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RESEARCH ARTICLE

FORMULATION DEVELOPMENT AND EVALUATION OF *IN SITU* NASAL GEL OF ZOLPIDEM TARTRATE

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ABSTRACT

Nasal drug delivery has attracted much attention as a promising alternative administration route, especially for peptide or protein drugs. The nasal route is an attractive alternative to drug administration and provides a direct access to the systemic circulation. In the present study various formulations were prepared by using carbapol 934P as gelling agent and HPMC K4M as controlled or sustained release polymer. All the formulations were evaluated for various parameters like pH, viscosity, drug content, gel strength, mucoadhesive strength and drug release. At optimized concentration of gelling agent and HPMC K4M showed *in situ* gelling with all parameter in range. *In vitro* release data revealed that the optimized formulation showed controlled and sustained drug release pattern.

Key Words: Carbapol 934P, in Situ, Hypnotic, Zolpidem Tartrate

INTRODUCTION

The nasal route is an attractive alternative to drug administration and provides a direct access to the systemic circulation. In this, drugs are administered through nasal cavity by different dosage forms such as solution, emulsion, gel etc. and useful method for drugs having low dose and shows no or minimal oral bioavailability such as proteins and peptides. One of the reasons for the low degree of absorption of peptides and proteins via the nasal route is rapid movement away from the absorption site in the nasal cavity due to the mucociliary clearance mechanism (Mahalaxmi R *et al*., 2007). Presently, commercially various nasal preparation is used for systemic absorption of drug in a different pathological conditions. Therapy through intranasal administration has been an accepted form of treatment in the Ayurvedic system of Indian Medicine (Chien, ?: Alagusundaram *et al*., 2010). *In situ* is a Latin word which means 'In its original place or in position'. In this type of drug delivery system, the preparation is in a solution form before administration in body, but it converts into a gel form after administration (Shah and Patel, 2010; Kute *et al*., 2013). An *in situ* gel is made of polymer materials that have a solution or semisolid state that responds to external stimuli at the administration site. These gels also have conformations that can undergo reversible conversion to form a semisolid or solid preparation. The *in situ* gelation compositions using ionic polysaccharides have been disclosed in U.S. Pat. No. 5,958,443, which discloses compositions comprising a drug, a film forming polymer and a gel forming ionic polysaccharide (such as an alginate) (Peppas and Langer, 1994; Nerkar *et al*., 2013). Zolpidem is a prescription short-acting nonbenzodiazepine hypnotic that potentiates gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding to benzodiazepine receptors which are located on the gamma-aminobutyric acid receptors.

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Zolpidem is used for the short-term treatment of insomnia. Zolpidem is rapidly absorbed from gastro intestinal tract but it is subjected to first pass metabolism. The main objective of present work is to enhance the bioavailability, reducing the dose and dosing frequency, gives patient compliance, increase the residence time so it gives the sustained drug release.

MATERIALS AND METHODS

Zolpidem tartrate was obtained as a gift sample from BAL Pharma, Bangalore. HPMC K4M was purchased from Colorcon Asia Pvt. Ltd, Mumbai. Carbapol 934P was purchased from Loba Chemicals, Mumbai. Propylene glycol, sodium chloride, propyl paraben was purchased from Loba Chemicals, Mumbai.

Method

Preparation of Standard curve of Zolpidem tartrate

Accurately weighed 50 mg sample of zolpidem tartrate was transferred to 100 ml volumetric flask and the volume was made upto 100 ml with phosphate buffer pH 6.4 to produce stock solution of 500 µg/ml. From above 5 ml solution was taken and diluted to 50 ml to form 50 µg/ml concentrations. The standard stock solution containing 500 µg/ml of zolpidem tartrate was prepared. From the stock solution pipette out (0.5-3) ml solution and dilute upto 10 ml with phosphate buffer 6.4 to get a concentration of 5, 10, 15, 20, 25, 30 µg/ml. The absorbance of the dilution were measured at 294.2 nm by using Shimadzu double beam UV visible spectrophotometer The highest concentration solution (30 µg/ml) was scanned over 200-800 nm wavelength range. Fourier Transform Infrared spectroscopy Fourier Transform Infrared (FTIR) spectroscopy was conducted. The procedure consisted of placing a zolpidem tartrate sample in FTIR sample holder. The drug sample was placed in the light path and scanned over the range of 4000-400 cm^{-1} on Shimadzu FTIR Prestige-21.The obtained spectrum was recorded.

Differential scanning colorimetry

The physical mixture of zolpidem tartrate with excipients sample was prepared by triturating pellets in a dried mortar pestle. The 2 mg of sample was weighed and sealed in aluminium pan. Empty aluminium pan was used as a reference. Using parameters DSC thermogram was recorded.

Preparation of nasal gel formulations

The formulations were prepared by dispersing carbopol 934 in distilled water with continuous stirring (Thermostatic hot plate with magnetic stirrer, Remi, Mumbai) until completely dissolved and allowed to hydrate overnight. For the preparation of solution, first HPMC K4M was added in distilled water and allowed to hydrate. Then carbopol was sprinkled over the solution and allowed to hydrate overnight. After complete hydration of polymers a separate solution of zolpidem tartrate and sodium chloride was added to the polymeric solution. The resultant solution was thoroughly mixed, methyl paraben was then added and mixed until a uniform and clear solutions were formed. Final volume was made by adding required volume of distilled water. All the formulations were adjusted to pH 4.5 to 5.5 by using freshly prepared 0.5 M sodium hydroxide solution.

The simulated nasal fluid (aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$) which had the cationic composition of nasal secretions; was added slowly with stirring. Gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked and graded. Each preparation was tested thrice to check the repeatability of the measurement.

Drug Content

Each formulation was spray (100 µl/spray) in a 50 ml volumetric flask diluted with phosphate buffer pH 6.4 and shaken to dissolve the drug in phosphate buffer pH 6.4.
The solution was filtered through 0.45µ PVDF syringe filter, 1ml of above filtrate was pipette out and diluted to 10 ml with phosphate buffer pH 6.4. The content of the drug was estimated spectrophotometrically (Shimadzu, UV-1800, Lab India) by using standard curve plotted at 294.2 nm. (Shrivastava *et al* 2008).

Gel strength determination

It is expressed in terms of time (in seconds) required by a 35 g piston for penetration of 5 cm distance, through the 50 g gel formulation. Test was performed using 'Gel strength apparatus' (Yong *et al*., 2001) modified in laboratory (Yong *et al*., 2001).

Table 1. Composition of nasal *in situ* gel formulation of Zolpidem tartrate

Ingredients	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zolpidem tartarate (mg)	175	175	175	175	175	175	175	175	175
Carbapol 934P	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
HPMC K4M	0.2	0.2	0.2	0.4	0.4	0.4	0.6	0.6	0.6
Propylene glycol (ml)	5	5	5	5	5	5	5	5	5
NaCl (mg)	180	180	180	180	180	180	180	180	180
Propyl Paraben (% w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	20	20	20	20	20	20	20	20	20

Characterization of nasal *in situ* gel

Appearance

The developed formulations were inspected visually for clarity in sol and gel form.

Viscosity and rheological behaviour studies

Viscosity of formulations before and after gelation were measured by Brookfield R/S CPS + Rheometer with software Rheo 3000 and using spindle CP-75 at 100 rpm shear rate. (Jiang *et al*., 2007) (Kim *et al*., 2002).

pH of the formulation

The pH of the each formulation was determined by using pH meter (Model No. CL 54, Make Lab india Pvt. Ltd.,India).

Gelation studies

Gelation is the process which is evaluated by transition of liquid phase to gel (Balasubramaniam *et al*., 2003). A 10 ml transparent vial containing a magnetic bar and 2 ml of each formulation was placed on a magnetic stirrer.

Formulation (50 g) was placed in a 100 ml measuring cylinder and gelation was induced by Simulated Nasal Fluid. The apparatus i.e. piston for measuring gel strength (35g) was then placed onto the gel. The gel strength was measured as the time (in seconds) required for moving the apparatus 5 cm down through the gel. In cases, that take more than 5 min to drop the apparatus into the gel, suitable weights were placed on top of the apparatus and gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel. Experiments were performed in triplicate (Lee *et al*., 1998).

Mucoadhesive strength study

Mucoadhesive force of nasal phase transition system was determined (Yong *et al*., 2001 and Zaki *et al*., 2003) using sheep nasal mucosa and phosphate buffer pH 6.4 as the moistening fluid (Majithiya *et al*., 2006; Varsha *et al*., 2010). At the time of testing, a section of tissue was secured, keeping the mucosal side out, onto each glass vial using a rubber band and aluminium cap. The diameter of each exposed mucosal membrane was 1.1 cm. On glass vials, tissues were fixed in a manner that the mucosal side became outer part and properly fixed. A vial with a section of tissue was connected to the modified balance and suitable height was maintained. The gel was applied to the exposed tissue of lower vial. The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of upper vial.

After applying constant weight for several minutes, suitable weights were added to the modified balance. Minimum amount of weight that detached two vials expressed as mucoadhesive force (dyne /cm^2).

Detachment stress (dynes /cm^2) $=Mg/A$

where, M is the weight added to balance in grams; g is the acceleration due to gravity taken as 980 cm/sec^2; A is the area of the tissue exposed and is equal to πr^2 (r, the radius of the circular hole in the aluminium cap).

In vitro release studies

In vitro diffusion study of formulated *in situ* gels was carried out on Franz diffusion cell having 2.4 cm diameter and 13 mL capacity. Dialysis membrane having cut off molecular weight 12000–14000 kDa (Himedia) was used as diffusion membrane. Pieces of dialysis membrane were soaked in phosphate buffer pH 6.4 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.4; dialysis membrane was mounted on cell. The temperature was maintained at 37 ± 0.5°C. The donor compartment contained 3 ml of artificial nasal fluid. After an equilibration of membrane, formulation equivalent to 1 mg of zolpidem tartrate was placed in the donor compartment. At predetermined time points (30,60,90,120,150,180,210,240 and 270 min), 1 ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with phosphate buffer pH 6.4 after each sampling to maintain a constant volume, for a period of 5hr (Zaki *et al* 2007). The samples withdrawn were filtered and used for analysis. Blank samples (without zolpidem tartrate were run simultaneously throughout the experiment to check for any interference. The amount of diffused drug was determined using UV visible spectroscopic method (Shimadzu, UV-1800, and Lab India).

RESULTS AND DISCUSSION

Standard calibration curve of Zolpidem tartrate was carried out in phosphate buffer 6.4 and absorbance measured at 294.2nm. Calibration curve is depicted in Figure 1.

Table 2. Absorbance values of Zolpidem tartrate in phosphate buffer 6.4 at 294.2 nm for preparation of standard curve

Sr.No.	Concentration (µg/ml)	Absorbance (λ_{max} 294.2 nm)
1	0	0
2	5	0.130
3	10	0.263
4	15	0.389
5	20	0.528
6	25	0.666
7	30	0.811

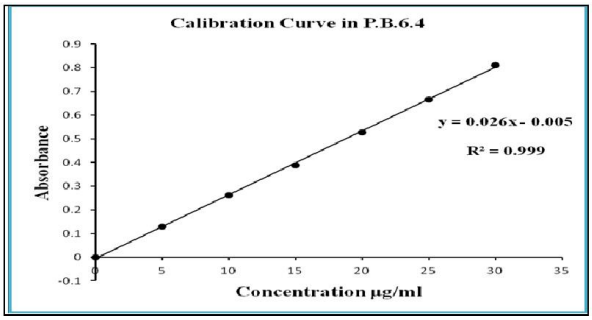

Figure 1. Standard curve of zolpidem tartrate

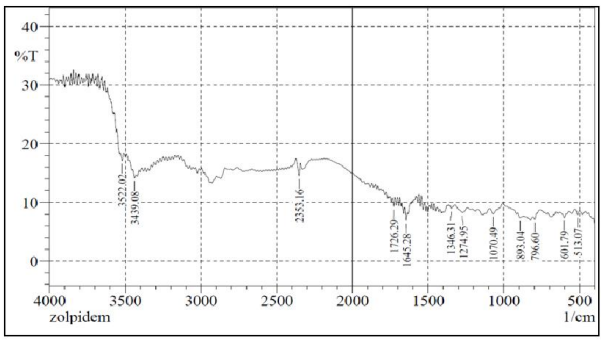

Figure 2. FTIR spectrum of zolpidem tartrate

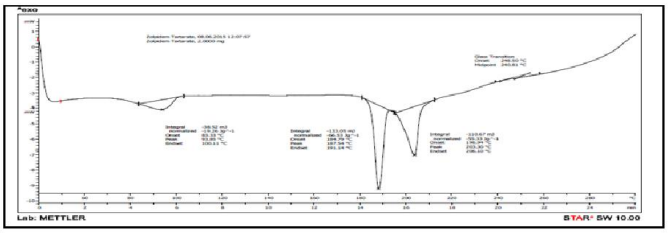

Figure 3. DSC thermogram of zolpidem tartrate

Appearance

The developed formulations were found clear in both sol and gel form.

Gelation studies

The gelling capacity of prepared formulations was observed by visual examination and graded on the basis of nature of gel formed.

- No gelation
+ Gelation occurred in few min and remained for few hrs.
++ Gelation immediate remained for few hrs.
+++ Gelation immediate, and for extended period
++++ Very stiff gel

Table 3. Gelling capacity of prepared formulations

Formulation code	Gelling Capacity
F1	+
F2	++
F3	++
F4	+++
F5	+++
F6	+++
F7	++++
F8	++++
F9	++++

From the gelation studies, it was observed that all the formulation showed instantaneous gelation. Formulation F1 does not form gel, F2, F3 showed weakest gelation while F9 showed very stiff gelation. Formulations F4 –F8 showed good gelation.

Viscosity measurements and rheological studies

Viscosities of all the formulation was determined at pH 4.4, the formulations exhibited low viscosity and were in solution

form. An increase in pH to 6.5 (pH of nasal fluid) using 0.5 M NaOH transformed the solution into gel and showed increase in viscosity.

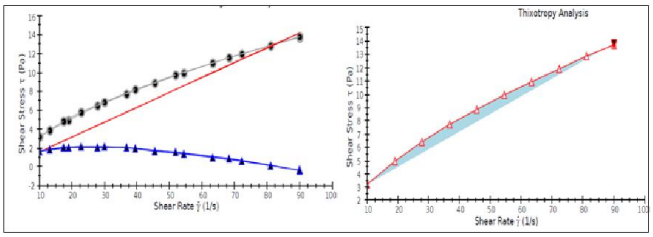

Figure 4: (A) showed the viscosity behaviour of zolpidem tartrate gel while (B) showed non-newtonian behaviour of zolpidem tartrate gel

Table 4. Viscosities of prepared formulation

Formulation code	Viscosity of sol^n (cps)	Viscosity of gel (cps)
F1	14.06 ± 0.23	1137 ± 0.05
F2	25.70 ± 0.65	1365 ± 0.12
F3	72.14 ± 0.25	1470 ± 0.20
F4	195.33 ± 0.36	1510 ± 0.85
F5	227.77 ± 0.56	1587 ± 0.46
F6	257.55 ± 0.58	1645 ± 0.39
F7	285.69 ± 0.46	1701 ± 0.78
F8	377.56 ± 0.31	1779 ± 0.77
F9	954.63 ± 0.92	1802 ± 0.69

pH studies

The normal physiological pH of nasal mucosa is 4.0 – 6.5, however the nasal mucosa can tolerate solutions within pH range of 3-10. The pH of all formulation was found to be in a range of 4.5-5.0 as shown in Table 5. The values are within the range which tolerate in the nasal mucosa.

Table 5. pH of prepared formulations

Formulation code	pH
F1	4.1 ± 0.2
F2	4.35 ± 0.05
F3	4.40 ± 0.3
F4	4.60 ± 0.1
F5	4.81 ± 0.08
F6	4.85 ± 0.6
F7	4.40 ± 0.48
F8	4.85 ± 0.23
F9	4.91 ± 0.3

Drug content

The percent drug content of all formulations (F1 to F9) was found to be in range of 92.08- 100.50%.

Table 6. Drug content of all prepared formulation

Formulation code	% Drug content (n=3)
F1	92.08 ± 0.76
F2	98.19 ± 0.48
F3	96.37 ± 0.23
F4	94.64 ± 0.28
F5	97.04 ± 0.69
F6	98.24 ± 032
F7	96.52 ± 0.24
F8	100.50 ± 0.19
F9	99.24 ± 0.76

Measurement of gel strength

The gel strength values between 25-50 seconds were considered sufficient as gel strength less than 25 seconds may not preserve its integrity and may erode rapidly while gels with strength greater than 50 seconds is too stiff and may cause discomfort. Formulation F1, F2, F3 showed gel strength value below 25 sec. From the results it was found that formulations F4 – F9 showed suitable gel value.

Table 7. Measurement of gel strength of prepared formulation

Formulation code	Gel Strength (sec)
F1	11.50 ± 0.36
F2	14.30 ± 0.54
F3	18.20 ± 0.10
F4	25.10 ± 0.29
F5	26.40 ± 0.24
F6	31.40 ± 0.85
F7	35.10 ± 0.95
F8	43.10 ± 0.17
F9	48.40 ± 0.42

Mucoadhesive strength

Mucoadhesive strength was determined in term of detachment stress i.e. force required to detach the formulation from mucosal surface. All formulations were subjected to *in vitro* mucoadhesion studies. Results indicated that the variation in concentration of HPMC K4M and carbapol 934P showed changes in mucoadhesive strength. The gradual increase was observed in mucoadhesive strength with the HPMC K4M level.

Table 8. Measurement of mucoadhesive strength of prepared formulation

Formulation code	Mucoadhesive Strength (dyne/cm^2)
F1	68.20 ± 0.15
F2	71.29 ± 1.23
F3	74.33 ± 0.79
F4	81.11 ± 0.96
F5	82.67 ± 0.75
F6	86.24 ± 0.84
F7	87.90 ± 0.66
F8	90.22 ± 0.45
F9	95.85 ± 0.36

In vitro diffusion study

From the results it was concluded that the initial release rate was very rapid due to incomplete gel formation, but the release became slow after complete gel formation and remained so. The release profiles exhibited an inflection point, which indicates gel formation on the diffusion membrane in donor compartment of diffusion cell. During gel formation, formulation got converted into the gel phase and thus drug release became slow. The results showed that the formed gels had the ability to retain zolpidem tartrate for the duration of 8 hours. *In vitro* release study indicated that the release of drug varied according to concentration of polymers, *in situ* gels were prepared using carbopol 934 and HPMC K4M. *In vitro* release of drug from formulations F8 indicated that a combination of carbopol 934 in HPMC K4M formulations is highly effective in sustaining the drug release up to 8 hours.

Table 9. Percent cumulative drug release of formulation F1 to F9

Time (min)	Sol^n	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0	0
30	36.06	12.88 ± 0.04	9.62±0.03	14.45±0.02	25.28±0.02	11.7±0.01	16.60±0.01	13.68±0.01	12.76±0.01	17.51±0.01
60	48.04	17.14±0.04	14.60±0.03	22.38±0.02	41.25±0.02	23.2±0.03	21.18±0.01	36.49±0.01	37.96±0.01	20.84±0.01
90	60.15	33.35±0.03	18.70±0.04	35.33±0.02	42.70±0.01	25.6±0.01	32.89±0.03	39.19±0.01	50.28±0.02	27.69±0.01
120	69.68	36.34±0.04	26.02±0.01	38.99±0.03	44.47±0.03	39.4±0.01	37.98±0.01	45.09±0.01	59.860.01±	34.14±0.01
150	80.69	44.30±0.02	39.28±0.02	45.99±0.02	50.78±0.02	55.6±0.01	45.24±0.01	46.68±0.005	70.92±0.01	39.79±0.02
180	89.26	100.8±0.26	65.060.03±	57.48±0.01	63.76±0.02	64.1±0.01	52.69±0.01	59.36±0.01	79.39±0.02	45.30±0.01
210	98.05		97.05±0.04	92.05±0.02	88.05±0.01	89.04±0.01	85.05±0.01	93.07±0.01	88.68±0.03	59.52±0.01
240	100								94.37±0.01	82.05±0.1
270									99.89±0.01	

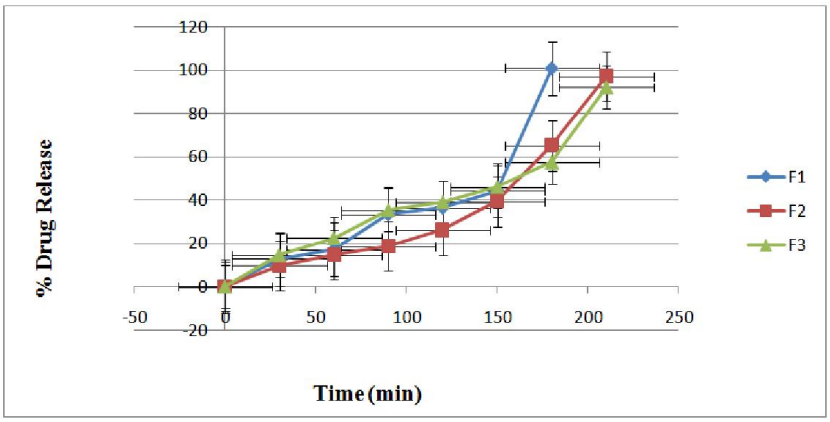

Figure 4. Release profile of drug from formulation F1-F3

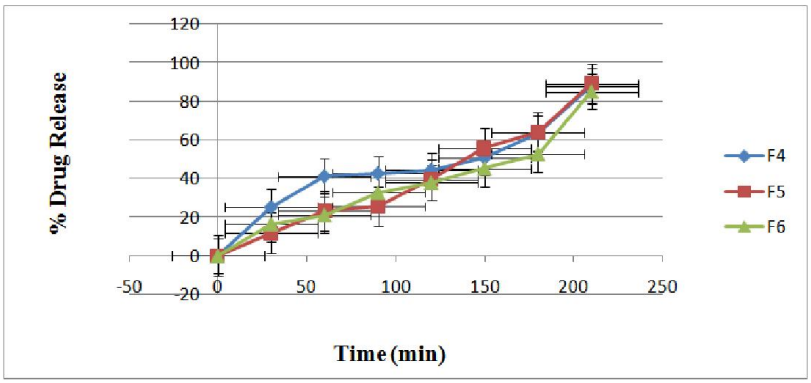

Figure 5. Release profile of drug from formulation F4-F6

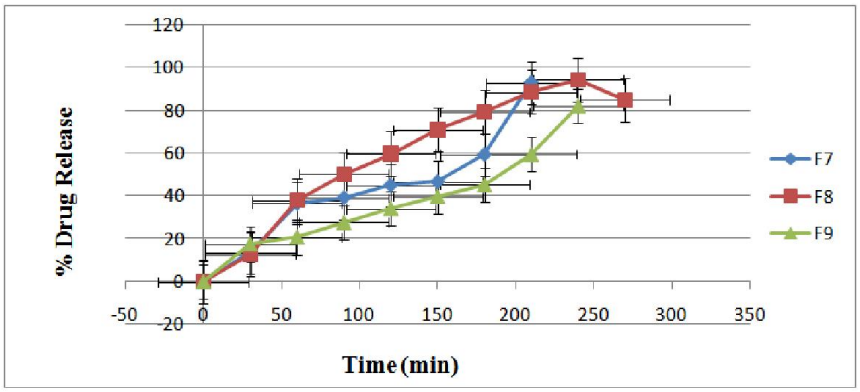

Figure 6. Release profile of drug from formulation F7-F9

Then formulation F8 was considered as optimized formulation, it showed the best *in vitro* release profile i.e. 99.898 %

Conclusion

Zolpidem tartrate was successfully formulated in pH triggered *in situ* gelling system using Carbopol 934 (0.4%, w/v) as a pH-triggered *in situ* gelling agent in combination with HPMC K4M (0.6%, w/v) as a viscosity enhancing agent. The optimized formulation F8 provided sustained *in vitro* release of drug over an extended period of 8 hrs. The optimized formulation can be a competent alternative to conventional nasal drops. As a consequence of its enhanced absorption due to longer residence time, it avoids the first pass effect and reduces the dosing frequency as well.

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