CHAPTER 3

ANALYTICAL METHODS (BUTORPHANOL TARTRATE)

3.0 Analytical Methods (Butorphanol Tartrate)

The analytical methods developed for testing of Butorphanol Tartrate (BT) in bulk powder and extended release formulation of are employed in this section. Table 1 - 3 and 2 - 3 show list of material and equipment used respectively. Method for Assay, content uniformity, dissolution studies and determination of drug during in-vivo studies are discussed in this section.

Sr. No.	Material	Source
1.	Butorphanol Tartrate	Theraquest Bioscience Corporations
2.	Dichloromethane	Merck Limited, Mumbai, India
3.	Methanol	Merck Limited, Mumbai, India
4.	Acetone	Merck Limited, Mumbai, India
5.	Isopropyl alcohol	Merck Limited, Mumbai, India
6.	Glacial acetic acid	S. D. Finechem Limited, Mumbai, India
7.	Hydrochloric acid	S. D. Finechem Limited, Mumbai, India
8.	Potassium dihydrogen phosphate	S. D. Finechem Limited, Mumbai, India
9.	Sodium hydroxide	S. D. Finechem Limited, Mumbai, India
10.	Sodium lauryl sulphate	S. D. Finechem Limited, Mumbai, India
11.	HPLC grade Methanol, Acetonitrile, Acetic acid	S. D. Finechem Limited, Mumbai, India
12.	Ethanol (99.5%V/V)	Baroda Chem. Ind. Ltd., Baroda, India
13.	Water (distilled)	Prepared in laboratory by distillation

Table 1 - 3 List of materials

	Equipments	Source/Make
1.	Digital weighing balance	AG-64, Mettler Toledo, Switzerland
2.	pH meter	Mettler Toledo, Switzerland
3.	Bath sonicator	DTC 503, Ultra Sonics
4.	HPLC system	LC 20-AT prominence, Shimadzu Corp., Japan

5.	UV-Visible Spectrophotometer	Shimadzu UV-1601, Japan
6.	Calibrated pipettes of 1.0 ml, 5.0 ml and 10.0 ml.	Schott & Corning (India) Ltd., Mumbai
7.	volumetric flasks of 10 ml, 25 ml, 50 ml and 100 ml capacity.	Schott & Corning (India) Ltd., Mumbai
8.	Funnels (i.d. 5.0 cm)	Schott & Corning (India) Ltd., Mumbai
9.	Beakers (250 ml) and other requisite glass wares	Schott & Corning (India) Ltd., Mumbai
10.	Nuclepore Polycarbonate membrane 2 µm 25mm	Whatman, USA

Table 2 - 3 List of Equipments

3.1. Preparation of reagents and buffers

3.1.1 Preparation of Simulated Gastric Fluid (SGF - pH 1.2)

2 gm of sodium chloride and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 8.5 ml of concentrated hydrochloric acid was added and the volume was adjusted with distilled water upto 1000 ml. pH was adjusted to 1.2. (USP 30, 2007).

3.1.2 Preparation of Acetate Buffer (pH 4.5)

2.99 gm of sodium acetate trihydrate and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 14 ml of acetic acid solution was added and the volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

3.1.3 Preparation of Simulated Intestinal Fluid (SIF - pH 6.8)

250 ml of 0.2 M potassium phosphate monobasic was placed in a 1000 ml volumetric flask, 112 ml of 0.2 M sodium hydroxide was added and volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

3.2. Estimation of Butorphanol tartrate

3.2.1. Estimation of Butorphanol tartrate in SGF pH 1.2, SIF pH 6.8 & PBS 4.5

Butorphanol tartrate shows strong absorbance in UV-Visible region. Hence, the estimation of Butorphanol tartrate was performed by UV-visible spectrophotometry. A UV-visible spectroscopic method for estimation of Butorphanol Tartrate for assay, uniformity of drug

content and *in-vitro* drug release in formulation was developed. Estimation method for the assay, uniformity of drug content was developed in water and method for *in-vitro* drug release was developed in SGF pH 6.8, SIF pH 1.2 & PBS pH 4.5.

3.2.1.1 Preparation of standard stock solutions of Butorphanol tartrate in SGF pH 1.2

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of SGF pH1.2 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with SGF pH1.2 to produce 1000 µg per ml of Butorphanol tartrate.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with SGF pH1.2 to prepare stock solution of 250 µg per ml of Butorphanol tartrate

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3.2.1.2 Calibration curve of Butorphanol tartrate in SGF pH 1.2

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 1, 2.5, 6.25, 12.5, 25, 50, 75, 100, 150, 200 µg/ml and analyzed by UV spectrophotometry at 280nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 1-3 show the linearity, of Butorphanol Tartrate in SGF pH 1.2, results are tabulated in table 3-3.

Concentration (µg/ ml)	**Average***	**SD**	**RSD**
1	0.0160	0.0002	1.2471
2.5	0.0320	0.0001	0.3622
6.25	0.0438	0.0006	1.3772
12.5	0.0581	0.0004	0.6891
25	0.1114	0.0010	0.9341
50	0.2232	0.0003	0.1544

75	0.3215	0.0004	0.1178
100	0.4238	0.0008	0.1885
150	0.6422	0.0008	0.1195
200	0.8437	0.0014	0.1655

Table 3 - 3 Calibration for Butorphanol tartrate in SGF pH 1.2

Regression equationY= 0.0042X + 0.0122; Correlation coefficient = 0.9999

*Mean of 3 values

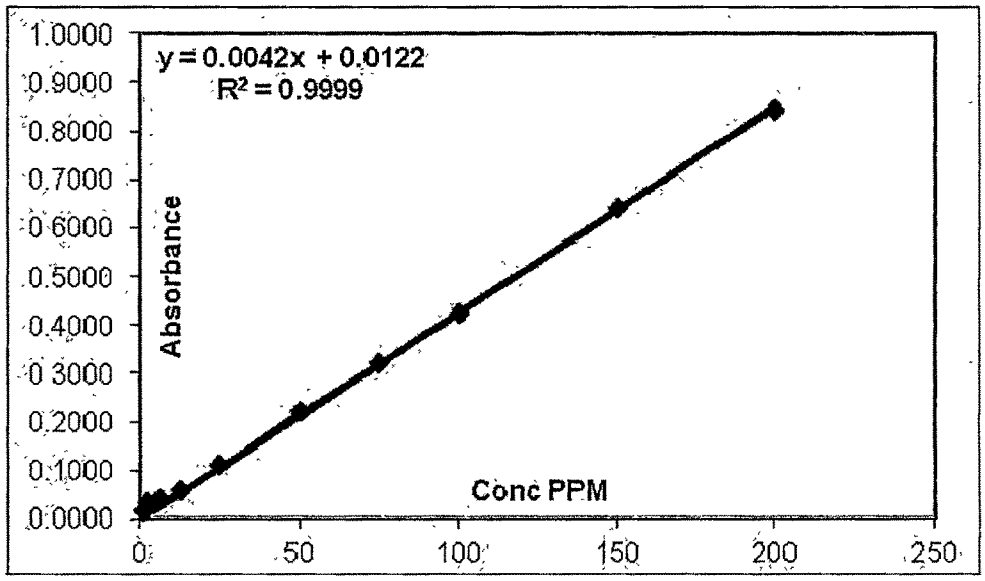

Figure 1 - 3 Calibration curve for estimation of Butorphanol tartrate in SGF pH 1.2

Accuracy and repeatability

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of butorphanol tartrate in SGF pH 1.2 is shown in Table 4 - 3.

In order to determine the accuracy of the developed method, known amounts of Butorphanol Tartrate (20µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (20, 100, 200 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 4 -3.

Conc. of BT (µg/ml) Std.	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
1.00	1.0	103.4	0.0230	0.0103	1.0 $\pm$ 0.0642	2.23	2.81
100	100.3	100.3	0.1914	0.0856	100.3 $\pm$ 0.5336	0.19	0.80
200	201.9	101.0	1.2571	0.5622	201.9 $\pm$ 3.5039	0.62	0.41

Table 4 - 3 Evaluation of accuracy and repeatability of the estimation method of BT in SGF pH 1.2

** At 95% Confidence level; t_{tab} = 3.18 for 4 degrees of freedom (n=5)*

3.2.1.3 Preparation of standard stock solutions of Butorphanol tartrate in SIF pH 6.8

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of SIF pH6.8 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with SIF pH 6.8 to produce 1000 µg per ml of Butorphanol tartrate. 25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with SIF pH 6.8 to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

3.2.1.4 Calibration curve of Butorphanol tartrate in SIF pH 6.8

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 100 ml with release media to give final concentrations of 3.125, 6.25, 12.5, 25, 50, 75, 100, 150, 200, 250 µg/ml and analyzed by UV spectrophotometry at 280nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed

analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Figure 2-3 show the linearity, of Butorphanol Tartrate in SIF pH 6.8, results are tabulated in table 5 - 3.

Concentration (µg/ ml)	**Average***	**SD**	**RSD**
3.125	0.0221	0.0010	4.5256
6.25	0.0460	0.0007	1.5584
12.5	0.0591	0.0010	1.6091
25	0.1200	0.0011	0.9177
50	0.2010	0.0017	0.8343
100	0.4050	0.0033	0.8026
150	0.6010	0.0040	0.6572
200	0.7990	0.0036	0.4549
250	0.9850	0.0035	0.3517

Table 5 - 3 Calibration for Butorphanol tartrate in SIF pH 6.8

Regression equation** Y= 0.0039X + 0.0141; Correlation coefficient = 0.9998

*Mean of 3 values

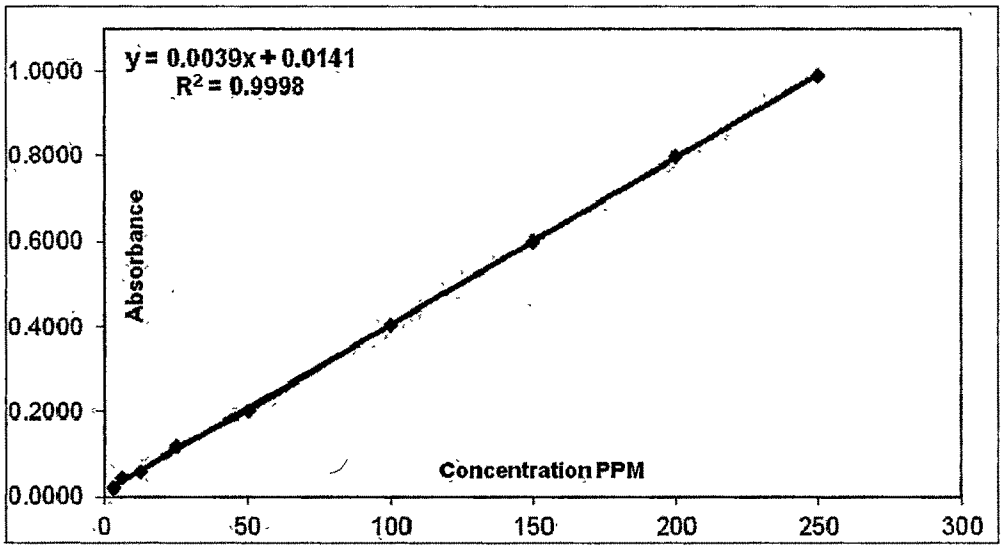

Figure 2- 3 Regressed calibration curve for estimation of Butorphanol tartrate in SIF pH 6.8

Accuracy and Precision

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate in SIF pH 6.8 is shown in Table 6 - 3.

In order to determine the accuracy of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 100, 200 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 6 - 3.

Conc. of BT (µg/ml) Std.	AVG Recovery (µg/ml)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
10	10.1	101.3	0.0283	0.0126	10.1 + 0.0788	0.28	0.74
100	100.3	100.3	0.1914	0.0856	100.3 + 0.5336	0.19	0.21
200	200.2	100.1	0.1002	0.0448	200.2 + 0.2794	0.05	0.09

Table 6 - 3 Evaluation of accuracy and precision of the estimation method of BT in PBS 6.8

** At Alphaa 0.05 Confidence level; t_{tab} = 3.18 for 4 degrees of freedom*

3.2.1.5 Preparation of standard stock solutions of Butorphanol tartrate in acetate Buffer pH 4.5

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of acetate Buffer pH 4.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and

then the final volume of the flask was made up to 50 ml with acetate Buffer pH 4.5 to produce 1000 µg per ml of Butorphanol tartrate.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS pH 4.5 to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

3.2.1.6 Calibration curve of Butorphanol tartrate in acetate buffer pH 4.5

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 20, 25, 50, 75, 100, 125, 150, 175, 200 µg/ml and analyzed by UV spectrophotometry at 280nm. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figures 3 – 3 show the calibration curve of Butorphanol Tartrate in acetate buffer pH 4.5, the values are tabulated in table 7 – 3.

Concentration (µg/ ml)	**Average***	**SD**	**RSD**
20	0.1292	0.0010	0.7753
25	0.1434	0.0007	0.5000
50	0.2291	0.0010	0.4149
75	0.3448	0.0011	0.3195
100	0.4373	0.0017	0.3836
125	0.5550	0.0033	0.5856
150	0.6574	0.0017	0.2551
175	0.7599	0.0036	0.4784
200	0.8424	0.0010	0.1128

Table 7 - 3 Calibration for Butorphanol tartrate in acetate buffer pH 4.5

Regression equation** Y= 0.0041X + 0.0392; Correlation coefficient = 0.999

*Mean of 3 values

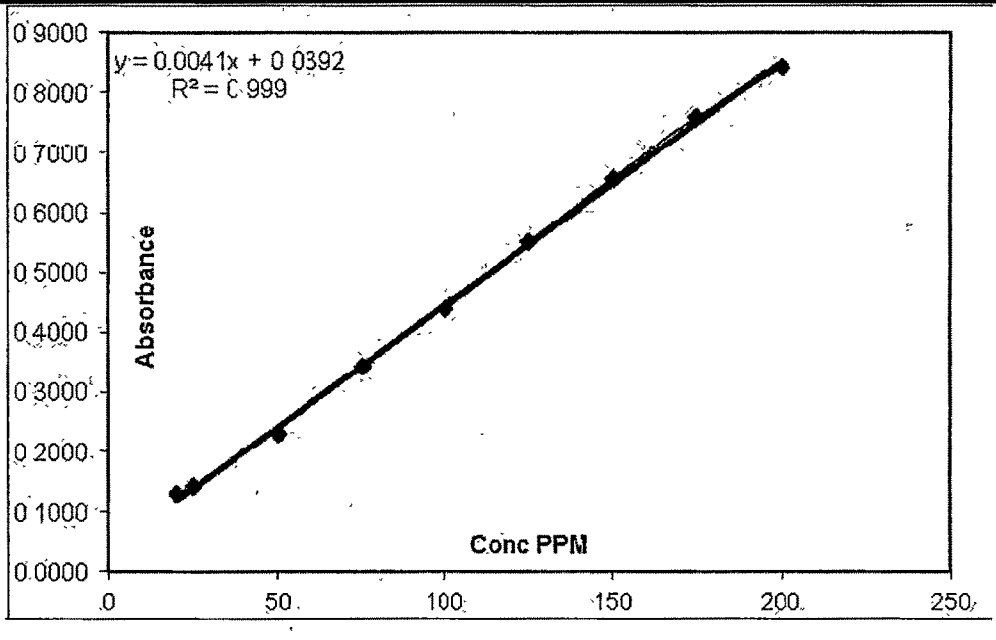

Figure 3 - 3 Regressed calibration curve for estimation of Butorphanol tartrate in acetate buffer pH 4.5

Accuracy and Precision

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate in acetate buffer pH 4.5 is shown in Table 8 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 3.3.

Theoretical Conc of BT (µg/mL)	AVG Recovery (µg/mL)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
20	20.1	100.7	0.1741	0.0779	20.1 ± 0.3254	0.86	0.91
100	100.7	100.7	0.3401	0.1521	100.7 ± 0.6355	0.34	0.80
200	201.4	100.7	0.8833	0.3950	201.4 ± 1.6506	0.44	0.41

Table 8 - 3 Evaluation of accuracy and precision of the estimation method of BT in acetate Buffer 4.5

** At Alphaa 0.05 Confidence level; t_{tab} = 3.18 for 4 degrees of freedom*

3.2.1.7 Preparation of standard stock solutions of Butorphanol tartrate in water

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of water was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with water to produce 1000 µg per ml of Butorphanol tartrate. 25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with water to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

3.2.1.8 Calibration curve of Butorphanol tartrate in water

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 10, 50, 100, 150, 200, 250 µg/ml and analyzed by UV spectrophotometry at 280nm. The above procedure was repeated three times. The data was recorded in Table 3.2 along with standard deviation. Figures 4 - 3 calibration curve of Butorphanol tartrate in water, the values are tabulated in table 9 -3.

Concentration (µg/ ml)	Average*	SD	RSD
10	0.0590	0.0004	0.6421
50	0.2289	0.0008	0.3491

100	0.4404	0.0008	0.1743
150	0.6516	0.0014	0.2143
200	0.8608	0.0003	0.0300

Table 9 - 3 Calibration curve of butorphanol Tartrate in water

Regression equation** Y= 0.0042X + 0.0190; Correlation coefficient = 1.0000

*Mean of 3 values

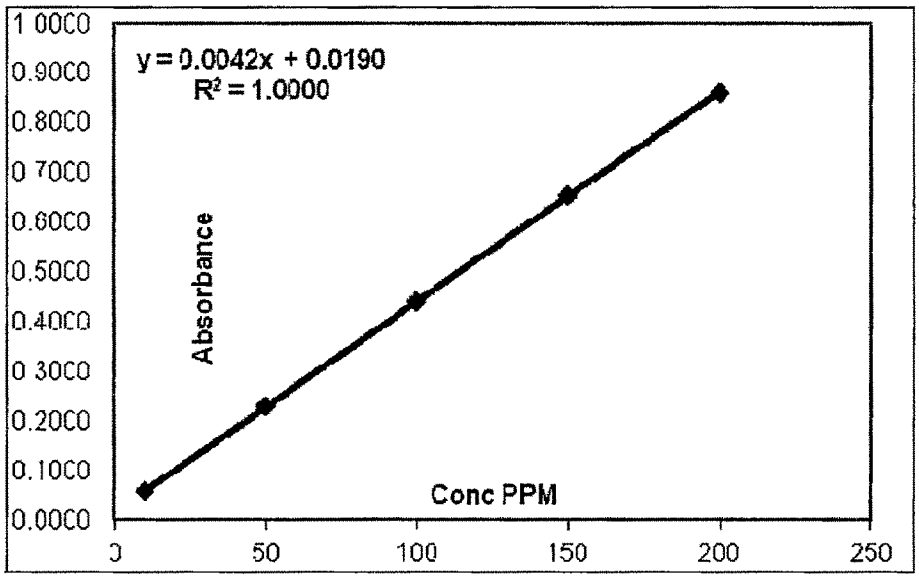

Figure 4 - 3 Regressed calibration curve for estimation of Butorphanol tartrate in water

Accuracy and Precision

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol tartrate in water is shown in Table 10 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 250µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 10.3.

Theoretical Conc of BT (µg/mL)	AVG Recovery (µg/mL)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
10	10.1	100.7	0.0891	0.0398	10.1 ± 0.1664	0.88	0.85
100	100.6	100.6	0.3750	0.1677	100.6 ± 0.7008	0.37	0.80
200	201.4	100.7	0.8833	0.3950	201.4 ± 1.6506	0.44	0.41

Table 10 - 3 Evaluation of accuracy and precision of the estimation method of BT in water

** At Alphaa 0.05 Confidence level; t_{tab} = 3.18 for 4 degrees of freedom*

3.3 Estimation of BT in Formulation for Assay

To determine the amount of BT in the Coated Tablet, 20 tablets were crushed and added to 100 ml of water and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients for extraction of the drug from the formulation. The filtered supernant was further diluted with water and estimated at 280 nm.

3.4 Estimation of BT in formulation for content uniformity

To determine the amount of BT in the Coated Tablet, 10 tablets were crushed individually and added to 100 ml of water and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients for extraction of the drug from the formulation. The filtered supernant was further diluted with water and estimated at 280 nm.

3.5 Estimation of BT for in-vitro release

The release studies for BT coated formulation in different release media. One tablet containing 10mg drug was placed in dissolution vessel containing 250 ml of release medium maintained using paddle at 50 RPM at 37 ± 2 °C. 5 ml aliquots were taken out at different time and replace with same quantity of release media. The dissolved drug in release medium analyzed as per the method above. The amount of the drug released and cumulative percentage release was calculated.

The spectrophotometeric determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Butorphanol tartrate the non-interference with Butorphanol tartrate indicates the specificity of the methods. Stability of the Butorphanol Tartrate in the solution was demonstrated to be stable in solvent during the period of 24 h since the change in the λ_{max} was not significant with the RSD value.

3.5.1 Test for alcohol dose dumping

In order to study the effect of alcohol on drug release or to verify weather alcohol leads to dose dumping 4 % V/V, 20 % V/V and 40 %V/V alcohol was added in the release media and drug release was verified against control (without alcohol).

3.5.2 Mechanism of drug release

To ensure the major mechanism of drug release, release studies of the optimized formulation were conducted in media of varying concentration of osmotically active solute. To increase the osmotic pressure of the release media, sodium chloride was added in dissolution medium because it's saturated solution produces osmotic pressure in the range of 356 atm. The pH of the medium was adjusted to 6.8 ± 0.05. Release studies were carried out in 250 ml of media using USP II dissolution test apparatus (50 rpm). Release profiles of the optimized formulations at different concentration of osmotically active agent was plotted and compared.

3.5.3 Multimedia Testing

The optimized formulation was subjected to in vitro release studies in buffers with different pH like SGF pH 1.2, SIF pH 6.8 and acetate buffer pH 4.5 for multimedia testing.

3.5.4 Effect of agitation intensity

To study the effect of agitation intensity of the release media, release studies of the optimized formulation of CPOP were carried out in USP dissolution apparatus type II at varying rotational speed (50, 100 and 150 rpm).

3.5.5 HPLC method for estimation of Butorphanol Tartrate

The plasma concentration of the butorphanol was determined by extraction and HPLC method. For determination of linearity 100 µg of Butorphanol Tartrate was accurately

weighed using single pan electronic balance and transferred to Mathanol to produce primary standard of Butorphanol, which was used to make working standard solutions of Butorphanol Tartarte by diluting primary standard solution with Mathanol. Rat plasma calibration standards (1.00, 5.00, 15.0, 20 µg/ml) of Butorphanol were prepared by spiking the working standard in to the drug free rat plasma. The samples were aliquoted (300 µL) into polypropylene tubes and stored at -20° C until analysis. 0.3 ml rat plasma was mixed with 1.0 ml 1N NaOH, and 7 ml chloroform. The samples were vortexed for 20 seconds, followed by centrifugation. The upper aqueous layer was aspirated to waste. The organic layer was transferred to a conical centrifuge tube and evaporated to dryness. The samples were reconstituted in 0.2 mL mobile phase to get 1, 5 15, 20 µg per ml concentration. 10 µl of the said stock was injected in HP in following condition. Butorphanol plasma standards were analyzed simultaneously with the samples.

Method Type : Isocratic System Method

Mobile Phase :Ammonium Acetate 0.05 M: Acetonitrile (3:1) adjusted pH to 4.1 with glacial acetic acid

Guard Column : Required. (For Plasma Analysing)

Column : Phenomenex - Luna 5µ C 18 (2) 100 A 250 x 4.60 mm x 5 µ (Size)

Flow Rate : 1.5 ml/min

λ_{max} : 280 nm

Thermostat : Not required (Room temperature)

Retention time:7.5 min

The mobile phase was prepared freshly, filtered through a 0.45 µm membrane filter.

Concentration (µg/ml)	Area µV .S	RSD
1	1134.0	1.0494
5	1092.1	0.8516
15	37703.0	0.6419
20	50722.0	0.5560

Table 11- 3 Calibration curve of Butorphanol tartrate using HPLC

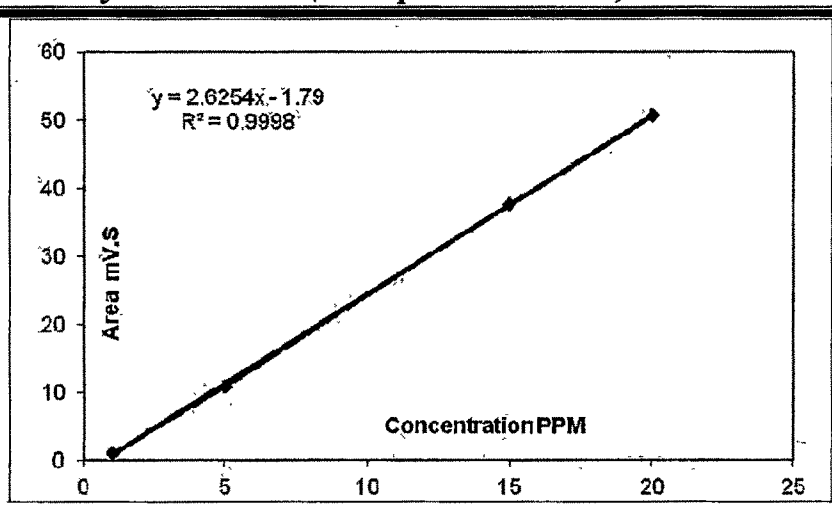

Figure 5 - 3 Regressed calibration curve for estimation of Butorphanol tartrate using HPLC

Figures 5 - 3 calibration curve of Butorphanol tartrate in using HPLC, the values are tabulated in table 11 -3.

Accuracy and Precision

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate is shown in table 12 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (1µg/mL, 5µg/mL and 20µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in following table.

Theoretical Conc of BT (µg/mL)	AVG Recovery (µg/mL)	% Recovery	SD	RME	Confidence	RSD Intra Day	RSD Inter Day
1	1.0	99.5	0.0098	0.0044	0.0184	0.99	0.84
5	5.0	101.0	0.0476	0.0213	0.0889	0.94	0.81
20	20.0	100.2	0.1477	0.0661	0.2760	0.74	0.76

Table 12 - 3 Evaluation of accuracy and precision of the estimation method of BT using HPLC

** At Alphaa 0.05 Confidence level; $t_{tab} = 3.18$ for 4 degrees of freedom*

The developed isocratic high performance liquid chromatographic method was rapid and suitable for the estimation of Butorphanol Tartrate in plasma. Specificity, linearity, precision, accuracy and robustness were verified. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method, the amount of Butorphanol Tartrate in plasma was determined. The chromatographic determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Butorphanol the non-interference with Butorphanol Tartrate indicates the specificity of the methods. Stability of the Butorphanol Tartrate in solution Butorphanol Tartrate was verified and found to be stable in solvents during the period of 24 h since the change in the λ_{max} was not significant with the maximum RSD value of 0.84%. It was also confirmed that retention time of Butorphanol Tartrate not shifted with the adjustment of the proportion of acetonitrile and the flow rate. But the final result did not show significant change. Considering the stability in the system suitability parameters, the method conditions would be concluded to be robust.

3.6 SEM Analysis

In order to elucidate the mechanism of drug release from the developed formulations, surface of coated tablets before and after dissolution studies, was studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12 mm diameter) with a double backed adhesive tape. Small sample of the coating membrane was carefully cut from the exhausted shells (after dissolution studies) and dried at 50°C for 2 h. Membranes were dried at 45-C for 12 hours and stored between sheets of wax waper in a dessicator until examination. The morphology of the exhausted osmotic pump shell was analyzed using SEM (Surface Electron Microscopy). The mounted samples were examined under SEM (JSM-6360, Jeol, Japan).

3.7 Discussion

The DSC of samples was carried out by scanning the samples using differential scanning colorimeter (Mettler) for pre-formulation studies to find out any incompatibility in advance.

The UV spectroscopic method was developed for the BT estimation in SGF pH 1.2, SGF pH 6.8, Acetate Buffer pH 4.5 and water. The measurement was done at λ_{max} 280 nm for all four solvents. There was no interference observed with any excipient used. The methods were validated for linearity, accuracy and repeatability. The validation parameters were found to meet the "readily pass criteria" specified in the USP and % RSD were found less than 1%.

The absorbance for BT in SGF pH 1.2 was found to be linear in the range of 1 - 200 µg /ml with r^2 value of 0.9999. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in SIF pH 6.8 was found to be linear in the range of 3.125 - 250 µg /ml with r^2 value of 0.9990. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in acetate buffer pH 4.5 was found to be linear in the range of 20 - 200 µg /ml with r^2 value of 0.9998. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in water was found to be linear in the range of 10 - 200 µg /ml with r^2 value of 1.0000. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The invitro release study was performed using type II dissolution apparatus using 250 ml release medium. At different time intervals, the samples were removed, replaced with same medium and analysed for the drug. The cumulative percentage drug released was calculated.

For Butorphanol Tartrate, the calibration curve was established using HPLC for estimation of drug in plasma with Ammonium Acetate 0.05 M: Acetonitrile (3:1) adjusted pH to 4.1 with glacial acetic acid as mobile phase and detection at 280nm. The linearity of Temozolomide was found to be 1-20 µg/ml (R^2=0.9998). The recovery studies for accuracy and precision were carried out at 1, 5 and 20 µg/ml and The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. 1 µg/ml of drug was spiked in plasma as concentration of the drug in plasma was below the quantification limit.

3.8 References

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