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Estimation of Thienorphine Drug present in PLGA microspheres using RP-HPLC Method

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Abstract: A reverse phase isocratic high performance liquid chromatographic method was developed for the estimation of thienorphine drug in microsphere formulation. The separation was achieved by C_{18} column (250mm×4.6mm, 5µm), a mixture of Acetonitrile- Methanol- Dihydrogen phosphate buffer pH3.0 (40:15:45) as mobile phase, at flow rate of 1.0ml/min. Detection was carried out at 220nm. Retention time of Thienorphine was found to be 4.3 min +or -0.5min. The mean recovery obtained for was 100.8%. Developed method was found to be accurate, precise, selective and rapid for estimation of Thienorphine drug in microspheres.

Key words: Thienorphine, Method development and Validation, HPLC.

Introduction:

Thienorphine[N-cyclopropylmethyl-7(-[(R)-1-hydroxy -1-methyl-3-(thien-2-yl)-propyl]-6,14-endo-ethano-tetrahydronororipavine] is a new compound **(Fig.1),** synthesized by our institute. The pharmacology studies showed that thienorphine is a potent, long-acting partial opioid agonist and may have a possible application in treating addiction[1].

As an analog of buprenorphine, thienorphine is a partial agonist of the µ-opioid receptor, as is buprenorphine[2], which has been widely used in the therapy of opioid addiction[3, 4].

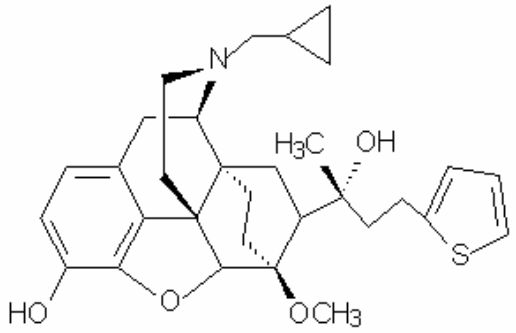

Figure 1: The chemical structures of thienorphine

Like buprenorphine, it has been reported that thienorphine is bound potently and non-selectively to μ-, δ-, and κ-opioid receptors stably expressed in CHO (Chinese hamster ovary) cells and behaved as a partial agonist at the μ-opioid receptor. Inspired by the result, we prepared the thienorphine loaded PLGA microspheres[5]. Our objective was to develop a controlled release system that is effective for a period of 1 month. Liquid chromatographic-tandem mass spectrometric method for the determination of the thienorphine in rat and beagle dog plasma was developed[6]. Till the date there is no HPLC method available for analysis of thienorphine.

Material and Methods:

Apparatus: High Performance Liquid Chromatographic system (Shimadzu) equipped with LC-10AT VP liquid pumps and UV detector, C_{18} column (250mm×4.6mm, 5μm, Phenomenex). Analytical balance (sartorius), pH meter (polmon) and Millie Q with (0.45. micron) filters for HPLC grade water.

Material: water HPLC grade, Acetonitrile and Methanol purchased from Caledon chemicals. Thienorphine Working standard and thienorphine PLGA microspheres were supplied by Beijing Institute of Pharmacology and Toxicology. All other materials or solvents were of reagent or analytical grade.

Chromatographic condition:

Equipment : Shimadzu HPLC and UV detector
Column : C_{18} column (250mm×4.6mm, 5μm, Phenomenex)
Flow rate : 1ml per min
Wavelength : 220nm
Injection volume: 20μl
Column oven : Ambient

Preparation of Phosphate buffer: Weigh 2.72 grams of Potassium Dihydrogen Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with mille Q water. Adjust the pH to 3.0 with Phosphoric acid.

Preparation of mobile phase: Mix a mixture of above buffer 450ml (45%), 400ml of Acetonitrile (40%) and 150ml of Methanol (15%) and degas in ultrasonic water bath for 5minutes. Filter through 0.45μm filter under vacuum filtration.

Standard Solution Preparation: Accurately weigh and transfer 10mg of thienorphine working standard into a 100ml volumetric flask add about 20ml of Acetonitrile and sonicate to dissolve it completely and make volume up to the mark with the diluents (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45μm filter.

Sample Solution Preparation: Accurately weigh and transfer the sample equivalent to 20mg of thienorphine PLGA microspheres into a 25ml volumetric flask. Add about 5ml of Acetonitrile and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45μm filter. Further pipette 1ml of the above filtrate into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45μm filter.

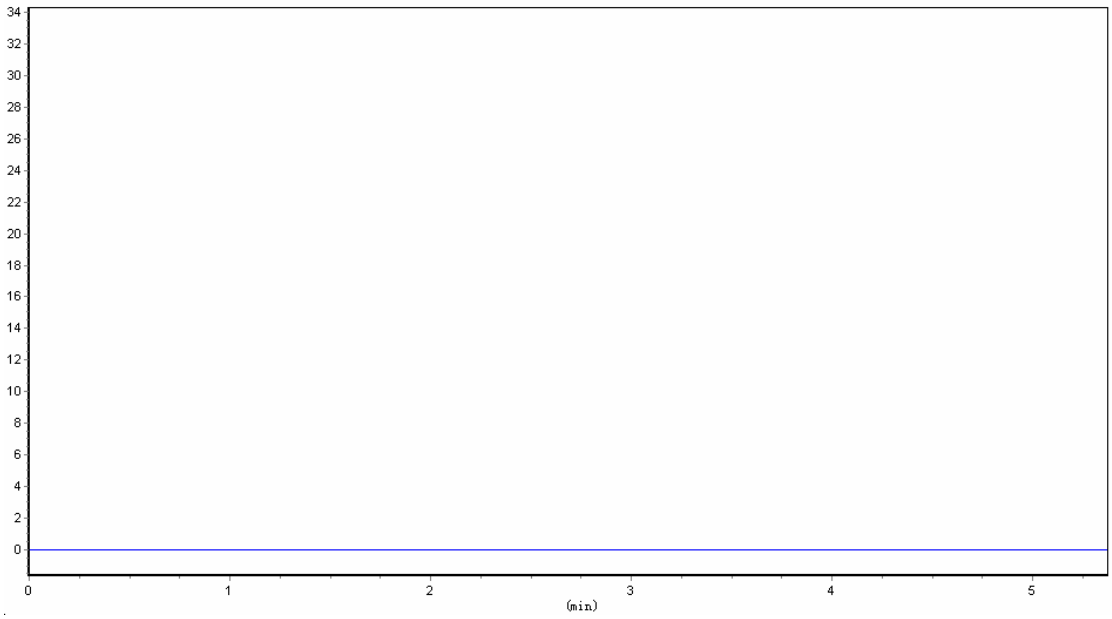

Figure 2: Chromatogram for blank

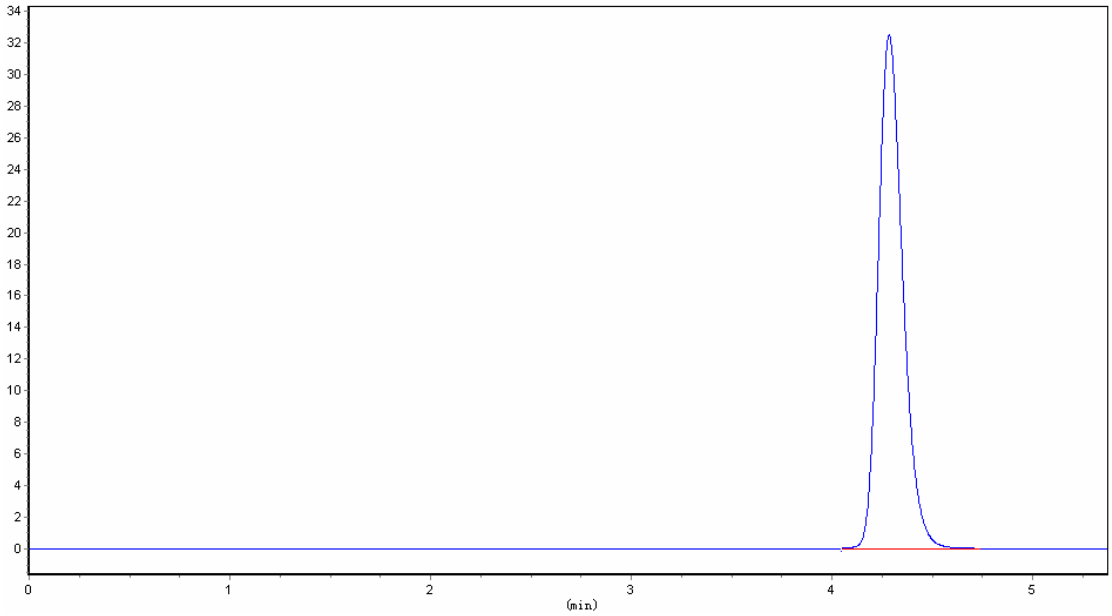

Figure 3: Chromatogram of Standard

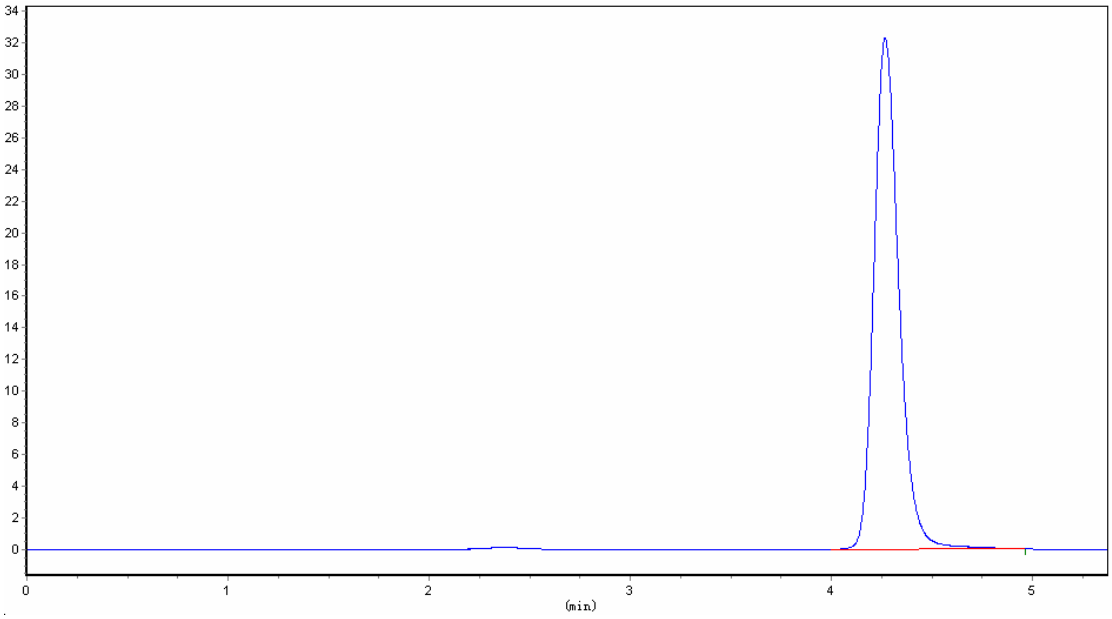

Figure 4: Chromatogram of the sample

Table 1: Linearity

Linearity levels mcg/ml)	Peak areas
1	28937
2	54036
5	130373
10	261000
25	654159
50	1301624
75	1923663
100	2552366

Table 2: Precision

Injection	Peak areas
Injection-1	288877
Injection-2	288463
Injection-3	288139
Injection-4	287841
Injection-5	288628
Average	288389.6
% RSD	0.12%

Table 3: Accuracy

% Concentration (at specification Level)	Peak areas	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	653951	5.2	5.23	100.6%	100.8%
100%	1300950	10.0	10.1	101.0%	
150%	1921816	15.0	15.1	100.7%	

Results and discussion:

Developed analytical method was a simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method for estimation of thienorphine. Different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination.

Method Validation:

The proposed RP-HPLC method was validated as per ICH guidelines.

System suitability:

Standard solution is injected five times and Flow rate was maintained at 1ml/min. temperature of column kept ambient and the column effluents were monitored at 220nm chromatograms were taken and System suitability parameters were computed. The system suitability was calculated as per ICH guidelines.

Calibration Curve:

Calibration curves were prepared by taking appropriate aliquots of thienorphine stock solution in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 1, 2, 5, 10, 25, 50, 75 and 100mcg/ml. These solutions were injected and chromatogram was taken. Flow rate was maintained at 1.0ml/min. Temperature of column kept ambient and the column effluents were monitored at 220nm. Calibration curve was constructed by plotting peak area *vs.* concentration and regression equation was computed. R^2 values of was found to be as 0.9999.

Specificity:

The peak purity of thienorphine was assessed by comparing the retention time of standard thienorphine sample good correlation was obtained between the retention time of standard and sample. Placebo and blank was injected and there were no peaks. There are no interferences hence method is specific.

Linearity:

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for thienorphine found to be as 1-100mcg/ml with correlation coefficient (R^2) 0.9999.

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage Relative Standard Deviation (% RSD) was found to be less than 2% that proves method is precise.

Accuracy (Recovery studies):

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 50%, 100% and 150% concentration levels. Known amounts of PLGA was added to the pre-analyzed samples and subjected to the proposed HPLC method. Results of recovery studies are shown in table no.3.

Table 4: Ruggedness

Injection	Peak areas
Injection-1	2887733
Injection-2	288121
Injection-3	287323
Injection-4	288544
Injection-5	288244
Average	288201
% RSD	0.14%

Table 5: Robustness (flow rate variation)

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.4	2187	1.1
2	0.5	2223	1.1
3	0.6	2072	1.12

Table 6: Robustness (Mobile phase variation)

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2012	1.1
2	*Actual	2230	1.1
3	10% more	2039	1.2

Table 7: Results of analysis of thienorphine PLGA microspheres

Batch	Drug content (%)
090220	4.60
090221	4.53
090224	4.57

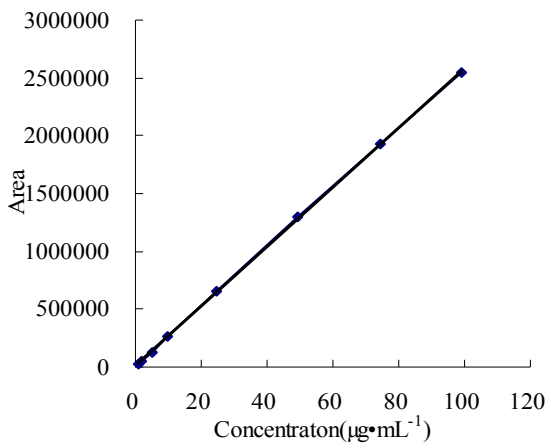

Figure 5: Linearity graph

Robustness:

The Robustness of method as carried out by changing the Chromatographic conditions such as Flow rate and Temperature variations. With the change of Flow rate of 0.4 ml, 0.5 ml and 0.6ml, change of organic solvent portion of the mobile phase with of 10% less, actual, 10% more and their tailing factor, plate count obtained within the limit.

Ruggedness:

The ruggedness of method carried out by using the different HPLC system with change of the system mean area found to be 98.5 and percentage RSD found to be 0.14.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD concentration obtained (based on three times the average noise level) is 0.013mcg/ml at a signal-to-noise ratio of 3, while LOQ concentration obtained is 0.098mg/ml at a signal-to-noise ratio of 10.

Application to analysis of thienorphine PLGA microspheres

The proposed HPLC method has been used in the analysis of the thienorphine PLGA microspheres and results of three samples are shown in table no.7.

Conclusion:

The proposed method is simple, specific, accurate and precise and hence can be used in routine for estimation of thienorphine in microsphere dosage. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The percentage RSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of thienorphine in microsphere dosage form.

Acknowledgement

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