Research Article

Fabrication and Characterization of Trandolapril Loaded Microspheres by ion Cross-linking Technique.

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ABSTRACT

The present research work was designed to formulate and evaluate microspheres of Trandolapril to develop a sustained release formulation using algino-eudragit RS100 system by ion-gelation technique. The various formulations containing varying concentrations of polymer blends were then evaluated for different parameters like percentage yield, particle size, micromeretic studies, entrapment efficiency of drug, in vitro drug release, along with quantitative analysis of prepared formulation by a novel, rapid, specific assay method using Ultra Performance Liquid Chromatography (UPLC). It was observed that all prepared formulations showed better flow behavior than pure drug, on increasing the polymer concentration, the entrapment efficiency and particle size also increased. The in-vitro release study indicates that the microspheres of Trandolapril were exhibiting sustained release behavior and its release was influenced by polymer concentration and it followed anomalous non-Fickian diffusion mechanism, it can also be said that the present method is most suitable for quality assurance of Trandolapril in prepared microspheres formulation. Therefore it can be concluded that Trandolapril loaded microspheres can be formulated for sustained drug delivery system.

Keywords: Trandolapril, Microspheres, Eudragit, Ion-gelation, Validation, System suitability.

INTRODUCTION

To prevent frequent drug administration and fluctuating drug level in the body, during treatment of diseases, it is essential to administer the drug by sustained release system. Sustained release formulations are mainly designed to maintain optimum therapeutic level of drug within the body for a longer duration with minimum side effects. Drugs with short elimination half life are suitable candidates for sustained release formulations.[1]Microsphere formulations are those dosage forms that deliver drug in sustained release manner. Disease like hypertension is one of the most prevalent vascular diseases and it is a major cause of public health issue at present. Treatment of hypertension by Angiotensin converting enzyme(ACE) inhibitor therapy is widely opted due to its blood pressure lowering property without effecting the cardiovascular reflexes.[2] Trandolapril (TD) is an ACE inhibitor used in the treatment of high blood pressure, Trandolapril is one of the newer drugs in this class, it is a non-sulfhydryl prodrug which after oral administration is easily hydrolyzed in liver to its biologically active diacid, that is trandolaprilat, which is a more potent and long-acting inhibitor of plasma and tissue ACE than quinaprilat, enalaprilat, captopril,[3] and isradipine.[4]Trandolapril (TD) if given at a dose of 2mg to 4 mg once daily, effectively controls blood pressure for at least 24 h in patients with mild to moderate hypertension.[5] It is well tolerated by the humans, with minimum adverse effects.[6,7]TD can be given safely to the patients for a prolonged period of time.[8] As the selected drug TD has short biological half life, so it is suitable candidate for sustained release formulation to achieve optimum drug level in body, microsphere formulation of Trandolapril with various combinations of natural and synthetic polymers have been tried to prepare in this experiment to obtain sustained release of drug.[9]The method to prepare microsphere formulation of Trandolapril by ion-cross linking has been selected, as it is most novel technique to dispense TD, because till now no such preparation of the selected drug has been reported in literature. Among various available delivery systems for sustained release formulations, here microspheres have been selected due to avoidance of dose dumping and enhanced solubility due to micron level particle size.[10]A natural polymer alginate, which is a linear polysaccharide, was selected due to its biocompatible and biodegradable nature, it is widely used in microspheres preparations. The commercially available salt form of alginate that is sodium alginate was taken, gelation of alginate on reacting with calcium ions forms ionic inter-chain bridges.[11]When alginate reacts with calcium ions, it undergoes gelation in aqueous solution due to binding of calcium ions. In present work ionic gelation technique was selected in fabrication of microspheres due to its simplicity, low cost and its high entrapment efficiency.[12] In present research work sodium alginate is used in combination with Eudragit RS 100 for sustained drug delivery of Trandolapril, Sodium alginate on exposure to dissolution fluids gets swelled & forms a viscous gel layer that sustained the drug release, where as Eudragit

RS 100 being water insoluble polymer retards drug release. So the objective of the present study was to develop a sustained release system of Trandolapril and evaluate the effect of polymer concentration on drug release kinetics [12, 13] along with its purity detected by Ultra Performance Liquid Chromatography (UPLC).

MATERIALS AND METHODS

Materials: Trandolapril (TD) was obtained as a gift sample from Hetero Healthcare Ltd. Hyderabad, India, Sodium alginate was a gift sample from Signet Chemical Co, India, Eudragit RS 100 was purchased from Natco, Hyderabad, India, Calcium chloride was obtained from Loba Chem Pvt. Ltd., India, Trifluoroacetic acid(TFA) and Acetonitrile was obtained from Merck Chemicals, Mumbai, India. All other chemicals used were of analytical and UPLC grade reagent.

Preparation of Trandolapril Loaded Microspheres

Microsphere preparation of Trandolapril was fabricated by ionic gelation technique using sodium alginate. Initially, specified amount of sodium alginate was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution and then specified quantity of Eudragit RS 100 was added to it and uniformly mixed with the help of magnetic stirrer at 200 revolutions per minute speed. Finally, the drug, Trandolapril was added to the polymers solution and mixed to form a smooth viscous dispersion, lastly the resulting dispersion was added drop wise with 24 G needle in 500 ml of 5% calcium chloride solution under continuous stirring at 200 rpm. This stirring was continued for 30 minutes to make the dispersion as fine as possible to obtain spherical microspheres. Then the mixture was filtered and product was dried at 40°C for 12 hour.[14]The prepared microspheres of Trandolapril with varying polymer coat composition are listed in Table 1.

Table 1: Composition of Trandolapril loaded microspheres.

Formulation Code	Drug (g)	Sodium alginate (g)	EudragitRS100 (g)	Curing Time (minutes)	Cross-linking agent ($cacl_2$ %w/v)	Stirring Speed(rpm)
F1	1	0.5	0.5	30	5	200
F2	1	0.5	1.0	30	5	200
F3	1	0.5	1.5	30	5	200
F4	1	0.5	2.0	30	5	200
F5	1	0.5	2.5	30	5	200
F6	1	0.5	3.0	30	5	200
F7	1	0.5	3.5	30	5	200
F8	1	0.5	4.0	30	5	200

Characterization of Trandolapril Loaded Microspheres

Percentage Yield: The percentage yields of all the prepared formulations were determined on weight basis with respect to the initial weight of material.[15]The calculated data are represented in Table-3.

Micromeretic Study

The flow behavior of prepared Trandolapril loaded microspheres were determined by calculating bulk density, tapped density, Carr's index and Hausner's ratio and angle of repose. Bulk density and tapped density were determined by using Bulk density apparatus (Electro lab India) and angle of repose was calculated by fixed base cone method.[16, 17]

Particle Size Analysis

The particle size of prepared Trandolapril (TD) loaded microspheres was determined by optical microscopic method. All readings were taken thrice[18] and the data shown in Table 3.

Entrapment Efficiency of Drug

The amount of drug entrapped in prepared TD microspheres was tested by taking 100 mg of the formulation in 50 ml of phosphate buffer of pH 7.4 in a volumetric flask and then it was stirred for 30 minutes in sonicator at 125W (Imeco Sonifier, Imeco Ultrasonics, India). Finally, the volume was made up to 100 ml with phosphate buffer of pH 7.4 and again stirred for 1 hour and kept overnight for 24 hours to extract the drug from microspheres. Then it was filtered and the filtrate was collected by passing through 0.45µ filter and required dilutions were made and the absorbance of resulting solution was measured at 210nm using UV-Visible spectrophotometer (UV- 2450 Shimadzu, Japan) against blank.[19]This study was conducted three times and the values are shown in Table 3**.** The drug entrapment was calculated by using the formula:

% Drug entrapment = (Calculated drug content/Theoretical drug content) x 100

In-Vitro Drug Release Study

The release of Trandolapril drug from the prepared microspheres was studied in 0.1N HCl and in phosphate

buffer pH 7.4(900ml) using a USP six station dissolution (LAB DISSO 2000) rate testing apparatus with a rotating paddle at 50 rpm and 25cm depth. Sample of 5 ml was withdrawn at various time intervals and subsequently diluted using pH 7.4 phosphate buffer. After suitable dilutions the absorbance was measured at 210nm using UV- visible spectrophotometer (2450 Shimadzu, Japan) against a blank. The dissolution study was conducted in triplicate.[20]The observed data is shown in Table 4.

Analytical Method Validation

Instrumentation and Chromatographic Conditions

A novel, rapid, specific and stable Reverse Phase- Ultra Performance Liquid Chromatography (RP-UPLC) method was developed and validated for the prepared microspheres formulation of Trandolapril. UPLC was performed by using Waters H Class System with Empower 3 software equipped with auto sampler and quaternary gradient pump with inline degasser. In present analytical method, separation was performed by using mobile phase of two solutions, Solution A comprises of 0.1% Trifluoroacetic acid (TFA) in water and Solution B consists of 0.1% TFA in Acetonitrile using Acquity UPLC BEH C18, 100 mm x 2.1 mm x1.7µm column.

Table 2: Optimized Chromatographic Conditions

S.No.	Parameter	Result
1	Column	Acquity UPLC BEH C18, 100 mm x 2.1 mm x1.7µm
2	Mobile phase	Solution A: 0.1% TFA in water Solution B: 0.1% TFA in Acetonitrile Gradient program was set as: Time (min)/ % solution B: 0/22, 2/22, 6/55, 8/55, 8.2/22 and 10/22
3	Flow rate	0.5 ml/min
4	Detection Wavelength	210 nm
5	Sample injector	02 µl loop
6	Run Time	10 min
7	Temperature	45^0 C

Sample and Solution Preparation

In present developed method for estimation of Trandolapril loaded microspheres a diluent mixture of water and Acetonitrile (8:2 V/V) was prepared for dissolving samples during entire experiment. For the preparation of standard Trandolapril solution, about 20mg of Trandolapril was dissolved in a 200ml with diluent. (100µg/ml)

Preparation of Trandolapril microsphere assay sample preparation

For the preparation of assay sample of Trandolapril loaded microspheres, about 25mg of equivalent powdered microspheres were taken and transferred to 10ml volumetric flask. To this added 4 mL of the diluent and sonicated for 30 minutes with intermediate shaking, the solution was then diluted to 10 mL with diluent and centrifuged at 3000 rpm for 10 min. The supernatant was collected, filtered through 0.22 µ filter and used as the sample solution to determine assay.

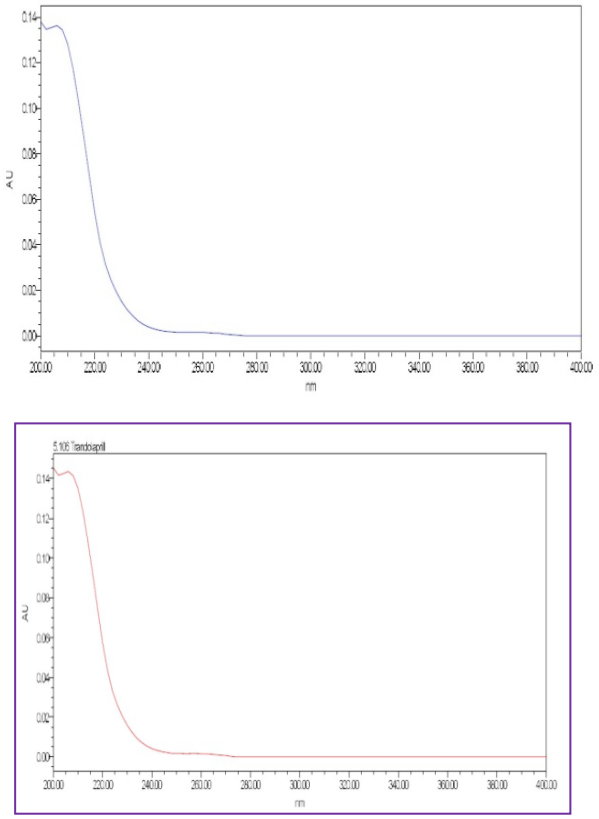

Figure 1: UV Spectra Standard and Sample solution at 210 nm

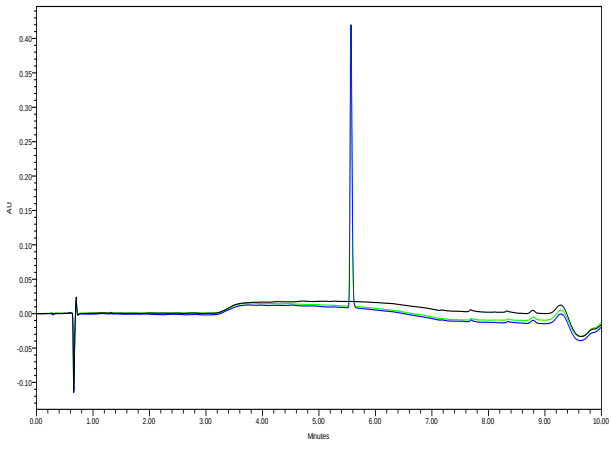

Figure 2: Overplayed Chromatogram for Blank, sample and Standard (Microspheres)

Linearity and Range

The ability of an analytical method to obtain results that are directly proportional to the concentration of the analyte in the sample is called as linearity. The range of an analytical method is the interval between the upper and lower levels of analyte that have been determined with precision, accuracy and linearity using the method as written. Linearity was determined by preparing five standard solutions of Trandolapril standard at concentration levels of 60% to 140 % of test concentration and each solution injected to be confirmed. Peak area was recorded, for all the peaks and calibration plot was constructed by plotting peak area Vs concentrations of Trandolapril which was found to be linear in the range of 2µg-10µg/ml. Coefficient of correlation was determined.

Accuracy

The measurement for exactness of the analytical method is known as accuracy. It was performed by preparing solutions at different levels 50 %, 100% and 150 % of test concentration using Trandolapril loaded microspheres Standard solution and added to placebo. Each solution was injected three times. The accuracy of the method was determined by spiking known amount of Trandolapril to placebo at 50%, 100% and 150% of test concentration in triplicate and analyzing as per the proposed method. The results were shown in Table 6 for Trandolapril formulation.

Precision

The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample under the prescribed conditions is known as precision. It may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation.

System suitability

System suitability parameters were performed to verify performance of the system, system precision was determined on six replicate injections of standard preparation, all essential chromatographic characteristics, including the relative standard deviation, peak tailing, theoretical plate number and resolution were measured. These all system suitability parameters covered the system, method and column performance. [21,22]

RESULTS AND DISCUSSION

The Trandolapril loaded microspheres were prepared using algino-eudragit RS 100 system by ionic gelation method with 5% calcium chloride as cross linking agent. This method was selected due to its novelty, ease of formulation, quick and cost effectiveness. The compositions of prepared formulations are represented in Table 1.

Percentage yield

The yield obtained from all the eight batches was good. The range for percentage yield was 92.50±0.04% to 98.80±0.05% for the prepared Trandolapril loaded microspheres, the obtained result showed a moderate increase in yield. Table 3 depicts the detail data of percentage yield.

Micromeretic Study

The flow properties of prepared Trandolapril formulations was determined by observing the obtained data for bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose as depicted from Table 3.

Bulk Density and Tapped Density

As observed from the Table 3, it can be concluded that the prepared microspheres have better flow properties as compared to the pure drug, as seen from data. The increase in tapped density as compared with each bulk density of the formulation for different Drug: Polymers ratios indicate better flow behavior of microspheres. The bulk density ranged from 0.27±0.01 to 1.31±0.29 and tapped density ranged from 0.30±0.02 to 1.50±0.21 for the Trandolapril loaded microspheres (F1 to F8), where as for pure drug the bulk density was 0.80±0.31 and tapped density was 1.30±0.28.

Hausner's ratio

The value of Hausner's ratio for pure drug Trandolapril was obtained as 1.62±0.15; it clearly indicates poor flow, the values for all the prepared microspheres formulation ranged from 1.02±0.10 to 1.27±0.02, as indicated in Table 3.

Carr's index

The values of all prepared microspheres from F1 to F8 indicates enhanced flow properties ranging from 02.32±0.78 to 21.56±0.85, where as for the pure drug TD the obtained values were 38.46±1.03, indicating poor flow, the values are represented in Table 3.

Angle of repose

Pure drug Trandolapril had poor flow with values 42.46^0, where as the prepared microspheres indicates better flow properties as shown in Table 3, ranging from 15.93^0 to 20.10^0.

Particle size analysis

The mean particle size of eight formulations from F1 to F8 ranged between 41.05±3.14µm to 77.87±2.01µm. It was observed that mean particle size of prepared formulations increased with increase in polymer concentration for the formulations F1, F2, F3, F4, F5, F6, F7 and F8, as seen in Table 3.

Entrapment Efficiency of Drug

Entrapment efficiency for different formulation of microspheres was found to vary between 68.71±0.86 to

78.89±0.01, it can be seen that with increase in polymer concentration more drug was entrapped with bigger particle size, it can be to enhanced availability of active calcium binding sites in polymeric cross-linking. The values represented in Table 3.

Table 3: Micromeretic, Particle size and Entrapment efficiency study of Trandolapril microspheres.

Formulation	%Yield	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose	Average Particle size	Entrapment Efficiency
Pure Drug	-	0.80±0.31	1.30±0.28	1.62±0.15	38.46±1.03	42.46	18.11±2.54	-
F1	92.5±0.04	1.31±0.29	1.50±0.21	1.14±0.85	12.66±0.95	20.10	41.05±3.14	68.71±0.86
F2	93.1±0.07	0.40±0.01	0.51±0.01	1.27±0.02	21.56±0.85	19.80	49.27±6.05	72.48±0.37
F3	93.7±0.03	0.42±0.02	0.43±0.02	1.02±0.10	02.32±0.78	17.80	52.08±7.04	75.91±0.03
F4	95.3±0.05	0.29±0.03	0.31±0.01	1.06±0.02	09.37±0.59	17.18	61.03±2.06	76.68±0.46
F5	98.2±0.06	0.27±0.01	0.30±0.02	1.11±0.01	10.00±0.45	15.93	66.15±4.02	78.89±0.01
F6	97.1±0.04	0.59±0.08	0.62±0.04	1.05±0.04	04.83±0.78	18.26	70.41±1.08	77.64±0.67
F7	98.8±0.05	0.61±0.02	0.73±0.03	1.19±0.01	16.43±0.09	17.91	74.94±0.39	78.81±0.08
F8	97.5±0.04	0.68±0.14	0.71±0.01	1.04±0.13	04.22±0.81	18.10	77.87±2.01	78.39±0.83

In-vitro drug release study

The influence of varying polymer concentrations on release of Trandolapril loaded microspheres was studied. Various prepared formulations of Trandolapril microspheres from F1 to F8 were able to sustain the release the drug for around 4,5,6,7,9,10 and 12 hours respectively. For F1 97.01% of drug was released after 4 hours, for F2 formulation of Trandolapril 95.31% of drug was released after 5 hours, for F3 formulation 94.04% of drug was released after 6 hours, for F4 93.87% was released after 7 hours, for F5 formulation 91.69% of drug was released after 9 hours, for F6 formulation 90.21% was released after 10 hours, for F7 formulation of Trandolapril microspheres 92.41% of the drug was released after 12 hours and for F8 formulation 80.01% of drug was released after 12 hours. It can be observed from the values of Table 3 that on increasing the quantity of Eudragit RS 100 to 3.5%, the release of drug from prepared microspheres was very slow after 12 hours. Formulations of microspheres F1 to F8 were more efficient in sustaining the drug due to Eudragit RS 100, as it forms rigid hydrophobic coat. Among all prepared formulations, F7 showed best dissolution profile, therefore it was selected to be the most optimized formulation as seen from Table 4.

The co-efficient of determination (R^2) was used as indicator of best fitting for each of the models considered. The kinetic data of all the formulations reached higher coefficient of determination (R^2 = 0.917 to 0.988) with zero order release, whereas exponent value (n) ranged from 1.33 to 2.018. From the release exponent in Korsmeyer-Peppas model, it can be concluded that the mechanism of release of Trandolapril loaded microspheres was an anomalous non-Fickian diffusion mechanism leading to the conclusion that a combination of release mechanism including drug diffusion and sphere erosion may be appropriate.

Table 4: Drug release profile for prepared Trandolapril loaded microspheres formulation.

Formulation	Zero Order(R^2)	First Order(R^2)	Higuchi Model(R^2)	Korsmeyer Model(R^2)	Release exponent(n)
F1	0.917	0.758	0.732	0.82	1.334
F2	0.976	0.758	0.821	0.978	1.524
F3	0.932	0.930	0.814	0.867	1.841
F4	0.924	0.957	0.843	0.832	1.867
F5	0.950	0.974	0.876	0.889	1.980
F6	0.919	0.974	0.905	0.914	2.018
F7	0.969	0.958	0.905	0.972	2.006
F8	0.988	0.959	0.876	0.987	1.892

Linearity and Range

The calibration curve showed linearity over a concentration range of 60% to 140% for Trandolapril, as shown in Figure 3 and was linear with a correlation coefficient of 0.999.

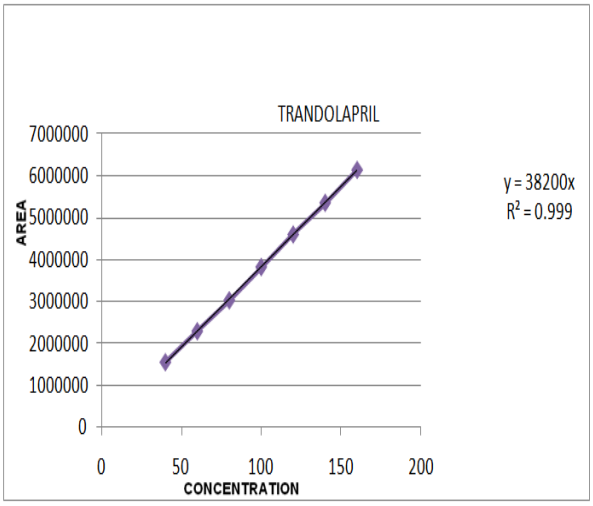

Figure 3: Calibration curve of Trandolapril.

Accuracy

The selected analytical method meets the pre-established acceptance criteria for recovery study as per protocol, hence the method is accurate. Recovery (%) of Trandolapril ranged from 100 to 101.0% for samples, as seen data from Table 5.

Table 5: Accuracy data for Trandolapril assay

S.No	Level (%) (n=3)	% Recovery	% RSD
1	50	100.1	0.3
2	100	100.3	0.4
3	150	100.6	0.5
n= Number of determinations			

Precision

The system precision or injection repeatability was performed by preparing standard stock solution as per test method and injected five times. The observation was shown in Table 6.

Table 6: Acceptance Criteria RSD should not be more than 2.0 %

Concentration	Injection	Area	Statistical Analysis
100 %	Injection 1	3826516.05	Mean : 3823757.8
	Injection 2	3824851.01	
	Injection 3	3821246.40	SD:5510.38
	Injection 4	3815826.50	
	Injection 5	3830348.80	RSD:0.14%

System suitability

System suitability study was performed on freshly prepared standard stock solution of Trandolapril formulation, under optimized chromatographic conditions and following parameters were studied to evaluate the suitability of the system. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are expressed in Table 7.

Table 7: System suitability parameters from assay standard of Trandolapril

Name	RT*	USP Tailing	USP Plate count	% RSD
Trandolapril	5.57	1.23	122119	0.22

*RT: Retention time

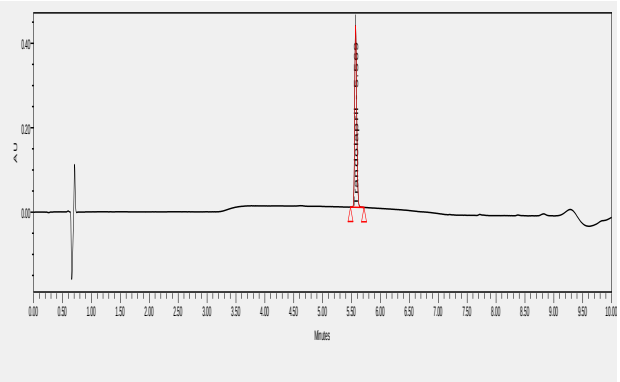

Figure 4: System suitability parameters from assay standard.

CONCLUSION

The Trandolapril loaded microspheres using algino-eudragit RS100 system showed good sustained release behavior with enhanced flow properties as compared to pure drug. The release of drug was sustained up to 12 hours for prepared formulation, the developed assay method was precise and meets with all the pre established acceptance criteria for recovery study. So, by this method we can formulate Trandolapril loaded microspheres for sustained, safe and effective drug delivery.

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REFERENCES

1. George M, Grass IV, Robinson JR, Sustained and Controlled release drug delivery systems, Marcel Dekker, 6, New York, 1978, 124-127.
2. Guyen LCDN, Brunner HR, Trandolapril in hypertension: Overview of a new Angiotensin converting enzyme inhibitor, American Journal of Cardiol, 70, 1992, 27-34.
3. Sakonjo H, Nakanishi J, Fukuda Y, Pharmacological action of a novel angiotensin converting enzyme(ACE) inhibitor RU

4457 namely (-)–(2S,3aR,7aS)-1-[(S)-N-[(S)-1-ethoxycarbonyl -3-phenylpropyl]alanyl]hexahydro-2indolinecarboxylic acid-in vivo ACE inhibiting effect and antihypertensive effect in various hypertensive models of rats in Japanese, Pharmacometrics, 45, 1993, 15-25.

4. Saleh A, Suwayeh Al, Effect of penetration enhancers on the transdermal delivery of isaradipine through excised rabbit skin, Saudi Pharmaceutical Journal, 16, 2008, 155-160.
5. Arakawa K, Limura O, Abe K, Clinical evaluation of RU44570 (trandolapril), a novel angiotensin converting enzyme inhibitor in the long-term treatment of patients with essential hypertension, Rinsho Iyaku, 8, 1992, 141-169.
6. Lynda RW, Donna MT, Trandolapril-A Review of its Pharmacodynamic and Pharmacokinetic properties and Therapeutic use in essential hypertension, Pharmacometrics, 42, 1994, 18-25.
7. Guay DRP, Trandolapril-a newer Angiotensin-converting enzyme inhibitor, Clinical Therapeutics, 25, 2003, 713-775.
8. Kober J, TorpPedersen C, Carlsen JE, A clinical trial of the Angiotensin converting enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infraction, The New England Journal of Medicine, 333, 1995, 1670-1676.
9. Patel J, Patel D, Raval J, Formulation and evaluation of Propranolol hydrochloride loaded carbopol-934/ethyl cellulose mucoadhesive microspheres, Iranian Journal of Pharmaceutical. Research, 9, 2010, 221-232.
10. Arora S, Ali J, Ahuja A, Khar RK, Baboota S, Floating Drug Delivery System-A Review, AAPS Pharm Sci Tech, 6, 2005, 372-390.
11. SkjakBræk G, Smidsrod O, Draget KI, Alginate as immobilization matrix for cells, Trends Biotechnol, 8, 1990, 71–78.
12. Khanam N, Alam IM, Sachan KA, Gangwar S, Fabrication and evaluation of propranolol hydrochloride loaded microspheres by ionic-gelation technique, Der Pharmacia Lettre, 4, 2012, 815-820.
13. Gattani YS, Kawtikwar PS, Sarkar DM, Formulation and evaluation of gastroretentive multiparticulate drug delivery system of aceclofenac, Int J Chem Tech Res, 1, 2009, 1-10.
14. Khanam N, Alam MI, Sachan AK, Gangwar SS, Anand C, Design and characterization of mucoadhesive microspheres of novel NSAID drug using algino-eudragit RS 100 system, Der Pharmacia Sininca, 2, 2011, 182-191.
15. Saraf S, Dashora K, Saraf S, Effect of processing variables on micro particulate system of aceclofenac, Pak J Pharm Sci, 19, 2006, 1-6.
16. Aulton ME, The Science of Dosage Form Design, 2nd Ed, Churchill Livingstone, New York, 2002, 113-138.
17. Lachman L, Liberman HA, Kanig JL, The Theory and Practice of Industrial Pharmacy, 3ed Ed, Varghese Publishing House, Bombay, 1991.
18. Shabaraya AR, Narayanacharyulu R, Design and evaluation of chitosan microspheres of metoprolol tartrate for sustained release, Indian J Pharm Sci, 65, 2003, 250-252.
19. Government of Indian, Ministry of Health and Family Welfare, Indian Pharmacopoeia, The controller of Publications, India, 1996, 2, 634-635.]
20. Napppinnai M, Kishore VS, Evaluation of microspheres of diltiazem hydrochloride, Ind J Pharm Sci, 69, 2007, 511-514.
21. IFPMA, "Stability ICH testing of new drug substances and products Q1A (R2)," in Proceedings of the International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2003.
22. Alam MI, Khanam N, Ganguly S, Barik S, Siddique AR, Development of assay method and forced degradation study of Dexibuprofen and Paracetamol by RP-HPLC in tablet formulation, Der Pharmacia Lettre, 6, 2014, 184-191.

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