

RESEARCH ARTICLE

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*Chemical Sciences
Journal, Vol. 2012:
CSJ-70*

Sensitive Spectrophotometric Method for the Determination of Olanzapine and Orphenadrine in Pure and Dosage Forms by Ternary Complex Formation with Eosin and Lead(II)

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Accepted: Aug 27, 2012; Published: Sep 21, 2012

Abstract

The present paper describes a simple and sensitive spectrophotometric method for the determination of olanzapine (OLZ) and orphenadrine (ORP) in pure and in dosage forms. The developed method is based on ternary complex formation of drugs under investigation with eosin and lead(II) using methyl cellulose as surfactant. The formed ternary complex which shows maximum absorbance (λ_{\max}) at 540 nm for both drugs OLZ and ORP, respectively. The optimum experimental conditions for the ternary complex formation were established. The Beer's law is obeyed in the concentration ranges, 0.0-35.0 and 0.0-55 $\mu\text{g/mL}$ for both methods. The molar absorptivity values for methods A and B were found to be 1.03×10^4 and $0.51 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, respectively with the corresponding Sandell's sensitivity values 0.0303 and 0.0602 $\mu\text{g cm}^{-2}$. The proposed methods were further applied to the determination of OLZ and ORP in bulk and in pharmaceutical preparations *via* standard addition technique. No interference was observed from the common adjuvants added to the tablets which are applicable for the assay of the investigated drugs in different dosage forms.

Keywords: Olanzapine; orphenadrine; ternary complex; spectrophotometry; dosage forms.

1. Introduction

Chemically, OLZ is 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine [1] (Figure 1a). Olanzapine (trade name Zyprexa or in combination with fluoxetine Symbyax) is an atypical antipsychotic, approved by the FDA for the treatment of schizophrenia and bipolar disorder [2]. Olanzapine is indicated for the acute and maintenance treatment of schizophrenia and psychotic disorders [3]. Olanzapine is structurally similar to clozapine, but is classified as a thienobenzodiazepine. ORP is chemically known as *N,N*-dimethyl-2-[(2-methylphenyl)-phenyl-methoxy]-ethanamine [4] (Figure 1b). Orphenadrine (sold under the brand names: Norflex, Mephenamin, Disipal, Banflex, Flexon, Biorphen, Brocasipal, Dolan, Norgesic and others) is an anticholinergic drug of the ethanolamine antihistamine class with prominent CNS and peripheral actions used to treat painful muscle spasms, other similar conditions, as well as the treatment of some aspects of Parkinson's Disease. It is closely related to diphenhydramine. Therefore, it is related to other drugs used for Parkinson's like benzotropine and trihexyphenidyl, and it is also structurally related to nefopam [5], which is a centrally-acting yet non-opioid analgesic. The combination of anticholinergic effects and CNS penetration make orphenadrine useful for pain of all etiologies, including pain from radiculopathy, muscle pain, and headaches.

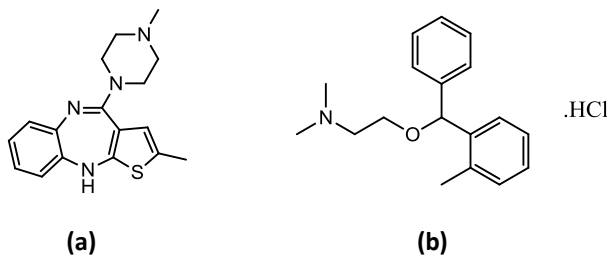


Figure 1: Structure of (a) Olanzapine (b) Orphenadrine hydrochloride.

Several analytical methods have been reported for the determination of OLZ and ORP in biological fluids and/or pharmaceutical formulations. The recommended method for determination of orphenadrine in USP [6] is HPLC while the British Pharmacopoeia (BP) recommends a potentiometric titration procedure for its assay of the raw material [7]. Literature survey on these two cited drugs reports the following methods, which include chromatographic techniques like HPLC–MS [8-14], GLC [15], potentiometry [16], LC with coulometric detection [17], HPLC with amperometric detection [18], HPLC with electrochemical detection [19], RP-HPLC [20], voltammetry [8, 21] and spectrophotometry [22-28]. Chromatographic techniques are highly expensive and require some derivatization steps before their determination. Some of the reported spectrophotometric methods are less sensitive, use costly reagents and/or extraction steps.

The purpose of the present work is the development of a simple, reliable and accurate, extraction free, spectrophotometric method for the determination of OLZ and ORP in bulk and in their pharmaceutical formulations through the formation of ion-association complex by the reaction of the studied drugs with Pb(II) and eosin.

2. Methods

2.1. Apparatus

All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer equipped with 1.0 cm matched quartz cells. An Elico 120 digital pH meter was used for pH measurements.

2.2. Materials and reagents

All chemicals and reagents used were of analytical reagent grade. Double distilled water was used to prepare all solutions throughout the investigation.

- Eosin Yellow* ($C_{20}H_6Br_4Na_2O_5$) (LOBA CHEMIE Pvt. Ltd., Mumbai, India) 2×10^{-3} M solution was prepared in double distilled water. The solution is found to be stable for 2 weeks.
- Lead acetate* 2×10^{-3} M. It was prepared by dissolving 0.076 g of lead acetate (E. Merck Limited, Worli, Mumbai, India) in 100 mL distilled water.
- Methyl cellulose (MC)* (350-550 cPs, Aldrich) 1% w/v was prepared by dissolving 1 g methyl cellulose in 100 mL distilled water.
- Working standard solutions of OLZ and ORP.*

Pharmaceutical grade OLZ and ORP certified to be 99.99 % and 99.52 % pure was received from Cipla India Ltd., Mumbai, India, as a gift and was used as received. A stock standard solution equivalent to 100 µg/mL of OLZ and ORP was prepared by dissolving 20 mg of the pure drug in 100 mL distilled water (use of few drops of conc. H_2SO_4 for dissolving OLZ). Working solutions were prepared as required by dilution. A pharmaceutical formulation of OLZ such as OLAN [Micro Synapse], and ORP such as ORPHIPAL [GSK] were purchased from local markets.

2.3. General procedure

Aliquots of standard drug solution ranging from 0-35.0 µg/mL (OLZ) and 0-55.0 µg/mL (ORP) were transferred into a series of separate 10 mL volumetric flasks using a micro burette. To this solution was added 1 mL methyl cellulose (1 %) and 2 mL of the buffer solution (pH 4.0). Then 0.7 mL each of eosin and lead solutions was added to each flask. Then, the contents of the flask were mixed well and heated up to about 65 °C (± 5 °C) for about 20 min, then cooled to room temperature for 5 min under tap water and the contents of the flask were diluted to the mark with distilled water and mixed well. The absorbance of the colored complex was measured at 540 nm after 10 min against distilled water as blank solution. The amount of OLZ and ORP present in the sample was computed from respective calibration curve or the regression equation.

2.4. Procedure for commercial samples

Olanzapine and orphenadrine tablet samples such as OLAN [Micro Synapse] and ORPHIPAL [GSK] were purchased from local markets. Twenty tablets each were weighed accurately and ground into fine powder. A quantity of the powder equivalent to 20 mg of OLZ and ORP was weighed accurately into a separate 100 mL calibrated flasks and 50 mL of distilled water was added (use of few drops of conc. H_2SO_4 for OLZ). The content was shaken for about 30

min; the volume was diluted to the mark with water and mixed well and filtered using a Whatman No. 41 filter paper. An appropriate dilute solution (OLZ and ORP) was subjected to analysis by the procedure described above.

3. Results and Discussion

The developed method involves the formation of ternary complexes between the drugs under investigation such as OLZ and ORP with eosin-lead, with absorption maximum at 540 nm for both OLZ and ORP. The absorption spectra of these complexes such as OLZ-eosin-lead and ORP-eosin-lead are presented in Figure 2.

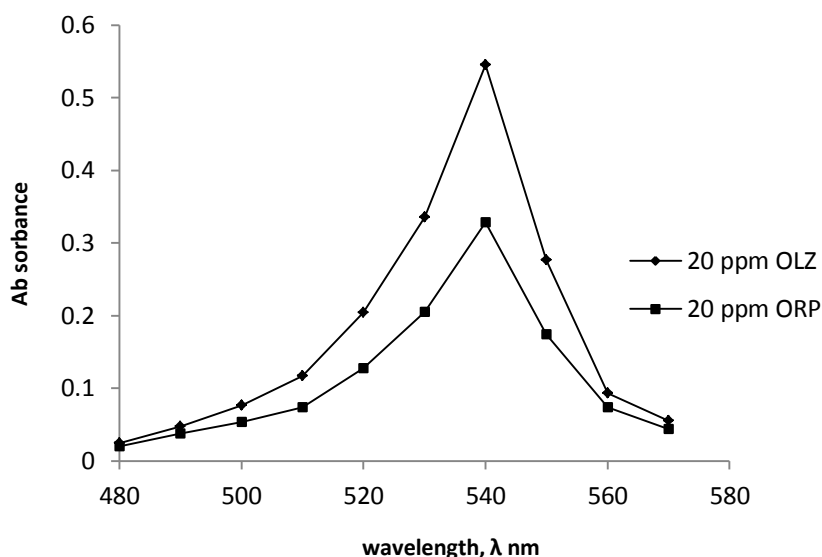


Figure 2: Absorption spectra of the ternary complex of olanzapine (20 µg/mL) and orphenadrine (20 µg/mL) with eosin and lead(II).

3.1. Chemistry

The main object of this study depends on the fact that, the ternary complex is formed between the studied drugs such as OLZ and ORP with eosin-lead(II). Ternary complex of general formula $(L_nM_xS_y)$ [or the studied drug-metal ion $[Pb(II) (drug)_n]$ as cation and (eosin) as anion have been widely used in spectrophotometric analysis [29-32]. The ternary complex formed in the present study is that their main ligand L is the drugs (OLZ and ORP), the second ligand S is eosin, and M is lead(II).

This work is undertaken in the view that the ternary complex has been formed between the drugs OLZ and ORP and eosin-lead(II). The ternary complex formed between the metal ion: electronegative ligand and organic base often have higher values of molar extinction coefficient than binary complexes of the same components. The formation of ternary complex not only improves the sensitivity of the method but also the selectivity as well.

3.2. Optimization of variables and method development

In order to develop a rapid, sensitive and stable method, several experimental parameters (the concentration of surfactant, pH, eosin, lead(II) acetate, reaction time and temperature) were studied separately by varying and observing the different parameters that affect absorbance of the ion-pair association complex at 540 nm, respectively by varying one parameter at a time and keeping the others constant. The optimum experimental conditions with the maximum color intensity were found to be as follows: 1 mL methyl cellulose (1 % w/v) and 2 mL of the buffer solution (pH 4.0) and 0.7 mL each of eosin and lead solutions.

3.2.1. Effect of Methyl Cellulose (MC)

Preliminary experiments were performed to study the effect of concentration of surfactant (MC) on the absorbance of the solution of the ternary complex at 540 nm. The addition of the non-ionic surfactant, MC was

found to be necessary to enhance the stability and sensitivity of the ternary complex prior extraction steps was found to be unnecessary. The addition of surfactants to solubilize and stabilize the ternary complex formation had been previously reported by Fujita *et al.* [33]. Thus, 1 mL MC (1 % w/v) was used in the present study, and the addition of MC was found to be necessary for the stability and sensitivity of the ternary complex and for prevention of precipitate formation.

3.2.2. Effect of pH

The effect of pH on the intensity of the color developed at the selected wavelength for the formation of the ion-pair association complex was also studied, it was found that the optimum pH is in the range of 3.7-4.3, using sodium acetate-acetic acid buffer; 2 mL of the buffer solution (pH-4) was adequate to obtain maximum and constant absorbance when measuring the test solution against the reagent blank, for OLZ and ORP.

3.2.3. Effect of reagents

The effect of concentration of reagents, eosin and lead(II) acetate, on the absorbance of the ternary complex was also studied. In order to select suitable metal ion as complexing agent with the drugs under investigation for the formation of ion-pair association complex, number of metal ions such as Cu(II), Pd(II) and Pb(II) were tested. Among the studied metal ions, Pb(II) was found to be the best which gave the highest sensitivity and reproducibility and thus selected as the suitable complexing agent in this study. Further, an appropriate reaction conditions were established for the colour reaction and for the eosin: Pb(II): drug to reach maximum sensitivity, and it was found 0.7 mL each of 2×10^{-3} M eosin and lead solutions were sufficient to produce maximum absorbance.

3.2.4. Effects of temperature, reaction time and stability of the ion-pair association complex

After the addition of 1 mL methyl cellulose and 2 mL of the buffer solution (pH 4.0) and 0.7 mL each of eosin and lead solutions to the cited drug solutions, the effect of standing time on the formation of ion-pair association complex was also studied. It was found that 10 min standing time was sufficient for the complete formation of the ion-pair association complex in both the drugs OLZ and ORP. In order to study the effect of temperature and reaction time on the absorbance of the ternary complex formation, the procedure mentioned under the construction of the calibration graph was carried out at different temperatures (room temperatures at 40, 50, 60, 70, 80 and 90 °C). At temperature of about 65 (± 5 °C), the absorbance of the formed ternary complex reached a maximum in 10 min with colouration remaining stable for a period of 60 min and 90 min for OLZ and ORP, respectively. At the temperature greater than 70 °C, the stability and sensitivity of the complex was affected.

3.3. Method validation

According to the International Conference on Harmonization (ICH) guidelines [34], the developed methods were validated for linearity, sensitivity, precision, accuracy, robustness, ruggedness, selectivity and recovery.

3.3.1. Linearity

Calibration graphs were constructed using standard solutions of OLZ and ORP. Under the optimized experimental conditions for both drugs under investigation, a linear correlation was found between the absorbance at 540 nm and concentrations of OLZ and ORP as given in Table 1. Regression analysis of the calibration curve using the method of least-squares was made to calculate the slope ($b = 0.0301$ and 0.0162 for OLZ and ORP, respectively), intercept ($a = 0.0143$ and 0.0052 for OLZ and ORP, respectively) and correlation co-efficient (r) for both OLZ and ORP and the values are presented in Table 1. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity values [35] of both the methods are also given in Table 1. The calibration curves of the OLZ and ORP are given in Figure 3a and b, respectively.

3.3.2. Sensitivity

The detection limits (LOD) and limits of quantitation (LOQ), for the proposed methods were evaluated as per ICH guidelines using the formula:

$$\text{LOD} = \frac{3.3 \times \sigma}{s} \quad \text{and} \quad \text{LOQ} = \frac{10 \times \sigma}{s}$$

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and s is the slope of the calibration graph. The high values of Molar absorptivity (ϵ) and low values of Sandell sensitivity, and LOD in both the methods indicate the high sensitivity of the proposed methods (Table 1).

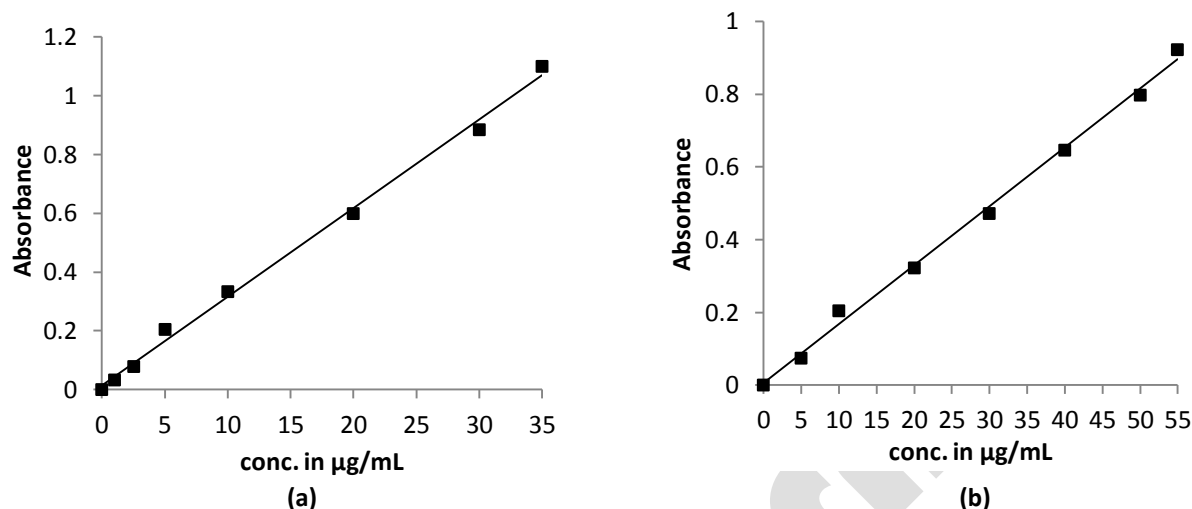


Figure 3: Calibration curve for (a) OLZ-eosin-lead and (b) ORP-eosin-lead.

Table 1: Analytical and regression parameters of the proposed methods.

Parameter	OLZ	ORP
λ_{max} nm	540	540
Colour stability	60 min	90 min
Beer's law range ($\mu\text{g/mL}$)	0.0 - 35.0	0.0 - 55.0
Linear range ($\mu\text{g/mL}$)	1.0 - 35.0	5.0 - 55.0
Molar absorptivity (ϵ), ($\text{L mol}^{-1}\text{cm}^{-1}$)	1.03×10^4	0.51×10^4
Sandell sensitivity ($\mu\text{g cm}^{-2}$)	0.0303	0.0602
Regression equation, Y^*		
Intercept (a)	0.0143	0.0052
Slope (b)	0.0301	0.0162
Correlation coefficient (r)	0.9980	0.9981
S_a	0.0399	0.0318
S_b	0.0011	0.0006
LOQ ($\mu\text{g/mL}$)	0.4547	0.9422
LOD ($\mu\text{g/mL}$)	0.1501	0.3109

* $y=a+bx$, where c is the concentration of MDZ in $\mu\text{g/mL}$ and y is the absorbance at the respective λ_{max} , S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope.

3.3.3. Intra-day precision and accuracy

To evaluate the intra-day accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analyzed, each determination being repeated five times. The relative error (%) and

relative standard deviation (%) values of intra-day studies were satisfactory and showed that the best appraisal of the procedures in daily use. The results obtained are presented in Table 2.

Table 2: Evaluation of intra-day accuracy and precision.

Tablet studied	Drug taken in µg/mL	Drug found* in µg/mL	RE %	SD	RSD %	ROE ** %
OLAN	10	9.97	0.34	0.10	1.00	±1.00
	20	20.03	-0.13	0.08	0.39	±0.39
	30	29.94	0.21	0.15	0.51	±0.51
ORPHIPAL	20	19.92	0.40	0.05	0.23	±0.23
	30	29.85	0.50	0.12	0.36	±0.36
	40	39.79	0.53	0.10	0.25	±0.25

RE: Relative error; SD: Standard deviation; SEM: Standard error of mean; RSD: Relative standard deviation; ROE: Range of error.

*Mean value of five determinations.

**At 95% confidence level for 4 degrees of freedom.

3.3.4. Interferences

The selectivity of the proposed method to pharmaceutical samples was tested by a systematic study under the optimum experimental conditions which were made for the effect of the additives and excipients. The recommended procedure was applied to the analysis of synthetic mixtures prepared in the laboratory. The usual diluents and excipients such as talc (20 mg), dextrose (20 mg), starch (10 mg), sodium alginate (20 mg), gelatin (15 mg) and acacia (10 mg) were found not to interfere with the analysis by the proposed methods and the results were obtained in the range 98.0 % to 101.5 % (both OLZ and ORP). These results further showed clearly the accuracy and precision of the developed method.

3.3.5. Application to analysis of pharmaceutical samples

To check the validity of the proposed method, OLZ and ORP was determined in some commercial formulations and the results are presented in Table 3. The results of an assay of OLZ and ORP tablets obtained were statistically compared with the reference methods [27, 28] by applying the Student's-t test for accuracy and F-test for precision. The results in the Table 4 showed that there is no significant difference between the proposed and reference methods [27, 28] at the 95 % confidence level with respect to accuracy and precision. The calculated t- and F- values (Table 4) did not exceed the tabulated values ($t=2.77$ and $F=6.39$), they could therefore be used easily for the routine analysis of pure OLZ and ORP and its dosage forms.

Table 4: Results of determination of MDZ in dosage form and statistical comparison with the reference method.

Tablet brand name	Nominal amount (mg per tablet)	Found* (% of nominal amount \pm SD)		
		Reference method [27, 28]	OLZ	ORP
OLAN	5	102.1 \pm 1.76	100.60 \pm 0.73 $t=0.95$, $F=5.89$	-
ORPHIPAL	50	99.00 \pm 0.62	-	99.78 \pm 0.25 $t=1.42$, $F=6.28$

*Mean value of five determinations.

Tabulated t - and F-values at 95 % confidence level are 2.77 and 6.39, respectively.

3.3.6. Evaluation of accuracy by recovery study (standard addition technique)

The accuracy of the proposed method was further ascertained by performing recovery studies via the standard addition technique. Pre-analyzed injections were spiked with pure drug at three different concentrations (50, 100 and 150 %) and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and the recovery percentage values ranged between 99.38 –

100.20 % with standard deviation in the range 0.01 – 0.11 % in both the methods were close to 100 % indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination. The results of recovery study are compiled in Table 5.

Table 5: Results of recovery experiments via the standard addition technique.

Tablet brand name	Labeled amount, mg/tablet	MDZ tablet, µg/mL	Pure MDZ added, µg/mL	Total found, µg/mL	Pure MDZ recovered* %±SD
OLAN	5	10	5	14.97	99.47±0.66
		10	10	20.06	100.63±0.94
		10	15	25.26	101.71±0.57
ORPHIPAL	50	10	10	19.98	99.81±0.20
		10	20	29.89	99.46±0.25
		10	30	40.02	100.07±0.29

*Mean value of three measurements.

4. Conclusion

The developed spectrophotometric method was successfully applied to the determination of OLZ and ORP in pure form and in dosage forms. The results obtained are compared statistically by student's *t*-test for accuracy and *F*-test for precision with the reference methods [27, 28]. The results showed that the *t*- and *F*-values were less than the tabulated value indicating that there is no significant difference between the developed and the reference method. Moreover, the developed method is more selective, sensitive, reproducible, rapid, cheap and simple. Therefore, the method developed here can be used for routine analysis in the majority of drug quality control laboratories where precision, time and cost effectiveness of analytical method is important.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Both authors contributed equally to this work.

Acknowledgement

The authors are grateful to Cipla India Ltd, for the generous supply of pure drug sample. One of the authors HND is thankful to the University of Mysore, Mysore for providing necessary facilities.

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