Original Research Article

Formulation and *In vitro/In vivo* Evaluation of Sustained Release Diltiazem Matrix Tablets

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Abstract

Purpose: To develop and optimise sustained release (SR) matrix tablets of diltiazem hydrochloride (DHL).
Methods: DHL tablets were prepared by direct compression and consisted of hydroxyprpoylmethyl cellulose, Kollidon SR and Eudragit RSPO. A 3^2 full factorial design was applied to study the effect of polymers used on drug release from the DHL. The tablets were also evaluated for physicochemical characteristics and release kinetics. In vivo human volunteer studies were carried out on the optimised formulation with a commercial sustained release product serving as reference.
Results: The physicochemical characteristics of all prepared tablets were satisfactory. The developed drug delivery system provided prolonged drug release rates over a period of 24 hours. The release profile of the developed formulation was described by the Higuchi model. Mean time of occurrence for maximum (peak) drug concentration (T_{max}) was 2.05 ± 0.52 and 2.30 ± 0.57 h for the optimized and commercial formulations, respectively, while mean maximum concentration of drug (C_{max}) was 501.74 ± 0.05 and 509.65 ± 0.06, ng/ml respectively. Good correlation between the dissolution profiles and bioavailability was observed using the method of linear regression analysis and correlation coefficient.
Conclusion: A fair correlation between in vitro dissolution and in vivo data was found. The results obtained indicate successful development of a sustained release formulation of diltiazem.

Keywords: Diltiazem, Matrix tablet, Hydroxypropyl methylcellulose Eudragit, In vitro/in vivo correlation, Optimization

INTRODUCTION

Compressed hydrophilic matrices have become popular as modified release dosage forms for oral administration in terms of clinical efficacy and patient compliance [1]. Matrix system is often used for manufacturing sustained-release dosage forms easy of production [2].

Diltiazem HCl is a water soluble calcium-channel blocker belonging to the benzothiazepine family. Calcium channel blockers affect the movement of calcium into the cells of the heart and blood vessels, relaxing the blood vessels and increasing blood supply and oxygen to the heart.

They are generally used to treat high blood pressure, arrhythmia and angina pectoris [3].

There are several studies where the polymer type, composition, and tablet characteristics have been studied in order to achieve desired DHL release profile [4-6]. Carbopols, polyvinyl alcohol, sodium carboxymethylcellulose, carrageenan and xanthan gums are among the most widely in this regard [7-9]. Matrix tablets can be prepared by wet granulation or by direct compression [10]. These tablets, when prepared using HPMC K15M CR, on contact with dissolution media or gastric fluids become hydrated to form a viscous gel layer through which drug will be released by diffusion and/or by erosion of the matrix [11].

However, the use of only hydrophilic polymers for extending drug release for highly water-soluble drugs is limited due to rapid diffusion of dissolved drug through hydrophilic gel networks. For such highly water soluble drugs, it becomes necessary to incorporate another type of polymer in the matrix system.

Among such polymers, Eudragit RLPO or RSPO have been used successfully to formulate appropriate sustained release matrix formulations [12-14]. The aim of the present study, therefore, was to design a sustained release matrix tablet capable of producing 20 - 24 h sustained release of diltiazem HCl using a combination of hydrophobic and gelling hydrophilic polymer. A secondary objective was to evaluate the influence of polymer content and type as well as polymer combination on drug release profile.

EXPERIMENTAL

Materials

Diltiazem hydrochloride and magnesium stearate were received as gifts from Golden Cross Pharma (India). Hydroxypropyl methylcellulose (HPMC) K15M CR Premium and starch (Sta-RX 1500) were obtained from Colorcon Asia Private Ltd. (India) while polyvinyl acetate/polyvinylpyrro-lidone (Kollidon SR) was supplied by BASF (India). Eudragit RSPO and the microcrystalline cellulose (MCC) were purchased from Evonik GmbH (Germany) and Ozone® International (India), respectively.

Preparation of diltiazem tablets

All the powder materials (drug and Excipients) were passed through a sieve of aperture size of 250 µm and then blended in a polybag manually. The blends were directly compressed (with compressing weight of 502 mg for tablets containing a single polymer and 452 mg for combination of polymers) on a Cadmach single punch tablet machine (Ahmadabad, India) using 8 mm concave punches.

Factorial design

A 3^2 factorial design was used in this study and two factors evaluated, each at three levels; experimental trials were performed at all nine combinations. Combinations of polymer HPMC K15 CR (X_1) and Eudragit RSPO (X_2) were selected as independent variables (Table 1). Drug release at 18 and 20 h was selected as dependent variables. The data were also subjected to 3D response surface methodology to determine the influence of polymer and binder on dependent variables.

All the batches each contained 100 mg diltiazem hydrochloride, 11.95 % Starch 1500, 2 % talc, 0.39 % magnesium stearate and MCC *q.s.* to produce tablets of 452 mg weight.

Table 1: Formulation variables in a 3^2 factorial design

Factor	Level		
	-1	0	1
X_1: amount of HPMC K15M CR (mg)	150	170	190
X_2: amount of Eudragit RSPO (mg)	60	80	100

Physical evaluation of diltiazem hydrochloride tablets

Tablet hardness was determined with a Monsanto tablet hardness tester (Campbell Electronics, Mumbai, India). The hardness of each of 10 tablets randomly selected from each batch was measured and then mean and standard deviation evaluation while friability was obtained with a friabilator (Hoffmann-La Roche Ltd., Basel, Switzerland). The weight of ten tablets before and after the test, and the percent loss in weight recorded as friability. The drug content of the tablets was evaluated spectrophotometrically (Shimadzu, model no-1700) at 235 nm.

In vitro drug release studies

In vitro drug release studies were carried out in 900 ml of 0.1M HCl for the first 2 h using a USP XXII type 1 dissolution apparatus (Electrolab TDT-08L) at 50 rpm and 37 ± 0.5 °C. Immediately after, the dissolution medium replaced with 900 ml of phosphate buffer (pH 6.8) that has been pre-equilibrated to 37 °C. At predetermined time intervals during the dissolution test, samples (5 ml) were withdrawn for assay and replaced with equivalent volume of fresh medium to maintain conditions. All dissolution studies were performed in triplicate. The samples were filtered, diluted appropriately was and then analyzed for diltiazem spectrophotometrically (Shimadzu, Japan) at 235 nm. The test was performed in triplicate.

Kinetic analysis of dissolution data

The release data were fitted to five kinetic models, viz, zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell to determine drug release mechanism with the aid of Expert 7.0.3 trial software, GraphPad Instat® (GraphPad Software Inc., San Diego, CA).

In-vivo evaluation studies

Bioavailability studies were carried out on the experimental formulation and Commercial formulation Diltime SR (Zydus medica, India) tablet which served as the reference. The human study was approved and carried out in compliance with the protocol of the institutional ethical committee (approval reference no. CPBEMV-112009 under CPU, India) following the guidelines for bioavailability and bioequivalence studies, CDSCO, DGHS, India. [16]. The subjects enrolled in the study were presented with full details of the investigation, both verbally and in written form, prior to providing written informed consent.

Study design

Six healthy non-smoking adult male subjects between 20 and 29 years old (Mean=24.7 years, SD± 3.4 years) and weighing from 55 to 65 kg (Mean =61 kg, SD±5.1 kg) were admitted to the intra-individual randomized three-period crossover study designed for single-dose blood level study. The subjects received diltiazem HCl in each trial period, i.e., the test formulation (DHE$_9$) or Diltime SR (reference). Dosage form administration strictly adhered to the study protocol.

Sample collection and analysis

Blood samples (5 ml) were collected from 0 to 48 h after administration of DHE$_9$ and Diltime SR. Plasma was harvested by taking 0.5 mL of plasma in ria vial, added 100 µl of 0.1 N HCL, vortexed the vials on vortexer, then added 3 mL of Ethyl Acetate, again vortexed for 5 mins, then centrifuged the vials in cold centrifuge for 10 min at 4500 rpm at 4°C. Separated 2 mL of upper layer of organic solvent in vial, evaporated to dryness at 50 °C under a steady stream of nitrogen and kept at -20 °C pending analysis by High pressure liquid chromatography (HPLC). Plasma diltiazem HCl concentration was determined by a HPLC method. Sample preparation was performed by liquid-liquid extraction in tert-butyl methyl ether. The organic solvent was evaporated and the residue dissolved in a mobile phase. Aliquots of 20 µL were injected automatically into the chromatograph. Separation of diltiazem was achieved on reversed phase (C18) column with a mobile phase consisting of 10 mM ammonium acetate: acetonitrile (55:45 v/v). Quantitation of diltiazem was performed by UV detector at 235 nm.

Each sample was analysed in triplicate and the mean computed. Pharmacokinetics parameters, i.e., C_{max} and T_{max}, were determined from plasa concentration versus time data, while AUC_{0-t} was calculated from the mean plasma diltiazem HCl concentration-time data.

Stability of test formulation

The optimized batch DHE$_9$ was kept for accelerated stability study at 40°C/75% R.H. as per ICH guidelines for up to 6 months. Accelerated stability study samples were analyzed for physical appearance, drug content and dissolution profiles with reference to initial results of the same.

Statistical analysis

For optimization of tablet formulations and data analysis, software, Opti-Stat (made in-house version 1.0.0), was developed. The resulting data were fitted to OPTI-STAT and Design Expert 7.0.3 (Stat-ease) software which analyzed statistically using analysis of variance (ANOVA). To compare the mean of all release data and to assess statistical differences between them, either single-factor analysis of variance (ANOVA) or unpaired two-tailed t-test was carried out at 95 % confidence level while multiple regression analysis was carried out using Design Expert 7.0.3 (Stat-Ease).

RESULTS

Physical characteristics of tablets

The physical appearance, hardness, friability and drug content were well within the prescribed limits (Table 2). All the physical parameters of the compressed tablets complied with the official requirements [15].

Drug release from DHL matrix tablets

The presence of polyvinyl acetate/polyvinyl-pyrrolidone (Kollidon SR) gave burst release profiles. Table 3 shows release profiles of RSPO-HPMC-based formulations. RPSO and HPMC concentration ranges of 22.1 to 30.3 % and 33.8 to 42.0 %, respectively, provided drug sustained release for over 20 h, with 90.56 ± 0.65 to 99.09 ± 0.89 drug released, irrespective of the polymer content. Formulation DHE$_9$ containing optimum blend of the polymers, sustained drug release for 24 h and therefore, was chosen for *in vivo* studies.

Table 2: Physical characteristics (mean ± standard deviation) of the prepared diltiazem HCl tablets

Formulation code	Variable level in coded form		Hardness (kg/cm^2)	Drug content (%)	Friability (%)
	Factor X$_1$	*Factor X$_2$*			
DHE$_1$	-1	-1	8.3±0.4	92.32±4.20	0.63
DHE$_2$	-1	0	8.5±0.5	90.25±2.40	0.66
DHE$_3$	-1	1	8.6±0.2	96.43±5.10	0.53
DHE$_4$	0	-1	8.5±0.1	97.24±3.30	0.72
DHE$_5$	0	0	8.2±0.3	94.45±5.20	0.58
DHE$_6$	0	1	8.3±0.1	95.14±3.30	0.69
DHE$_7$	1	-1	8.3±0.2	91.45±1.50	0.59
DHE$_8$	1	0	8.4±0.3	93.24±2.40	0.65
DHE$_9$	1	1	8.5±0.4	97.32±4.20	0.61

Table 3: Release of diltiazem HCl from the test formulations as per factorial design

Time (h)	Formulation code									
	DHE$_1$	DHE$_2$	DHE$_3$	DHE$_4$	DHE$_5$	DHE$_6$	DHE$_7$	DHE$_8$	DHE$_9$	Diltime SR
1	22.0	20.3	18.9	19.3	18.5	17.9	18.3	17.9	15.9	17.20
2	32.5	30.9	25.8	27.1	25.6	25.6	29.9	29.9	25.9	20.94
3	37.8	35.9	32.6	38.6	36.0	34.0	38.0	35.9	31.9	24.48
4	41.2	39.2	36.8	41.9	40.3	34.3	42.9	43.2	39.2	28.15
5	46.8	45.2	42.8	48.4	46.8	43.8	52.2	49.2	43.2	31.86
6	54.1	51.6	46.3	51.0	49.3	45.3	56.0	53.3	48.3	35.39
7	60.2	58.2	53.7	55.7	53.7	50.7	60.7	57.2	52.2	38.75
8	65.8	64.1	59.1	59.0	58.8	55.8	63.4	61.1	56.1	42.52
9	69.4	67.0	65.4	67.7	65.9	63.9	65.5	66.0	61.0	46.89
10	73.9	71.7	68.1	71.5	69.9	65.9	71.1	70.7	65.7	50.11
11	78.8	76.3	71.3	74.1	72.8	68.8	73.8	74.3	69.3	53.89
12	82.6	80.6	76.3	78.3	76.8	73.8	76.3	78.0	72.0	57.24
14	86.8	85.3	82.8	83.1	81.3	78.3	82.8	82.3	78.3	63.95
16	93.9	92.9	89.3	88.7	87.9	84.9	87.5	86.5	82.5	71.85
18	97.6	95.4	94.8	94.4	92.8	90.8	91.1	89.2	87.2	77.98
20	99.1	98.5	97.9	97.5	96.5	95.5	94.8	93.6	90.6	85.69
22							98.7	97.9	93.9	93.82
24									98.0	99.54

Drug release kinetics

When the *in vitro* release data were subjected to kinetic analysis, Higuchi and Korsmeyer-Peppas model gave the highest correlation coefficient (R^2) 0.996 and 0.995 and n value of 0.564, thus indicating that the release mechanism was mixed of diffusion-erosion so called anomalous drug diffusion.

In vivo availability

Drug release from DHE$_9$ was almost similar to that from commercial formulation which was selected. Mean plasma concentration profiles of DHE$_9$ and commercial formulation are presented in Figure 1.
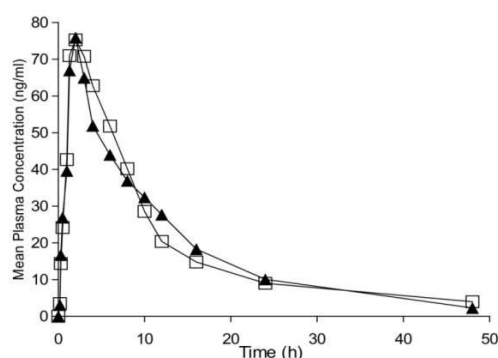

Fig 1: Mean plasma concentration of optimized diltiazem HCl formulation (▲) and commercial (reference) product (□)

Both the formulations exhibited as sustained release *in vivo*. All the pharmacokinetics

parameters displayed in Table 4 Mean Time of occurrence for maximum (peak) drug concentration (T_{max}) was 2.05 ± 0.52 h and 2.30 ± 0.57 h for the optimized and commercial formulations, respectively, while mean Maximum concentration of drug (C_{max}) was 501.74 ± 0.05 ng/ml and 509.65±0.06 ng/ml, respectively. $AUC_{(0-\infty)}$ is an area under the drug concentration in plasma versus time curve. The values for Cmax, Tmax, AUC (0-t) and AUC (0-∞) were found to be comparable indicating formulation exhibited comparable sustained release pattern.

Table 4: Pharmacokinetic parameters of optimized Diltiazem HCl formulation

Parameter	DHE$_9$	Commercial Formulation
C$_{max}$ (ng/ml)	501.74±0.054	509.65±0.057
T$_{max}$ (h)	2.05±0.52	2.3±0.57
AUC$_{(0-t)}$ (ng/ml*h)	5357.75±0.214	5424.38±0.219
AUC$_{(0-\infty)}$ (ng/ml*h)	5622.77±0.221	5653.22±0.226

In vitro/in vivo

Correlation

When the in-vitro dissolution of diltiazem HCl was compared with *in-vivo* data using the method of linear regression analysis, a correlation coefficient (r^2) of 0.8551 was found, indicating only a fair correlation

Stability of test formulation

The stability of the test formulation, stored at 40 °C/75 %RH, was monitored over a period of 6 months as per ICH guidelines and not noticeable changes in its physical appearance, drug content and dissolution profiles were found.. Drug content and dissolution at the end of 24 h was initially found to be 97.32±4.20 and 98.0±4.30 % respectively while same was observed to be 96.22±3.80 and 97±3.72 % after the period of six months accelerated stability study.

DISCUSSION

A suitable sustained release diltiazem HCl dosage form would not only enhance therapeutic efficacy and patient compliance but also produce more desirable blood drug levels and lower incidence of adverse effects.

The drug content of the formulations ranged from 90.25 ± 2.40 - 97.32 ± 4.20, indicating good content uniformity for some of the formulations. Furthermore, all the formulations showed satisfactory mechanical characteristics based on the hardness and friability data.

The presence of polyvinyl acetate/polyvinylpyrrolidone (Kollidon SR) caused a 'burst' release phenomenon. This can be explained by the high water permeability of Kollidon SR while the presence of Eudragit RSPO and HPMC promoted slow release.as drug is released by erosion of the Eudragit matrix and swelling and gelling property of the HPMC. Hence, Eudragit RSPO and HPMC K15 M CR were selected for the optimization of drug release.

As the concentration of Eudragit RSPO increased, there was decrease in dissolution rate due to the extremely low water solubility of RSPO whose hydrophobic character caused a delay in the penetration of dissolution medium into the matrix, thereby decreasing drug dissolution and diffusion rate. Since RSPO remains undissolved at various pH conditions, the polymer also acts as an insoluble barrier to the release of drug from within the matrix.

Generally, the Higuchi order model gave the highest correlation coefficient (R^2) value for the dissolution data. This indicates that the *in vitro* drug release pattern is not controlled by the amount of drug left in the tablet as dissolution progresses or by the disintegration of the tablet, but by the diffusion. Since Eudragit is also swells, penetration of water does occur due to polymer chain relaxation after prolonged contact with water.

The Korsemeyer-Peppas release exponent (n) value of the optimised formulation (DHE$_9$) is 0.564, which indicates anomalous drug diffusion [7]. This is means that drug release was both by diffusion and erosion, and this is probably so because HPMC swells to form a gel through which the drug diffuses while the drug is also released by erosion of Eudragit matrix. The optimised formulation DHE$_9$ showed a correlation coefficient of 0.9961 for the Higuchi model indicating a largely diffusion-dependent drug release. The *in vitro* and *in vivo* correlation was fair with a correlation coefficient (r^2) value of 0.8551.

CONCLUSION

Sustatined release diltiazem HCl matrix tablet formulation that released drug up to 24 h has been developed. The tablets showed good bioavailability, stability and physicochemical characteristics

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