

## Development and Validation of Analytical Method for Simultaneous Estimation of Lamotrigine and Clozapine in Synthetic Mixture by Absorbance Correction Method

Priyanka P. Atodariya\*, Hasumati A. Raj, Vineet C. Jain  
Department of QA, Shree Dhanvantary Pharmacy Collage, Kim,  
Surat, Gujarat, India

\*atodariya.priyanka@yahoo.com

### ABSTRACT

The simple spectroscopic method has been developed for simultaneous estimation of Lamotrigine and Clozapine in synthetic mixture. Absorbance Correction Method involves the measurement of absorption at two wavelengths 307 nm ( $\lambda_{\text{max}}$  for Lamotrigine) and 360 nm ( $\lambda_{\text{max}}$  for Clozapine). The method was found linear between the range of 1-5  $\mu\text{g/ml}$  for Lamotrigine and 6-30  $\mu\text{g/ml}$  for Clozapine for method. The accuracy and precision was determined and validated statistically. Both the method showed good reproducibility and recovery with %RSD less than 1. The method was found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis for Lamotrigine and Clozapine in bulk and combined dosage form.

Keywords: Abrasive action, fluoride, human health, kidney disease, reproductive functioning

### INTRODUCTION

Lamotrigine is an epileptic drug which is believed to suppress seizures by inhibiting the release of excitatory neurotransmitters<sup>[1]</sup>. IUPAC name of Lamotrigine is 3, 5-diamino-6-(2, 3-dichlorophenyl)-1, 2, 4 triazine<sup>[2]</sup>. Clozapine is benzodiazepine derivative and use in treatment of schizophrenia<sup>[3]</sup>. The IUPAC name of Clozapine is 8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b, e] [1, 4] diazepine<sup>[4]</sup>. It acts by inhibiting presynaptic voltage sensitive sodium channels and excitatory neurotransmitter release. Dopamine is one of the important neurotransmitter and plays a significant role in the functioning of central nervous system<sup>[5]</sup>. The inhibition of dopamine transmission through blockade of dopamine D2 receptors is considered to be essential for antipsychotic efficacy, but it is postulated that modulation of glutamate transmission may be equally important. In support of this, symptoms similar to schizophrenia can be induced in healthy volunteers using N-methyl-D-aspartate (NMDA) antagonist drugs that are also known to enhance glutamate transmission.<sup>[6]</sup>

Furthermore, Lamotrigine, which can modulate glutamate release, may add to or synergise with atypical antipsychotic drugs, some of which may themselves modulate glutamate transmission<sup>[7]</sup>. So, Lamotrigine use with Clozapine in treatment of schizophrenia.

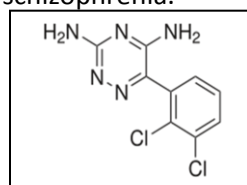


Fig-1 Lamotrigine<sup>[8]</sup>

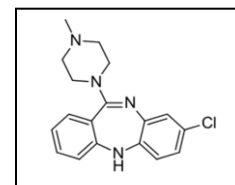


Fig-2 Clozapine<sup>[9]</sup>

### Chemical structure of Lamotrigine and Clozapine

### MATERIAL AND METHODS

#### APPARATUS AND INSTRUMENT

- A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.
- Spectra were automatically obtained by UV-Probe system software.

- An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples.
- Sonicator (D120/2H, TRANS-O-SONIC)
- Class 'B' volumetric glassware were used (Borosilicate)
- All instruments and glass wares were calibrated.

#### REAGENTS AND MATERIAL

- Lamotrigine raw material was received as gift sample from Praveen Laboratories.
- Clozapine raw material was received as gift sample from ZCI Pharmaceuticals Pvt. Ltd.
- Methanol AR Grade (Rankem), Distilled Water, 0.1 N HCl, 0.1 N NaOH were used for development purpose.

#### FOR ABSORBANCE CORRECTION METHOD

##### PREPARATION OF STANDARD SOLUTION:

##### Standard solution of Lamotrigine (LAMO)

**Preparation of stock solution of LAMO:** Accurately weighed quantity of LAMO 10 mg was transferred to 100ml volumetric flask, dissolved, and diluted up to mark with methanol to give a stock solution having strength 100µg/ml.

##### Standard solution of Clozapine (CLO)

**Preparation of stock solution of CLO:** Accurately weighed quantity of CLO 10 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength 100µg/ml.

**Preparation of Standard Mixture Solution (LAMO + CLO):** 1ml of standard stock solution of LAMO (100µg/ml) and 6 ml of standard stock solution of CLO (100µg/ml) was pipette out into two 10ml volumetric flasks and volume was adjusted to the mark with methanol to get 10µg/ml of LAMO and 60µg/ml of CLO.

##### Preparation of test solution:

The preparation of synthetic mixture was as per patent:

- Lamotrigine: 10mg
- Clozapine: 60mg
- Sodium Starch Glycolate: 10 mg
- Starch: 10 mg

- Magnesium stearate: 10 mg

All the excipients were mixed in 100ml volumetric flask then make up the volume with methanol and sonicated for 15min. after sonicate make up the volume up to 100 ml with methanol. The solution was filtered through What man filter paper No. 42. Finally the solution had concentration 100µg/ml for LAMO and 600µg/ml for CLO.

#### VALIDATION OF PROPOSED METHOD<sup>[10]</sup>

Parameters to be considered for the validation of methods are:

##### 1) LINEARITY AND RANGE

###### Procedure:

**Calibration curves for Lamotrigine:** The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 1-5µg/ml OF LAMO. This series consisted of five concentrations of standard LAMO solution ranging from 1-5 µg /ml. The solutions were prepared by pipetting out Standard LAMO stock solution (0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with Methanol. A zero order spectrum, measured the absorbance at 307 nm against a reagent blank solution (Methanol).

**Calibration curve for Clozapine:** The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 6-30 µg /ml CLOZ. This series consisted of five concentrations of standard CLOZ solution ranging from 6-30 µg/ml. The solutions were prepared by pipetting out Standard CLOZ stock solution (0.6ml, 1.2ml, 1.8ml, 2.4ml, and 3.0ml) was transferred into a series of 10 ml volumetric flask and volume was adjusted up to mark with Methanol. A zero order spectrum measured the absorbance at 360 nm against a reagent blank solution (Methanol).

#### PRECISION

##### Intraday precision: Procedure

- The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three Standard solutions

containing concentrations 1,3,5µg/ml for LAMO and 6,18,30 µg/ml for CLO and three replicate (n=3)each on same day.

- For zero order spectra was measured at 307 nm for LAMO and 360 nm for CLO.

- The % RSD value of the results corresponding to the absorbance was expressed for intra-day precision.

## II. Interday Precision: Procedure

- The precision of the developed method was assessed by analyzing samples of the same batch in nine determinations with three Standard solutions containing concentrations 1,3,5 µg/ml for LAMO and 6,18,30µg/ml for CLO and three replicate (n=3)each on different day.

- For zero order spectra was measured at 307 nm for LAMO and 360 nm for CLO.

- The % RSD value of the results corresponding to the absorbance was expressed for inter-day precision.

## ACCURACY

- It was determined by calculating the recovery of LAMO and CLO by standard addition method.

- Accuracy was done by adding both API standard solution and test solution. Total concentration was as per table.1

Table 1: Solution for accuracy study

Concentration of Formulation (µg/ml)		Concentration of API in spiking solution (µg/ml)		Total concentration	
LAM	CLO	LAM	CLO	LAMO	CLO
2	12	1.8	10	3.8	22
2	12	2	12	4	24
2	12	2.2	14	4.2	26

## Procedure

Each solution was taken and diluted with methanol up to 10ml volumetric flask and scanned between 200nm to 400nm against methanol as a blank. The amount of LAMO and CLO was calculated at each level and % recoveries were computed.

## LIMIT OF DETECTION AND QUANTITATION RESULT AND DISCUSSION

- The Limit of detection and quantitation of the developed method was assessed by analyzing ten replicates of standard solutions containing concentrations 1 µg/ml for LAMO and 6 µg/ml of CLO.

- The LOD and LOQ were calculated as  $LOD = 3.3\sigma/S$ , and  $LOQ = 10\sigma/S$ , where  $\sigma$  is the standard deviation of the lowest standard concentration and  $S$  is the slope of the standard curve.% RSD was calculated.

## ROBUSTNESS AND RUGGEDNESS

- Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:

- Change in Stock Solution Preparation,

- Stock-1(10mg LAMO in 100ml methanol-100µg/ml and 10mg CLO in 100ml methanol –100 µg/ml)

- Stock-2(10mg LAMO in 50ml methanol-200µg/ml and 10mg CLO in 50ml methanol - 200 µg/ml)

- Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450),

- Three replicates were made for the concentration (1, 3, 5 µg/ml of LAMO and 6, 18, 30 µg/ml of CLO) with different stock solution preparation.

## ANALYSIS OF EDA AND ARG IN SYNTHETIC MIXTURE (ASSAY)

### Composition of synthetic mixture

The preparation of synthetic mixture was as per patent:

- Lamotrigine: 10mg

- Clozapine: 60mg

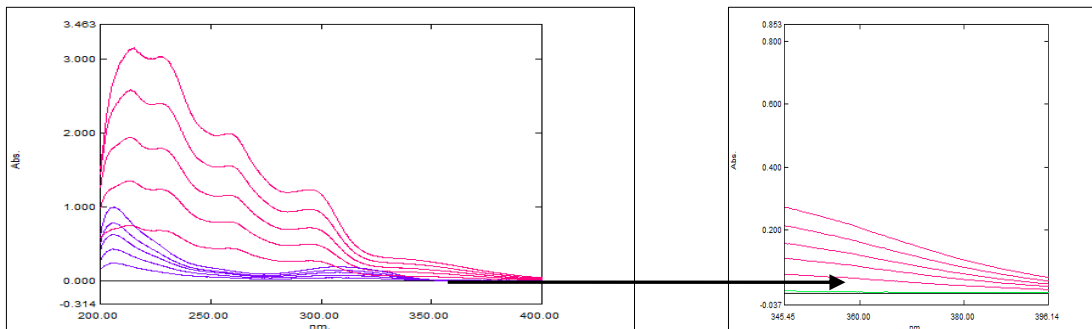
- Sodium Starch Glycolate: 10 mg

- Starch: 10 mg

- Magnesium stearate: 10 mg

All the excipients were mixed in 100ml volumetric flask then make up the volume with methanol and sonicated for 15 min. after sonicate make up the volume up to 100 ml with methanol. The solution was filtered through what man filter paper No. 42. Finally the solution had concentration 100µg/ml for LAMO and 600µg/ml for CLO.

**SELECTION OF WAVELENGTH AND METHOD DEVELOPMENT FOR DETERMINATION OF LAMOTRIGINE AND CLOZAPINE**



Overlain zero order spectra of Lamotrigine and Clozapine

- From spectra at 360 nm ( $\lambda_{max}$  of Clozapine) Lamotrigine has zero absorbance so Clozapine is directly estimate at 360 nm.
- At 307 nm ( $\lambda_{max}$  of Lamotrigine) both drugs have some absorbance so Lamotrigine is estimate at 307 nm using absorbance correction method.

( $r_2$ ) of 0.997 and 0.997 for LAMO and CLO, respectively. This method obeyed beer’s law in the concentration range 1-5  $\mu\text{g/ml}$  and 6-30 $\mu\text{g/ml}$  for LAMO and CLO, respectively.

- Correlation coefficient( $r_2$ ) form calibration curve of LAMO and CLO was found to be 0.998and 0.998, respectively.

**VALIDATION PARAMETERS**

**Linearity and Range**

- The Zero order spectra (fig.7) showed linear absorbance at 307 nm for LAMO (1-5  $\mu\text{g/ml}$ ) and 360 nm for CLO (6-30  $\mu\text{g/ml}$ ) with correlation coefficient

The regression line equation for LAMO and CLO areas following

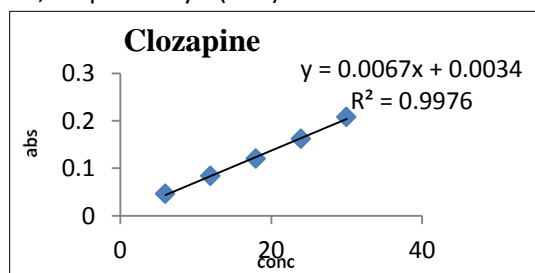
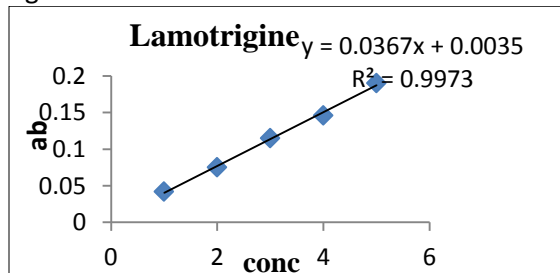
$Y=0.0367X+0.0035$  for LAMO----- (1)

$Y=0.0067+0.0034$  for CLO----- (2)

Table 2: Calibration data for LAMO and CLO at 307 nm and 360nm, respectively. \*(n=6)

Lamotrigine Conc.	Absorbance $\pm$ SD*	Clozapine Conc.	Absorbance $\pm$ SD*
1	0.042 $\pm$ 0.0010	6	0.046 $\pm$ 0.0011
2	0.075 $\pm$ 0.0012	12	0.084 $\pm$ 0.0009
3	0.115 $\pm$ 0.0012	18	0.120 $\pm$ 0.0015
4	0.146 $\pm$ 0.0012	24	0.162 $\pm$ 0.0006
5	0.190 $\pm$ 0.0009	30	0.208 $\pm$ 0.0006

Fig-4 Calibration data for LAMO and CLO at 307 nm and 360 nm, respectively. \*(n=6)



**MIXTURE LINEARITY [FOR ABSORPTION CORRECTION METHOD]**

Fig-5 Mixture Linearity in range of 1-5 µg/ml of LAMO and 6-30 µg/ml CLO (1:6 Ratios)

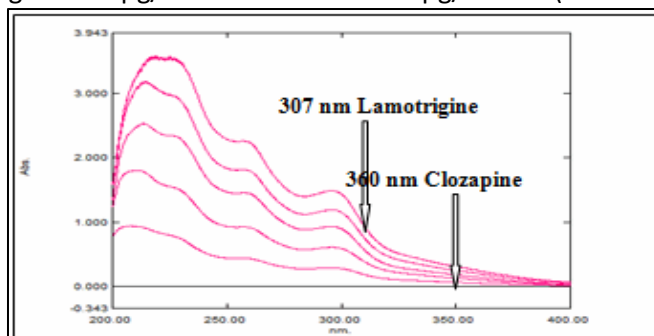
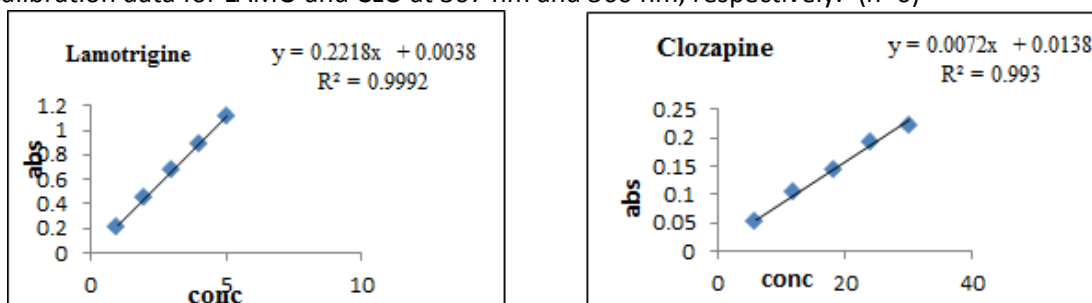


Table 3: Mixture Linearity in range of 1-5 µg/ml of LAMO and 6-30 µg/ml CLO (1:6 Ratios)

S.N	CONCENTRATION IN MIXTURE (LAMO:CLO) (1:6)µg/ml	ABSORBANCE (307 nm)	ABSORBANCE (360 nm)
1	1 : 6	0.214	0.052
2	2 : 12	0.456	0.104
3	3: 18	0.680	0.146
4	4 :24	0.890	0.194
5	5 : 30	1.106	0.224

Fig-4 Calibration data for LAMO and CLO at 307 nm and 360 nm, respectively. \*(n=6)



### Precision

**I. Intraday precision:** The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 1, 3, 5 µg/ml for LAMO and 6, 18, 30 µg/ml for CLO. Three replicate (n=3) each on same day for intraday

Table-4: Intraday precision data for estimation of LAMO and CLO \*(n=3)

Precision	Conc.	LAMO(307 nm)	Conc.	CLO(360 nm)
<b>INTRADAY (n=3)</b>				
Abs. ±% RSD	1µg/ml	0.215±0.57	6 µg/ml	0.052±0.90
	3 µg/ml	0.681±0.24	18 µg/ml	0.142±0.33
	5 µg/ml	1.104±0.19	30 µg/ml	0.227±0.71

**II. Interday Precision:** The precision of the developed method was assessed by analyzing combined standard solution containing three different concentrations 1, 3, 5 µg/ml for LAMO and 6, 18, 30 µg/ml for CLO. Three replicate (n=3) each on different day for interday precision.

Table-5: Interday precision data for estimation of LAMO and CLO \*(n=3)

Precision	Conc.	LAMO(307 nm)	Conc.	CLO(360 nm)
<b>INTERDAY (n=3)</b>				
Abs. $\pm$ % RSD	1 $\mu\text{g/ml}$	0.216 $\pm$ 0.75	6 $\mu\text{g/ml}$	0.051 $\pm$ 0.91
	3 $\mu\text{g/ml}$	0.682 $\pm$ 0.31	18 $\mu\text{g/ml}$	0.148 $\pm$ 0.84
	5 $\mu\text{g/ml}$	1.106 $\pm$ 0.15	30 $\mu\text{g/ml}$	0.228 $\pm$ 0.35

### ACCURACY

- Accuracy of the method was determined by recovery study from synthetic mixture at three levels (80%, 100%, and 120%) of standard addition.
- Percentage recovery for LAMO and CLO by this method was found in the range of 99.96 to 101.65% and 99.77-101.90%, respectively,
- The value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

Table 6: Recovery data of LAMO \* and CLO\* (n=3)

Initial conc. ( $\mu\text{g/ml}$ )		Level of recovery	Quantity of Std. Added ( $\mu\text{g/ml}$ )		Total Amount ( $\mu\text{g/ml}$ )		Result of recovery study			
LAM	CLO		LAM	CLO	LAM	CLO	Total Quantity Found* ( $\mu\text{g/ml}$ ) $\pm$ %RSD		% recovery $\pm$ %RSD	
LAM	CLO		LAM	CLO	LAM	CLO	LAM	CLO	LAM	CLO
2	12	0 %	-	-	2	12	1.98 $\pm$ 0.81	12.03 $\pm$ 0.38	99.03 $\pm$ 0.18	100.26 $\pm$ 0.30
2	12	80 %	1.8	10	3.8	22	3.7 $\pm$ 0.19	22.23 $\pm$ 0.09	99.03 $\pm$ 0.20	101.08 $\pm$ 0.38
2	12	100 %	2	12	4	24	4.00 $\pm$ 0.28	24.09 $\pm$ 0.14	100.24 $\pm$ 0.39	100.37 $\pm$ 0.19
2	12	120 %	2.2	14	4.2	26	4.21 $\pm$ 0.46	25.75 $\pm$ 0.19	100.23 $\pm$ 0.29	99.06 $\pm$ 0.55
Mean of 3 Determination									100.72%	100.37%

### LIMIT OF DETECTION AND QUANTITATION

Parameter	LAMO*	CLO*
LOD ( $\mu\text{g/ml}$ )	0.259	0.205
LOQ ( $\mu\text{g/ml}$ )	0.786	0.679

Table 7: LOD and LOQ data of LAMO and CLO\* (n=10)

### 5. ROBUSTNESS AND RUGGEDNESS

- Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:

- Change in Wavelength

LAMO at 306.80nm and 307.20 and CLO at 359.80nm and 360.20nm

- Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450),

- Three replicates were made for the concentration (1, 3, 5  $\mu\text{g/ml}$  of LAMO and 6, 18, 30  $\mu\text{g/ml}$  of CLO) with different stock solution preparation.

Table 8: Robustness and Ruggedness data of LAMO and CLO\* (n=3)

Conc. ( $\mu\text{g/ml}$ )	Lamotrigine (Mean* $\pm$ % RSD) (n=3)			
	Instrument		Wavelength	
	UV-2450	UV-1800	306.80	307.20
1	0.216 $\pm$ 0.75	0.217 $\pm$ 0.57	0.214 $\pm$ 0.38	0.216 $\pm$ 0.75
3	0.684 $\pm$ 0.38	0.685 $\pm$ 0.29	0.683 $\pm$ 0.23	0.686 $\pm$ 0.23
5	1.105 $\pm$ 0.14	1.106 $\pm$ 0.15	1.106 $\pm$ 0.11	1.105 $\pm$ 0.15
Clozapine (Mean* $\pm$ % RSD) (n=3)				
	UV-2450	UV-1800	359.80	360.20

6	0.053±0.88	0.055±0.85	0.052±0.89	0.054±0.86
18	0.146±0.55	0.147±0.55	0.147±0.55	0.148±0.31
30	0.222±0.56	0.225±0.72	0.224±0.36	0.226±0.71

#### APPLICATION OF THE PROPOSED METHOD FOR ANALYSIS OF EDA AND ARG IN SYNTHETIC MIXTURE (ASSAY)

- In that **synthetic mixture (100 mg)** the excipients like **Sodium Starch Glycolate, Starch, Magnesium Stearate** were taken as per the required weight. With the Lamotrigine and Clozapine with the ratio (1:6) dissolved in Methanol with small volume of Solvent, Sonicate for 15 min, then make up to 100ml with methanol and filter it.  
- Finally the solution had concentration 100µg/ml for LAMO and 600µg/ml for CLO.

DRUGS	RESULT OF SYNTHETIC MIXTURE ANALYSIS (n=3)		
	AMOUNT OF DRUG	%ASSAY± S.D.	%R.S.D.
LAMO	2 µg/ml	99.03 ±0.09	0.34
CLO	12 µg/ml	100.26±0.04	

Table-9: %Assay of synthetic mixture analysis (n=3)

#### SUMMARY OF VALIDATION PARAMETRS

Table 11: Summary of validation parameter

SR.NO	PARAMETER	LAMOTRIGINE	CLOZAPINE
1	Wave length Max.	307 nm	360 nm
2	Linearity (µg/ml) (n=6)	1-5 µg/ml	6 -30 µg/ml
3	Regression equation	Y=0.0367x + 0.0035	Y= 0.0067x + 0.0034
4	Correlation coefficient (r <sup>2</sup> )	0.997	0.997
5	Accuracy(%Recovery) (n=3)	100.72 %	100.37 %
6	Precision		
	Intra-day (%RSD)(n=3)	0.19-0.57	0.33-0.90
	Inter-day (%RSD)(n=3)	0.15-0.62	0.35-0.91
7	LOD (µg/ml) (n=10)	0.259	0.205
8	LOQ (µg/ml) (n=10)	0.786	0.679
9	Robustness and Ruggedness (%RSD)	0.11-0.75	0.31-0.89
10	Assay	99.03 %	100.26 %

#### CONCLUSION

All parameters are validated as per ICH guidelines which use for found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. This method is completely use for future analysis of dosage form.

**ACKNOWLEDGEMENT:** We are sincerely thankful to Shree Dhanvantary Pharmacy College, Kim, Surat, for providing us Infrastructure facilities and moral support to carry out this research work. We are also thankful to SDPARC for giving us their special time and guidance for this research work. We also thank our colleagues for their helping hand.

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