CHAPTER- 5 PREPARATION AND EVALUATION OF SMEDDS FOR OLMESARTAN

5.1 ANALYTICAL METHODS DEVELOPMENT AND VALIDATION

A number of analytical methods based on ultra-violet spectroscopy, spectrofluorimetric and high performance liquid chromatography were developed and validated to estimate olmesartan for various *in vitro* and *in vivo* studies.

5.1.1 Spectroscopic Methods:

5.1.1.1 Simple UV spectroscopy

Instrument

Shimadzu UV – 1700 UV visible spectrophotometer with 10 mm quartz cells was used for spectral measurements.

Reagents

Methanol (analytical reagent grade) was used to prepare the primary stock solution and for subsequent dilutions for the estimation of olmesartan.

1. Experimental

Preparation of Primary stock solution

Olmesartan was weighed (approx. 10 mg) and transferred to 100 mL volumetric flask. About 70 mL of the methanol was added to volumetric flask. The solution was vortex mixed for 2 min to allow the dissolution of suspended drug molecules at ambient temperature. The final dilution was made to 100 mL (i.e. 100 µg/mL) using methanol. The primary stock solution was stored at room temperature.

Determination of UV Absorbance Maxima of Olmesartan:

Olmesartan standard solution (10 µg/mL) was scanned for determination of absorbance maxima (λ_{max}) on a spectrophotometer. The scanning was carried out in the range of 200-400 nm.

Method Validation: (1)

1. Linearity:

The linearity of the assay was determined by plotting the calibration curve.

Preparation of Test solution

Secondary stock solution (100 µg/mL) was prepared by diluting 10 ml of primary stock solution (100 µg/mL) to 100 mL with methanol. Aliquots of the secondary stock

solutions of olmesartan ranging from 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 mL were transferred into 50mL volumetric flask and volume were made up to mark using methanol to obtain final concentrations of 6, 8, 10, 12, 14, 16 and 18 µg/mL. Six different sets of primary stock solutions were prepared and final dilutions were made using methanol. The absorbance of samples was measured on three consecutive days. Methanol was used as a blank. Calibration curves were obtained by plotting mean absorbance vs. concentration. Linear least-square regression analyses of the calibration graphs were performed and the values are noted in Table 5.1.1. Calibration curve was obtained by plotting mean absorbance vs. concentration which is shown in Figure 5.1.1.

2. Accuracy and precision

2.1. Accuracy

2.1.1. Intra-day Accuracy of the Assay:

Primary stock solutions were appropriately diluted using methanol to obtain final concentrations of 6 (LQC), 12 (MQC) and 18 µg/mL (HQC). Six different sets of primary stock solutions were prepared and diluted in the similar manner. The absorbance of samples was measured at λ_{max} 256 nm three times on the same day. The solutions were prepared freshly on each time. Methanol was used as a blank. The amount of drug found was calculated as percent of true value of drug analyzed and the results are recorded in Table 5.1.3.

2.1.2. Inter-day Accuracy of the Assay:

Primary stock solutions were appropriately diluted using methanol to obtain final concentrations of 6 µg/mL (LQC), 12 µg/mL (MQC) and 18 µg/mL (HQC). Six different sets of primary stock solutions were prepared and diluted in the similar manner. The absorbance of samples was measured at λ_{max} 256 nm on three consecutive days. The solutions were prepared freshly on each day. Methanol was used as a blank. The amount of drug found was calculated as percent of true value of drug analyzed and the results are recorded in Table 5.1.4.

2.2. Precision:

The intra- and inter day precisions were calculated by replicate analysis of the

solutions of known concentrations of olmesartan at three quality control concentration (LQC, MQC, and HQC) levels. The observed concentrations of the drug were calculated (from absorbance) using the equation of standard calibration curve. The variations between the observed concentrations were determined by calculating the percent Relative Standard Deviation (% RSD).

2.2.1. Intra-day Precision of the Assay:

Aliquots from primary stock solutions were appropriately diluted using methanol to obtain final concentrations of 6 µg/mL (LQC), 12 µg/mL (MQC) and 18 µg/mL (HQC). Six different sets of primary stock solutions were prepared and diluted in the similar manner. The absorbance of samples was measured at λ_{max} 256 nm three times on the same day. The solutions were prepared freshly on each time. Methanol was used as a blank. The precision was calculated as % relative standard deviation and the results are recorded in Table 5.1.3.

2.2.2. Inter-day Precision of the Assay:

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3. Robustness and Ruggedness:

Robustness and ruggedness of the method was evaluated by changing solvents, analyzing samples using different spectrophotometer and different analyst. To check this parameter solution having intermediate concentrations (MQC) of 12 µg/mL was prepared and absorbance was measured at 256 nm. The concentration was calculated from the linearity curve (mean of n=6) against observed absorbance of the sample.

4. Stability:

The stability of WRS samples were checked at room temperature for 24 hrs. The absorbance was measured.

5. Limit of Detection and Limit of Quantification:

Six random readings (absorbance) for analytical blank signal after "Auto Zero" were as follows 0.001, 0.002, 0.001, 0.003, 0.001 and 0.002.

LOD and LOQ were determined using the following equation.

$$LoD(or)LoQ = \frac{kS_B}{S} \ (Equation\ 5.4)$$

Where k = *a constant* (3 *for LoD and* 10 *for LoQ*),

S_B = *the standard deviation of the analytical blank signal,*

S = *the slope of the concentration/response graph,*

The results are listed in table 5.1.5.

2. Result and Discussion

Absorbance maxima of Olmesartan

The maximum absorbance (λ_{max}) of Olmesartan was found at 256 nm.

Calibration Curve of Olmesartan:

Calibration curve of olmesartan was found as linear with slope, intercept and correlation coefficient of 0.046, 0.018 and 0.9999.

Method Validation:

1. Linearity

Sr. No.	Concentration (µg/mL)	Absorbance ± SD (n=6)
1	6	0.257 ± 0.06
2	8	0.349 ± 0.02
3	10	0.439 ± 0.05
4	12	0.535 ± 0.04
5	14	0.632 ± 0.03
6	16	0.721± 0.04
7	18	0.804± 0.05

Table 5.1.1 Absorbance of Olmesartan at different concentrations

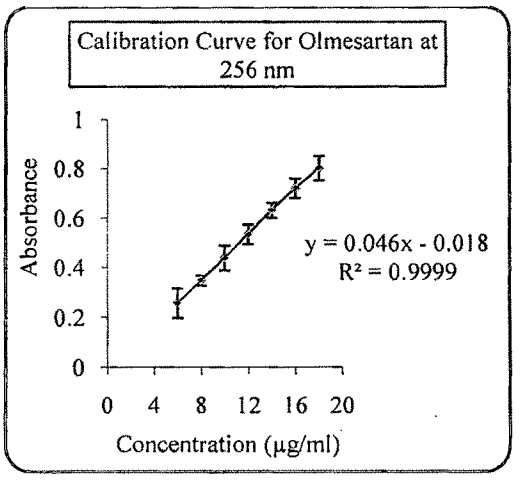


Figure 5.1.1 Calibration curve for Olmesartan

The calibration curve was found to be linear in the concentration range form 6 µg/mL to 18 µg/mL. The values of absorbance obtained at each concentration are listed in Table 5.1.1 and calibration graph is shown in Fig 5.1.1.

Day	Number of Runs (n)	Slope	Intercept	Linear Least Square Regression (r^2)
1	6	0.046	0.018	0.9999
2	6	0.045	0.017	0.9999
3	6	0.047	0.018	0.9998
Mean	**6**	**0.046**	**0.018**	**0.9999**

Table 5.1.2 Calibration curves of Olmesartan in Methanol at 256 nm on different days

2. Accuracy and Precision

Intra day and inter day accuracy and precision are resulted in Table no 5.1.3 and 5.1.4 at three concentration levels.

Parameters	Olmesartan Concentration		
	Low QC, 6 µg/mL	Medium QC, 12 µg/mL	High QC, 18 µg/mL
Mean	6.07	12.08	18.02
SEM	0.02	0.06	0.06
Precision as % RSD	0.59	0.88	0.59
Accuracy (%)	101.17	100.64	100.13

Table 5.1.3 Intra day Accuracy and Precision for Olmesartan determination

It is observed that % RSD is less than 2 for all three concentrations hence intra day precision in the range of 2 ± 0.5% and accuracy is in the range of 100 ± 2% which complies with the limit of ICH guideline. So it can be concluded that developed method is accurate and precise for entire calibration range.

Parameters	Olmesartan Concentration		
	Low QC, 6 µg/mL	Medium QC, 12 µg/mL	High QC, 18 µg/mL
Mean	5.97	12.03	18.19
SEM	0.04	0.04	0.04
Precision as % RSD	1.24	0.55	0.39
Accuracy (%)	99.44	100.28	101.07

Table 5.1.4 Inter day Accuracy and Precision for Olmesartan determination

The similar results are observed in case of inter day accuracy and precision. Theses results indicate that method is accurate and precise to give the correct results.

3. Robustness and Ruggedness

On repeatability of method, it produces similar results irrespective of change in instrument and person performing the method which proves robustness and ruggedness of method.

4. Stability

The samples were found to be stable at room temperature for 24 hrs.

5. Limit of Detection and Limit of Quantification

Limit of detection and limit of quantification were determined based on blank reading of solvent. LOD and LOQ for olmesartan at 256 nm are 0.053 µg/mL and 0.177 µg/mL respectively.

Sr. No.	Absorbance of blank
1	0.001
2	0.002
3	0.001
4	0.003
5	0.001
6	0.002
Mean	0.0017
SD	0.0008
LOD (µg/mL)	0.053
LOQ (µg/mL)	0.177

Table 5.1.5 Determination of LOD and LOQ

3. Conclusion

It can be concluded from above discussion that the developed method is accurate, precise, repeatable, reproducible, linear, simple and inexpensive. This method can be used for estimation of olmesartan in bulk drug and marketed formulations. Some critical parameters for the method are enumerated in Table 5.1.6.

PARAMETERS	RESULTS
λ_{max}	256
Beer's range (µg/ml)	6 to 18
Regression Equation ($Y = mx + C$) Slope (m) Intercept(c)	 0.046 -0.018
Limit of Detection (µg/ml)	0.053
Limit of Quantification (µg/ml)	0.177
Coefficient of determination	0.9999
Precision Intra day Inter day	 < 1% < 2%
Accuracy Intra day Inter day	 100-102% 99-102%

Table 5.1.6 Parameters of method validation for UV spectroscopy

Application of UV method for *In vitro* study

1. Experimental

Linearity:

The linearity of the assay was determined by preparing calibration curve.

Preparation of Working Standard Solution

Primary and secondary stock solutions were prepared as mentioned above. Aliquots of the secondary stock solutions of olmesartan ranging from 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 22.5 mL were transferred into a 100mL volumetric flask and volume were

made up to mark with pH 6.8 buffer to obtain final concentrations of 7.5, 10, 12.5, 15, 17.5, 20 and 22.5 µg/mL. Six different sets of primary stock solutions were prepared and final dilutions were made using methanol. Buffer was used as a blank. Calibration curves were obtained by plotting mean absorbance vs. concentration. Linear least-square regression analyses of the calibration graphs were performed and the values are noted in Table 5.1.7. Calibration curve is obtained by plotting mean absorbance vs. concentration which is shown in Figure 5.1.2.

2. Results

Linearity was set in pH 6.8 buffer and it was found linear with slop of 0.036 and intercept of 0.061. Coefficient of regression was found to 0.999. The solutions were found liner for the concentration range of 7.5 µg/mL to 22.5 µg/mL. This calibration plot was used to determine concentration of sample of each time interval during in-vitro diffusion study.

Sr. No.	Concentration (µg/mL)	Absorbance ± SD (n=6)
1	7.5	0.257 ± 0.05
2	10	0.349 ± 0.04
3	12.5	0.439 ± 0.04
4	15	0.535 ± 0.02
5	17.5	0.632 ± 0.06
6	20	0.721± 0.06
7	22.5	0.804± 0.05

Table 5.1.7 Absorbance of Olmesartan at different concentrations

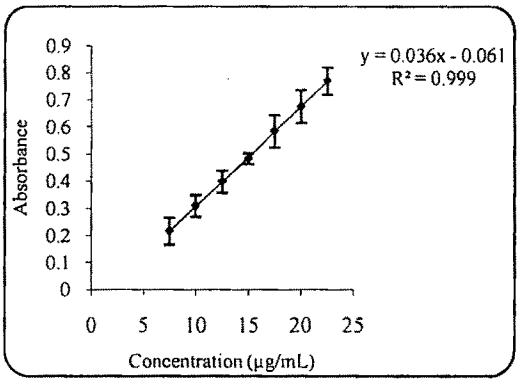


Figure 5.1.2 Calibration curve for Olmesartan in pH 6.8 buffer

5.1.1.2 Spectrofluorometric Method:

Instrument

A Shimadzu Spectrofluorophotometer (Model RF-540 with DR-3 data recorder), equipped with a 1cm fluorescence free quartz cell having four transparent side was used for all spectral and fluorescence measurements.

Reagents

Methanol (analytical reagent grade) was used to prepare all the solutions for the estimation of olmesartan.

1. Experimental

Method Development

Preparation of Primary stock solution

100 mg of olmesartan was weighed and transferred to 100 mL volumetric flask. About 70 mL of the methanol was added to volumetric flask. The solution was vortex mixed for 2 min to allow the dissolution of suspended drug at ambient temperature. The final dilution was made to 100 mL (i.e. 1 mg/mL) using methanol. The primary stock solution was stored at 2°C to 8°C till assayed.

Preparation of Secondary stock solution and Test solution

Secondary stock solution of concentration 10 µg/mL was prepared by diluting 1 ml of primary stock solution (1 mg/mL) to 100 mL with methanol. Aliquots of the secondary stock solutions of olmesartan ranging from 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25 and 2.5 were transferred into a 10mL volumetric flask using micropipette and volume were made up to 10 mL with methanol to obtain final concentrations of 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25 and 2.5 µg/mL respectively.

Determination of absorption and emission maxima of Olmesartan

Standard solution (1 µg/mL) of Olmesartan was scanned in the range of 200-350 nm for determination of excitation wavelength and same solution was scanned for determination of emission wavelength in the range of 310-450 nm. Prepared standard solutions were scanned in above range for their relative fluorescence intensity and calibration curve was prepared by plotting concentration against fluorescence intensity.

Preparation of Calibration Curve

Standard solutions prepared from secondary stock solution in the range of 0.75-2.25 µg/mL were used to prepare calibration curve and linearity was determined by using straight line equation.

Method Validation

Developed method was validated by using analytical parameters such as precision, linearity, limit of detection (LOD), limit of quantification (LOQ) as per ICH guideline and procedure was followed as mentioned in section 4.1.1.1.

2. Results and Discussion

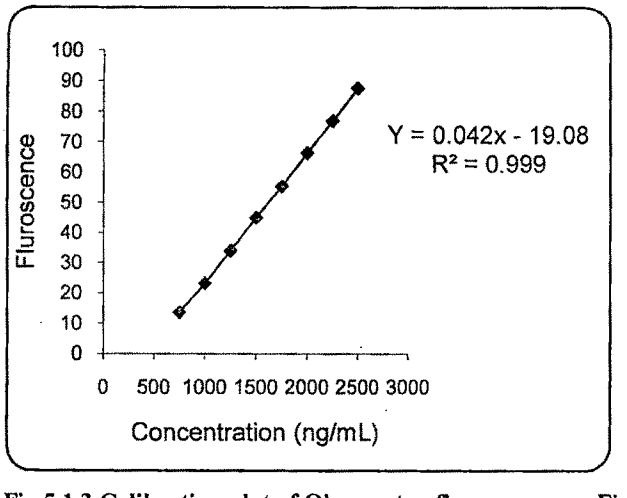
Method Development

Excitation and Emission Maxima

Excitation (λ_{ex}) and emission (λ_{em}) wavelength for standard olmesartan solutions were found to be 280 nm and 360 nm respectively. Further confirmation of these wavelength was done by analyzing series of standard solutions.

Calibration Curve for Olmesartan

The calibration curve plotted for fluroscence against concentration of standard solutions was found to be linear in the range of 7.5- 2.5 µg/mL. Linearity is shown in Fig. 5.1.3 and spectra for intensity are shown in Fig. 5.1.4.



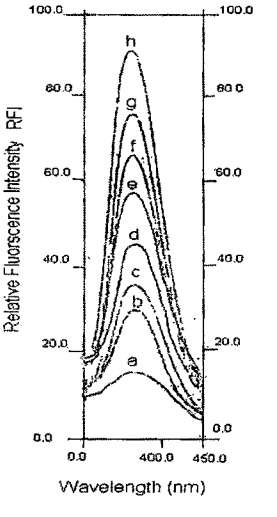


Fig 5.1.3 Calibration plot of Olmesartan fluorescence **Fig 5.1.4 Fluorescence spectra of Olmesartan**

Calibration curve was repeated six times and RSD of each concentration level was found to be less than 2%. Fig 5.1.3 shows linearity curve and Fig 5.1.4 shows typical spectra of fluorescence.

Method Validation

Different validation parameters were measured for developed fluorimetric method and results are summarized in Table 5.1.6. The excitation wavelength was set at 286 nm and emission peak was obtained at 360 nm. The method was found to be linear for the range of 0.75 µg/mL-2.5µg/mL. The coefficient of determination was found 0.999. Validation parameters are summarized in Table 5.1.8.

Parameters	Results
Excitation wavelength, λ_{ex} (nm)	286
Emission wavelength, λ_{em} (nm)	360
Linearity range (µg/mL)	0.75 – 2.5
Regression equation (Y*)	Y = 0.042X - 19.08
Slope (b)	0.042
Intercept (a)	19.08
Coefficient of determination (r^2)	0.9996
Correlation coefficient (r)	0.9998
Limit of detection, LOD (µg/mL)	0.38
Limit of quantification, LOQ (µg/mL)	0.67
Inter-day % RSD	<1.31%
Intra-day % RSD	<1.06%
% Assay	100.52%

Table 5.1.8 Validation Parameter of Spectrofluorometric method of Olmesartan

Standard, stock and working standard solutions of Olmesartan were found to be stable up to 6 hrs. The developed method was applied to marketed formulation and the assay was found 100.52% which conclude that method can be applicable to formulation also.

3. Conclusion

It can be concluded from above discussion that the developed method is accurate, precise, repeatable, reproducible, linear, simple and inexpensive.

5.1.2 Chromatographic Methods:

High Performance Liquid Chromatography (HPLC)

Instrument

Shimadzu HPLC consists of UV-VIS detector (SPD-20A Prominence UV-VIS detector), solvent delivery pump (LC-20AT Liquid Chromatography) and manual injector (Rhyod injector).

Reagents

Methanol, Acetonitrile and Distilled Water of HPLC grade were used to prepare primary stock solution and for all other subsequent dilutions.

1. Experimental

Method Development

Development of Chromatographic conditions

Since valsartan and olmesartan are structurally similar compounds, the method used for analyzing valsartan was adopted with slight modifications. Preparation of mobile phase and chromatographic conditions was set same as mentioned in section 4.1.3.1.

Optimization of Chromatographic conditions

The method was optimized using different composition of mobile phase and flow rate. Different peak parameters were observed like peak shape, tailing factor, theoretical plates which are summarized in Table 5.1.9.

Preparation of Primary Stock Solution

Exactly 100 mg of Olmesartan was weighed in to 100 ml volumetric flask. Approximately 70 ml of Methanol was added and vortex mixed to allow the dissolution of drug into methanol. The final volume was adjusted up to the mark with methanol to prepare the primary stock solution of concentration 1 mg/ml. This primary stock solution was used as a standard for further dilutions.

Preparation of Secondary Stock Solution

To cover entire linearity range two secondary stock solution were prepared from

primary stock solution of standard olmesartan drug. 5 mL of primary stock solution was transferred to 200 ml volumetric flask by using bulb pipette and diluted with methanol up to the mark to make a solution of 25 µg /mL and another one prepared by diluting 1 mL of primary stock solution to 10 mL with methanol to produce solution of 100 µg /mL. These solutions were used as secondary stock solutions for preparation of working standard solutions.

Determination of UV Detection Wavelength

Secondary stock solution was used to determine the absorbance maxima in UV detector. The detector was set at two different wavelength i.e. 215 nm followed by 250 nm and injections of secondary stock solution (25 µg/mL) were injected in HPLC pump for both the wavelength. The detector responses in terms of peak area at both wavelengths were compared. The wavelength at which maximum area was obtained was selected as detection wavelength.

Preparation of Working Reference Standards (WRS)

WRS solutions were prepared to plot a calibration graph. Different aliquots 1, 2, 4 and 20 mL of secondary stock solution having concentration of 25 µg/mL were pipette out by using pipettes and transferred to 100 mL volumetric flask to produce WRS of concentration 0.25, 0.5, 1 and 5 µg/mL. The solutions of 10, 20, 30, 40 and 50 µg/mL were prepared by diluting 1, 2, 3, 4 and 5 mL to 10 mL secondary solution having concentration of 100 µg/mL. The final volumes were made up to the mark with diluents (water: methanol in ratio of 50:50).

Method Validation (1)

Method validation was performed as per ICH guideline. Different parameters of method validation like linearity, accuracy (inter day and intra day), precision, % recovery, limit of detection (LOD) and limit of quantification (LOQ) were determined.

1. System suitability

To determine system suitability 5 injections of secondary solution having concentration of 50µg/mL was injected and different peak parameters were observed like retention time, tailing factor, theoretical plates and % relative standard deviation (% RSD) of area which are summarized in Table 5.1.10.

2. Linearity

WRS solutions of concentrations 0.250, 0.500, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0μg/mL were injected in HPLC and detector responses were measured at 215 nm in terms of peak area. Six different sets of primary WRS solutions were prepared and absorbances of samples were measured on three consecutive days. Calibration curves were obtained by plotting area vs. concentration. Linear least-square regression analyses of the calibration graphs were performed and the values are noted in Table 5.1.11.

3. Precision

3.1. Intra-day Precision

Three concentrations from range of WRS i.e. 0.25(LQC), 10.0(MQC) and 50.0 μg/mL (HQC) were selected for determination of accuracy. Six different sets of primary stock solutions were prepared and diluted in the similar manner. The response areas of samples were measured at 215 nm wavelength (λ_{max}) three times on the same day. The solutions were prepared freshly every time. The precision and accuracy were calculated and the results are recorded in Table 5.1.12.

3.2. Inter-day Precision

Primary stock solutions are appropriately diluted using methanol to obtain final concentrations of 0.25 (LQC), 10.0 (MQC) and 50.0 μg/mL (HQC). Six different sets of primary stock solutions were prepared and diluted in the similar manner. The absorbance of samples was measured at 215 nm wavelength (λ_{max}) on three consecutive days. The solutions were prepared freshly on each day. The precision and accuracy were calculated and the results are recorded in Table 5.1.13.

4. Accuracy

Accuracy was determined by spiking the preanalyzed sample solution with the aliquots of standard drug solution in the range of 80%, 100% and 120% of the claimed amount. Recovery solutions were prepared as method mentioned in method validation for valsartan. The area of all the solutions was noted at 215 nm wavelength. The % recovery was calculated on basis of drug found to drug added. The results are listed in Table 5.1.14.

5. Ruggedness

5.1. System to system variability, Column to Column Variability and Analyst to Analyst Variability:

The study was conducted for system to system, column to column and analyst to analyst variability study on two HPLC systems of different manufacturer by using different column and by different analyst. Standard drug solution having concentration of 10µg/mL was injected as per the test method and different system suitability parameters were measured which are listed in Table 5.1.15.

5.2. Stability

Standard of concentration 10µg/mL was prepared in duplicate as per the test method and keep them on bench top. Inject standard into the HPLC system following the conditions described in test method at 0, 1, 6, 8, 12 and 24 hrs. The % assay of standard solutions against a fresh standard was calculated each time. The peak response was measured and results are recorded in Table 5.1.16.

6. Limit of Detection and Limit of Quantification

Limit of Quantification (LOQ) is the lowest concentration of analyte in a sample that may be measured in a sample matrix such as impurities in bulk drug substances and degradation products in finished pharmaceuticals.

The solution was prepared with the approximate concentrations which gave signal to noise ratio about 3 based on 25% of Linearity solution injection. To prepare this, 5 mL of lowest concentration solution 0.25 µg/mL was taken and diluted to 20 mL with methanol which resulted in solution of 62.5 ng/mL concentration. This solution was injected into the HPLC system and response was observed. Limit of delectation was determined by identifying the concentration which gave a signal to noise ratio about 3. In similar manner, limit of quantification was determined by identifying the concentration which gave a signal to noise ratio about 10. Results of LOD and LOQ are given in Table 5.1.17

7. Robustness

The reliability of an analysis with respect to deliberate variations in method parameters measures robustness of method

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers);
- Temperature;
- Flow rate.

7.1 Effect of variation of pH in mobile phase:

The effect of pH variation was studied by preparing two mobile phases with ± 0.2 of the method pH. Then a system suitability solution (Standard preparation) as per the test method was injected into the HPLC system using both mobile phases. The system suitability values were determined by the test method for both the mobile phases.

7.2 Effect of variation in flow rate :

The system suitability solution of standard preparation as per the test method was injected into the HPLC system with ± 0.2 mL of method flow. The system suitability values were evaluated by the test method for both the flow rates.

2. Result and Discussion

Method Development

Optimization of Chromatographic conditions

When injection of standard solution of 50µL solution was injected and peak was observed at two different wavelengths, maximum area was obtained at 215 nm.

Sr. No.	*Mobile phase composition*	*Flow rate mL/min*	*Approximate Retention time (min)*	*Peak observation*
1	Water: ACN (50:50)	1.0	2	Sharp with early elution
2	Water: ACN (50:50)	0.5	4	Broad with tailing
3	Water: ACN (60:40)	1.0	16	late elution
4	Water: ACN (40:60)	1.0	5	Sharp but early elution
5	**Water: ACN (35:65)**	**0.7**	**12.2**	**Sharp and good**

Table 5.1.9 Effect of variation in mobile phase composition and flow rate

Hence 215 nm was selected for further optimization and validation. Different combinations of Mobile phase were used at flow rate 0.5 to 1.0 ml/min. The variation in mobile phase composition and flow rate showed significant changes in peak shape. As the aim was to achieve good peak shape with low tailing at optimum retention time (R_t). Hence finally mobile phase having composition of water: acetonitrile in ratio of 35:65 was found to produce good peak at 12 min (R_t) with low tailing. Therefore this composition and flow rate was finalized for further validation. The final chromatographic conditions are mentioned below:

Column	: Hypersil BDS, C_{18} (250 X 4.6mm), 5µ
Mobile phase	: Water: Acetonitrile (35:65) with 0.2% TEA at pH 3.0
Injection volume	: 20 µl
Flow rate	: 0.7 ml/min
Needle wash	: Double Distilled Water
Detector	: UV Detector-215 nm
Temperature	: Ambient

Method Validation

1. System Suitability

The values of system suitability parameter are mentioned in Table 5.1.10 given below.

Parameter (Mean ± SD)	Values
R_t	12.2 ± 0.03
Tailing factor	0.7 ± 0.05
Theoretical plates	12130 ± 2.8
% RSD of area	0.86

Table 5.1.10 System Suitability parameters for olmesartan

2. Linearity

Linearity was checked by using WRS solution from 0.250 to 50.0 µg/mL and mean area obtained for 3 injections of each concentration are presented in Table 5.1.11.

Concentration (µg/ml)	Mean Area
0.25	16.07
0.5	31.90
1	59.22
5	321.70
10	714.40
20	1392.63
30	2117.14
40	2837.21
50	3509.35

Table 5.1.11 Mean area of different concentrations Olmesartan

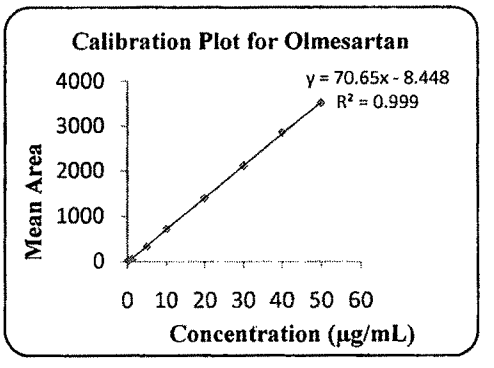


Fig 5.1.5 Linear Calibration Plot of HPLC method for Olmesartan

Developed HPLC method was found to be linear over the calibration range. The correlation of coefficient was 0.999, Y-intercept (constant of regression) was 8.448 and slope of line was 70.65.

3. Precision

Intra day and inter day precision were determined by preparing three different concentration (Low, Medium and High) on the same day (intra day) and on next three consecutive day (inter day). It is resulted in Table no 5.1.12 and 5.1.13 at three concentration levels.

Parameters	Olmesartan Concentration		
	Low QC, 0.25 µg/mL	Medium QC, 10 µg/mL	High QC, 50 µg/mL
Mean	0.249	10.02	50.17
SEM	0.008	0.06	0.31
Precision as % RSD	1.52	0.94	1.16

Table 5.1.12 Intra day Precision for Olmesartan determination

As per ICH guideline, precision can be recorded in terms of % relative standard deviation (%RSD) and it should be less than 2%.

Parameters	Olmesartan Concentration		
	Low QC, 0.25 µg/mL	Medium QC, 10 µg/mL	High QC, 50 µg/mL
Mean	0.251	9.98	50.52
SEM	0.005	0.08	0.14
Precision as % RSD	1.02	0.83	0.94

Table 5.1.13 Inter day Precision for Olmesartan determination

Table 5.1.12 and 5.1.13 shows that for both the cases, % RSD was found to be less than 2% and which complies with specified limit. Theses results indicate that method is precise and reproducible to give the correct results.

4. Accuracy/ % Recovery

The % recovery was calculated on the basis of amount of drug recovered. Accuracy was assessed using a minimum of 9 determinations over a minimum of 3 concentration levels.

Concentration of Olmesartan(µg/m			Amount found#(µg/ml)	% Recovery#
Initial	Added	Total		
60	20	80	79.58 ± 0.29	99.47
60	40	100	100.98 ± 0.57	100.98
60	60	120	121.02 ± 0.84	100.85

#Mean ± S.D. of three determinations

Table 5.1.14% Recovery for olmesartan

As shown in above Table 5.1.14, the concentration of marketed formulation was kept constant to 60µg/ml and to that standard drug solution was added to get total concentration of 80, 100 and 120 µg/ml. This resulted in accuracy of 99 to 101% which complies with ICH specification limit of 100 ± 3%.

5. Ruggedness

5.1. System to system variability, Column to Column Variability and Analyst to Analyst Variability:

The method should be considered rugged for system to system, column to column and

analyst to analyst variability, if the RSD of assay results is not more than 2.0%.

Ruggedness Parameters	Concentration (µg/mL)	% RSD
System to System	10	1.04
Column to column	10	0.54
Analyst to analyst	10	1.38

Table 5.1.15 Ruggedness parameters for olmesartan solution

5.2. Stability

Bench top Stability	Found Concentration (µg/mL)	% Assay
Initial	10.02	100.2
After 12 hrs	9.88	98.8
After 24 hrs	9.95	99.5

Table 5.1.16 Bench top stability of olmesartan standard solution

The stability of solution was determined on bench top. The results shown in Table 5.1.16 confirm that solution was stable even after 2 days. The solutions were found stable as concentration of test solution was constant for 24 hrs of preparation.

6. Limit of Detection and Limit of Quantification

	Concentration (µg/mL)	S/N ratio
LoD	0.0625	3.2
LoQ	0.25	15.8

Table 5.1.17 Limit of Detection and Limit of Quantification for olmesartan solution

When solution of concentration 0.0625 µg/mL was injected, the value of S/N ratio was 3.2. So it was detected as LOD. Similarly the solution having concentration 0.25 µg/mL considered LOQ as S/N ratio value was found to 15.8.

7. Robustness

7.1 Effect of variation of pH in mobile phase:

Effect of variation in pH on system suitability parameters are listed in Table 5.1.18. The method was found robust for pH variation.

7.2 Effect of variation in flow rate :

The method was found robust for variation in flow rate. Table 5.1.18 shows results for this test.

Parameter (Mean ± SD)	Variation in pH of mobile phase	Variation in Flow rate
R_f	12.4 ± 0.5	12.3 ± 0.2
Tailing factor	0.82 ± 0.05	1.24 ± 0.4
Theoretical plates	12340 ± 16.19	11894 ± 20.41
% RSD of area	0.75 ± 0.6	1.17. ± 0.8

Table 5.1.18 Robustness parameters

3. Conclusion

The developed method was validated for different parameters and it was found to be linear, accurate, precise, rouged and robust. All the parameters are summarized in Table 5.1.19 to give the overview of method.

Method validation parameters	Results
Linearity and Range	
• Linearity (µg/ml)	0.25 -50.00
• Slope	70.65
• Intercept	8.44
• Regression co-efficient (r)	0.9999
Precision (% RSD)	
• Intraday precision	0.9 – 1.6
• Interday precision	0.8 – 1.1
Accuracy	99-101 %
Limit of Detection (µg/ml)	0.0625
Limit of Quantification (µg/ml)	0.25

Table 5.1.19 Summary of Validation Parameter

Application of HPLC for Bioavailability Study

Developed HPLC method was applied for analysis of olmesartan in spiked plasma samples.

1. Experimental

Primary and secondary stock solution

To prepare primary stock solution, 100 mg of drug was transferred into 100 mL of volumetric flask. To that 70 mL of methanol was added, sonicated for about 5 min and diluted up to volume with methanol. 10 mL of above primary stock solution was transferred into 100 mL volumetric flask and diluted to mark with methanol. Secondary stock solution was stable for 48 hrs.

Calibration Standards Solution

Aliquots of secondary stock solution of 0.4, 0.6, 0.8, 1, 1.2 and 1.4 mL were transferred in 100 mL volumetric flask and diluted with diluents (water: methanol in ratio of 1:1) to prepare calibration standard solutions of 400, 600, 800, 1000, 1200 and 1400 ng/ml of olmesartan.

Plasma sample preparation

0.1 mL of calibration standard solutions of each concentration was added in 5 mL polypropylene centrifuge tube with flat caps separately containing 1.0 mL of drug-free human plasma. To each tube 0.1 mL of losartan potassium solution having concentration of 10 µg/ml was added as an internal standard and mixed for 3 minutes on vortex shaker to allow through mixing. Set of ten centrifuge tube was prepared containing calibration standard solution concentration ranging from 400 to 1400 ng/ml.

Sample extraction Procedure

Sample extraction was done by protein precipitation method. In each centrifuge tube 1 mL of acetone was added to precipitate plasma proteins. Centrifuge tubes were centrifuged at 10000 RPM for 10 min in high speed homogenizer (Sigma Hi Speed Homogenizer, Japan). Supernatant from each tube was separated using micro pipettes and 20 µL solutions were injected into HPLC system.

Chromatographic conditions

Chromatographic conditioned are same as mentioned in section 5.1.2. Additional guard column was attached prior to HPLC column to prevent blockage due to plasma components.

2. Results and Discussion

Concentration (ng/ml)	Area of Drug	Drug/IS
400	107.44	0.224
600	234.74	0.490
800	349.43	0.729
1000	470.25	0.981
1200	598.3	1.248
1400	725.4	1.513

Table 5.1.6 Mean Area for standard solutions of different concentrations of olmesartan

Calibration curve plotted for olmesartan was found to be linear within concentration range of 400 ng/mL to 1400 ng/mL. This linearity was used as tool to determine unknown concentration of valsartan in plasma matrix.

The regression of coefficient was found to be 0.9990 which indicates significant linearity of method. Linearity was obtained by plotting graph of ratio of drug area to area of internal standard.

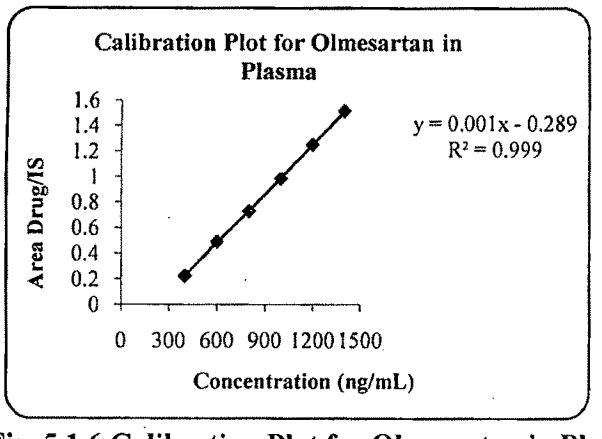


Fig. 5.1.6 Calibration Plot for Olmesartan in Plasma

The lowest concentrations range i.e. 400 ng/mL shows that method can be applied to determine the low concentration of olmesartan in plasma matrix.

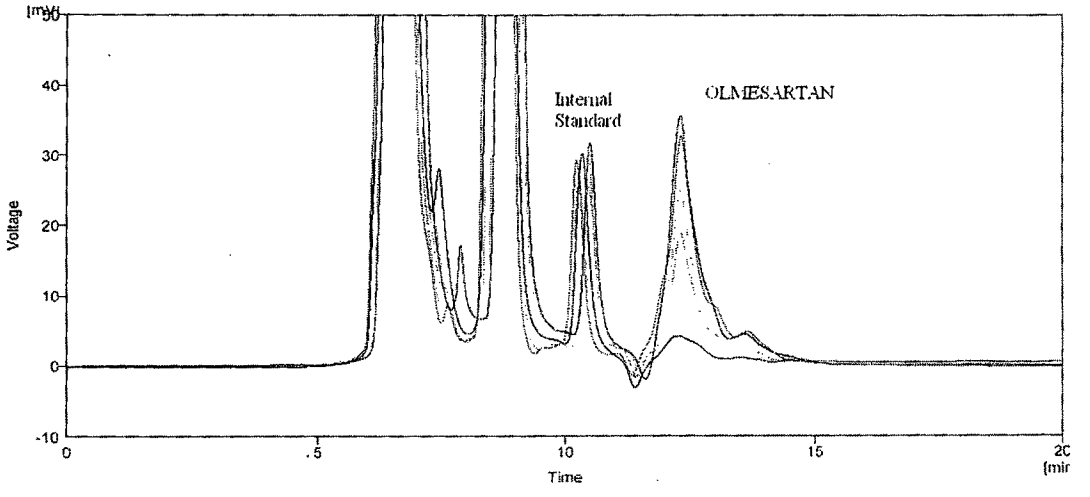


Fig 5.1.7: Chromatogram showing Linearity of Olmesartan in Plasma.

The Fig. 5.1.7 shows the overlay chromatogram of different concentration of valsartan recovered from plasma. The peak for olmesartan shows increase in area along with increase in concentration of drug. The peak is of internal standard shows consistency as its concentration was constant in all the samples. The results of linearity indicate that developed HPLC has application in plasma matrix.

5.2 PREPARATION OF SELF MICROEMULSIFYING DRUG DELIVERY SYSTEM (SMEDDS)

5.2.1 Experimental

5.2.1.1 Selection of Excipients

Solubility of olmesartan was checked in different oils like vegetable oils (e.g. Peanut oil, Cotton seed oil), medium Chain Triglycerides (e.g. Labrafil M 2125, Labrafac PG, Captex 200 P, Captex 355 NF). Other excipients like surfactants (e.g. Cremophore RH 40, Tween 80, Tween 20) and co- surfactants (e.g. Polyethyleneglycol 400, Transcutol P, Plurol olique) were added to facilitate the solubility enhancement of drugs. The procedure used was same as mentioned in section 4.2.1.1.

5.2.1.2 Pseudo-ternary phase diagram study

On the basis of the solubility studies of drug, Captex 200 P, Labrafac PG and Labrafil M 2125 were selected as oil phase. Tween 80, Cremophore EL and Cremophore RH 40 were used as surfactants and Transcutol P used as cosurfactants. Standard buffer solution (pH 6.8) (2) was used as an aqueous phase for the construction of phase diagrams. Oil, surfactants and cosurfactants were grouped in three different combinations for phase studies (Fig. 5.2.2). System 1 (OSMS 1) was prepared with Labrafil M 2125 as oil, Cremophore RH 40 as surfactant and Tanscutol P as cosurfactant. Another system 2 (OSMS 2) was prepared with Labrafac PG as oil, Cremophore EL as surfactant and Tanscutol P as cosurfactant. Captex 200 P, Cremophore EL and PEG 400 as an oil, surfactant and cosurfactant were used to form system 3 (OSMS 3). Surfactant and cosurfactant (S_{mix}) in each group were mixed in different weight ratios (1:1, 2:1, 3:1) to produce three formulation forms each system. These S_{mix} were chosen in increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams for formulation of microemulsion (Fig. 5.2.3). Phase diagrams were prepared as mentioned in section 4.2.1.2. Aqueous titration method was adopted for phase diagram study. Slow titration with aqueous phase was done to each weight ratio of oil and S_{mix} and visual observation was carried out for transparent and easily flowable o/w microemulsions. The physical state of the microemulsion was marked on a pseudo-ternary phase

diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant (S_{mix}) at fixed weight ratios. Fig 5.2.3 represents pseudo-ternary phase diagrams for olmesartan SMEDDS which show self microemulsion region in black color and self emulsion region in gray color.

5.2.1.3 Preparation of SMEDDS:

Based on the results of pseudo-ternary phase diagram, different SMEDDS were prepared in such a way that each system gave three formulations (Table 5.2.2, 5.2.3, 5.2.3) with varying ratios of oil, surfactant and cosurfactant. Formulation A, B and C were prepared from OSMS 1, formulations D, E and F were prepared using excipients of OSMS 2 and OSMS 3 was used to make formulations G, H and I. In all the formulations, the level of olmesartan was kept constant (i.e. 13 mg/0.2 mg of SMEDDS). Preparation procedure of SMEDDS was same as mentioned in section 4.2.1.3. Prepared SMEDDS were observed for the turbidity to transparency and transparency to turbidity upon dilution with pH 6.8 buffer. Prepared SMEDDS were optimized for clarity and in-vitro characterization. The excipient profiles of each SMEDDS for olmesartan are recorded in Table 5.2.2 (OSMS 1), Table 5.2.3 (OSMS 2) and Table 5.2.4 (OSMS 3).

5.2.2 Result and Discussion

5.2.2.1 Selection of Excipients

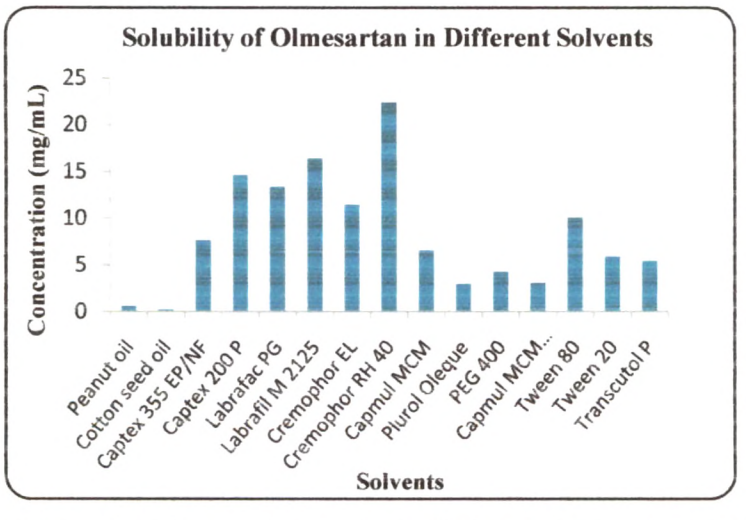
Solubility data of olmesartan in different oils, surfactant and cosurfactant is given in Table 5.2.1.

Since SMEDDS consists of oil, surfactant and co-surfactant as constructing component of the system, solubility of drug in oil, surfactant and cosurfactant is important tool for selection of excipients. To form a stable SMEDDS, drug should be completely in solubilized form. Olmesartan has good solubility in synthetic oils as compared to vegetable oils (Table 5.2.1). So, Captex 200 P, Labrafac PG and Labrafil M 2125 were selected as oil phase. Tween 80, Cremophore EL and Cremophore RH 40 which act as surfactant, also showed good solubility of Olmesartan. Hence they were selected as other components of SMEDDS. The third component of SMEDDS

may be cosurfactant which is not necessarily required but may help surfactant to stabilize the system.

Sr. No.	Name of oil/surfactant/cosurfactant	Solubility of Olmesartan (mg/mL) in oil /surfactant/cosurfactant
1	Peanut oil	0.59
2	Cotton seed oil	0.21
3	Captex 355 EP/NF	7.64
4	Captex 200 P	16.41 √ (O)
5	Labrafac PG	13.36 √ (O)
6	Labrafil M 2125	14.6 √ (O)
7	Cremophor EL	11.47 √ (S)
8	Cremophor RH 40	22.42 √ (S)
9	Capmul MCM	6.55
10	Plurol Olieque	2.93
11	PEG 400	4.28
12	Capmul MCM (C10)	3.5
13	Tween 80	10.06 √ (S)
14	Tween 20	5.88
15	Transcutol P	5.39 √ (Co S)

Table 5.2.1 Solubility of Olmesartan in different oils, surfactant and cosurfactant



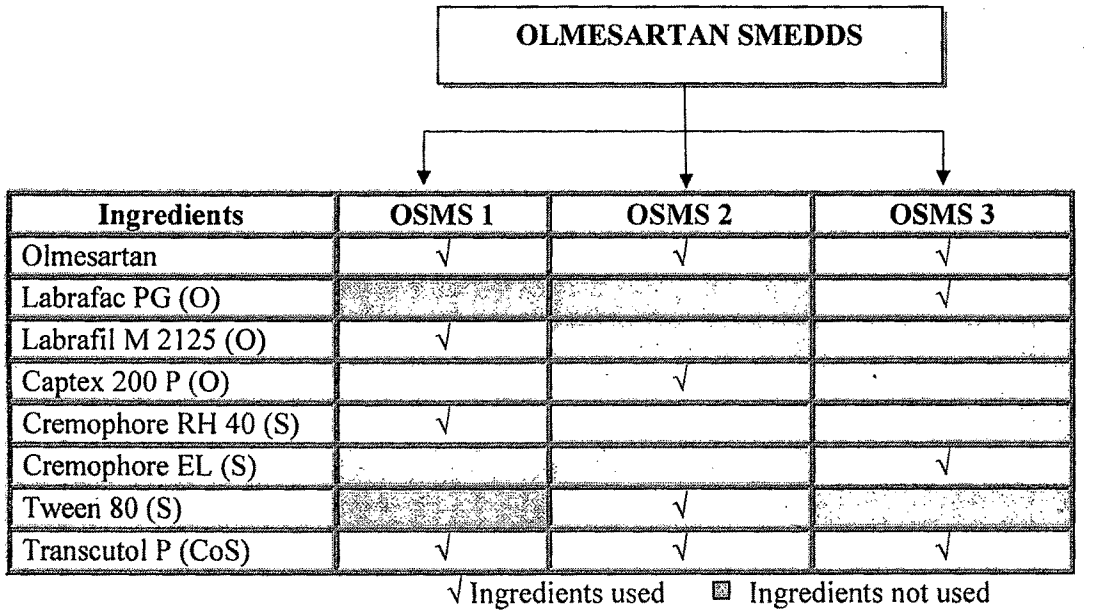
5.2.1 Graphical Presentation of Solubility of Olmesartan in different solvents

The main use of cosurfactant is as film expander. In our study, Transcutol P showed significantly higher solubility than PEG 400 for olmesartan and hence force used as

cosurfactant.

5.2.1.2 Pseudo-ternary phase diagram study

Olmesartan SMEDDS were prepared using three different system are summarized in Figure 5.2.2.

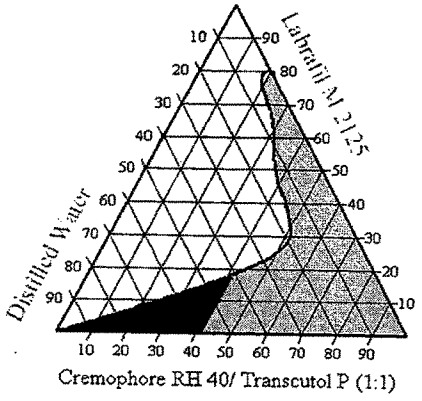


Ingredients	OSMS 1	OSMS 2	OSMS 3
Olmesartan	√	√	√
Labrafac PG (O)			√
Labrafil M 2125 (O)	√		
Captex 200 P (O)		√	
Cremophore RH 40 (S)	√		
Cremophore EL (S)			√
Tween 80 (S)		√	
Transcutol P (CoS)	√	√	√

√ Ingredients used ▨ Ingredients not used

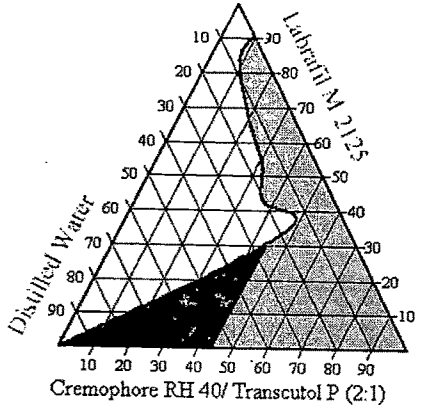
Figure 5.2.2 Excipient profiles for three different systems of Olmesartan SMEDDS

OSMS 1



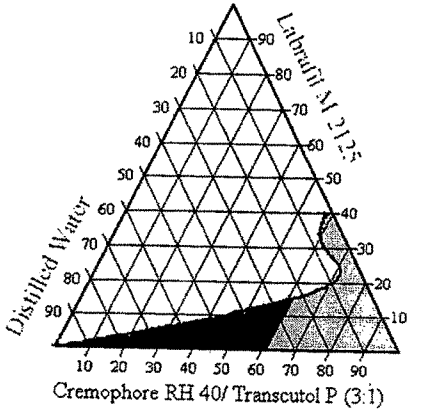
(A)

OSMS 2



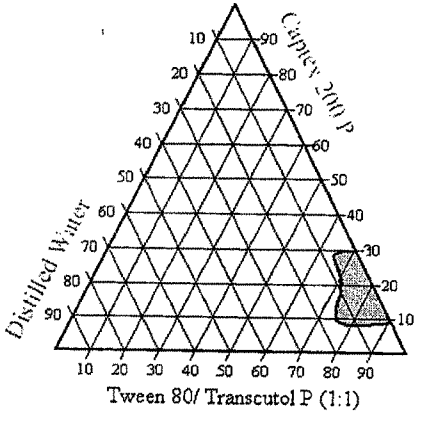
(B)

OSMS 3



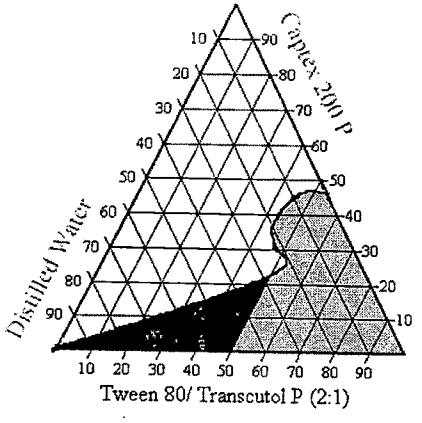
(C)

OSMS 2



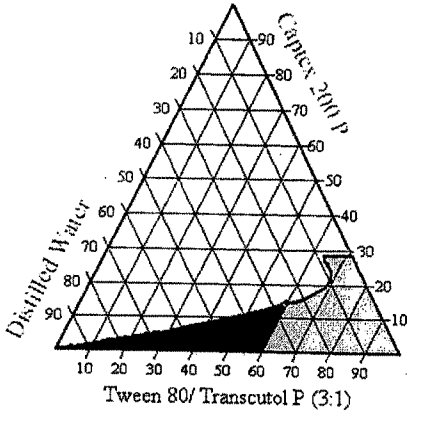
(D)

OSMS 2



(E)

OSMS 2



(F)

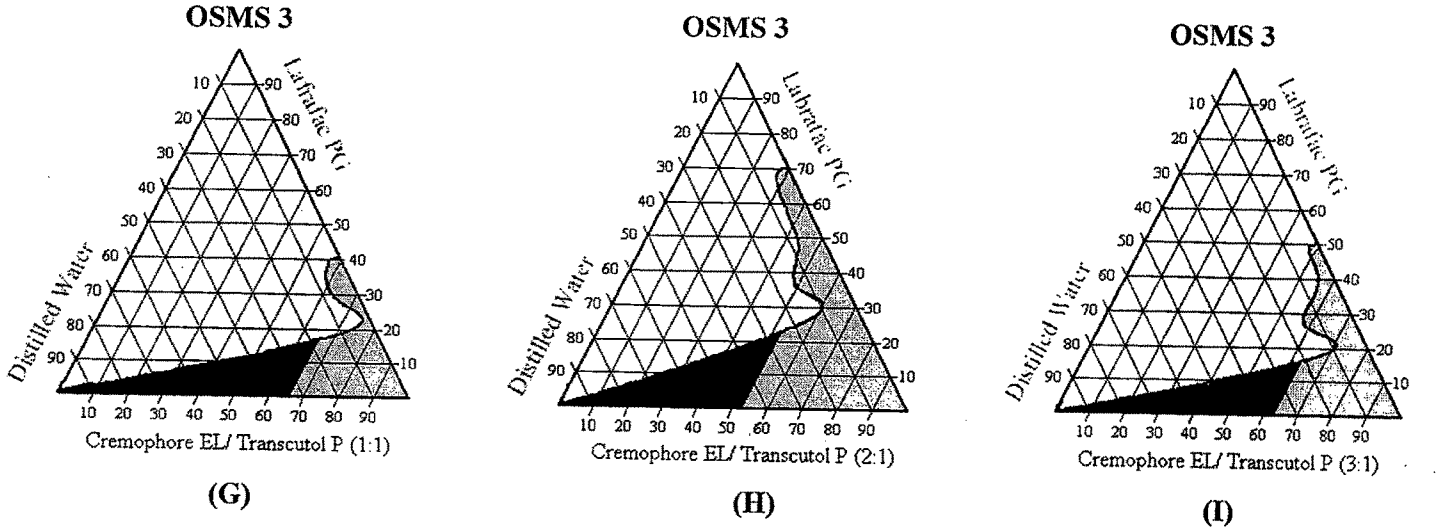


Figure 5.2.3 Pseudo-ternary phase diagrams for OSMS (System 1, 2 and 3) showing ME and emulsion regions at S: CoS ratio 1:1, 2:1 and 3:1 ◆ Self Microemulsion ◇ Microemulsion

Psudo-ternary phase diagram for each formulation shown in Fig. 5.2.3 represents presence of microemulsion and emulsion regions. Black region represents self microemulsion domain where as gray region indicates formation of coarse emulsion.

In pseudo-ternary phase diagrams shown in Fig. 5.2.3, black region is self microemulsion region i.e. even upon infinite dilution microemulsion remains stable. The region above these boundaries indicates formation of emulsion upon further dilution. Formulation A, B and C were prepared from system 1 (OSMS 1) using Labrafil M 2125 as oil phase, Cremophore RH 40 as surfactant and Transcutol P as cosurfactant with surfactant/cosurfactant (S/CoS) ratio of 1:1, 2:1 and 3:1. In this system Cremophore RH 40 was used which is chemically polyoxyl 40 Hydrogenated castor oil having HLB value between 14 and 16. Due to its high HLB value, this acts as a surfactant which rapidly forms o/w droplets and spreads in the aqueous media resulted in good self emulsification. For an effective absorption, the precipitation of the drug compound within the GI lumen should be prevented and the drug should be kept solubilized for a prolonged period of time at the site of absorption. Surfactants are amphiphilic in nature and they can dissolve or solubilize relatively high amounts of hydrophobic drug compounds. The lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS (3). Formulation A (figure 5.2.3 (A)) contains 20% of oil, 40% of S_{mix} and 80% of water. The black boundary covers self microemulsion region and even upon dilution it remains stable. At any point beyond this boundary, microemulsion if formed initially, become turbid on further dilution of solution. This indicates formation of self emulsion with higher particle size (<100 nm). The formulation A contains S_{mix} ratio 1:1 i.e. it contains 20% surfactant and 20% cosurfactant. Another experiment was tried by increasing concentration of surfactant to S_{mix}. Formulation B was prepared with S_{mix} ratio of 2:1. As the concentration of surfactant increased, self microemulsion forming region was also increased (Fig. 5.2.3 (B)). This could entrap up to 30% of oil. This formulation has an added advantage over the previous one that more amount of drug could be solubilized in the oil droplet. Moreover total concentration of S_{mix} was also up to 45% only therefore risk of toxicity due to high surfactant can be avoided. Further concentration of surfactant was increased by increasing the S_{mix} ration to 3:1 to observe the effect of surfactant on microemulsion. Since concentration of surfactant was increased it was expected that more microemulsion region will be covered with increased oil amount. But it was observed in Fig. 5.2.3 (C), that self forming microemulsion region was decreased and oil

concentration after dilution also decreased to 20%. Further during titration with water, time required to form microemulsion was also increased to more than 2 min. The concentration of S_{mix} ratio was reached to 61% which is at higher side and may lead to toxicity related problems. Beyond the boundary of microemulsion region there was a formation of transparent gel like structure. On comparing above three formulations A, B and C, only formulation B was found satisfactory with more microemulsion area, lower particle size and high amount of oil solublization. Therefore to prepare SMEDDS form system 1 (OSMS 1) with most suitable S_{mix} ratio of 2:1 was explored for further study.

The system 2 for olmesartan SMEDDS (OSMS 2) was prepared with combination of Captex 200 P as oil, Tween 80 as surfactant and Transcutol P as cosurfactant. It produced three formulations D, E and F with three S_{mix} ratios of 1:1, 2:1 and 3:1 respectively. It is clear form Fig. 5.2.3 (D) that formulation D did not form microemulsion region. The gray region in the figure identifies only presence of emulsion. Further surfactant concentration was raised to ratio 2:1 to get microemulsion with same excipients. Here microemulsion existence was observed 22% of oil entrapment (Fig 5.2.3 (E)). When particle size of this microemulsion was measured it was found to be around 100 nm (data shown in characterization) suggesting that it may increase on storage and may convert into emulsion. When S_{mix} was increased to 3:1 to get better microemulsion, existence area was decreased and at the same concentration of S_{mix} was also increased which creates again safety issues. Also amount of oil get reduced to 15% which did not solubilize required amount of drug which further on storage precipitated drug and solution became hazy which can be considered as stable microemulsion. So with case of formulation D, E and F stable microemulsion was not obtained with any S_{mix} ratio.

To prepare OSMS 3 different excipients were used i.e. Labrafac PG was used as oil, Cremophore EL was used a surfactant and Transcutol P as cosurfactant. Formulation G was prepared by using S_{mix} ratio to 1:1 (Fig 5.2.3 (G)). This covers considerable region in pseudo-ternary phase diagram for microemulsion formation. The oil can be entrapped upto 18% while concentration of S_{mix} ratio was reached to 65%. This is again high value for surfactant concentration. Though this formulation was most stable, high amount of surfactant is not recommended. It is important to maintain surfactant concentration at low

level as large amount of surfactant may cause GI irritation (3). This problem of high surfactant concentration was tried to solve by increasing ratio of S_{mix} to 2:1. For this ratio phase diagram was obtained as shown in Fig 5.2.3 (H). As per the expectation, the concentration of S_{mix} ratio was reduced to 52% and oil amount was also increased to 22%. So this SMEDDS could solubilize more amount of drug with low concentration of surfactant. To improve the efficiency of SMEDDS, S_{mix} ratio was further increased to 3:1, but opposite to later case reduction in oil concentration was observed (Fig 5.2.3 (I)). Though microemulsion existence area was increased, there was also increase in surfactant concentration which was not desirable. When this formulation was stored at room temperature, the solution became hazy suggesting that system was not stable.

4.2.1.3 Preparation of SMEDDS

Pseudo-ternary phase diagrams were considered as base to decide the concentration of each component of SMEDDS. A SMEDDS was prepared by selecting such a composition form microemulsion region which contains maximum amount of oil because solubility of drug is main factor to accommodate dose of drug as well as to maintain stability of microemulsion. Hence form each phase diagram, formulation was selected which covers maximum microemulsion region. The composition of OSMS 1, OSMS 2 and OSMS 3 are listed in Table 5.2.2, 5.2.3 and 5.2.4 respectively. In each formulation concentration of olmesartan was kept constant to 13 mg/mL.

Vehicle (% w/w)	**A (1:1)**	**B (2:1)**	**C (3:1)**
Olmesartan (mg)	13	13	13
Labrafil M 2125	20	30	10
Cremophore RH 40	45	46.67	67.5
Transcutol P	45	23.33	22.5

Oil- Labrafil M 2125, Surfactant- Cremophore RH 40, Co-surfactant- Transcutol P

Table 5.2.2 Compositions of System 1 (OSMS 1)

Vehicle (% w/w)	D (1:1)	E (2:1)	F (3:1)
Olmesartan (mg)	13	13	13
Captex 200 P	10	20	10
Tween 80	45	53.33	67.5
Transcutol P	45	26.67	22.5

Oil- Captex 200 P, Surfactant- Tween 80, Cosurfactant- Transcutol P

Table 5.2.3 Compositions of System 2 (OSMS 2)

Vehicle (% w/w)	G (1:1)	H (2:1)	I (3:1)
Olmesartan (mg)	13	13	13
Labrafac PG	10	20	10
Cremophore EL	45	53.33	67.5
Transcutol P	45	26.67	22.5

Oil- Labrafac PG, Surfactant- Cremophore EL, Cosurfactant- Transcutol P

Table 5.2.4 Compositions of System 3 (OSMS 3)

5.3 CHARACTERIZATION AND SELECTION OF SMEDDS

5.3.1 Experimental

25 µL of SMEDDS were diluted with 25 mL of distilled water to get 1000 times dilution. These microemulsion solutions were considered for assessment of various in vitro parameters. Appearance, particle size, zeta potential, viscosity and refractive index, conductance, % transmittance and assay content were determined for olmesartan SMEDDS using methods as mentioned in section 4.3.1.

5.3.2 Result and Discussion

5.3.2.1 Appearance

Diluted SMEDDS appeared as transparent blue colored solution due to presence of synthetic oils. In some cases, initially upon dilution, SMEDDS formed clear microemulsion but while studying bench top stability it was converted to gel form or emulsion suggesting system to be unstable SMEDDS.

Vehicle (% w/w)	A (1:1)	**B (2:1)**	C (3:1)
Mean Particle size (nm)	47.8	**23.5**	15.3
Zeta Potential (mV)	-0.031	**-0.0105**	-0.021
Poly Dispersivity Index (PDI)	0.242	**0.204**	0.299
% Transmittance	99.8	**99.6**	98.6
pH	6.75	**6.81**	6.72
Viscosity (cP)	0.995	**0.985**	0.981
Conductivity (μ semence)	99.81	**97.13**	96.43

Table 5.3.1 Characterization of System 1 (OSMS 1)

Vehicle (% w/w)	**D (1:1)**	**E (2:1)**	**F (3:1)**
Mean Particle size (nm)	126.8	79.2	53.9
Zeta Potential (mV)	-0.396	-0.482	-0.523
Poly Dispersivity Index (PDI)	0.325	0.310	0.306
% Transmittance	86.63	96.44	97.28
pH	6.74	6.87	6.91
Viscosity (cP)	1.127	0.987	0.981
Conductivity (μ semence)	80.35	95.67	96.99

Table 5.3.2 Characterization of System 2 (OSMS 2)

Vehicle (% w/w)	**G (1:1)**	**H (2:1)**	**I (3:1)**
Mean Particle size (nm)	120.8	115.1	97.2
Zeta Potential (mV)	-0.133	-0.108	-0.241
Poly Dispersivity Index (PDI)	0.449	0.370	0.332
% Transmittance	94.23	93.90	94.88
pH	6.67	6.85	6.94
Viscosity (cP)	0.963	0.948	0.932
Conductivity (μ semence)	93.97	94.88	94.49

Table 5.3.3 Characterization of System 3 (OSMS 3)

Hence to determine the stability of SMEDDS, other characters were checked, results of which are listed in Table 5.3.1, 5.3.2 and 5.3.3 for different formulations.

5.3.2.2 Particle Size

In literature SMEDDS was considered having particle size less than 100 nm (4). Therefore after selection of microemulsion region from phase diagram, first evaluation tool was to determine particle size. The particle size of microemulsion should not increase above 100 nm. The values listed in Tables 5.3.1, 5.3.2 and 5.3.3 are the values obtained measured immediately after dilution to 1000 times. Among all values, particle size for formulation B and C were found lowest. The stability of microemulsion with respect to particle size was also checked and it was found consistent on storage.

Formulation A was also prepared from the same system OSMS 1 but it showed higher particle size 47.8 (Fig. 5.3.1) as compared to 23.5 for formulation B and 15.3 (Fig. 5.3.2) for formulation C (Fig. 5.3.3). Though it is less than 100 nm and fits in the criteria of SMEDDS but particle size increases on further storage. Formulation A contained less amount of oil (based of pseudo-ternary phase diagram) and thereby could not solubilize required amount of drug. On comparing particle size for formulation A, B and C, a reduction in particle size was observed. The ratio of surfactant/ cosurfactant was gradually increased starting from 1:1 to 2:1 followed by 3:1 for formulation A, B and C respectively and with the increase in concentration of surfactant, decrease in particle size were observed. Constantinides also reported lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS with less particle size (5). Same trends were observed for other formulations D, E and F prepared form system OSMS 2 and formulations G, H and I prepared from OSMS 3 with increase of S_{mix} ratio.

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 51.2	Peak 1:	11.4	70.1	2.68
PdI: 0.242	Peak 2:	153	29.9	40.4
Intercept: 0.925	Peak 3:	0.00	0.0	0.00

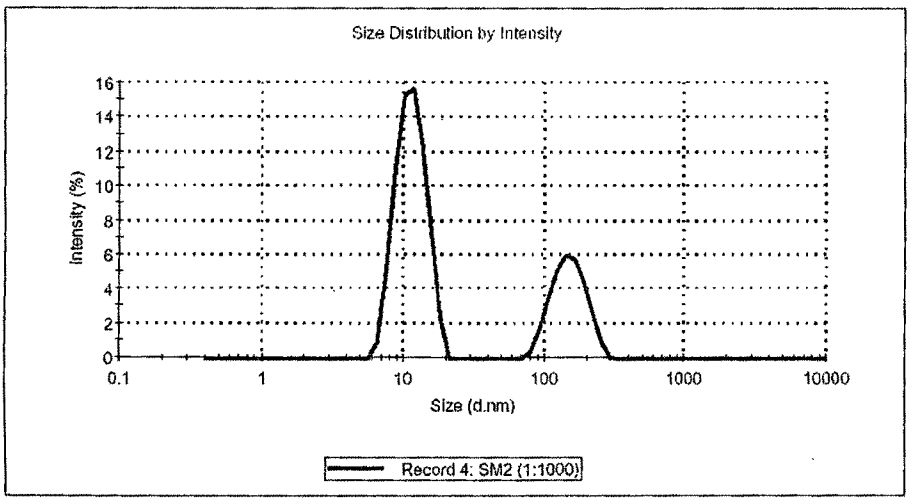


Figure 5.3.1: Particle size and Polydispersivity index (PDI) of Formulation A

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 23.5	Peak 1:	22.9	98.5	5.97
PdI: 0.204	Peak 2:	5380	1.5	322
Intercept: 0.944	Peak 3:	0.00	0.0	0.00

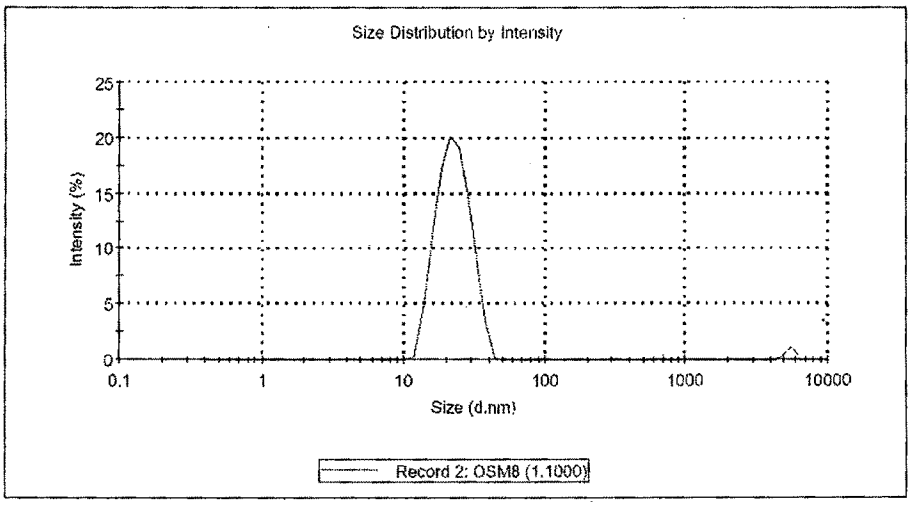


Figure 5.3.2: Particle size and Polydispersivity index (PDI) of Formulation B

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 15.3	Peak 1:	13.0	95.6	4.77
PdI: 0.299	Peak 2:	3850	4.4	1130
Intercept: 0.942	Peak 3:	0.00	0.0	0.00

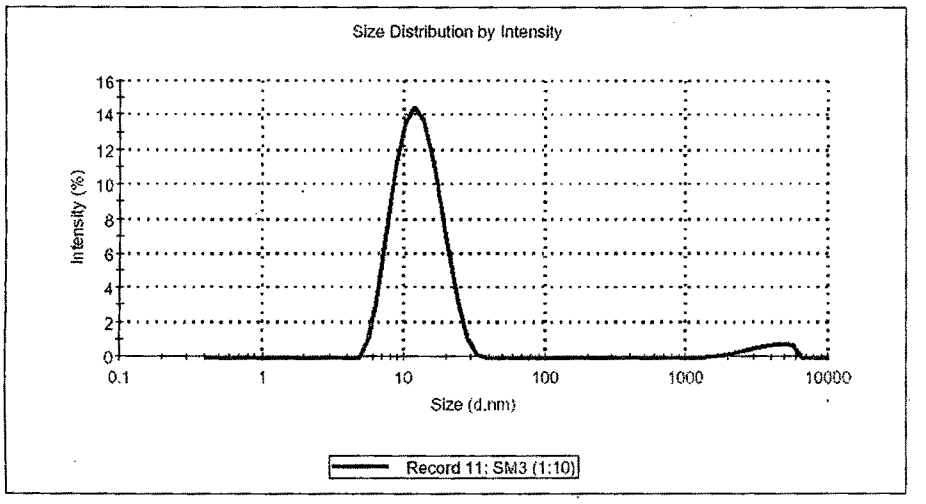


Figure 5.3.3: Particle size and Polydispersivity index (PDI) of Formulation C

5.3.2.3 Zeta Potential

The charge of the oil droplets of SMEDDS is another property that should be assessed. The charge of the oil droplets in conventional SMEDDS is negative due to the presence of free fatty acids. Incorporation of a cationic lipid, such as oleylamine will lead a positive zeta potential value of about 35-45 mV (6, 7, 8). Non-ionic surfactants can be widely used and polyoxyethylene sorbitan *n*-acyl esters (Tweens), have been reported to have minimal toxicity. Furthermore, the insensitivity of nonionic microemulsions to pH and electrolyte concentration relative to their ionic counterparts represents an added benefit (9).

The zeta potential values for all the formulations studied were found to be approximately zero as the surfactant used in preparation of SMEDDS are non-ionic. Some negativity in values was due to fatty acids of oil used.

5.3.2.4 Polydispersivity Index (PDI)

The PDI is measure for uniformity of particle size. Low PDI indicates uniform dispersion of oil droplets within microemulsion. PDI was determined for all formulations and it was

found lowest (0.190) for formulation B.

5.3.2.5 % Transmission

Clarity of microemulsion can be assessed by passing UV light through formulation to measure % Transmission. As microemulsion forms transparent solution on dilution it should be as clear as water and give %T value neat up to 100% but presence of synthetic oil reduce %T values. Formulations of OSMS 1 produced %T value above 98% which indicates the clarity and transparency of solution. The same for other formulations were found below 98% which indicates haziness of the solution.

5.3.2.6 pH

pH value of solutions measures the ionization of drug molecule in microemulsion. If drug is stable at pH of dilution media there won't be any change in pH of resultant microemulsion. Microemulsion is thermodynamically driven by the requirement of surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration (CMC) under the prevailing conditions of temperature, pH and ionic strength. Because nonionic surfactants typically have lower CMCs than their ionic counterparts, o/w microemulsion dosage forms based on non-ionic surfactants and designed for oral use are likely to offer superior in vivo stability (10).

All formulations diluted with pH 6.8 buffer, resultant microemulsions also showed same pH with variation of ±0.1. This shows the stability of drug as well as formation of water continuous microemulsion.

5.3.2.7 Viscosity

Viscosity measurements indicate formation of oil/water continuous microemulsion. Phase inversion on storage reflects in change in viscosity. All the formulations except formulation D showed viscosity value near to that of water indicates in all case there was formation of water continuous microemulsion. Even on storage no phase inversion or phase separation was observed as there was no change in viscosity. In case of formulation D viscosity was found to be quite higher side which may be either due to increase in particle size or formation of self emulsion.

5.3.2.8 Conductivity

Conductivity measurements provide a means of determining whether a microemulsion is

oil-continuous or water-continuous as well as providing a means of monitoring percolation or phase inversion phenomena (11, 12, 13). It is already discussed above that all formulations were water continuous and no phase inversion were observed.

On the basis of above data, three formulations A, B and C from OSMS 1 were considered to be suitable as SMEDDS. The main two criteria, particle size and zeta potential of these systems fulfill the requirement of SMEDDS. The other characteristics also support the formation of o/w microemulsion. Therefore stability studies were performed for formulations A, B and C.

5.4 STABILITY STUDIES OF OLMESARTAN SMEDDS

5.4.1 Experimental

5.4.1.1 *Robustness to dilution*

Robustness of SMEDDS to dilution was studied using the method of Date et al., with slight modifications (14). SMEDDS were diluted to 10, 100 and 1000 times with various dissolution media viz. water, pH 1.2 buffer, pH 4.5 buffer and pH 6.8 buffer. The diluted microemulsions were stored for 12 hours and observed for any signs of phase separation or drug precipitation.

5.4.1.2 Physical Stability

Formulation A, B and C were subjected to physical stability (15). The prepared microemulsions were subjected to accelerated centrifugation for the assessment of physical phase separation, if any between the oil and aqueous phase.

Criteria for selection of batches (16)

1. Microemulsions having mean globule size below 100 nm; and
2. Low Polydispersivity index.

Microemulsions having least globule size are expected to have larger surface area and therefore, may get absorbed or may transverse rapidly across the gastric mucosa. Low PDI measures uniform dispersion of oil droplet through microemulsion. Therefore, both the selection criteria were used as a filter prior to assessment of accelerated physical stability.

Method

The study was carried out using the procedure mentioned in section 4.4.1.2. The results

of globule size following accelerated centrifugation for formulation A, B, and C are recorded in Table 5.4.1.

5.4.1.2 Chemical Stability

SMEDDS for olmesartan were subjected to accelerated temperature and stress conditions (17). The stress stability was conducted at 60° C ± 2° C in an incubator. The accelerated stability was performed at 30° C ± 2° C / 65% ± 5% relative humidity (R.H.) and 40° C ± 2° C / 75% ± 5% R.H. The duration of stability was 6 months and samples were withdrawn at predetermined time intervals after 1 month, 2 months, 3 months and 6 months (14). The parameters monitored for physical separation at accelerated gravitational force were active ingredient content, globule size determination, zeta potential measurement, appearance. The results for olmesartan chemical studies are recorded in Table 5.4.2, Table 5.4.3 and Table 5.4.4.

5.4.2 Result and Discussion

5.4.2.1 Robustness to Dilution

Diluted SMEDDS did not show any precipitation or phase separation on storage in various dilution media. This revels that all media were robust to dilution.

5.4.2.2 Physical Stability

Microemulsions are inherently unstable from physical standpoint. Poor physical stability is ultimately exhibited by phase separation, which can be visually monitored. Certain properties of emulsions will start to change long before this separation is visually apparent. An increase in particle size is particularly indicative of physical instability, since this monitors the coalescence or flocculation that is part of the process involved in ultimate phase separation. Increases in viscosity (due to flocculation) and changes in zeta potential (arising from a decrease in droplet surface area) are both indicative of poor physical stability. The presence of drug and/or cosolvents can potentially hasten the phase separation.

Various parameters like particle size, zeta potential and viscosity of formulation A, B and C were determined to predict the stability of prepared system. Stability data are shown in Table 5.4.1.

Formulation	Globule size (nm)		
	Top layer	Middle layer	Bottom layer
A	43.7 ± 0.58	65.25 ± 0.25	88.31 ± 0.41
B	15.64 ± 0.36	27.85 ± 0.22	25.90 ± 0.13
C	10.57 ± 1.10	38.94 ± 0.94	69.76 ± 1.06

Table 5.4.1 Physical Stability of SMEDDS for Olmesartan

In physical stability study, formulation A and B showed continuous increase in particles size. Due to centrifugal force oil droplets may agglomerate and settle down to bottom of tube resulting in increase in particle size in bottom layer. But consistency was observed for particle size of formulation B in all layers. The initial value of PDI (0.190) was not altered after stability study.

5.4.2.3 Chemical Stability

Temperature and humidity has impact on stability of microemulsion. Since SMEDDS contains oil, high temperature and humidity may cause oxidative degradation leading to formation of free fatty acids. Microemulsions stabilised by non-ionic surfactants, especially those based on polyoxyethylene, are very susceptible to temperature because a decrease in surfactant solubility occurs with increasing temperature. So the systems stabilized by non-ionic surfactants or mixtures thereof often have characteristic phase inversion temperatures (PITs) which alters physical and in-vitro characteristics formed microemulsion (18).

Formulation A	Ratio of S:CoS	Period (month)	40°C/75% RH				30°C/65% RH			
			Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%)	Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%) ± SEM
	1:1	0	47.8 ± 10.95	-0.03 ± 2.05	99.8 ± 0.1	101.2	47.8 ± 11.40	1.15 ± 1.95	99.8 ± 0.2	101.2 ± 0.35
		1	51.3 ± 9.20	-0.02 ± 3.89	98.3 ± 0.3	99.5	53.1 ± 7.90	0.06 ± 2.10	98.3 ± 0.1	98.9 ± 0.33
		2	60.7 ± 10.63	0.04 ± 1.87	97.7 ± 0.4	97.7	62.6 ± 4.95	1.10 ± 1.98	98.6 ± 0.3	97.1 ± 0.84
		3	78.4 ± 5.10	0.09 ± 3.35	98.1 ±0.2	94.5.4	77.3 ± 10.78	-1.07 ± 3.10	97.4 ± 0.4	96.6 ± 0.19
		6	108.6 ± 3.56	-1.08 ± 4.58	96.74± 0.5	92.9	95.2 ±4.96	0.08 ± 1.96	98.1 ± 0.5	95.9 ± 0.22

Table 5.4.2 Stability data of Formulation A

Formulation B	Ratio of S:CoS	Period (month)	40°C/75% RH				30°C/65% RH			
			Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%)	Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%) ± SEM
	2:1	0	23.5 ± 8.23	-0.75 ± 0.95	99.6 ± 0.3	99.18 ± 0.30	24.8 ± 5.35	-1.52 ± 0.82	99.9 ± 0.3	98.2 ± 1.23
		1	25.2 ± 6.96	-0.27 ± 3.10	99.8 ± 0.4	100.8 ± 1.05	29.3 ± 6.52	-3.10 ± 2.63	99.1 ± 0.5	102.1 ± 0.25
		2	27.9 ± 10.95	-0.66 ± 1.85	98.7 ± 0.2	98.7 ± 0.85	27.7 ± 5.94	-2.90 ± 1.45	98.4 ± 0.2	100.5 ± 1.01
		3	26.7 ± 9.33	-0.18 ± 3.22	99.3 ± 0.5	99.5 ± 0.63	31.6 ± 5.03	-3.74 ± 1.88	98.7 ± 0.6	98.3 ± 0.36
		6	30.1 ± 8.96	-0.69 ± 2.55	98.2 ± 0.1	98.7 ± 0.70	29.9 ± 24.74	-1.51± 1.05	98.9 ± 0.1	99.6 ± 1.37

Table 5.4.3 Stability data of Formulation B

Formulation C	Ratio of S:CoS	Period (month)	40°C/75% RH				30°C/65% RH			
			Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%) ± SEM	Globule size (nm) ± SEM	Zeta potential (mV) ± SEM	Trans-mittance (%) ± SEM	Drug content (%) ± SEM
	3:1	0	15.2 ± 5.36	-0.12 ± 2.56	99.7 ± 0.2	99.4 ± 0.84	15.2 ± 5.46	-0.12 ± 0.70	98.9 ± 0.2	99.4 ± 0.20
		1	28.9 ± 7.25	-0.10 ± 3.39	99.3 ± 0.1	98.1 ± 0.27	25.7 ± 8.95	-0.87 ± 2.00	99.5 ± 0.1	97.9 ± 0.54
		2	49.7 ± 8.95	-0.77 ± 1.85	99.1 ± 0.3	97.6 ± 0.31	40.1 ± 4.26	-0.46 ± 1.92	99.2 ± 0.5	96.1 ± 0.69
		3	71.6 ± 8.56	-0.43 ± 1.77	98.8 ±0.5	96.3 ± 0.68	58.2 ± 9.78	-1.07 ± 2.10	98.6 ± 0.3	93.8 ± 0.20
		6	94.5 ± 12.93	-0.29 ± 2.05	97.9 ± 0.4	95.7 ± 0.19	73.6 ± 1.32	-0.32 ± 1.90	98.4 ± 0.4	91.5 ± 0.19

Table 5.4.4 Stability data of Formulation C

The stability study was performed as per ICH guideline. According to that conditions for stability studies can be decided based on climatic condition of that particular zone. As per guideline, stability should be studied under three different conditions. They were:

(a)	25°C/60% RH	12 months	Long term stability
(b)	30°C/65% RH	6 months	Intermediate stability
(c)	40°C/75% RH	6 months	Accelerated study

Stability study is important tool to decide shelf life of the formulation. If samples are stable under stability conditions for at least 6 months, it will remain stable through its shelf line. The stability study for prepared SMEDDS was carried out under two conditions i.e., intermediate condition at 30°C/65% RH and accelerated condition at 40°C/75% RH for 6 months. The results of these studies are shown in Table 5.4.2, Table 5.4.3 and Table 5.4.4. The results showed formulation B was the most stable system. Stability of microemulsion were observed at different time intervals i.e., 0 (initial), 1, 2, 3 and 6 months. All the characteristics for formulation B were found to be stable even after 6 months period. The results for other two systems (Table 5.4.2 and 5.4.4) showed that values for particle size are continuously increased over the period of months. The zeta potential, found to be stable for all the system. %Transmission for the formulation A and C was gradually decreased and increasing particle size or formation of unstable microemulsion may be the reason for this. The effect of stability conditions on drug was assessed by determining assay contents. At stability conditions formation of fatty acid may cause degradation of drug and results in reduction in actual drug content of the formulation. The present drug content of formulation was determined by estimating assay value using HPLC method. Table 5.4.3 showed that assay for formulation B was found in the range of 98-102% throughout 6 months period. The drug content was decreased from 101% to 92% for formulation A and 99% to 91% for formulation C over the period of time. These also give the idea about instability of systems.

The physical and chemical stability data shows that formulation B is most stable SMEDDS and gives microemulsion with low particle size and good zeta potential values. Formulation B has low particle size and significant potential to keep system suspended. Low PDI values shows uniformity of globule size, %T indicates the clarity of

microemulsion, viscosity and conductivity confirms water-continuous microemulsion system and constant pH represents solubility and stability of drug in oil droplet. Even in pseudo-ternary phase diagram formulation B showed maximum microemulsion region. Therefore formulation B was selected for further *in vitro* and *in vivo* studies.

5.5 *IN VITRO* DIFFUSION STUDY

In Vitro drug diffusion study was carried by using two different methods.

1. Dialysis bag study.
2. Intestinal permeability study.

In this investigation, all the test formulations were assessed for *in vitro* diffusion across the dialysis technique and *in vitro* permeation across Male Sprague Dawley rat's duodenum in triplicate and the physicochemical parameters were calculated as mentioned in section 4.5.

5.5.1 Experimental

5.5.1.1 Dialysis Bag Method

In vitro release of formulation B was tested by using dialysis bag method (19). Dialysis bag was soaked for over night into a phosphate buffer pH 6.8 for saturation purpose and then was used for further experimental study. Method mentioned in section 4.5.1.1 was used. The revolution speed of the paddle was maintained at a rate of 50 rpm (20). At predetermined time intervals (0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12 hrs), 5 mL samples were drawn out and the same volume of fresh dissolution medium was replenished. The release of olmesartan from SMEDDS was compared with that of from marketed capsule formulation and standard drug solution. Samples were analyzed quantitatively for olmesartan dialyzed across the membrane at corresponding time by using UV as mentioned in section 5.1.1.1. The experiments were run in triplicate and data are presented in terms of % cumulative release of drug along with SD. The release from SMEDDS was compared with that of pure olmesartan suspension and marketed formulation (Olmetor 40®). The results are shown in Table 5.5.1. and represented graphically in Figure 5.5.1.

5.5.1.2 Intestinal Permeability Study (21, 22)

The experimental procedure was similar to the procedure mentioned in section 4.5.1.2.

The receiver compartment was filled with 30 mL of Phosphate buffer pH 6.8. Aliquots of 5 mL were withdrawn at different time intervals and volume of aliquots replaced with fresh dialysis medium each time. The samples were analyzed quantitatively for olmesartan dialyzed across the membrane at corresponding time by using UV-Visible spectrometric method as mentioned in section 5.1.1.1. The experiments were run in triplicate and the mean cumulative % drug diffused along with SD for pure olmesartan, marketed formulation (Olmetor 40) and prepared formulation B are shown in Table 5.5.2. and graphically it is represented in Figure 5.5.2.

3.5.2 Result and Discussion

3.5.2.1 Dialysis Bag Method

Time (Hrs)	% Cumulative Drug Release				
	Formulation B in pH 1.2	Formulation B in pH 4.5	Formulation B in pH 6.8 Buffer	Marketed Formulation in pH 6.8 Buffer	Standard Drug Solution
0.08	4.33	7.84	15.05	6.39	4.21
0.17	6.08	8.88	26.81	13.12	8.15
0.25	8.80	10.26	37.57	22.37	12.76
0.33	11.09	13.05	42.44	35.44	18.30
0.5	13.27	14.65	47.77	43.77	21.34
0.75	14.92	16.58	52.56	47.56	24.22
1	17.51	18.09	60.69	53.69	27.60
1.5	18.20	19.18	68.09	55.09	29.18
2	19.01	21.34	74.87	57.87	30.39
3	18.95	22.36	81.96	58.19	31.06
4	19.14	22.60	86.22	58.63	31.47
8	19.06	22.84	89.32	58.55	31.5
12	19.17	22.73	90.17	58.89	31.41

Table 5.5.1 % Cumulative Drug Release In-vitro Release Study (Dialysis Bag Method)

On comparing data of *in vitro* release for olmesartan from formulation A in three different medias i.e. pH 1.2, pH 4.5 and pH 6.8, results indicates that formulation B showed highest release in pH 6.8 buffer (Table 5.5.1). This variation in release was due to pH dependent solubility of olmesartan. Based on these results, further *in vitro* release profile of formulation B was compared with conventional formulation (Olmetor 40®) and standard drug suspension in pH 6.8 media by using dialysis bag. The graphical presentation for olmesartan release is shown in Fig 5.5.1. The formulation B showed

highest release i.e. 90% while conventional formulation released 59% of drug and standard drug solution released only upto 32% of drug after 12 hrs.

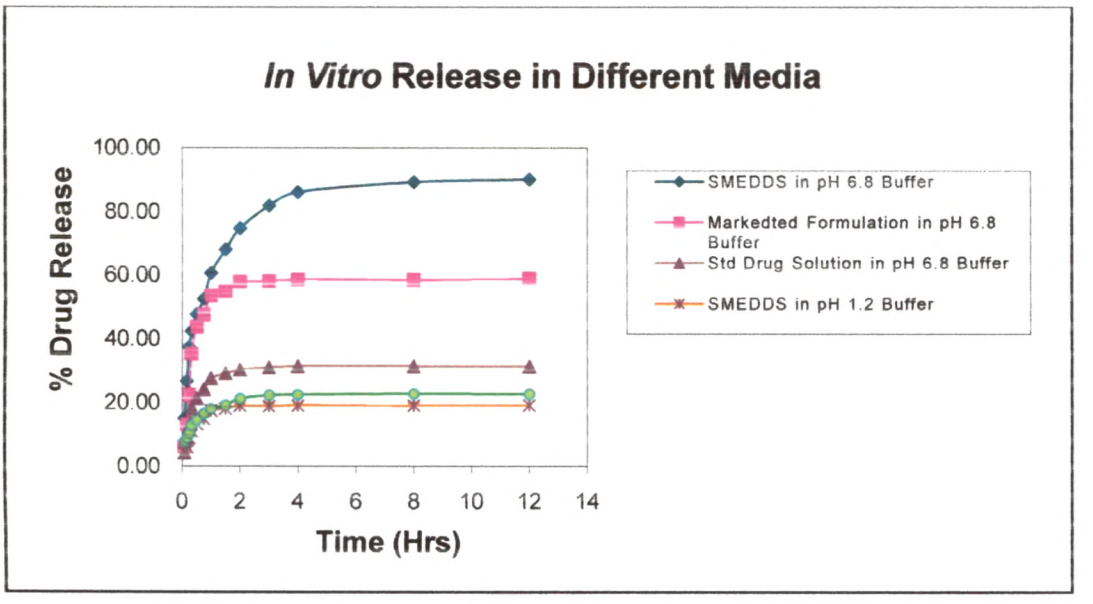


Fig 5.5.1 Comparative *In vitro* Release Study (Dialysis Bag Method)

SMEDDS formulation released more than 60% drug within one hour while release rate is slow in case of conventional formulation i.e. only upto 53% and same for drug suspension is only up to 27% within first hour. SMEDDS must be in diluted form to get good release. Since microemulsion was water continuous, oil droplet suspended in water phase come in contact with dialysis bag membrane. Due to presence of surfactant, drug suspended in oil droplet easily diffuse through membrane and high solubility of drug in receiving compartment help to solubilize drug. This in-vitro study gives primary assumption to predict bioavailability of drug. The above data concludes that *in vitro* release of olmesartan was greatly enhanced by SMEDDS.

3.5.2.2 Intestinal Permeability Study

Another approach of intestinal permeability study was also adopted to correlate drug diffusion form SMEDDS. In this study results found were different than that of dialysis bag method. Obviously SMEDD showed higher release than conventional formulation and pure drug suspension, but amount of drug release was less compared to dialysis bag method for all there system.

Time (Hrs)	% Drug Release		
	Olmesartan SMEDDS	Market Formulation	Standard Drug Solution
0.08	5.12	2.61	1.23
0.17	9.57	4.83	2.06
0.25	14.90	7.96	3.61
0.33	22.23	11.08	5.07
0.5	32.35	15.19	6.52
0.75	41.11	21.02	9.04
1	50.33	28.39	11.75
1.5	57.82	31.22	12.98
2	62.68	36.21	13.08
3	68.57	39.47	13.72
4	72.48	40.62	14.00
8	73.44	41.10	14.05
12	73.50	41.33	13.99

Table 5.5.2 % Cumulative Drug Release in Intestinal Permeability Study

SMEDDS of formulation B released 73.5% of drug while market formulation released only up to 41.33% and pure drug suspension showed least release of 14%. The overall release of the drug through intestinal membrane may be due to its higher thickness.

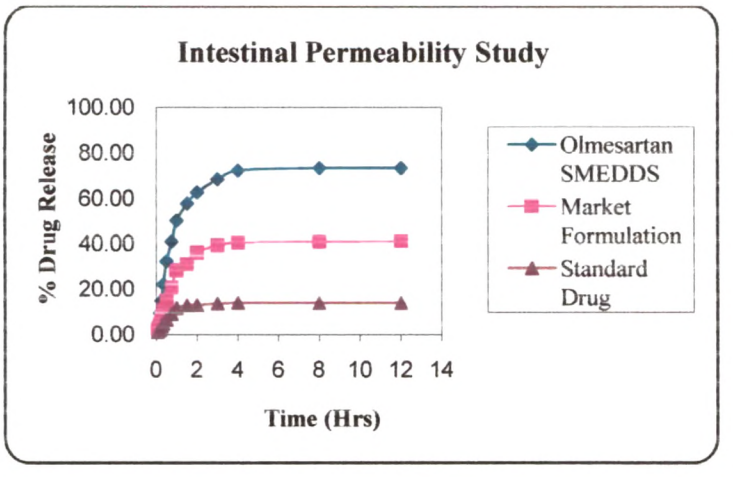


Fig. 5.5.2 Comparative *In vitro* Release Study (Intestinal Permeability Method)

Although difference were observed in two diffusion studies, it is confirmed that olmesartan SMEDDS showed best release as compare to other two formulations.

5.6 BIOAVAILABILITY STUDY

5.6.1 Experimental

Bioavailability of SMEDDS for olmesartan was compared with suspension of marketed formulation (Olmetor 40, Torrent Pharmaceutical, Ahmedabad). The experimental procedure adopted was similar to that mentioned in section 4.6.1.

5.6.2 Result and Discussion

Bioavailability for olmesartan was measured on rabbits. In-vivo pharmacokinetic behavior of olmesartan was observed with formulation B and marketed formulation (Olmetor 40). Dose of olmesartan was administered as 2.8 mg/kg body weight. Mean plasma concentration profile of olmesartan after oral administration of formulation B and conventional marketed formulation plotted as a function of time is shown in fig 5.6.1. The non compartment model was used to evaluate pharmacokinetic parameters of olmesartan absorption which are summarized in Table 5.6.1.

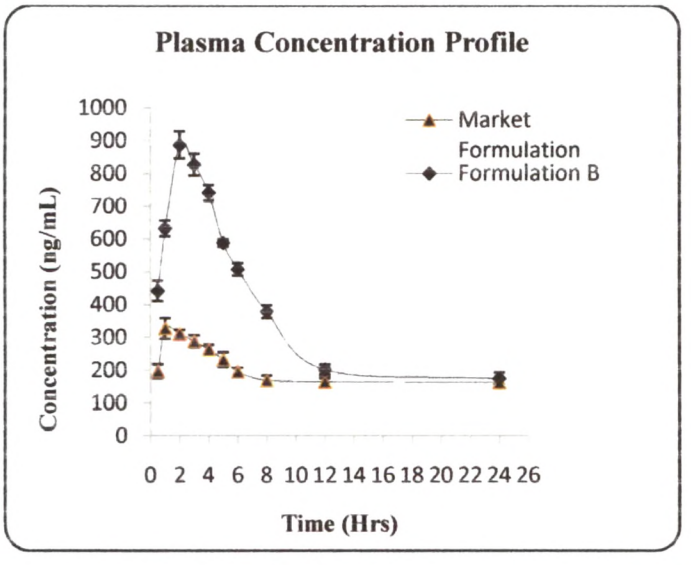


Fig 5.6.1 Plasma concentration profile for olmesartan after oral administration of Formulation B and conventional market formulation in rabbit (n=6)

Significant rise can be observed in concentration of olmesartan from formulation B which consists of microemulsion as compare to marketed formulation. Although T_{max} were same i.e. 1 Hr for both formulation, SMEDDS formulation showed sudden raise in C_{max}

to 887 ng/mL which was 328 ng/mL for marketed formulation. This rise in concentration represents increase in bioavailability with SMEDDS formulation. Other pharmacokinetic parameters were calculated for both formulations which are listed in Table 5.6.1. Significant rise were also observed in Area Under Curve (AUC) which is important tool to calculate bioavailability.

Parameters	**Formulation B**	**Market Formulation**
t_{max} a(h)	1±0.35	1±0.41
C_{max} b(ng/ml)	887.7±42.6	328.16±24.56
$AUC_{0\rightarrow t}$ c(ng h/ml)	7942.94±98.25	4545.340±88.32
$AUC_{0\rightarrow \infty}$ d(ng h/ml)	11947.55±112.4	83110.629±189.63
$AUMC_{0\rightarrow t}$ e(ng h/ml)	63791.18±178.98	49198.683±302.74
$AUMC_{0\rightarrow \infty}$ f(ng h/ml)	283832.25±1709.75	39751851.05±2035.5
$MRT_{0\rightarrow \infty}$ g(h)	21.61±1.23	478.300±2.44
Relative bioavailability h(%)	216.00	-

a Time of peak concentration.
b Peak of maximum concentration.
c Area under the concentration time profile curve until last observation.
d Area under the concentration time profile curve extrapolated to infinity.
e Area under moment curve computed to the last observation.
f Area under moment curve extrapolated to infinity.
g Mean residence time.
h Relative bioavailability.

Table 5.6.1 Relative Bioavailability and pharmacokinetic parameter of olmesartan after oral administration of Formulation B and conventional marketed formulation

The linear trapezoidal rule was used to calculate the area under curve ($AUC_{0\rightarrow t}$). Relative bioavailability was calculated using following formulae:

$$Relative\ BA(\%) = \frac{A \cup C_{test}}{A \cup C_{reference}} \times \frac{Dose_{reference}}{Dose_{test}}$$

Relative bioavailability was also increased to 216% compared to market formulation.

On the basis of in-vitro and in-vivo correlation it can be concluded that increase in release profile of olmesartan from SMEDDS can lead to increase in bioavailability of

olmesartan. The study of several factors like low particle size, optimized concentration and ratio of surfactant and cosurfactant suggest that SMEDDS seems to be a promising approach to increase solubility and bioavailability of olmesartan.

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