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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND INDAPAMIDE IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract

The Present manuscript describes two simple, accurate, precise, economical, reproducible and specific spectrophotometric methods have been developed for simultaneous determination of Olmesartan Medoxomil (OLM) and Indapamide (IND) in its combined tablet dosage form by using methanol as a solvent. The first method is Q absorption method which involves λ_{max} 241 nm (λmax of IND) and isoabsorptive point at 285.24 nm. The second method simultaneous method in which absorption at 286.78 nm (λ_{Max} of IND) was used for quantification of OLM and 256.40 nm (λ_{Max} of OLM) was used for quantification of IND. Two methods follow Beer's linearity in the range of 5-55 µg/ml and 1-25 µg/ml for OLM and IND both. The mean % recoveries were found to be in the range of 99.06 – 101.11 % and 98 – 100.77 % for OLM and IND respectively for Q- absorption method and in the range of 99.72 – 102.88% and 98 – 101.58% OLM and IND respectively for simultaneous method. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of OLM and IND in their combined Tablet dosage form. The results of analysis have been validated statistically and also by recovery studies. Thus the present study gives an excellent method for the determination of all the two drugs in combined dosage formulation without their prior separation.

INTRODUCTION

Olmesartan medoxomil is a prodrug, which, after ingestion, liberates the only active metabolite, Olmesartan. It is a competitive and selective AII type 1 receptor antagonist that is used alone or with other Antihypertensive agents to treat hypertension**,** the hydrolysis of olmesartan medoxomil occurs readily by the action of esterases, which are present abundantly in the gastrointestinal tract, liver and plasma. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle
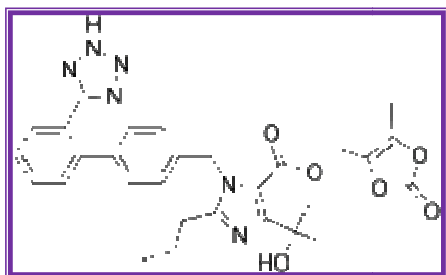
Figure 1 Chemical structure of Olmesartan Medoxomil

Indapamide (INDA), chemically, 3-(Aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H indol-1-yl)benzamid, is a diuretic which is used as an antihypertensive agent(fig.-2). Indapamide is official in B.P. and U.S.P. Indapamide, in vitro, directly inhibits pressure stimuli probably through a reduction of calcium flux in vascular smooth muscle, whilst diuretics are inactive. Indapamide does have mild diuretic activity at therapeutic doses, has both diuretic and vasodilator properties. A low urinary excretion and specific accumulation into arterial smooth muscle of this lipophilic molecule may provide a rationale for this dual activity.
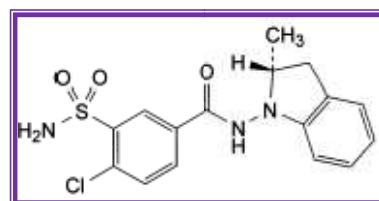
Figure 2 Chemical structure of Indapamide

The review of literature revealed that various analytical methods involving spectrophotometry6-11, HPLC12-19, HPTLC20 have been reported for OLM in single form and in combination with other drugs. Several analytical methods have been reported for IND in single form and in

combination with other drugs including spectrophotometry21-25, HPLC26-32, LC-MS33 and HPTLC 34.

To the best of our knowledge, there is no published spectrophotometric method for this combination. So, the present paper describes a simple, accurate and precise method for simultaneous estimation of OLM and IND in combined tablet dosage form by two UV Spectrophotometric methods (Q-absorption method, Simultaneous estimation method). The developed methods were validated in accordance with ICH Guidelines36 and successfully employed for the assay of OLM and IND in their combined dosage form.

MATERIALS & METHODS

REAGENTS AND CHEMICALS

Analytically pure OLM and IND were kindly provided by Zydus Cadila Healthcare Ltd, Ahmedabad, Gujarat, India and Ami Life science, Baroda, Gujarat, India respectively as gratis samples. Analytical grade methanol was purchased from Astron Chemical limited, Ahmedabad, India. Tablet of OLM and IND in combined dosage form, OLMY-D 20, was procured from Zydus cadila healthcare Ltd. Purchase from local market.

INSTRUMENTS

Two spectrophotometers were used for study, A Shimadzu UV/Vis 1800 double beam spectrophotometer with a wavelength accuracy (±0.3 nm), 1 cm matched quartz cells and UV probe 2.32 software was used for all the spectral measurements and Shimadzu UV/Vis 1601 double beam spectrophotometer with a wavelength accuracy (±0.3 nm) and 1 cm matched quartz cells was used for reproducibility study. Calibrated analytical balance Shimadzu Instrument Pvt. Ltd was used for weighing purpose.

Q- ABSORPTION METHOD

It uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that OLM and IND show an isoabsorptive point at 285.24 nm. The second wavelength used is 241 nm, which is the λ-max of IND.

Six working standard solutions having concentration 5,15, 25,35,45,55 μg/ml for OLM and 5,10,15,20,25 μg/ml for IND were prepared in methanol and the absorbances at 285.24 nm (isoabsorptive point) and 241

nm (λ-max of IND) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

CA = [(QM − QI) / (QA -QI)] × A1/aX1........... **(1)**

CI = (A1/aX1) − CA .... **(2)**

Where, A1 and A2 are absorbances of mixture at 285.24 nm and 241 nm;

aX1 and aY1 are absorptivities of OLM and IND at 285.24 nm;

aX2 and aY2 are absorptivities of OLM and IND respectively at 241 nm;

QM = A2 / A1,

QA = aX2 / aX1 and

QI = aY2 / aY1.

Preparation of Standard Stock Solutions

Accurately weighed 100 mg of OLM and IND standard were transferred to a separate 100 ml volumetric flask and dissolved in 50 ml methanol. The flasks were shaken and volume was made up to the mark with methanol to give solutions containing 1000 µg/ml OLM and 1000 µg/ml IND. From this solution 10 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100µg/ml of OLM and 100µg/ml IND.

SIMULTANEOUS EQUESTION METHOD

For the simultaneous equation method wavelengths selected were λmax of both the drugs, at the λmax of the IND,OLM shows the considerable absorbance and at the λmax of OLM, IND shows considerable absorbance. The study of spectra also reveals that OLM and IND have λmax at nm 256.40 and at 286.78 nm respectively. Both the drugs were found to have considerable absorbance at λmax of each other. The wavelengths selected for analysis were 256.40 nm and 286.78 nm respectively for OLM and IND. A series of standard solutions ranging from 5-55 µg/mL for OLM and from 1-25 µg/mL for IND both were prepared and the absorbance of solutions was recorded at selected wavelengths. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range

under study. The concentration of two drugs in mixture was calculated by using following equations:

$A_2 a_{y1} – A_1 a_{y2}$ $a_{x2} a_{y1} – a_{x1} a_{y2}$ …. **(3)**

$A_1 a_{x2} – A_2 a_{x1}$ $a_{x2} a_{y1} – a_{x1} a_{y2}$ …. **(4)**

Where A1 and A2 are the absorbances of mixture at 256.40nm and 286.78 nm and ax1, ay1, ax2 and ay2 were absorptivity of OLM and IND at 256.40 nm and 286.78 nm respectively.

VALIDATION OF THE METHOD:

These methods were validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ), in accordance with ICH guideline.

LINEARITY

Linearity was taken for Olmesartan Medoxomil and Indapamide in the concentration range of 5-55µg/ml and 1-25µg/ml respectively. The calibration curve was obtained by plotting absorbance →concentrations.

PRECISION

For Intraday precision, it was carried out by preparing 3 replicates of Olmesartan Medoxomil and Indapamide 25,35,45µg/ml and 10, 15, 10 µg/ml concentrations respectively concentrations, within the linearity range and measuring the absorbance of each solution. % RSD (% relative standard deviation) was calculated. For Interday precision, 3 different concentration solutions within the linearity range were measured for 3 different days. % RSD (% relative standard deviation) was calculated.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y- intercept) and S, is the mean of the slope of calibration plot.

ACCURACY

To study accuracy of the method, recovery studies were carried out by addition of standard drug in a tablet sample at 0%, 80%, 100% and 120%. The percentage of recovery was calculated for both methods.

ASSAY

It was tested by analysis of commercially available marketed formulation.

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 20 mg of Olmesartan medoxomil was transferred to 50 ml volumetric flask containing 40 ml of methanol, gentle shaking was carried out for 5 min and ultra sonicated for 5 min. The volume was made up to the mark with methanol.

The tablet sample solution was filtered through Whatman filter paper no. 41. 5 ml of filtrate was further diluted to 25 ml of methanol to get 100 µg/ml concentrations.
 From the 100 µg/ml of sample stock solution take 2 ml of solution and diluted up to the mark in 10 ml volumetric flask. So the final solution was made which contains 20 µg/ml and 0.15 ml of solution and diluted up to the mark in 10 ml volumetric flask. So the final solution was made which contains 1.5 µg/ml of Indapamide absorbances were measured at 241 nm and 285.24 nm and 256.40 and 286.78nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2 (Q-absorption analysis) and equation 3 and 4.(simultaneous method).

RESULTS AND DISCUSSION

Method I: Simultaneous Equation Method

UV-spectrophotometric method using simultaneous equation was developed. OLM showed absorbance maxima at 256.40 nm and IND at 286.78 nm. Linearity was observed in the concentration rage of 5 - 55µg/ml for IND and 1-25 µg/ml respectively. Correlation coefficient was found to be 0.999 and 0.999 at 256.40nm and 286.780nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for OLM and IND was found to be99 .00 and 101.33 respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis.

Method II: Absorbance Ratio Method

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 285.24 nm (isoabsorptive point) and 241.00 nm (λ max of indapamide) Linearity was observed in the concentration range of 5-55 µg/ml and 1-25 µg/ml correlation coefficient was found to be 0.999 and 0.999 respectively The proposed method was applied for pharmaceutical formulation; % label claim

for OLM and IND was found to be 100.2 and 99.33 respectively.

For parameters like linearity, precision, accuracy, LOD, LOQ. the data for which are presented in the Table 1, 2. Analytical recovery experiments were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives (Table1.5 and2.5). The validated method was successfully applied for the determination of in tablets mixture of OLM and IND the results are given in Table1.6 and2.6 indicate that the amount of drug in tablet samples met with requirements.

CONCLUSION

The proposed Q absorption method and simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of OLM and IND in combined tablet dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The method can be used for routine analysis of OLM and IND in combined dosage form.
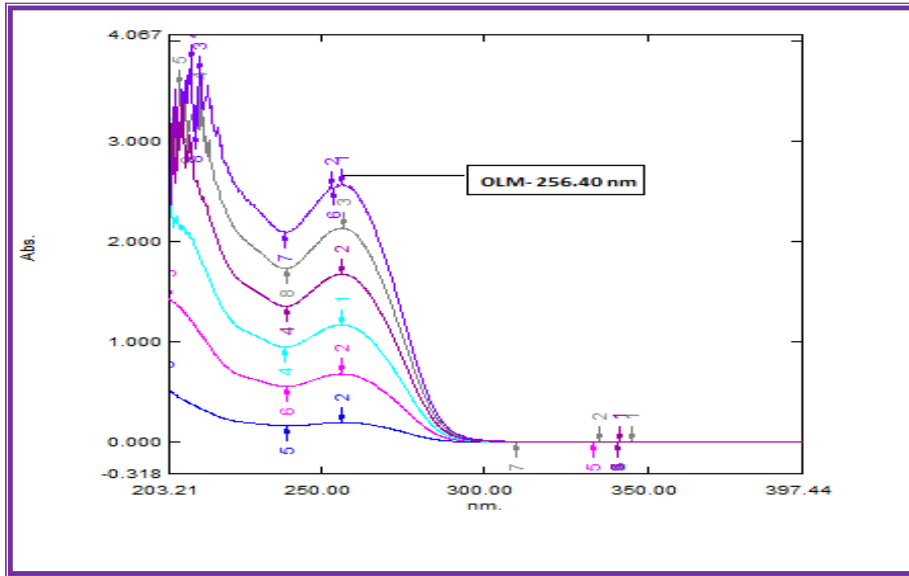
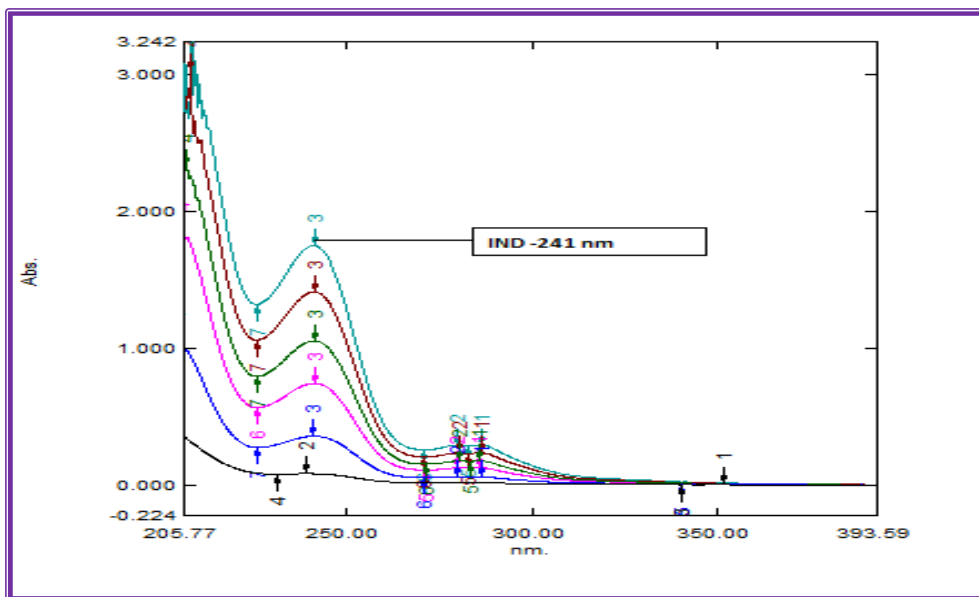**Figure3 Overlay spectra of OLM at 256.40 nm**

Figure 4 Overlay Spectra Of IND at 241 nm
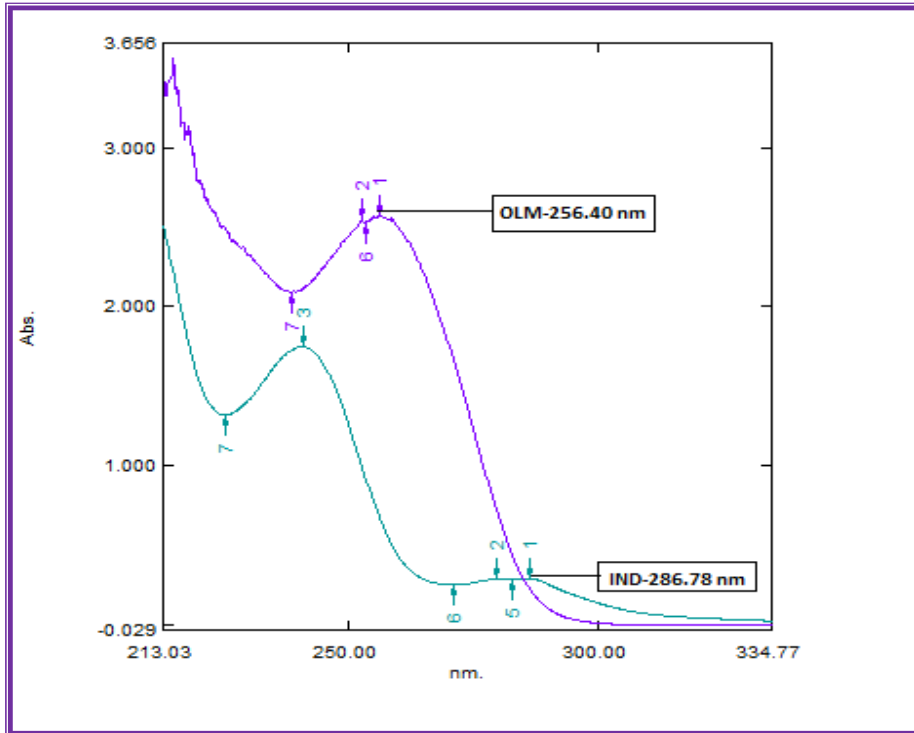
Figure 5 Overlay spectra of OLM (55 µg/ml) and IND (25 µg/ml) for simultaneous method
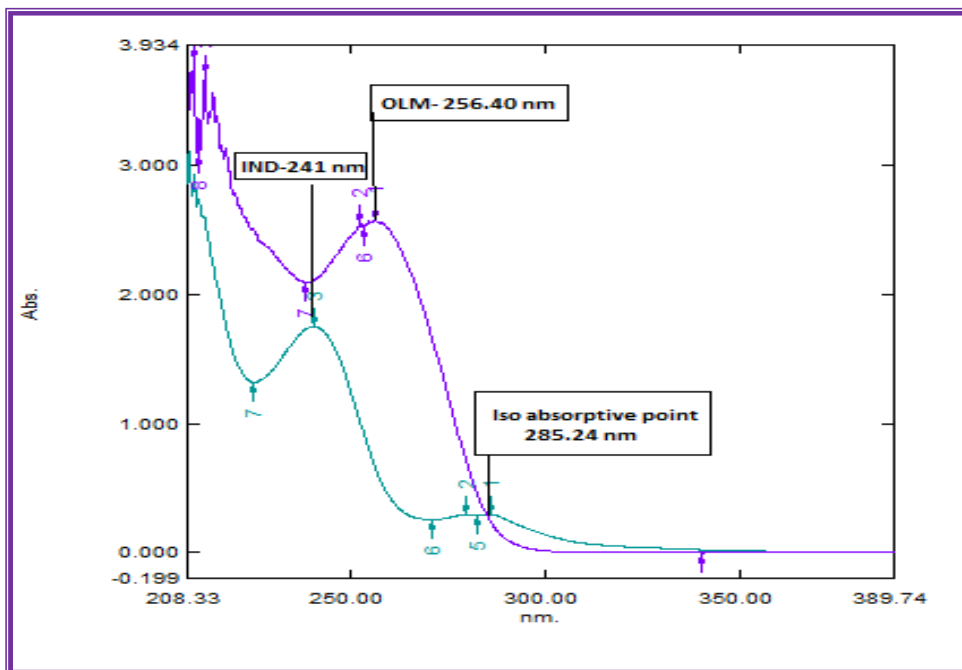
Figure 6 Overlay spectra of OLM (55 µg/ml) and IND (25 µg/ml) for Absorbance Ratio Method

Table 1.1

Summary of Validation Parameters of Q- Absorption Method

Parameters	OLM		IND	
	241nm	**285.24nm**	**241nm**	**285.24nm**
Recovery %	99.03-100.43	100.90-101.1	100.38-100.7	98-98.98
Precision				
Intra-day (n=3)	0.956-1.744	0.126-0.234	0.712-1.412	0.122-0.233
Inter-day (n=3)	0.956-1.745	0.126-0.234	0.743-1.412	0.121-0.233
LOD (µg/ml)	0.34	1.65	0.56	0.66
LOQ (µg/ml)	1.036	5	1.72	2
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 1.2

Statistical Data OLM and IND by Q- Absorption Method

Parameters	OLM		IND	
	241	**285.24**	**241**	**285.24**
Analytical Wavelength	241	285.24	241	285.24
Range	5-55(µg/ml)	5-55(µg/ml)	(1-25(µg/ml)	(1-25) µg/ml)
Slope	0.038	0.005	0.069	0.0115
Intercept	-0.004	-0.004	0.020	0.0039
Regression Coefficient (r2)	0.999	0.999	0.999	0.999

Table 1.3

Precision Data for OLM Absorbance at 241nm & 285.24 nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)		%R.S.D		Interday (Abs. ± S.D)		%R.S.D	
	241	**285.24**	**241**	**285.24**	**241**	**285.24**	**241**	**285.24**
25	0.956±0.001	0.126±0.001528	0.4026	1.21	0.957±0.001	0.127±0.001	0.1	0.78
35	1.364±0.00057	0.182±0.00057	0.28	0.313	1.363±0.0017	0.183±0.001	0.12	0.62
45	1.744±0.00057	0.234±0.001528	0.14	0.65	1.743±0.002	0.234±0.001	0.114	0.42

Table 1.4

Precision data for IND absorbance difference at 241 nm & 285.24 nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)		%R.S.D		Interday (Abs. ± S.D)		%R.S.D	
	241	**285.24**	**241**	**285.24**	**241**	**285.24**	**241**	**285.24**
10	0.743±0.0015	0.122±0.0015	0.4026	1.25	0.743±0.00152	0.121±0.00152	0.2	1.2
15	1.053±0.001	0.1746±0.0015	0.28	0.878	1.052±0.0020	0.1736±0.00152	0.190	0.88
20	1.412±0.001	0.233±0.0005	0.14	0.244	1.408±0.007	0.231±0.001	0.497	0.43

Table 1.5

Accuracy Data for OLM and IND by Q- Absorption Method

% Level	Amount of Drug taken		Amount of Drug Added		Amount Recovered		% Recovery	
	0LM	**IND**	**OLM(µg/ml)**	**IND(µg/ml)**	**OLM(µg/ml**	**IND(µg/ml)**	**% OLM**	**% IND**
80	20	1.5	16	1.2	36.4	2.66	101.11%	98.76%
100	20	1.5	20	1.5	40.4	2.93	101%	98%
120	20	1.5	24	1.8	44.4	3.266	100.90%	98.98%

Table 1.6

Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Olmy-D 20	Olmesartan medoxomil	20	20.04	100.2
	Indapamide	1.5	1.49	99.33

Table 2.1
Summary of Validation Parameters OF Simultaneous Method

Parameters	OLM		IND	
	256.40nm	**286.78nm**	**256.40nm**	**286.78nm**
Recovery %	99.72-101.75%	102.77-102.88%	100.95-101.58%	98-98.98%
Precision				
Intra-day (n=3)	1.1663-2.133	0.091-0.169	0.292-0.544	0.1236-0.231
Inter-day (n=3)	1.1646-2.133	0.089-0.169	0.292-0.544	0.0.123-0.234
LOD (µg/ml)	0.37	3.3	0.35	0.924
LOQ (µg/ml)	1.145	10.18	1.083	2.8
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 2.2

Statistical Data OLM and IND By Simultaneous Method

Parameters	OLM		IND	
	256.40	**286.78**	**256.40**	**286.78**
Analytical Wavelength	256.40	286.78	256.40	286.78
Range	5-55(µg/ml)	5-55(µg/ml)	1-25(µg/ml)	1-25(µg/ml)
Slope	0.047	0.003	0.026	0.011
Intercept	-0.033	-0.006	0.012	0.002
Regression Coefficient (r2)	0.999	0.999	0.999	0.999

Table 2.3

Precision Data for OLM Absorbance at 256.40nm &286.78 nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)		%R.S.D		Interday (Abs. ± S.D)		%R.S.D	
	256.40	**286.78**	**256.40**	**286.78**	**256.40**	**286.78**	**256.40**	**286.78**
25	1.166±0.0015	0.09133±0.0005	0.6321	0.0057	1.1633±0.0015	0.0913±0.001	0.1313	1.6724
35	1.6733±0.0015	0.131±0.001	0.7633	0.0066	1.6736±0.0015	0.118±0.002	0.0912	1.6949
45	2.133±0.0015	0.169±0.001	0.5917	0.005	2.133±0.0017	0.167±0.001	0.0812	0.5988

Table 2.4

Precision Data for IND Absorbance at 256.40nm & 286.78nm

Conc. (µg/ml)	Intraday (Abs. ± S.D)		%R.S.D		Interday (Abs. ± S.D)		%R.S.D	
	256.40	**286.78**	**256.40**	**286.78**	**256.40**	**286.78**	**256.40**	**286.78**
10	0.292±0.001	0.123±0.00057	0.3424	0.4668	0.293±0.002	0.1246±0.001	0.6825	1.1225
15	0.407±0.001	0.171±0.0015	0.2832	0.4668	0.407±0.002	0.173±0.002	0.4914	1.1560
20	0.544±0.001	0.231±0.0015	0.1838	0.4667	0.542±0.001	0.233±0.002	0.1845	0.8921

Table 2.5

Accuracy Data for OLM and IND by Simultaneous Method

% Level	Amount of Drug taken		Amount of Drug Added		Amount Recovered		% Recovery	
	0LM	IND	OLM(μg/ml)	IND(μg/ml)	OLM(μg/ml	IND(μg/ml)	% OLM	% IND
80	20	1.5	16	1.2	37	2.66	102.77	98.76
100	20	1.5	20	1.5	41	2.93	102.77	98
120	20	1.5	24	1.8	45.25	3.26	102.88	98.98

Table 2.6

Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Olmy-D20	Olmesartan medoxomil	20	19.80	99
	Indapamide	1.5	1.52	101.33

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