 50µg was subjected to UV spectrophotometry using methanol as blank. The absorbance was scanned from200-320nm. It showed three maximas i.e. at 205nm, 226nm and 272.5nm. However 226nm was selected as working maxima. Employing standard olanzapine solution a calibration curve was prepared (Fig.3). The method obeys Beer's Law from 0.1µg to 50µg/ml of olanzapine.

Non-aqueous Titration

50mg of olanzapine was dissolved in 20ml of glacial acetic acid in a 250 ml conical flask. Two drops of o.2% naphthobenzene indicator were added. The resulting solution was titrated against 0.1 N standardized perchloric acid solutions until the color changed from orangish yellow to green. A blank titration was also performed. Each ml of 0.1N perchloric acid is equal to 15.622mg of olanzapine.

Procedure for Studying Interfering Organic Compounds

To an aliquot (10µg) of olanzapine for UV and 50mg for non-aqueous titration different amounts of various organic compounds having similar action were added until the solution showed the same (± 0.01) absorbance for UV procedure and factor obeyed for non-aqueous titration as that of the pure olanzapine solution with out the addition of the organic compound under experimental condition as described under general procedure. The value was calculated as the percentage of the organic compound with respect to the amount of olanzapine.

Procedure for Determination of Olanzapine in Pharmaceutical Preparations

UV Spectrophotometry

Tablets: Tablets containing 5mg, 7.5mg and 10mg of olanzapine were powdered weighed, dissolved in methanol and filtered. The filtrate was diluted with methanol to get 1mg/ml solution of olanzapine. An aliquot containing 0.1µg to 50 µg/ml was taken, the above procedure was followed and the absorbance was measured at 226nm. The quantity was either determined from the calibration graph or calculated from the formula given below

Absorbance X 100/ Concentration X A (1%, 1cm)

A----760 for olanzapine in methanolic solution

Non- aqueous Titration

Tablets: Tablets containing 5mg, 7.5mg or 10mg of olanzapune were powdered. An accurately weighed amount of the powdered equivalent to 0.3mg to 100mg of olanzapine was dissolved in 20ml of glacial acetic acid. The procedure was followed as described under the general procedure for non-aqueous titration and the quantity per tablet was calculated. Each ml of 0.1N perchloric acid is equivalent to 15.622mg of olanzapine.

Conclusions

Olanzapine has been determined in pure and pharmaceutical preparation using two different analytical techniques viz UV and non-aqueous titration. It is concluded that though the non-aqueous titration provides better results and is more suitable to carry out the quantitative determination than the UV method, yet the use of acid like perchloric acid and acetic acid makes the procedure costly and tedious. However for the quicker analysis the UV procedure is more appropriate as it is also precise, accurate and does not involve many chemicals or solvents.

Both the methods are not reported in literature [10], however mostly HPLC procedures are employed for the determination of olanzapine in blood plasma [3, 5, 6, 8], blood serum [7, 9], breast milk [4] and urine [7] which are not only long and tedious but also involves many reagents and solvents

References

1. Van Tran, P. Olanzapine for the treatment of insomnia. Eur.Pat. Appl. EP 795, 330 (Cl.A61K31 155) 17 Sep 1997 GB Appl. 96/6, 731, 29, March 1996, 12 P.
2. R. R. Conley, H.Y. Meltzer, *J. Clin. Psychiatry*, **61** (Supply) 26 (2000).
3. M. A. Raggi, G. Casamenti, R. Manrioli, Fanali, S; De Ronchi, D. V. Volterra, *Chromatographia,* **51** (9/10), 562 (2000)
4. S.C. Kasper, E.L. Mattiuz, S.P. Swanson, J. A. Chiu, J. T. Johnson, C. O. Garnera, *J. Chromatogr., B.Biomed. Appl.*, **726** (1-2), 203 (1999).
5. J. A. Chiu, R. B. Franklin, *J. Pharm. Biomed. Anal.*, **14** (5), 609 (1996).
6. J.T. Catlow, R. D. Barton, M. Clemens, T. A. Gillespie, M. Goodwin, S. P. Swanson, *J. Chromatogr. B: Biomed. Appl.*, **668**(1), 85 (1995).
7. M. J. Bogusz, K. D. Kruger, R. D. Maier, R. Erkwoh, F. Tuchtenhagen, *J.Chromatogr. B. Biomed. Sci. Appl.*, **732** (2), 257 (1999).
8. M. Berna, R. Shugert, J. Mullen, *J. Mass Spectrom.*, **33** (10), 1003 (1998).
9. O. V. Olesen, K. Linnet, *J. Chromartogr. B. Biomed. Sci. Appl.*, **714** (2), 309 (1998).
10. Chemical Abstracts 1906-2000.
11. G. D. Christian, Data Handling: In Analytical Chemistry, 5th Ed.; John Wiley and Sons Inc.; New York, 47 (1994).
12. British Pharmacopoeia, The Pharmaceutical Press, 17 Bloomsbury Square London WCI. p. 1290 (1968).