ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preparation and Evaluation of Sustained Release Matrix Tablets of Tramadol Hydrochloride Using Compritol 888 ATO by Melt Granulation Technique

Kavitha K, Rakesh K Deore* and Theetha G Tamizhmani

Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar, Karanataka –571422, India.

ABSTRACT

The purpose of this work was to prepare oral sustained release matrix tablet of highly water soluble drug and to evaluate the effect of concentration of hydrophobic polymer and effect of methods of preparation on the release of drug**.** The tablets were prepared by direct compression and melt granulation methods. The drug: wax interaction was determined by IR spectroscopic method. Drug release from the matrix drug delivery system was studied using USP II .The release data were subjected to different models in order to evaluate their release kinetics and mechanisms. The hardness of all formulations was found to be in the range of 4.8 ± 0.1000- 6.8 ± 0.0577kg/cm^2. Among these all formulations (MG1 to MG4) prepared by melt granulation, batch MG4 was best formulation and showed very slow release i.e. 56.6767 ± 0.6299% in 12 h ($p < 0.05$). The drug release (72.1453 ± 0.6688% in 8 h) was higher from the DC1 formulation prepared by direct compression as compared to MG2 formulation (78.8683 ± 0.6307 in 12 h) prepared by melt granulation method. The drug release was observed by fickian diffusion mechanism. Compritol 888 ATO can be used as matrix forming agent to sustain the release of water soluble drugs. Melt granulation was found to be better technique than direct compression. Drug release kinetics indicates the drug release by fickian diffusion mechanism. Formulation MG4 was better in comparison with marketed SR formulation. The developed matrix tablets of Tramadol hydrochloride may be used for prolonged drugrelease.

Keywords: Tramadol Hydrochloride; Compritol 888 ATO; Melt granulation; Direct compression

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Sustained- release dosage form is defined as "any drug or dosage form modification that prolongs the therapeutic activity of the drug" [1].The primary objectives of sustained drug delivery are to ensure safety and enhancement of efficacy of drug with improved patient compliance. So the use of these dosage forms is increasing in treatment of acute and chronic diseases as they maintain the concentration of drug in plasma above minimum effective concentration and below the minimum toxic level for extended period of time. Thus, sustained drug delivery results in optimum drug therapy with reduced frequency of dosing and side effects [2]. Glycerides are family of excipients, which have generated considerable interest for the preparation of oral dosage forms. Some glycerides like compritol (glyceryl behenate) can be used for the preparation of sustained release dosage forms [3]. The esterification of glycerol by long chain fatty acid gives them a pronounced hydrophobic character with low HLB value of 2 [4]. Several techniques like melt granulation [5], melt pelletization [6], hot melt extrusion [7] and hot melt coating [8] have been used to obtain sustained release dosage forms from glycerides based formulations. Melt granulation is a solvent free process, which involves the use of a substance, which melts at relatively low temperature. This substance can be added in the molten form over the substrate or in the solid form, which is then heated above its melting point. In melt granulation substance act as liquid binding agent, but the melt granulation does not require use of organic solvents. Moreover, in melt granulation the drying step is not necessary, thus the process is less consuming in terms of time and energy compared to other methods [9]. The sustained release matrix tablets were produce using compritol 888 ATO by melt granulation method [10, 11], hot melt extrusion method [12], melt pelletization method [6] and direct compression method [13].Tramadol hydrochloride, a synthetic opioid of aminocyclohexanol group, centrally acting analgesic. It was approved by USFDA in 1995. Tramadol has been proved to be effective in both experimental and clinical pain without causing serious cardiovascular or respiratory side effects [14]. Tramadol hydrochloride has plasma elimination half life of 4-6 hrs. The usual dosage regimen is 50-100 mg every 4-6 hrs. So, to reduce the frequency of administration and to improve patient compliance, a sustained release dosage formulation of tramadol is desirable. Tramadol hydrochloride is associated with certain side effects, like abdominal pain, anorexia and it may also induce psychic and physical dependence. Therefore properly designed Sustained Release Dosage Form of this drug will minimize the fluctuation in blood concentration, decreasing the risk of side effects and will show uniform pharmacological response [15]. Hydrophobic matrix tablet were produced to sustain formulation of tramadol using hydrogenated castor oil, ethylcellulose [16] and glyceryl behenate [17] .Various monoolein-water system were also formulated [18]. Tramadol has also been complexed with a sulfonic acid cation-exhange resin in a microencapsulation process by the spray- drying method [19].The objective of the present study was to prepare oral sustained release matrix tablet of highly water soluble drug by melt granulation method and to evaluate the effect of concentration of hydrophobic polymer on the release of drug.

ISSN: 0975-8585

EXPERIMENTAL

Materials

Tramadol hydrochloride was received as a gift sample from Neon Laboratories Limited, Mumbai, India. Compritol 888ATO (Glyceryl behenate) were obtained as a gift sample from Gattefosse Corp., France.

Methods

Preparation of Tramadol hydrochloride Matrix Tablets

By melt granulation method: Compritol 888 ATO (comp.) were exactly weighed as per formulation design then melted in porcelain dish on a water bath maintained at 75°C for three minutes. Tramadol hydrochloride (TH) were exactly weighed and gradually added to the melted comp. with continuous stirring till uniformly mixed. The molten mixture was allowed to cool and solidify at room temperature. The solidified mass was crushed in mortar and passed through a 16 mesh sieve. The granules are compressed into a flat – faced tablet using KBr press at pressure 1.0 Ton. Sustained release matrix tablets of Tramadol Hydrochloride with compritol 888 ATO were prepared with the different ratios viz.1:1,1:2,1:3,1:4 and these denoted by formulation code like MG1,MG2,MG3,MG4 respectively (Table 1).

By direct compression of physical mixture: Tramadol hydrochloride 100mg and compritol 888 ATO 200mg for formulation DC1 (1:2) proportion were mixed in a mortar for 10 minutes, that physical mixture were compressed into flat faced tablets by direct compression method(Table 1).

Compatibility Studies The pure drug, wax and prepared matrix tablet were subjected to IR spectroscopic study using FT-IR spectrophotometer (IRAffinity-1, Shimadzu). The spectra were scanned over the wave number range from 4000 – 400 cm^{-1}.

Percentage drug content study Drug content was determined by dissolving sustained release granules equivalent to100 mg of Tramadol hydrochloride in 70 ml of distilled water. It was shaken for 15 min. and then diluted to 100 ml with distilled water. It was filtered through Whatman filter paper no. 41. One ml of this solution was transferred to 10 ml volumetric flask and final volume was made 10 ml. Absorbance of the resulting solution was measured at 271 nm. The drug content was determined by referring to the calibration curve.

In Vitro Drug Release Study The in vitro dissolution study of Tramadol hydrochloride tablets was determined spectrophotometrically using USP II dissolution apparatus (Electrolab, TDT-08L). The 500 ml of 0.1 N HCl was used as dissolution media for 2 h followed by 8 h study in 6.8 pH phosphate buffer. The pH of the medium was adjusted to 6.8 after 2 h by adding 2.4 g of sodium hydroxide and 3.38 g of potassium dihydrogen phosphate dissolved in 5 ml water [20].

ISSN: 0975-8585

Temperature was maintained constant at 37 ±0.5 °C. The paddle rotation speed was kept at 50 rpm. At predetermined time interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h, 5 ml sample was withdrawn, filtered, suitably diluted and assayed at 271 nm by UV spectrophotometer (Shimadzu 1800). All the parameters were run 3 times (n=3) and the calibration curve specification were $y = 0.0053X + 0.0133$ (r^2=0.9996, n=9) ($p < 0.05$).

Drug Release kinetics To determine the values of correlation coefficient (R^2) and the mechanism of drug release from the formulations, the data were treated according to zero-order (cumulative percentage drug released *vs.* time, Eq 1), first order (Log cumulative percentage drug retained *vs.* Time, Eq 2), the Highuchi equation [21] (Cumulative percentage drug released *vs.* square root of time, Eq 3) models.

$$M_t = M_0 + k_0 t \quad \text{..... (1)}$$
$$\ln M_t = \ln M_0 + k_1 t \quad \text{..... (2)}$$
$$M_t = M_0 + k_H t^{1/2} \quad \text{..... (3)}$$

Where M_t is the cumulative amount of drug released at any time, t and M_0 is the dose of the drug incorporated in the delivery system. k_0, k_1 and k_H are rate constants for zero-order, first order and Higuchi models, respectively.

The dissolution data were also fitted according to the well-known exponential equation of Peppas [22] (Log of fraction of drug released *vs.* Log time, Eq 4) which is often used to describe drug release behaviour from polymeric systems.

$$\frac{Mt}{M\alpha} = kt^n \quad \text{..... (4)}$$

Where, $Mt / M\alpha$ is the fraction of drug released at time, t, k is the kinetic constant, and n is the diffusional exponent for drug release. The diffusional exponent, n, is dependent on the geometry of the device as well as the physical mechanism for release. Zero order release describes the release rate independent of drug concentration. Higuchi square root kinetic model describes, release rate is time dependent process. The values of n indicating drug release mechanism, if $0.1 < n < 0.5$ indicating fickian diffusion mechanism and $0.5 < n < 1$ indicating non-fickian diffusion mechanism.

Statistical analysis: The Statistical analysis was performed using GraphPad InStat3 and GraphPad Prism 5 software. All the parameters were run 3 times (n=3) except the pre-compression parameter. Experimental results were expressed as mean ± SD., Student's t-test and one-way analysis of variance (ANOVA) were applied to check significant differences in mean of post-compression parameter, %Cumulative Release, Zero order, First order, Higuchi kinetics, Peppas Equation between batch series 'MG', batch 'DC1' and marketed SR preparation. Differences were considered to be statistically significant at $p < 0.05$.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Pre-compression evaluation: The granules of various formulations containing drug and meltable binders were evaluated for the angle of repose, loose bulk density(LBD), tapped bulk density(TBD), void volume, bulkiness, total porosity and Compressibility(carr's) index (Table 2). The angles of repose for all formulations were found to be in the range of 16 to 20^0 indicating excellent flow properties. The values for LBD and TBD were found to be in the range of 0.57 to 0.68 g/cm^3 and 0.64 to 0.76 g/cm^3 indicating good packing capacity. The void volume for all formulation was found to be in the range of 0.3 to 0.4 ml. The bulkiness for all formulations was found to be in the range of 1.61 to 1.75 ml. The total porosity for all formulations was found to be in the range of 10.35 to 11.75% indicating slower the rate of dissolution. Carr's indexes for all formulations were found to be in the range of 10.52 to 13.04 % indicating excellent flow properties, cohesiveness.

Post-compression evaluation: The each formulation type (MG1 to MG4 and DC1) were evaluated for parameters such as thickness, diameter, weight variation, drug content, hardness and friability (Table 3). The weight of all formulation tablets were within the range according to IP. The hardness were in range of 4.8 ± 0.1000 to 6.8 ± 0.0577kg/cm^2,which indicating that the increase in polymer content increase the interparticulate bonding during compaction which results in increase in crushing strength of tablets. The thickness of all formulation was found to be in the range of 1.260 ± 0.0584 to 3.527 ± 0.0970 mm. Friability was found to be 0.71 ± 0.0361 to 0.92 ± 0.0300%. As friability was below 1% tablets in each formulation can withstand the mechanical shocks. Percentage drug content in formulations MG1 to MG4 and DC1 were found to be in the range of 95.31 ± 0.5160 to 97.13 ± 0.8155%. It showed uniform distribution of drug in matrix. All the parameters were run 3 times (n=3). The difference in mean of thickness, diameter, weight variation, drug content, hardness and friability between batch series 'MG' and batch 'DC1' was significant ($p < 0.05$).

Compatibility Studies: The major IR peaks observed in matrices were 1736 (C=O stretching, carbonyl group), 1623 (C=C stretching) of compritol 888 ATO and 2730 (C-H stretching), 3402 (N-H stretching vibration, ter. amine), 1298 (C-N stretching vibration) of Tramadol hydrochloride (Fig 1 C). From IR spectroscopic study, it was found that there was no evidence of interaction between drug and polymer.

In Vitro Drug Release Study

Effect of different drug: wax ratios on release of drug effect of different drug: wax ratios (MG1 to MG4) on the release profile of Tramadol HCL studied (Table 4). The drug release revealed that formulation MG1, MG2, MG 3 MG4 showed 85.4683 ± 1.098%, 78.8683 ± 0.6307%, 67.9217 ± 0.6278% and 56.6767 ± 0.6299% of Tramadol hydrochloride within 12 h. From study, it was found that as concentration of wax increases, release of drug from matrices decreases (Fig 2). It may be due to slower penetration of dissolution medium in the matrices due to increase lipophilicity of waxy substances [6]. The drug release profiles for tablets made from

ISSN: 0975-8585

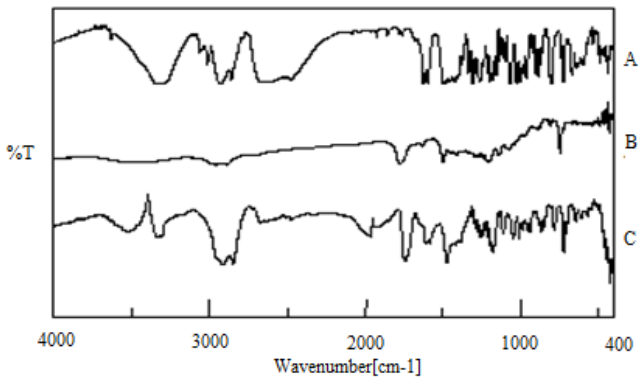

Fig 1: IR spectrum of Tramadol hydrochloride (A), compritol 888 ATO (B) and TH:comp(C).

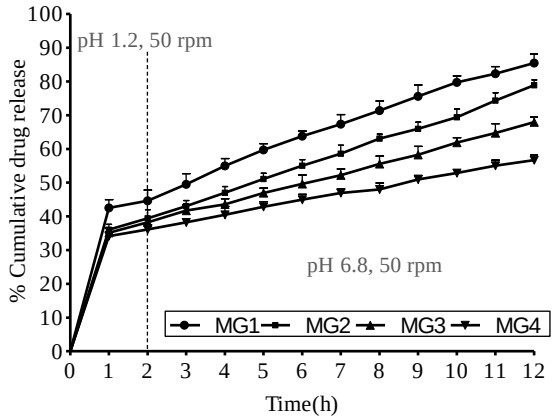

Fig 2: Effect of different concentration of Compritol 888 ATO on in vitro release of Tramadol hydrochloride matrix tablets.

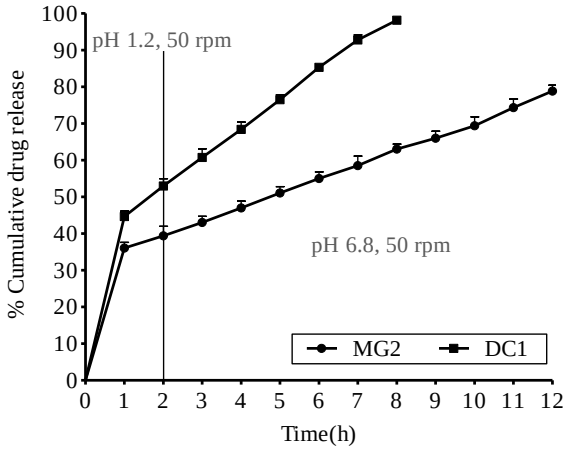

Fig 3: Comparative release profiles of sustained release matrix tablet made by melt granulation and direct compression in the ratio of 1:2.

ISSN: 0975-8585

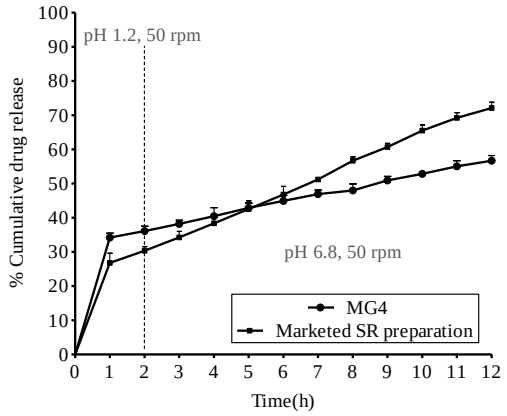

Fig 4: Comparative release profiles of best batch MG4 and marketed SR preparation

melt granulation are shown in Fig 2. In preliminary studies it was observed that at lower concentration of meltable binder the matrices were disintegrated during dissolution test. Disintegration properties of these matrices were dependant on content of matrix forming agent. Hence different ratios of drug: lipophilic binder was designed to prepare matrices and drug release retardation. This study revealed that as concentration of lipophilic binder increases, release from matrices decreases. It may be due to slower penetration of dissolution medium in waxy matrices. That is increasing the ratio of drug: Lipophilic binder from 1:1 to 1:4 resulted in decreasing release of drug. The effect of meltable binder content on the release characteristics were found to be irrespective of their chemical nature. In the formulation MG4 shows maximum drug release retardation because of high concentration (1:4) i.e. 56.6767%. Tramadol hydrochloride release occurred by diffusion mechanism. Increasing the ratio of Compritol 888 ATO in the granules resulted in decreasing the release of drug. (Fig 2).The drug release revealed that formulation MG1, MG2, MG 3 and MG4 showed 42.5030%, 36.066%, 35.119% and 34.1830% of Tramadol hydrochloride within first hrs. This finding may be attributed to fast dissolution of drug on the surface [23]. Reza et al[23], reported that the drug particles present on the surface of matrix were initially released into surrounding media generating many pores and cracks which facilitates further release of drug and also formation of channels within the matrix in case of water soluble drug. From the above observations the drug release retardation from tablets was in the following order:

MG4 >MF3 >MG2>MG1

All the parameters were run 3 times (n=3). The difference in mean of drug release of batch series 'MG' was significant ($p < 0.05$).

Effect of method of preparation: The matrices were prepared by physical mixtures by direct compression and melt granulation .The release of drug (72.1453 ± 0.6688 in 8 h) is faster from matrix tablet prepared by direct compression (DC1) than melt granulation method (Fig. 2). To studying the effect of method of preparation of sustained release tablets on drug release

ISSN: 0975-8585

Table 1: Formulation composition of Tramadol hydrochloride in matrix System

Formulation code	Drug: Polymer ratio	Total weight (mg)
MG1	TH: Comp (1:1)	200
MG2	TH: Comp (1:2)	300
MG3	TH: Comp (1:3)	400
MG4	TH: Comp (1:4)	500
DC1	TH: Comp (1:2)	300

TH: Tramadol hydrochloride Comp: Compritol 888ATO

Table 2: Pre-compression evaluation matrix tablets of Tramadol Hydrochloride

Formulation code	Angle of Repose (°)	Loose Bulk Density (g/cm3)	Tapped Bulk Density (g/cm3)	Void Volume (ml)	Bulkiness (ml)	Total Porosity (%)	Carr's Index (%)
MG1	18.00	0.58	0.66	0.4	1.72	11.75	12.12
MG2	19.57	0.68	0.76	0.3	1.47	10.35	10.52
MG3	16.17	0.57	0.64	0.4	1.75	11.43	10.93
MG4	20.14	0.60	0.69	0.4	1.66	10.78	13.04
DC1	18.70	0.62	0.71	0.4	1.61	11.17	12.67

Table 3: Post-compression evaluation matrix tablets of Tramadol Hydrochloride

Formulation code	Thickness (mm)±SD	Hardness (kg/cm^2) ±SD	Friability (%)±SD	Tablet Weight (mg) ±SD	Drug Content (%)±SD
MG1	1.260±0.0584	4.8±0.1000	0.81±0.0208	197.55±0.6411	96.87±0.3988
MG2	1.972±0.1212	5.5±0.1000	0.92±0.0300	297.50±0.7006	96.18±0.8413
MG3	2.648±0.0843	6.2±0.1000	0.71±0.0361	395.40±0.5204	95.70±0.5145
MG4	3.527±0.0970	6.8±0.0577	0.87±0.0451	496.34±0.4747	95.31±0.5160
DC1	2.063±0.3167	5.5±0.3015	0.87±0.0617	298.01±0.6773	97.13±0.8155

SD=Standard deviation (n=3) The difference in mean of Thickness, Hardness, Friability, Tablet Weight, Drug Content between batch series 'MG' and batch 'DC1' was significant ($p < 0.05$).

Table 4: In vitro release kinetics of matrix tablets of Tramadol Hydrochloride

Formulation code	%Cumulative Release ±SD	Zero order (R^2) ±SD	First order (R^2) ±SD	Higuchi kinetics (R^2) ±SD	Peppas Equation	
					(n)	(R^2) ±SD
MG1	85.47±1.100	0.993±0.003	0.98±0.004	0.98±0.002	0.3076	0.95±0.003
MG2	78.87±0.631	0.997±0.001	0.97±0.003	0.97±0.004	0.3259	0.94±0.009
MG3	67.92±0.628	0.996±0.003	0.98±0.007	0.97±0.006	0.2713	0.93±0.010
MG4	56.68±0.630	0.994±0.005	0.99±0.006	0.98±0.005	0.2129	0.93±0.009
DC1	98.18±0.199	0.997±0.000	0.85±0.009	0.98±0.007	0.3868	0.97±0.009
Marketed SR prepration	72.15±0.669	0.997±0.013	0.96±0.027	0.95±0.003	0.3754	0.93±0.009

SD=Standard deviation (n=3) The difference in mean of %Cumulative Release, Zero order, First order, Higuchi kinetics, Peppas Equation between batch series 'MG', batch 'DC1' and marketed SR preparation was significant ($p<0.05$).

properties of Tramadol hydrochloride, the matrices were prepared by two methods i.e. physical mixtures by direct compression and melt granulation. By using above said lipophilic binders for ratio of 1:2 drug: meltable binder which was optimized from the previous studies. Fig 3. shows

comparative release profiles of matrix tablet prepared by direct compression of physical mixtures and matrix tablet prepared by compression of granules prepared by melt granulation. The release was higher from the matrix tablet made by direct compression of physical mixtures as compared to matrices made by compression of granules by melt granulation. The result attributed to this formation of coating of lipophilic binder over drug particles is more uniform in melt granulation technique than matrix tablet prepared by direct compression. Also integrity of matrix tablet formed by melt granulation was found to be better than matrix tablet prepared by direct compression of physical mixtures. The probable mechanism of faster release of drug from matrix tablet prepared by direct compression of physical mixtures is erosion controls drug release from these matrices. The retardation of drug release was due to more effective coating of drug particles with lipophilic binders in melt granulation technique and cause slow diffusion as main mechanism which controls drug release. From the above observations the drug release retardation from tablets was in the following order.

Melt granulation > Direct compression

All the parameters were run 3 times (n=3) ($p < 0.05$).

Drug Release kinetics

The drug release data were explored for the type of release mechanism followed. The best fit with the highest determination R^2coefficients was shown by both the zero order and Higuchi models. Zero order release describes the release rate independent of drug concentration. Higuchi square root kinetic model describes, release drug from the insoluble matrix as square r of time dependent process. It describes release of drug by simple diffusion mechanism. The value of *n* with regression coefficient for all the formulations is shown in Table 4. The values of *n* were in the range of 0.2129 to 0.3868 (n is less than 0.5) indicating fickian release governed by the drug diffusion. However as indicated by the values of R^2 both of the models (Higuchi and Peppas) were found to be efficient in describe the release of Tramadol hydrochloride from the matrix tablets. All the parameters were run 3 times (n=3). The difference in mean of Zero order, First order, Higuchi kinetics and Peppas Equation between batch series 'MG', batch 'DC1' and marketed SR preparation was indicating significant ($p < 0.05$).

CONCLUSION

The study showed that compritol 888ATO is appropriate waxy material, which can be used as matrix forming agent to sustain the release of water soluble drug such as Tramadol Hydrochloride. As concentration of compritol 888 ATO was increased, the drug release rate was decreased. Matrix tablets of Tramadol hydrochloride prepared by melt granulation technique shows retardation of drug release more effectively than tablet prepared by direct compression of physical mixture. Among these all formulations, MG4 (56.6767 ± 0.6299%) was found to be best formulation. The formulations followed zero order, Higuchi kinetics and Peppas Equation

while the drug release was found to be fickian diffusion mechanism. Formulation MG4 was better in comparison with DC1 and marketed SR formulation. The developed matrix tablets of Tramadol hydrochloride may be used for prolonged drug release, thereby improving the bioavailability and patient compliance.

ACKNOWLEDGEMENTS

Authors are thankful to Neon Laboratories Limited, Mumbai, India for the gift sample of Tramadol Hydrochloride and Gattefosse Corp. France for providing compritol 888ATO.

REFERENCES

[1] Gudsoorkar VR, Rambhau D. The Eastern Pharmacist 1997; 40: 111-113.
[2] Wise DL. In Handbook of Pharmaceutical Controlled Release Technology, Marcel Dekker, New York and Basel, 2005, pp. 435-440.
[3] Bodmeier RA, Swarbrick J, Boylan JC. Eds. Encyclopedia of pharmaceutical technology, 2nd ed., Vol. III, New York Marcel Dekkerlnc, 1998, pp. 2988-3000.
[4] Hamdani J, Moes AJ, Amighi K. Int J Pharm 2003; 260: 47 – 57.
[5] Zang YE, Schwartz JB. Drug Dev Ind Pharm 2003; 29: 131- 138.
[6] Hamdani J, Moes AJ, Amighi K. Int J Pharm 2002; 245: 167 - 177.
[7] Lloanusi NO, Schwartz JB. Drug Dev Ind Pharm 1998; 24: 37-44.
[8] Barthelemy P, Laforet JP, Farah N, Joachim J. Eur J Pharm Biopharm 1999; 47: 87- 90.
[9] Chaudhari PD. Melt Granulation Technique: A Review, 2006 [cited 2006 Jan 8]. Available from Http://www. pharmainfo.net./ exclusive/ reviews.
[10] Mahaparale PR, Kasture PV, Deshmukh SS, Kuchekar BS. J Pharmaceut Res 2006; 5: 10-14.
[11] Paradkar AR, Maheshwari M, Chauhan MB. Indian Drugs 2004; 41: 350 – 353.
[12] Liu J, Zhang F, McGinity JW. Eur J Pharm Biopharm 2001; 52: 181 – 190.
[13] Feng QL, Jin HH, Jia XD, Hua S, Shu X, Ji YL. Int J Pharm 2006; 30: 1-6.
[14] Lehmann KA. Drugs 1997; 53: 25-33.
[15] rxlist [Internet]. tramadol hydrochloride-drug [cited 2009 Aug 25]. Available from http:// www.rxlist.com
[16] Tiwari SB, Murthy TK, Pai MR, Mehta PR, Chowdhary PB. AAPS PharmsciTech 2003; 4: 110-116.
[17] Obaidat AA, Obaidat RM. Eur J Pharm Biopharm 2001; 52: 231-235.
[18] Malonne H, Fontaine J, Moes A. Biol Pharm Bull 2000; 23: 627- 631.
[19] Zhang ZY, Ping QN, Xiao B. J Control Rel 2000; 66: 107-113.
[20] Patel NM, Soniwala MM. Indian Drugs 2008; 45: 98-104.
[21] Higuchi T. J Pharm Sci 1963; 52: 1145-1149.
[22] Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Int J Pharm 1983; 15: 25-35.
[23] Reza MS, Quadir MA, Haider SS. J Pharm Pharma Sci 2003; 6: 282 – 291.