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QUANTITATIVE ESTIMATION OF LEVOFLOXACIN AND ORNIDAZOLE BY UV SPECTROPHOTOMETER: A MIXED HYDROTROPY SOLUBILIZATION APPROACH

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ABSTRACT: Two simple, accurate, novel, safe and precise methods developed for the simultaneous estimation of poorly water-soluble drugs Levofloxacin and Ornidazole in tablet dosage form using 2M sodium acetate and 8M urea solution (50:50% W/W) as mixed hydrotropic solution. Sodium acetate and urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs were seen. LEVO and OZ follows the Beer's law in the concentration range of 5-25 µg/ml ($r^2 = 0.9997$ and 0.9998). Method-A simultaneous equation method employs 287 and 320 nm as two analytical wavelengths, method-B is absorption ratio method, which uses 301 and 320 nm as two analytical wavelengths were used for estimation of LEVO and OZ. The mean percent label claims of tablet dosage were found to be 98.528 ± 0.431 and 97.916 ± 0.732 in method A, 97.586 ± 0.821 and 98.642 ± 0.293 in method B for LEVO and OZ respectively. The standard deviation, coefficient of variance and standard error were obtained for LEVO and OZ was satisfactorily low. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values therefore the both methods can be used for routine monitoring of LEVO and OZ in industry in the assay of bulk drug and tablets.

INTRODUCTION: Levofloxacin hemihydrates (LEVO) chemically (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate (**Figure 1A.**), is a fluoroquinolone antimicrobials, is the active S-isomer isolated from the racemic ofloxacin¹⁻².

Ornidazole (OZ) chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazole-1-yl) propane-2-ol (**Figure 1B.**), is a 5-nitroimidazole derivative used as an anti-infective agent^{1, 2}. Levofloxacin hemihydrate is official in IP³. Numerous GC-MS⁴, HPLC^{4,9}, Capillary electrophoresis and nuclear magnetic resonance spectroscopy¹⁰, HPLCMSMS¹¹ and HILICMSMS¹² has been used to determine drugs in biological fluids.

Ornidazole is official in IP³ and USP¹³. The assay procedure mentioned in these pharmacopoeias uses non-aqueous titration for estimation of ornidazole. A literature survey reveals that ornidazole is estimated by glassy carbon electrode¹⁴, UV¹⁵

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method, HPLC¹⁶ method, GC¹⁷ method and calorimetry¹⁸ method in solid dosage form and in biological fluids. Some spectrophotometric^{19, 20}, HPLC²¹ and HPTLC²² methods have been reported for their simultaneous estimation with ofloxacin in the tablet dosage form.

Various techniques have been employed to enhance the aqueous solubility and hydrotophy is one of them. Maheshwari and Jain et al has used sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of drug²³⁻³⁴. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents.

Therefore, it was thought worthwhile to employ this mixed hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. There are no reports yet for determination of this combination by proposed methods. Present work emphasizes on the quantitative estimation of LEVO and OZ in their combined dosage form by UV Spectroscopic methods.

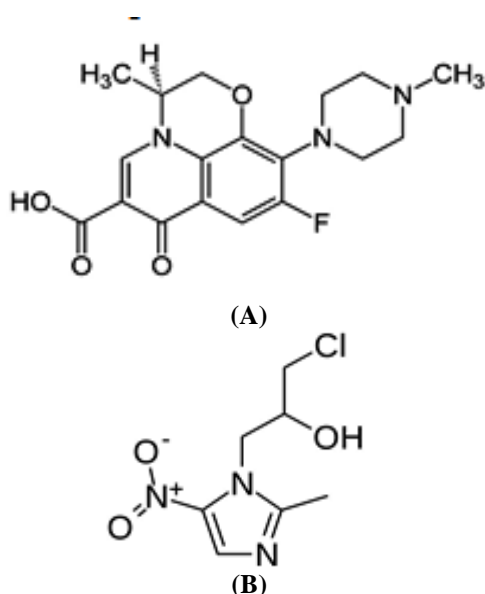


FIGURE 1: STRUCTURE OF (A) LEVOFLOXACIN (B) ORNIDAZOLE

EXPERIMENTAL:

Instrument: UV-Visible double beam double detector spectrophotometer, Shimadzu model-1700 having spectral bandwidth 3 nm and of wavelength accuracy ± 1 nm, with 1cm quartz cells was used.

Reagents and chemicals: Pure sample of LEVO and OZ was obtained as gift sample from Intas Laboratories Pvt Ltd, and GSK Ltd. Mum Swan pharmaceutical, respectively. Sodium acetate and urea obtained from Merck Chemical Division, Mumbai. Reverse Osmosis Water was used throughout the study.

Preliminary solubility studies of drugs:

Solubility of both drugs was determined at $25 \pm 1^\circ\text{C}$. An excess amount of drug was added to two screw capped 25 ml of volumetric flask containing different aqueous systems viz different combination of hydrotropic agent and 2M sodium acetate and 8M urea solution. The volumetric flasks were shaken mechanically for 12 h at $25 \pm 1^\circ\text{C}$ in a mechanical shaker. These solutions were allowed to equilibrate for next 24 h and then centrifuged for 5 min at 2000 rpm.

The supernatant liquid was taken for appropriate dilution after filtered through Whatman filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of LEVO and OZ was found to be more than and 49 and 45 folds respectively in mixture of 2M sodium acetate and 8M urea solution (1:1) as compared to solubility studies in other solvents.

Selection of hydrotropic agent: LEVO and OZ was scanned in hydrotropic agent in the spectrum mode over the UV range (200-400) and mixture of 2 M sodium acetate and 8 M urea (50:50% V/V) solution were found to be most appropriate because:

- LEVO and OZ is soluble in it (49 and 45 fold enhancement of solubility)
- LEVO and OZ is stable in hydrotropic agent
- LEVO and OZ, both exhibit good spectral characteristics in it.
- Sodium acetate and urea solution has no interference with the λ_{max} of LEVO and OZ i. e. 287 nm and 320 nm respectively (**Figure 2**).

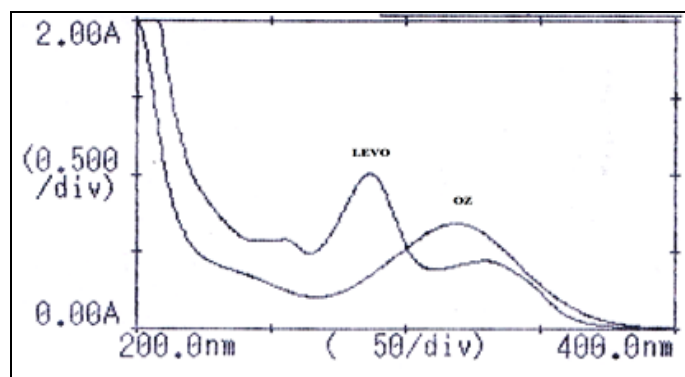


FIGURE 2: OVERLAY SPECTRA OF LEVO AND OZ

Establishment of Stability Profile: Stability of LEVO and OZ was observed by dissolving in mixed hydrotropic agent. Solution of LEVO and OZ was scanned under time scan for 30 min. Spectra of drug under time scan shows that drug are stable in hydrotropic solution.

Linearity range and Calibration graph:

- Preparation of Standard Stock Solutions of LEVO and OZ:** Standard stock solutions were prepared by dissolving separately 100 mg of each drug in mixed hydrotropic solution and the flask was sonicated for about 10 min to solubilize the drug (Stock-A).
- Preparation of Working Standard Solution for calibration curve:** The standard solution (1000 $\mu\text{g/ml}$) was further diluted in ranging from 5-25 $\mu\text{g/ml}$ for LEVO and 5-25 $\mu\text{g/ml}$ for OZ. Calibration curve was plotted between concentrations versus absorbance **Figure 3**,

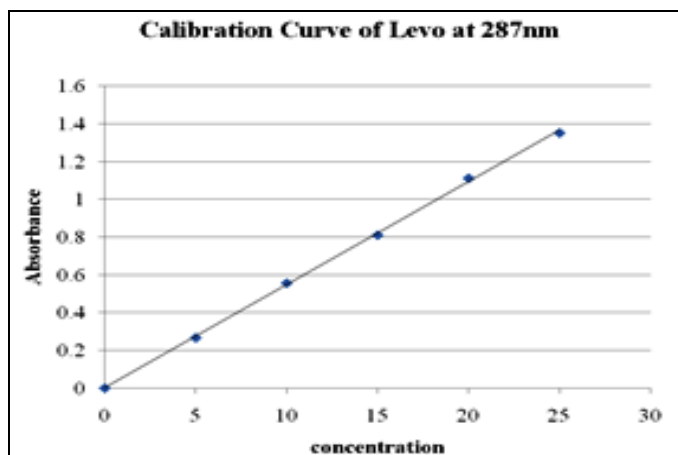


FIGURE 3: CALIBRATION CURVE OF LEVO

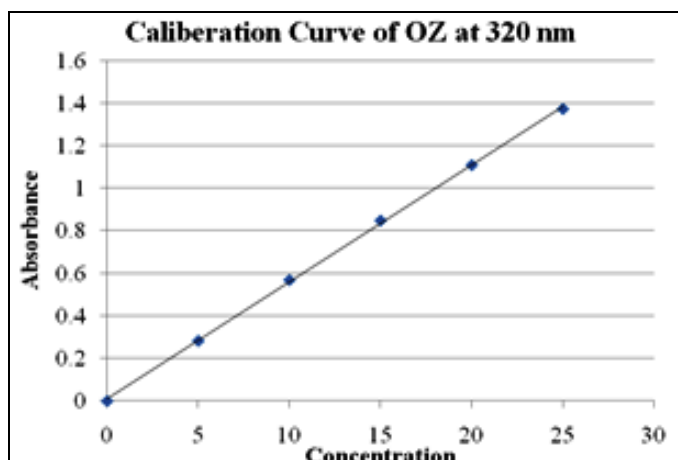


FIGURE 4: CALIBRATION CURVE OF OZ

TABLE 1: LINEARITY OF LEVO AND OZ

LEVO ($\lambda_{\text{max}} = 287 \text{ nm}$)			OZ ($\lambda_{\text{max}} = 320 \text{ nm}$)		
Standard Conc. ($\mu\text{g/ml}$)	Absorbance \pm S.D (n=5) at 287 nm	% RSD	Standard Conc. ($\mu\text{g/ml}$)	Absorbance \pm S.D (n=5) at 320 nm	% RSD
0	0	0	0	0	0
5	0.267 \pm 0.0041	1.55	5	0.283 \pm 0.0045	1.60
10	0.556 \pm 0.0076	1.37	10	0.569 \pm 0.0048	0.84
15	0.811 \pm 0.0038	0.47	15	0.848 \pm 0.0043	0.51
20	1.112 \pm 0.0101	0.91	20	1.109 \pm 0.0043	0.39
25	1.352 \pm 0.0112	0.83	25	1.373 \pm 0.0117	0.85

TABLE 2: OPTICAL CHARACTERISTICS AND LINEARITY DATA OF LEVO AND OZ

Sr. No.	Parameters	LEVO	OZ
1	Working λ	287 nm	320 nm
2	Beer's law limit ($\mu\text{g/ml}$)	5-25	5-25
3	Correlation Coefficient (r^2)*	0.9997	0.9998
4	Slope (m)*	0.0546	0.0550
5	Intercept (c)*	0.0008	0.0097

*Average of five determination

Study of Overlay Spectra of drugs and selection of method:

The spectra exhibit major absorbance maxima at 287 nm and 320 nm for LEVO and OZ respectively and isobestic point at 301 nm (Figure 2). Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Method A) and Q-analysis method (Method B)

1. Vierordt’s simultaneous equation method (Method A):

The wavelength 287 nm (λ_{max} of LEVO) and 320 nm (λ_{max} of OZ) was selected. The absorbencies of LEVO and OZ were measured at 287 nm and 320 nm. This method of analysis is based on the absorption of drugs X and Y at the wavelength maxima of the other. The quantification analysis of LEVO and OZ in a binary mixture was performed by using Eqn-1 and Eqn-2. Where C_X and C_Y are the concentrations of LEVO and OZ respectively in the diluted sample, ax_1 and ax_2 are absorptivities of LEVO at λ_1 and λ_2 , ay_1 and ay_2 are absorptivities of OZ at λ_1 and λ_2 respectively Table 3. A_1 and A_2 are the absorbances of samples at the 287 and 320 nm respectively³⁵.

$$C_X = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \dots\dots\dots \text{Eqn.1}$$

$$C_Y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \dots\dots\dots \text{Eqn.2}$$

2. Q-analysis method (Method B): In this method, absorbances of both the drugs were calculated at two selected wavelengths; among which λ_1 is the wavelength of isoabsorptive point of both drugs and λ_2 is the λ_{max} of either drug among both drugs. From the overlain spectra wavelength 301 nm (isoabsorption point) and 320 (λ_{max} of OZ) were selected for study. The absorbencies at 301 nm and 320 nm for LEVO were obtained and similarly for OZ absorbencies are measured at 301 nm and 320 nm. The concentrations of the individual components were calculated by using the following equations;

$$C_X = Q_m - Q_y / Q_x - Q_y \times A_1 / ax_1 \dots\dots\dots \text{Eqn.3,}$$

$$C_Y = Q_m - Q_y / Q_y - Q_x \times A_1 / ax_1 \dots\dots\dots \text{Eqn.4}$$

Where $Q_m = A_2 / A_1$, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components. ax_1 and ax_2 represent absorptivities of LEVO at λ_1 and λ_2 and ay_1 and ay_2 denote absorptivities of OZ at λ_1 and λ_2 respectively Table 3.; C_X and C_Y be the concentration of LEVO and OZ respectively³⁵⁻³⁶.

TABLE 3: ABSORPTIVITIES OF LEVO (X) AND OZ (Y) AT λ_1 and λ_2

Drug	Method- I				Method- II			
	287 nm (λ_1)	320 nm (λ_2)	301 nm (λ_1)	320 nm (λ_2)	301 nm (λ_1)	320 nm (λ_2)	301 nm (λ_1)	320 nm (λ_2)
LEVO	ax_1	0.0552	ax_2	0.0232	ax_1	0.0291	ax_2	0.0232
OZ	ay_1	0.0261	ay_2	0.0554	ay_1	0.0417	ay_2	0.0554
					Q_x	0.7969	Q_y	1.3285

N=5

Analysis of Marketed Formulation: Twenty marketed tablets of LEVO and OZ (Levoflox –OZ, Cipla Limited) were weighed and ground to a fine powder; amount equal to 250 mg of LEVO was taken in 100 ml volumetric flask. The OZ present in this amount of tablet powder was 500 mg. Then 80 ml of sodium acetate and urea solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with

hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method and absorbance ratio method. The statistical evaluation of tablet analysis has reported in Table 4.

TABLE 4: RESULTS AND STATISTICAL PARAMETERS FOR TABLET ANALYSIS (LEVOFLOX –OZ)

S. No	Drug	Label Claim	Amount Found	MEAN*	S.D.*	%COV*	Std. Error*
Method A	LEVO	250	246.32	98.528	0.431	0.437	0.080
	OZ	500	487.93	97.586	0.821	0.841	0.154
Method B	LEVO	250	244.79	97.916	0.732	0.748	0.137
	OZ	500	493.21	98.642	0.293	0.297	0.054

*Average of five determination

Validation Parameters: The developed methods for simultaneous estimation of EPS and HCZ were validated as per ICH guidelines (Linearity, Accuracy, Precision and Robustness)³⁷.

1. **Linearity:** Linearity of LEVO and OZ was established by response ratios of drug.

TABLE 5: RESPONSE RATIO OF LEVO AND OZ

LEVO			OZ		
Conc. (µg/ml)	ABS	Response Ratio	Conc. (µg/ml)	ABS	Response Ratio
5	0.269	0.018	5	0.284	0.056
10	0.547	0.018	10	0.561	0.056
15	0.817	0.018	15	0.842	0.056
20	1.11	0.018	20	1.108	0.056
25	1.361	0.018	25	1.386	0.056

Response ratio of both drugs was calculated by dividing the absorbance with respective concentration **Table 5**. Then a graph was plotted between concentration and response ratio **Figure 5, Figure 6**.

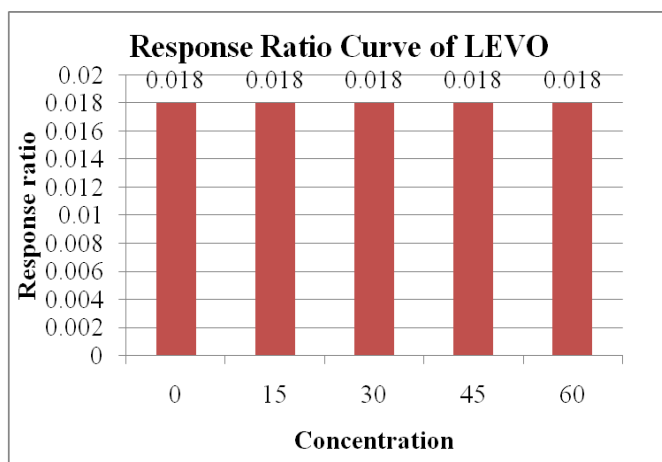


FIGURE 5: RESPONSE RATIO CURVE OF LEVO

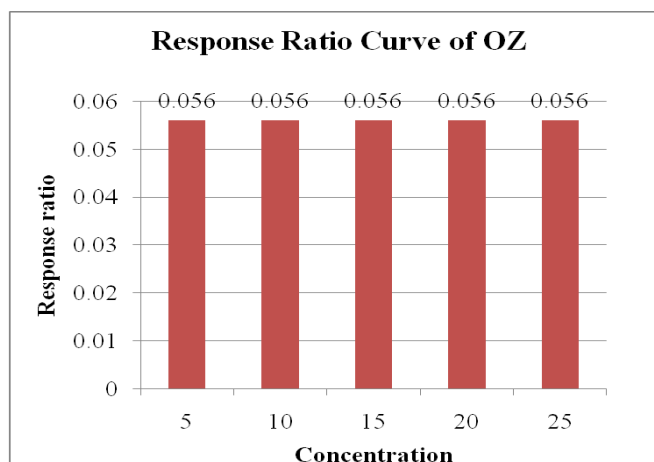


FIGURE 6: RESPONSE RATIO CURVE OF OZ

2. **Accuracy:** The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. The recovery studies were carried out by adding known amount of standard solution of LEVO and OZ to preanalysed tablet

solutions. The resulting solutions were then re-analysed by proposed methods. Total amount of drug found and percentage recovery was calculated. Result of recovery studies are reported in **Table 6**.

TABLE 6: RESULTS OF RECOVERY STUDIES ON MARKETED FORMULATIONS

Recovery Level %	% Recovery (Mean±SD)*			
	Method A		Method B	
	LEVO	OZ	LEVO	OZ
80	97.21±0.121	98.43±0.229	97.77±0.530	98.84±0.293
100	98.34±0.102	98.62±0.492	98.88±0.802	98.74±0.365
120	98.11±0.217	98.86±0.140	97.66±0.332	97.40±0.649
Mean	97.88±0.146	98.63±0.861	99.77±0.55	98.32±0.435

*Average of five determination

3. **Precision:** Precision of the methods was studied at three levels as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility **Table 7**.
4. **Robustness:** For the robustness of the analytical method we changed the ratio of hydrotropic solution. Instead the 50:50 ratios of sodium acetate and urea 60:40 sodium acetate and urea were used as solvent (**Table 7**).

TABLE 7: RESULTS OF VALIDATION (MEAN±SD)

Parameter	Method – A				Method - B			
	LEVO	%RSD	OZ	%RSD	LEVO	%RSD	OZ	%RSD
Precision (Mean±SD)*								
Repeatability	98.45±0.75	0.761	98.28±0.93	0.946	98.77±0.19	0.192	98.48±0.13	0.132
Day to Day	98.56±0.39	0.395	98.30±0.84	0.854	98.79±0.94	0.951	99.81±0.25	0.250
Analyst to Analyst	97.92±0.37	0.377	97.88±0.50	0.510	97.60±0.37	0.379	98.43±0.40	0.406
Reproducibility	98.12±0.91	0.927	98.84±0.92	0.930	98.59±0.44	0.446	98.75±0.62	0.627
Robustness*	98.64±0.63	0.38	97.71±0.77	0.788	97.88±0.70	0.715	97.93±0.74	0.755

*Average of five determination

RESULTS AND DISCUSSIONS: Based on the solubility and stability and spectral characteristics of the drugs, 2M sodium acetate and 8M urea solution (50:50% W/W) as mixed hydrotropic solution. It was found that solubility enhanced of LEVO and OZ was more than 49 and 45 fold respectively in mixed hydrotropic solution as compare with distilled water. LEVO and OZ were show maximum absorbances at 287 and 320 nm respectively. Sodium acetate and urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs were seen. LEVO and OZ follows the Beer's law in the concentration range of 5-25 µg/ml ($r^2 = 0.9997$ and 0.9998).

Method-A simultaneous equation method employs 287 and 320 nm as two analytical wavelengths, method-B is absorption ratio method, which uses 301 and 320 nm as two analytical wavelengths were used for estimation of LEVO and OZ. The optimized methods showed good reproducibility and recovery with ranging from 97.88 ± 0.146 and 98.63 ± 0.861 in method A and 99.77 ± 0.55 and 98.32 ± 0.435 in method B for LEVO and OZ respectively.

The mean percent label claims of tablet dosage were found to be 98.528 ± 0.431 and 97.916 ± 0.732 in method A, 97.586 ± 0.821 and 98.642 ± 0.293 in method B for LEVO and OZ respectively. The standard deviation, coefficient of variance and standard error were obtained for LEVO and OZ was satisfactorily low. Result of precision at different level were found be within acceptable limits ($RSD < 2$).

CONCLUSION: There was no interference of 2M sodium acetate and 8M urea solution (50:50% W/V) in the estimation and hence the two UV spectrophotometric methods were found to be simple, accurate, economic and rapid for

simultaneous estimation of LEVO and OZ in bulk and tablet dosage form. The proposed method can be successfully employed for the routine analysis of LEVO and OZ containing dosage forms.

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