CHAPTER 3

Analytical Methods

3.1 INTRODUCTION

The analytical method used in the study of drug loaded mucoadhesive periodontal thermoreversible gel, strip and microspheres, should be compatible to the formulation additives along with accuracy, precision and reproducibility. Techniques which ensure minimum or no interference from other formulation additives were optimized to be the method followed to draw attention to any potential incompatibility between the various formulation additives.

3.2 EXPERIMENTAL

3.2.1 Drugs

Minocycline hydrochloride was received as gift sample from IPCA Laboratories, Mumbai, India. Clindamycin phosphate was gifted by Bharat Parenterals Limited, Baroda, India.

3.2.2 Reagents

Hydroxy propyl methyl cellulose, HEC, PVA were procured from S.D. Fine Chem. India, poly vinyl alcohol from Loba Chemi Pvt. Ltd., Mumbai, India. Polycarbophil and poly acrylic acid from Noveon USA, pluronic F127 from Sigma Chemicals, St. Louis, MO, U.S.A, polyethylene glycol 1000 from The Dow Chemical Company, New Milford, USA and sodium hydroxide from Loba Chemi Pvt. Ltd., Mumbai, India. Carbopol 934p was received as gift from B.F. Goodrich, USA. Ethyl cellulose was obtained from The Dow Chemical Corporation, USA. Hydrogenated soya phosphatidyl choline (HSPC) was obtained from Lipoid (Lipoid GmBH, Ludwigshafen, Germany) and cholesterol and span 80 from S. D. Fine chemicals, Mumbai, India and other chemicals and plasticizers used are of A.R. grade.

3.2.3 Apparatus

Shimadzu UV-Visible Spectrophotometer (UV-1601) (Shimadzu Corporation, Japan).

3.2.4 Estimation of minocycline hydrochloride using UV- Visible spectroscopy

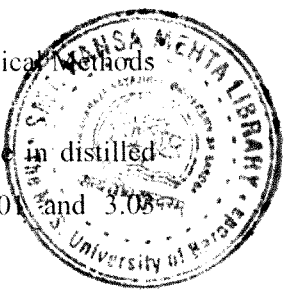
This method of estimation is based on the observation that minocycline hydrochloride shows strong absorbance in both distilled water and phosphate buffer saline (pH 6.75) in the UV region of the electromagnetic spectrum.

3.2.4.1 Reagents and solutions

- Phosphate buffer saline (pH 6.75): About 2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 g of sodium chloride was dissolved in sufficient distilled water to produce 1000ml and adjusted with phosphoric acid to get pH 6.75.
- Minocycline hydrochloride in distilled water and in phosphate buffer saline yields characteristic curves when scanned in the ultraviolet region between 200-400 nm. The λmax of minocycline hydrochloride in both distilled water and in phosphate buffer saline is at 246nm.
- Stock solution of minocycline hydrochloride (100µg/ml) in the distilled water and in phosphate buffer saline pH 6.75 was prepared by dissolving 10mg of minocycline hydrochloride in 100ml of PBS pH 6.75.

3.2.4.2 Preparation of calibration curve

- Suitable aliquots of the stock solution of minocycline hydrochloride were pipette and transferred separately into 10ml volumetric flasks. The volume was made up with the same solvent i.e. in the distilled water and in phosphate buffer saline pH 6.75 respectively. The solutions were shaken well and the absorbance of the resulting solutions were measured at 246nm using a Shimadzu UV-Visible spectrophotometer (UV-1601) with cells of 10mm length against the same solvent used as blank (Florey et al, 1977). The procedure was repeated for six times. Mean absorbance values along with regression values for distilled water and in phosphate buffer saline pH 6.75 are shown in table no 3.01 and 3.02 respectively. The calibration curve in distilled water and in PBS pH 6.75 are shown in figure 3.02 and 3.04 respectively. Absorptivity scans over the wave



length range of 200-400 nm for the solution of minocycline hydrochloride in distilled water and in phosphate buffer saline pH 6.75 is shown in figure 3.01 and 3.02 respectively.

3.2.5 Stability and selectivity

Changes in absorbance of the solutions of minocycline hydrochloride in distilled water and in phosphate buffer saline pH 6.75, used for preparing calibration curve at analytical wave length of 246nm over a period of 72 hrs, was used as a means to study the stability of the solutions with time.

Minocycline hydrochloride was estimated in presence of other constituents used in the formulations (pluronic F127, carbopol 934P, PVA, PVP, HPMC, PAA) in the same concentrations in which they were included in the formulations, to obtain an understanding of the selectivity of the developed method for estimation of minocycline hydrochloride.

Table No. 3.01: Mean absorbance value, regressed value and statistical data of the calibration curve for the estimation of minocycline hydrochloride in distilled water

Concentration in mcg/ml	Mean Absorbance ±S.E.	Regressed value
1	0.04± 0.0012	0.041
3	0.1± 0.0021	0.1146
5	0.197± 0.0019	0.1882
10	0.365± 0.0023	0.3722
15	0.557± 0.0025	0.5562
20	0.735± 0.0018	0.7402
25	0.916± 0.0029	0.9242
30	1.112± 0.0037	1.1082

Regression equation **Y = 0.0368x + 0.0042

Correlation coefficient = 0.9996

* Mean of 6 values

** Using regression equation

n =48

Table No. 3.02: Mean absorbance value, regressed value and statistical data of the calibration curve for the estimation of minocycline hydrochloride in phosphate buffer saline pH 6.75

Concentration in mcg/ml	Mean Absorbance ±S.E.	Regressed value
1	0.053± 0.0012	0.0581
3	0.12± 0.0021	0.1259
5	0.197± 0.0019	0.1937
10	0.37± 0.0023	0.3632
15	0.542± 0.0025	0.5327
20	0.7± 0.0018	0.7022
25	0.874 ± 0.0029	0.8717
30	1.037 ± 0.0037	1.0412

Regression equation **Y = 0.0339x + 0.0242

Correlation coefficient = 0.9998

* Mean of 6 values

** Using regression equation

n =48

Figure 3.01: Absorptivity scan of minocycline hydrochloride in distilled water.

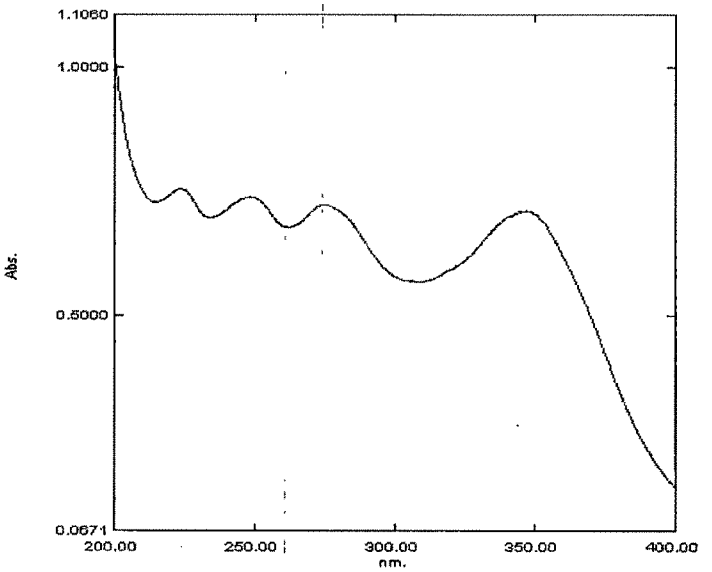
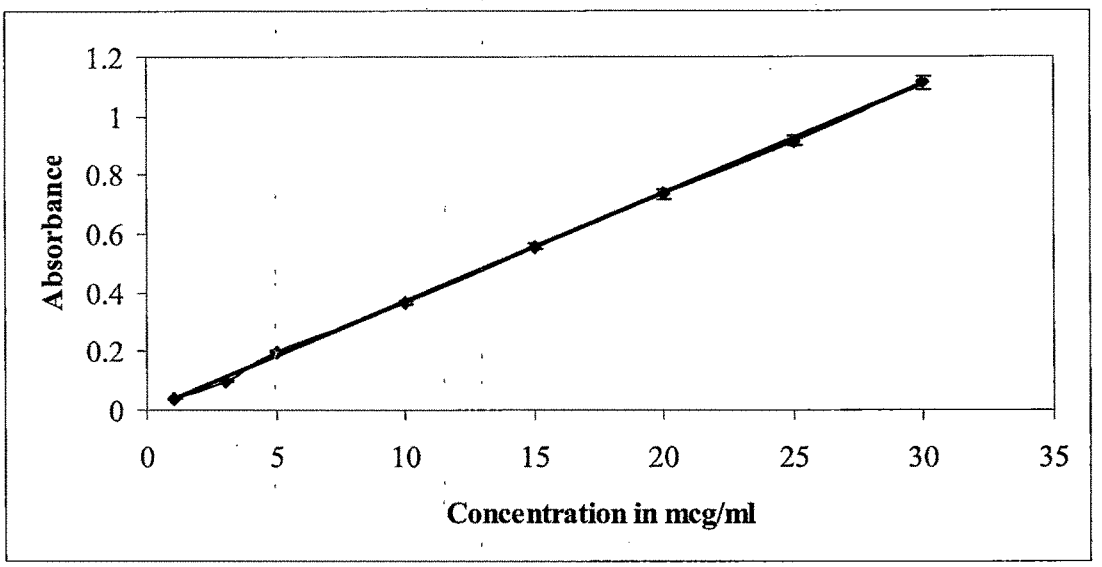


Figure 3.02: Calibration curve of minocycline hydrochloride in distilled water



n=6

Figure 3.03: Absorptivity scans of minocycline hydrochloride in PBS 6.75

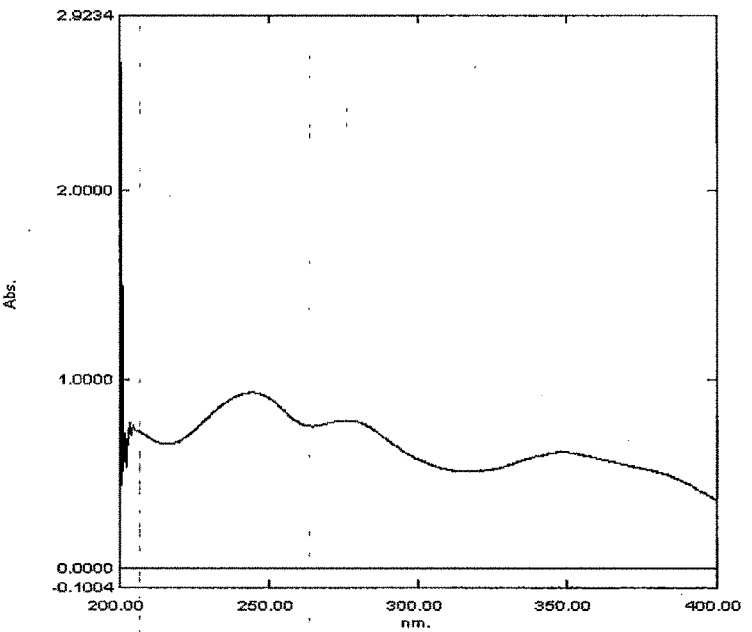
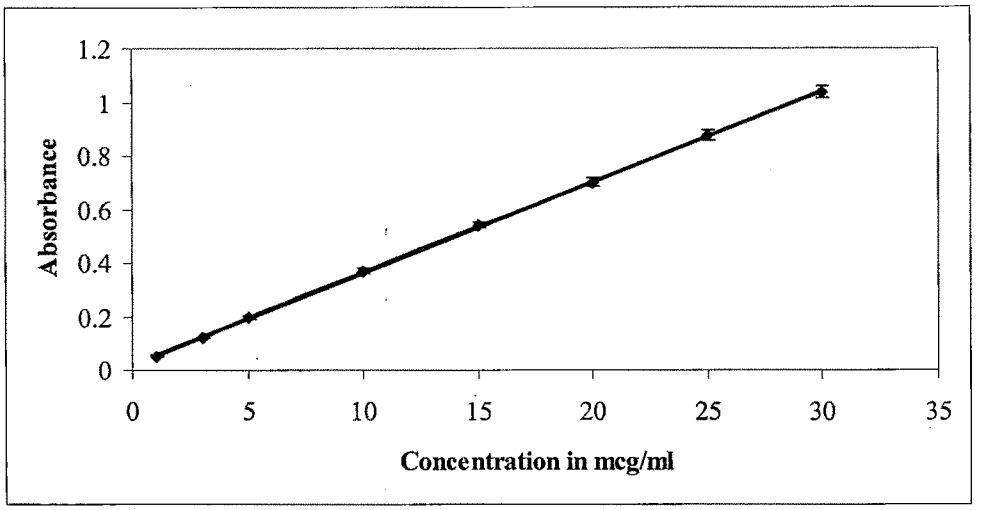


Figure 3.04: Calibration curve of minocycline hydrochloride in PBS 6.75



n=6

3.2.6 Estimation of clindamycin phosphate using UV- Visible spectroscopy

This method of estimation is based on the observation that clindamycin phosphate shows strong absorbance in both distilled water and phosphate buffer saline (pH 6.75) in the UV region of the electromagnetic spectrum.

3.2.6.1 Reagents and solutions

- Phosphate buffer saline (pH 6.75): About 2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 g of sodium chloride was dissolved in sufficient distilled water to produce 1000ml and adjusted with phosphoric acid to get pH 6.75.
- Clindamycin phosphate in distilled water and in phosphate buffer saline yields characteristic curves when scanned in the ultraviolet region between 200-400 nm. The λmax of clindamycin phosphate in both distilled water and in phosphate buffer saline is found to be at 210 nm.
- Stock solution of clindamycin phosphate (100μg/ml) in the distilled water and in phosphate buffer saline pH 6.75 was prepared by dissolving 10mg of clindamycin phosphate in 100ml of PBS pH 6.75.

3.2.6.2 Preparation of calibration curve

- Suitable aliquots of the stock solution of drug were pipette out and transferred separately into 10ml volumetric flasks. The volume was made up with the same solvent i.e. in the distilled water and in phosphate buffer saline pH 6.75 respectively. The solutions were shaken well and the absorbance of the resulting solutions were measured at 210nm using a Shimadzu UV-Visible spectrophotometer (UV-1601) with cells of 10mm length against the same solvent used as blank (Florey et al, 1981). The procedure was repeated for six times. Mean absorbance values along with regression values of clindamycin phosphate in distilled water and in PBS are shown in table no 3.03 and 3.04 respectively. The calibration curve in distilled water and in PBS pH 6.75 are shown in figure 3.06 and 3.08 respectively. Absorptivity scans over the wave length range of 200 to 400 nm of the solution of clindamycin phosphate is shown in figure 3.05 and 3.07 respectively.

3.2.7 Stability and selectivity

Changes in absorbance of the solutions of clindamycin phosphate in distilled water and in phosphate buffer saline pH 6.75, used for preparing calibration curve at analytical wave length of 210nm over a period of 72 hrs, was used as a means to study the stability of the solutions with time.

Clindamycin phosphate was estimated in presence of other constituents used in the formulations (pluronic F127, carbopol 934P, PVA, PVP, HPMC, PAA) in the same concentrations in which they were included in the formulations, to obtain an understanding of the selectivity of the developed method for estimation of clindamycin phosphate.

Table No. 3.03: Mean absorbance value, regressed value and statistical data of the calibration curve for the estimation of clindamycin phosphate in distilled water

Concentration in mcg/ml	Mean Absorbance ±S.E.	Regressed value
5	0.018 ± 0.0012	0.0185
10	0.036 ± 0.0019	0.0355
15	0.053 ± 0.0023	0.0525
20	0.07 ± 0.0016	0.0695
25	0.087 ± 0.0009	0.0865
30	0.103 ± 0.0025	0.1035

Regression equation **Y = 0.0034x + 0.0015

Correlation coefficient = 0.9997

* Mean of 6 values

** Using regression equation

n=36

Table No. 3.04: Mean absorbance value, regressed value and statistical data of the calibration curve for the estimation of clindamycin phosphate in phosphate buffer saline pH 6.75

Concentration in mcg/ml	Mean Absorbance ±S.E.	Regressed value
5	0.023 ± 0.0034	0.0224
10	0.041 ± 0.0026	0.0414
15	0.061 ± 0.0017	0.0604
20	0.08 ± 0.0015	0.0794
25	0.1 ± 0.0037	0.0984
30	0.118 ± 0.0026	0.1174

Regression equation **Y = 0.0038x + 0.0034

Correlation coefficient = 0.9998

* Mean of 6 values

** Using regression equation

n=36

Figure 3.05: Absorptivity scan of clindamycin phosphate in distilled water

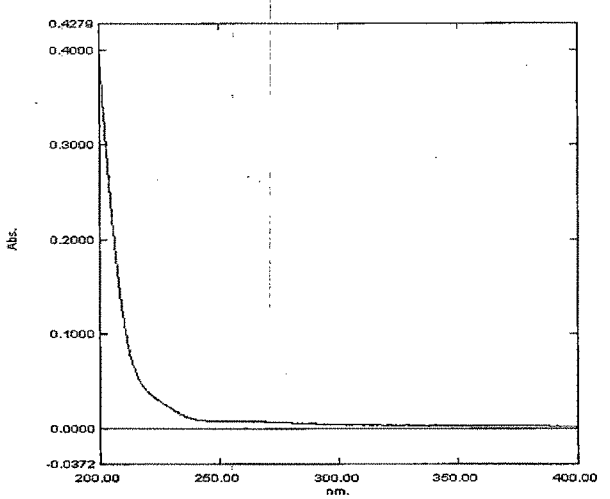


Figure 3.06: Calibration curve of clindamycin phosphate in distilled water

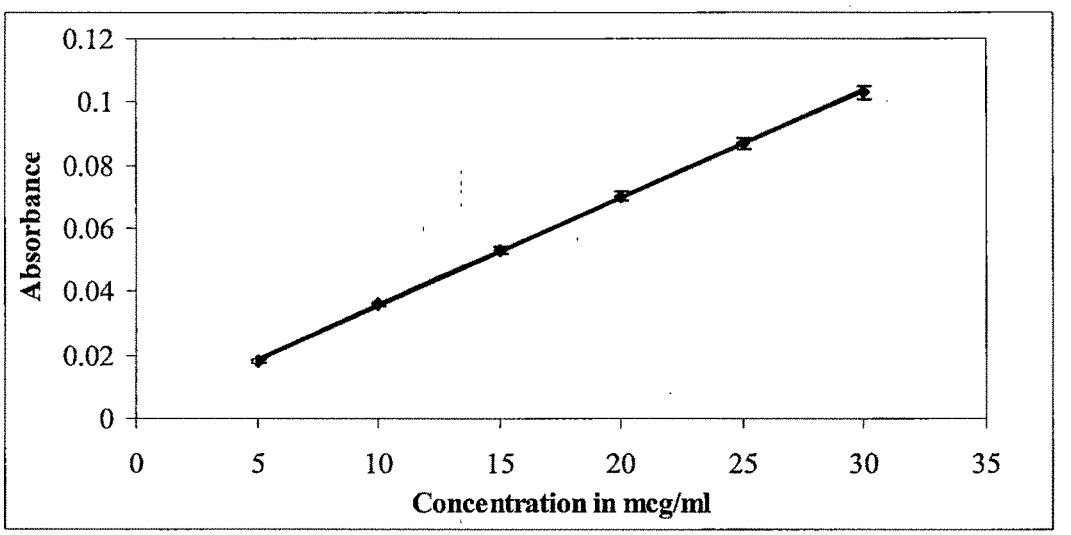


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Figure 3.07: Absorptivity scan of clindamycin phosphate in PBS 6.75

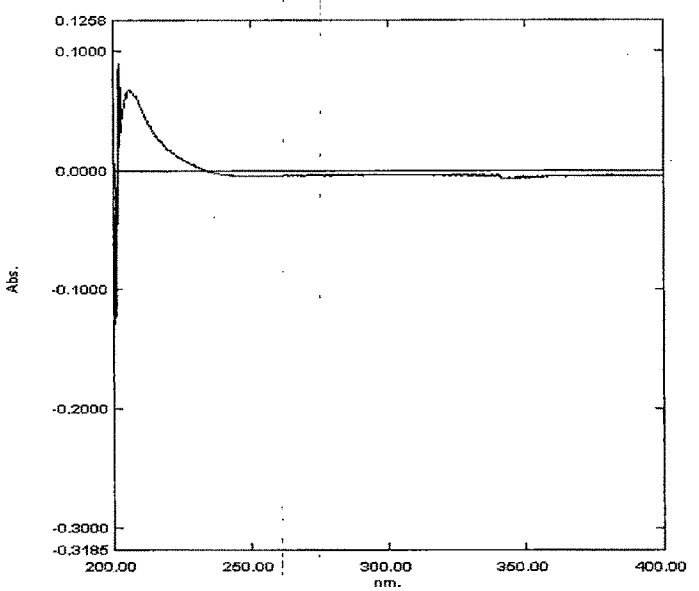
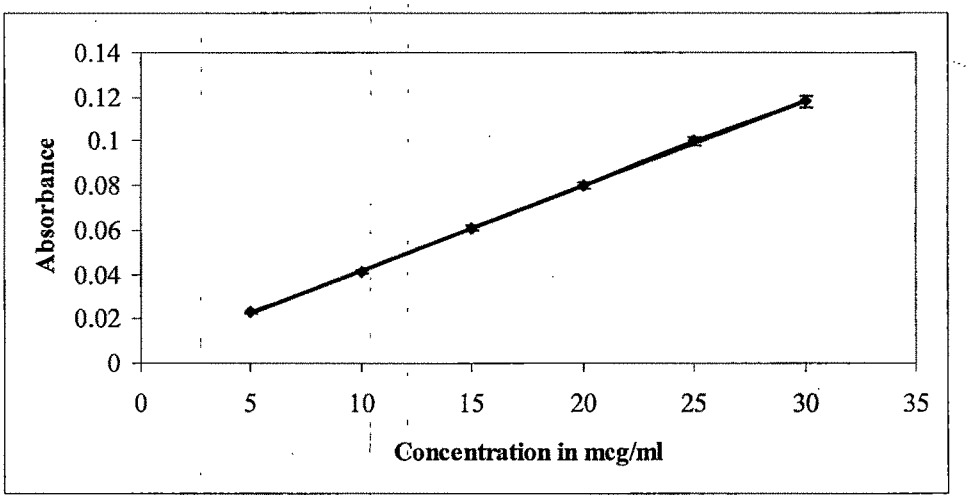


Figure 3.08: Calibration curve of clindamycin phosphate in PBS 6.75



n=6

3.3 RESULTS AND DISCUSSION

In distilled water and phosphate buffer saline pH 6.75, minocycline hydrochloride yield characteristic curves when scanned in UV- Visible wave length in the range between 200-400 nm. However, as the absorptivity at the wave length 246nm was found to be satisfactory the same was selected as the analytical wave length and used for further studies.

However, in distilled water and phosphate buffer saline pH 6.75, clindamycin phosphate also yield characteristic curves when scanned in UV- Visible wave length in the range between 200-400 nm of which, the absorptivity at the wave length 210nm, was found to be satisfactory and was selected as the analytical wave length for further studies for clindamycin phosphate.

The high correlation coefficients (table no.3.01 and 3.02) in both distilled water and in phosphate buffer saline pH 6.75 in case of minocycline hydrochloride indicated that absorbance and concentration are linearly related. Beer's law was found to be obeyed in the range of 1.0-20.0 µg/ml in distilled water (table no.3.01) and in the range of 1.0-25µg/ml in phosphate buffer saline pH 6.75 (table no.3.02). Regression analysis of the experimental data was carried out and is shown in table no.3.01 and 3.02.

As shown in the data from table no. 3.03 and 3.04, the high correlation coefficients of clindamycin phosphate in both distilled water and in phosphate buffer saline pH 6.75 indicated that absorbance and concentration are linearly related. Beer's law was found to be obeyed in the range of 5.0-30 µg/ml in distilled water (table no.3.05) and in the range of 5.0-30µg/ml in phosphate buffer saline pH 6.75 (table no.3.06). Regression analysis of the experimental data was carried out and is shown in table no. 3.07and 3.08.

The stability of both minocycline hydrochloride and clindamycin phosphate was done in both distilled water and in phosphate buffer saline pH 6.75 and was ascertained over a period of 72 hrs. Analysis of variance (ANOVA) of the mean absorbance values of both the solutions of different concentrations at various time intervals revealed that there was no significant difference between the readings. From this it was concluded that minocycline hydrochloride and clindamycin phosphate are stable over the determined time period in both the solvents.

Estimation of both the drugs were carried out in presence of other constituents used in the formulations such as pluronic F127, carbopol 934P, PVA, PVP, HPMC, PAA, etc. at appropriate levels in which they were present in the final formulations. None of the materials interfered in the estimation of minocycline hydrochloride/ clindamycin phosphate using the above methods.

3.4 REFERENCES

Vladimir Zbinovsky and George P. Chrekian , Minocycline hydrochloride, In Analytical profile of drug substances, 6th Edn., Florey K., Academic Press, New York,1977.

Leo W. Brown and William F. Beyer, Clindamycin phosphate, In Analytical profile of drug substances, 10th Edn., Florey K., Academic Press, New York, 1981.

2006 USPC, Inc. Official 1/1/06- 3/31/06 USP Monographs: Clindamycin phosphate; page 539.

2005 USPC, Inc. Official 8/1/05- 12/31/05 USP Monographs: Minocycline hydrochloride; page 1298.