SECTION II
EXPERIMENTAL WORK

4. EXPERIMENTAL WORK

4.1. Materials

4.1.1. DRUGS AND FORMULATIONS

Glipizide (99.79% pure) was kindly supplied by Apostle remedies (Vadodara, India). Nateglinide (99.87% pure) was obtained from Alembic Ltd. (Vadodara, India) as a gift sample.
Gliclazide (gliclazide, purity of 99.69%), used as an internal standard (IS) in HPLC quantitation were obtained as gift from Relax Pharmaceuticals (Vadodara, India). Samples of Glytop 10 SR® (RPG Life Sciences, India) and Natelide-60 (alembic Ltd, India) were obtained from retail pharmacy and used as reference products.

4.1.2. POLYMERS AND EXCIPIENTS

Ethylcellulose (EC, Ethocel® standard 7 FP premium), hydroxypropylmethyl cellulose (HPMC, Methocel® K100LV, K4M, K15M, K100M grade) and Starch 1500® were kindly gifted by the Colorcon® Asia Pacific (India). The degree of substitution for methoxy and hydrdoxypropoxy groups in HPMC were 22 and 8, respectively for all above mentioned grades. Typical viscosity values for 2% (w/v) aqueous solutions of Methocel® K100LV, K4M, K15M, K100M at 20°C were 100, 4000, 15000, and 100000 mPa·s, respectively. Microcrystalline cellulose (MCC, Avicel® PH 102, PH 301, PH 302), carrageenan (Gelcarin GP-379 and Gelcarin GP-911) and sodium alginate (Protonal LF 120M) were gifted by the FMC Biopolymers® (Ireland). Different grades of Carbomers (Carbopol® 931P, Carbopol® 934P, Carbopol® 941P, Carbopol® 971P and Carbopol® 974P, BF Goodrich Specialty Chemicals, Cleveland, OH) were generous gifts from Maruti Chemicals (Ahmedabad, India). Eudragit L100 and Eudragit S100 (Rohm Pharma, Darmstadt, Germany) were generous gifts from Embrosiya Remedies (Vadodara, India). Xanthan gum USP XXVIII (Jungbunzlaver, Austria), lactose spray-dried (Flowlac 100®, Meggle, Germany), colloidal silicon dioxide (Aerosil SD 200®, Degussa AG, Germany), magnesium stearate (Mallinckrodt, USA, used as a lubricant in tableting) were obtained as gift samples from Relax Pharmaceuticals (Vadodara, India). Calcium dibasic phosphate ($CaHPO_4 \cdot 2H_2O$), calcium gluconate, disodium hydrogern phosphate ($Na_2HPO_4 \cdot 2H_2O$) and potassium chloride (KCl) were purchased from S. D. Fine Chem, Ltd. (Mumbai, India) and used as one of the excipient in matrix tablet formulations.

4.1.3. CHEMICALS AND REAGENTS

Hydrochloric acid (HCl), sodium hydroxide (NaOH), tri-sodium phosphate ($Na_3PO_4 \cdot 12H_2O$) were purchased from commercial supplier, S. D. Fine Chem, Ltd. (Mumbai, India). Deionized double-distilled water was used through out the study. All other chemicals and solvents were of analytical grade and used without further purification. For HPLC analysis, potassium dihydrogen ortho-phosphate (KH_2PO_4), 85% ortho-phosphoric acid (H_3PO_4), as well as acetonitrile, methanol and water (HPLC grade) were obtained from S. D. Fine Chem, Ltd. (Mumbai, India) and used as received. All buffers and solutions were prepared with HPLC grade water. Unless otherwise specified, all solutions were filtered through a 0.2 µm Ultipor® N66® Nylon 6, 6 membrane filter (Pall Life Sciences, USA) prior to use.

4.1.4. EQUIPMENTS/INSTRUMENTS

Analytical balance (Mettler AE-50 electronic balance, Greifensee, Switzerland) was used for accurate and precise weighing. The UV-visible spectroscope/spectrophotometer (Shimadzu UV-1601) was used for all in vitro quantitation of drugs. Fourier transform infra-red (FTIR) spectrophotometer (FTIR-8300, Shimadzu, Tokyo, Japan) and differential scanning caloriemeter (DSC; DSC-60, Shimadzu, Tokyo, Japan) were used to check the drug-excipient compatibility study. An automatic eight station rotary tablet machine (Modern Engineering Works, New Delhi, India) was used to prepare the hydrophilic matrix tablets. The USP XXVI dissolution test apparatus (TDT-60T, Electrolab, Mumbai, India) type II (paddle) was used for dissolution throughout the study. Scanning electron microscope (JSM-5610LV; Jeol Ltd., Tokyo, Japan) was used to obtain the surface images of the tablet before and after dissolution study.

4.2. Analytical Method Development

Spectrophotometric methods have been developed for estimation of glipizide and nateglinide from physical mixture, tablets, and dissolution studies. Moreover, HPLC methods for estimation of both the drugs have been developed and validated for quantitation from rabbit plasma samples.

4.2.1. ESTIMATION OF GLIPIZIDE (SPECTROSCOPIC METHOD)

For estimation of glipizide content of powder mixture, matrices and in vitro dissolution study, a calibration curve of glipizide in phosphate buffer pH 6.8 (prepared as per IP 1996 using KH_2PO_4-NaOH) was constructed over an analytical range of 1–50 µg ml^{-1} at 276 nm. Each concentration was made in triplicate, the absorbance of the solutions was determined spectroscopically (Shimadzu UV-1601, Shimadzu Corporation, Kyoto, Japan) using matched 1 cm quartz cells using phosphate buffer pH 6.8 as control.

4.2.2. ESTIMATION OF NATEGLINIDE (SPECTROSCOPIC METHOD)

Similar to glipizide, calibration curve of nateglinide in phosphate buffer pH 6.8 (prepared as per IP 1996 using KH_2PO_4-NaOH) was constructed over an analytical range of 1–50 µg ml^{-1} at 210 nm for estimation of nateglinide content of powder mixture, matrices and in vitro dissolution study.

4.2.3. ESTIMATION OF GLIPIZIDE (HPLC METHOD)

In the present study, the effect of mobile phase pH (which influences the ionisation state of the compounds), the mobile phase composition (which influences the peaks separation), and the flow rate (which influences the peaks shape, retention time, and resolution, etc) were studied on various chromatographic parameters such as resolution, plates, height equivalent to theoretical plates, symmetry factor, retention time, peak areas, etc.

4.2.3.1. Equipment and Chromatographic Conditions

A reverse phase HPLC method was developed, optimized, and validated for the analysis of glipizide from rabbit plasma for in vivo estimations. The HPLC system (Shimadzu, Kyoto, Japan) was consisted of the pump (LC-20AT), the UV-Vis spectrophotometric detector (SPD-20A Prominence) with 20-µl fixed loop and the reverse phase column Phenomenex C_{18} column (paricle size 5 µm; 250 mm×4.6 mm i.d.; Phenomenex, Torrance, USA) preceded by an ODS guard column (particle size 10 µm, 10 mm×5 mm i.d.) at an ambient temperature. Chromatographic data were recorded and processed using a Spinchrom Chromatographic Station® CFR Version 2.4.0.195 (Spinchrom Pvt. Ltd., Chennai, India). Analysis was isocratic at 1.0 ml/min flow rate with 70:30 v/v mixture of acetonitrile : 10 mM KH_2PO_4 buffer solution adjusted to pH 3 using phosphoric acid (PBS) as mobile phase (MP). The mobile phase was prepared freshly everyday, premixed, filtered through a 0.2 μm membrane filter to remove any particulate matter and degassed by sonication before use. Glipizide concentration was determined at the wavelength of 225 nm. A previous UV (190–280 nm) scanning was done in order to select the optimal absorbance wavelength. The sensitivity of the detector was set at 0.01 AUFS. Each solution was injected in triplicate, and the relative standard deviation (RSD) was required to remain below 1.0% on glipizide/internal standard (IS, gliclazide) peak area ratio basis. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. For the estimation of glipizide, the plasma samples obtained from the rabbits were spiked with known concentration of IS and treated with equal volume of methanol (1 ml) to precipitate plasma proteins followed by addition of 0.75 ml of acetonitrile for complete extraction of drug and IS. The mixture was vortexed for 3 min and centrifuged (C-24.2/2002, Remi Cooling Centrifuge, Mumbai, India) at 3000 × *g* for 10 min. The supernatant transparent liquid was transferred into a glass tube and evaporated to dryness at room temperature under a stream of nitrogen. The residues were reconstituted with 0.2 ml of mobile phase, and filtered through a 13 mm Ø 0.2 μm membrane filter using syringe filter holder. An aliquot of 20 μl was injected into the rheodyne injector of an HPLC system.

4.2.3.2. Preparation of Standard Solutions

Accurately weighed glipizide and IS were dissolved individually in methanol to obtain the stock solution concentration of 500 μg/ml and were stored at 4°C and were stable for at least 3 months according to the FDA (US FDA CDER Guidance for the Industry, 2001). Suitable aliquots of these solutions were stepwise diluted with the mobile phase to obtain final concentration of the 30 μg/ml of both glipizide and IS. This solution was used in the preliminary experiments and for the optimization of the proposed method.
The spiking solutions of glipizide and IS (each of 8 μg/ml) were prepared by serial dilution of the above working stock solutions with HPLC grade water. These solutions were used to spike the blank plasma in order to obtain the glipizide concentrations in the analytical range of 10 to 2500 (10, 25, 100, 250, 500, 750, 1000, and 2500) ng/ml and that of IS to be 2500 ng/ml for calibration curve.

4.2.3.3. Optimization of Chromatographic Separation Conditions

The optimization of experimental conditions was aimed to obtain chromatograms with better resolution of adjacent peaks as well as sharp symmetrical peaks. Suitable chromatographic conditions were achieved after several trials with the elution systems composed of acetonitrile-water, methanol-water, acetonitrile-methanol-water, acetonitrile-acetate buffer, acetonitrile-phosphate buffer, etc. at different proportions and pH. From the above preliminary experiments, the combination of acetonitrile and phosphate buffer resulted in suitable retention time and separation of the peaks. Moreover, when phosphoric acid was used to adjust the acidic pH of phosphate buffer, a significant improvement on the peak symmetry and sharpness was observed.

As chromatographic separations are significantly affected by the mobile phase conditions like the type and composition of the organic modifiers (Kanji and Shigeya, 1998), the developed method was optimized before going for validation of chromatographic conditions. To reach at the optimized condition, systemic studies were conducted by changing: (i) mobile phase pH at 2, 3, and 4 keeping the mobile phase composition (acetonitrile:PBS, 70:30 v/v) and the flow rate (1 ml/min) constant; (ii) mobile phase (acetonitrile:PBS) composition was varied from 60:40, 70:30, to 80:20 v/v at same pH of 3 and flow rate of 1.0 ml/min; (iii) flow rate was changed (0.8, 1.0, and 1.2 ml/min) at fixed mobile phase composition (acetonitrile:PBS, 70:30 v/v) and pH of 3. All mobile phases used in the study were prepared by mixing the buffer system with the organic solvent in the desired proportions and apparent pH of the mixtures were adjusted to desired value using H_3PO_4.

4.2.3.4. Estimation of Glipizide from Rabbit Plasma

To allow for high-throughput in pharmaceutical and biomedical analysis in support of pharmacokinetic evaluation or clinical trials on glipizide, a developed method was tried to estimate the drug from rabbit plasma. Accurate and reliable quantitation of any drug from biological samples needs the extraction method that is sufficiently robust and flexible in its application with high extraction efficiency. With regard to the complexity of the various biological matrices, sample pretreatment procedure mainly liquid-liquid extraction was chosen in order to avoid endogenous interference and thus prolong the lifetime of the column. Different extraction solvents tried were acetonitrile, ethylacetate, methanol, butan-1-ol alone or in combination at different proportions to achieve complete deproteinization and the highest recovery of glipizide and IS from spiked plasma samples. The best extraction recovery with high precision was obtained using the combination of acetonitrile and methanol as the extraction solvent.

Blank plasma of white albino rabbit was spiked with known spiking standards of glipizide and IS and mixed well by vortexing. In one 2–ml micro-centrifuge tube (Ependorff, USA), spiked plasma along with equal volume of methanol (1 ml) was added to precipitate plasma proteins followed by addition of 0.75 ml of acetonitrile for complete extraction of drug and IS. The mixture was vortexed for 3 min and centrifuged (C-24.2/2002, Remi Cooling Centrifuge, Mumbai, India) at 3000 *g* for 10 min. The supernatant transparent liquid was transferred into a glass centrifuge tube and evaporated to dryness at room temperature under a stream of nitrogen. The residue was reconstituted with 0.2 ml of mobile phase. Then, the samples were filtered through a 13 mm ø 0.2 μm membrane filter using syringe filter holder. An aliquot of 20 μl was injected into the rheodyne injector of an HPLC system for the separation and quantitation.

4.2.3.5. Validation of Proposed Method

The developed method was validated in accordance with the ICH Guidelines.

4.2.3.5.1. Calibration Curve (Linearity)

The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations (quantities) of an analyte in the sample (Hubert et al., 1999; Hubert et al., 2003). Calibration standards were prepared by spiking the blank rabbit plasma to give plasma concentrations 10, 25, 100, 250, 500, 750, 1000, 1250, and 2500 ng/ml for glipizide and fixed IS concentration of 2500 ng/ml. The linear regression of the glipizide/IS peak area ratio versus concentration was fitted by replicate analysis (n=6) over the selected concentration range using least square method with Microsoft Excel® program.

4.2.3.5.2. Accuracy and Precision

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The precision of the test procedure expresses the agreement between the results (degree of scatter) obtained when replicate determinations are conducted using the procedure on multiple samples of the same homogenous sample under the prescribed conditions (Carr et al., 1994). Inter-day precision and accuracy were determined from the analyses of spiked plasma samples (at 10, 100, 500, 1000, and 2500 ng/ml of glipizide) on three different days, whereas intra-day precision and accuracy were determined by analyzing spiked plasma samples that were run in random order six times over the course of one day. The precision of the method at each concentration level was determined by comparing the mean and relative standard deviation (RSD), as a percentage of the calculated mean concentration, whereas the accuracy estimated for each spiked sample was obtained as the percent of ratio of the nominal concentration to the assayed concentration.

4.2.3.5.3. *Sensitivity*

In accordance with the IUPAC (Currie, 1995) and ISO (ISO Standard 8466-2, 1994), the instrumental response sensitivity is the slope of the calibration line because a method with a large slope is better able to discriminate between small differences in an analyte content. Limit of detection (LoD) is the lowest amount of an analyte in a sample which can be detected, but not necessarily quantitated as an exact value. Limit of quantitation (LoQ) is the lowest non-zero amount of an analyte in a sample that can be quantitatively determined with defined precision and accuracy under the stated experimental conditions (US FDA CDER Guidance for the Industry, 1997). LoD and LoQ were determined based on the signal to noise ratio of 3 and 10, respectively.

4.2.3.5.4. *Selectivity*

The CGMP guidelines on analytical method validation define the term selectivity as an ability of the method to detect qualitatively the analyte in the presence of the components that may be expected to be present in the sample matrix. The selectivity of the method was investigated by comparing the chromatograms of blank plasma samples from different rabbits with that of blank plasma spiked with standard solutions and the samples collected after oral administration of developed SR tablet. Five blank plasma samples from different rabbits were processed with and without the internal standard to evaluate to which extent the endogenous plasma components may contribute to the peak interference at retention time of glipizide and IS.

4.2.3.5.5. *Stability*

Blank plasma was spiked with the known amount of glipizide to achieve the concentration of 50, 1000, and 2000 ng/ml (n=3) and stored at –20°C. The stability of these samples was checked for up to 1 month by comparing the results with fresh stock prepared on the day of analysis. Samples were considered to be stable, if the assay values were within the acceptable limits of accuracy and precision. No internal standard was added prior to the analysis.

4.2.3.5.6. *Extraction Efficiency*

The recovery of an analyte is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method (Milena et al., 2005).

Different organic extraction solvents (ethyl acetate, dichloromethane, acetonitrile and butan-1-ol) were tried in the experiment, and acetonitrile proved to be the most efficient in extracting glipizide from rabbit plasma and had a small variation in extraction recoveries over the concentration range. Spiked plasma samples were prepared in triplicate at four concentrations 50, 500, 1000, and 2000 ng/ml of glipizide and 2500 ng/ml of IS, and assayed as described above. The recovery of glipizide was determined by comparing the peak areas measured after analysis of spiked plasma samples with those found after direct injection of non-biological (non-extracted) samples (representing 100% extraction efficiency) into the chromatographic system at the same concentration levels.

4.2.3.6. Pharmacokinetic Analysis of Glipizide from Rabbits

The method described above was applied to quantify the plasma concentration of glipizide in a single-dose pharmacokinetic study conducted on white albino rabbits. The protocol was approved by the Institutional ethical committee at the M. S. University of Baroda, India. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. The rabbits weighing 1.5 to 2.5 kg were housed with free access to food and water, except for the final 12 h before experimentation. The group 1 rabbits were administered glipizide oral solution (1.87 mg in 20 ml aqueous solution) by oral-gastric intubation for estimation of pharmacokinetics parameters. Following the administration, 2.0 ml of blood samples were collected from the marginal ear vein at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h time-points into heparinized collection tubes. The blood was immediately centrifuged (1900 *g*) for 10 min at an ambient temperature. The supernatant plasma layer was separated and stored at –20°C until analyzed. The plasma samples were analyzed for glipizide concentrations as described above. The first order elimination rate constant (k_{el}) was estimated by the least-square regression of plasma concentration–time data points of the curves describing the terminal log-linear decaying phase. $T_{½}$ (Eq. 4.1) was derived from k_{el} ($T_{½}=\ln 2/k_{el}$). The area under the plasma concentration–time curve from zero to the last measurable plasma concentration at time t (AUC_{0-t}) was calculated using the linear trapezoidal rule. The area was extrapolated to infinity ($AUC_{0-\infty}$) (Eq. 4.3) by addition of C_t/k_{el} (Eq. 4.2) to AUC_{0-t}, where C_t is the last detectable drug concentration. The absorption rate constant (k_a) was determined by residual method. The maximum observed glipizide concentration (C_{max}) and the time at which C_{max} was observed (T_{max}) were reported directly from the profile. The *AUMC* is the area under the plot of time vs. the product of time and concentration extrapolated to infinity and was calculated by trapezoidal rule. Volume of distribution (V_d) and total clearance rate (*TCR*) were calculated using eq. 4.4 and 4.5, respectively. The mean residence time (*MRT*) was determined by *AUMC* divided by *AUC* as shown in eq. 4.6. The clearance (*Cl*) was calculated as dose divided by *AUC* with extrapolation to infinity ($AUC_{0-\infty}$) in accordance to eq. 4.7. All the pharmacokinetic parameters were calculated using Microsoft® Office Excel 2003 (Microsoft Corporation, USA) software application.

$$T_{\frac{1}{2}} = \frac{\ln 2}{k_{el}} \qquad 4.1$$

$$AUC_{(t\to\infty)} = \frac{C_t\ (t = t\ \text{last})}{k_{el}} \qquad 4.2$$

$$AUC_{(0\to\infty)} = AUC_{(0\to t)} + AUC_{(t\to\infty)} \qquad 4.3$$

$$V_d = \frac{D_G^0 \cdot AUMC}{(AUC)^2} \qquad 4.4$$

$$TCR = k_{el} \cdot V_d = \frac{V_d \cdot 0.693}{t_{1/2}} \qquad 4.5$$

$$MRT_{(t=12hr)} = \frac{AUMC}{AUC} \qquad 4.6$$

$$Cl = \frac{D_G^0}{AUC_{0\to\infty}} \qquad 4.7$$

4.2.4. ESTIMATION OF NATEGLINIDE (HPLC METHOD)

4.2.4.1. Equipment and Chromatographic Conditions

All parameters remained same as glipizide. The only difference is that instead of glipizide, here nateglinide was the compound of interest, and it was detected at 203 nm with the same internal standard (gliclazide).

4.2.4.2. Preparation of Standard Solutions

A stock solutions of nateglinide (500 µg/ml) and gliclazide (500 µg/ml) were prepared in methanol and were stored at 2-8°C until used. Aliquots of these solutions were diluted stepwise with the mobile phase to obtain 30 µg/ml of both nateglinide and gliclazide. This solution was used for the optimization of the proposed method.

The spiking solutions of nateglinide (8 µg/ml) and gliclazide (8 µg/ml) were prepared by diluting the suitable aliquots of stock solutions with HPLC grade water. Accurate aliquots of the spiking solutions were spiked to plasma in order to obtain the nateglinide concentrations in the analytical range of 10 to 2500 (10, 25, 100, 250, 500, 1000, and 2500) ng/ml and that of gliclazide to be 2500 ng/ml for calibration curve.

4.2.4.3. Optimization of Chromatographic Separation Conditions

The optimization strategy followed here was similar to that of glipizide. The mobile phase composition, pH and flow rate studied were the same with the change that instead of glipizide, nateglinide was the compound to be separated and quantified.

4.2.4.4. Estimation of Nateglinide from Rabbit Plasma

In order to investigate the practical applicability of the method in biological analysis, the present method was applied to the estimation of nateglinide from rabbit plasma. Blank plasma of white albino rabbit was spiked with known concentrated aqueous standards of nateglinide and IS followed by the same extraction procedures as described for glipizide.

4.2.4.5. Validation of Proposed Method

The developed nateglinide quantitation method was validated for accuracy and precision, linearity, sensitivity, selectivity, stability and extraction efficiency in accordance with the ICH Guidelines as described above for glipizide.

4.2.4.6. Pharmacokinetic Analysis of Nateglinide from Rabbits

The method described above was applied to quantify the plasma concentration of nateglinide in a single-dose pharmacokinetic study conducted on three white albino rabbits. After a single oral administration of 15 mg of nateglinide (Natelide™-60), 2.0 ml of blood samples were collected from the marginal ear vein at 0, 0.5, 1, 2, 3, 4, 5, 6, 9, and 12 h time-points into heparinized collection tubes. The collected blood samples were immediately centrifuged and the supernatant plasma layer was separated and stored at −20°C until analyzed. The plasma samples were analyzed for nateglinide concentrations and different pharmacokinetic parameters for nateglinide were calculated as discussed earlier.

4.3. Experimental Work

4.3.1. DRUG–EXCIPIENT INTERACTION STUDIES

The possibility of drug–excipient interaction was investigated by differential scanning calorimetry. The DSC thermograms of pure drug, individual excipients and drug–excipient mixtures were recorded. The samples were separately sealed in aluminium cells and set in Shimadzu DSC-60 thermal analyzer. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 30-400°C. An empty aluminium cell was employed as the reference standard.

4.3.2. PREPARATION & CHARACTERIZATION OF PHYSICAL MIXTURE

Before compression, the mixtures were sieved through ASTM sieve #24 sieve (0.710 mm) and their fluidity as well as compressibility was determined by calculating the Hausner ratio (Hausner, 1972) and Carr's compressibility index (Carr, 1965). The bulk density (ρ_b) and the tap density (ρ_t) of the powder mixture were determined with a bulk/tap density test apparatus (model : VTAP-MATIC-II, Veego, Mumbai) that dropped 10 and 250 times, respectively. For each sample, the Carr's index was calculated according to the following equation:

$$I_c = \left(\frac{\rho_t - \rho_b}{\rho_t} \right) \times 100 \qquad 4.8$$

and the Hausner ratio was calculated according to the following equation (Moneghini et al., 2000):

$$R_H = \rho_t / \rho_b \qquad 4.9$$

The flow of the powder mixture for all the batches were investigated by measuring angle of repose (θ) according to the fixed funnel and free standing cone method (Train, 1958). A funnel with the end of the stem cut perpendicular to its axis of symmetry is secured with its tip 2 cm above a graph paper placed on a flat horizontal surface. Powder is carefully poured through the funnel until the apex of the cone thus formed just reaches the tip of the funnel. The mean radius (r) and height (h) of the heap were measured and the angle of repose was calculated from following equation.

$$\tan(\theta) = \frac{h}{r} \qquad 4.10$$

The uniformity of mixing was assessed by conducting content uniformity test on the samples of the powder mixture before tabletting.

4.3.3. PREPARATION & CHARACTERIZATION OF MATRIX TABLETS

Matrix tablets were prepared by conventional direct compression of the mixture of glipizide/nateglinide with different combination of excipients at different ratios (an overview of all formulations evaluated during the study is shown in individual matrix sections). Briefly, weighed quantity of drug was physically mixed with all the auxiliary excipients by geometric addition using a glass mortar and pestle for about 10 min. Then magnesium stearate and aerosol were added as the glidants/lubricant and further thoroughly blended for 2 min. The homogeneous powder mixture for a single matrix was weighed, fed manually into the die of an automatic eight station automatic rotary tablet machine (Modern Engineering Works, New Delhi, India) equipped with flat faced die-punch set of 6.81 mm diameter (for glipizide tablets) or that of 13 mm diameter (for nateglinide tablets), and compressed to a target weight (as per the composition of the matrix shown in individual tables) and an average hardness of 6-7 kg/cm^2 for all the tablets. Checks were carried out during tabletting to ensure that both weight and hardness levels complied with these targets. The obtained cylindrical matrices were subjected to various physico-chemical investigations. A theoretical content of glipizide, silicon dioxide and magnesium stearate per tablet was 10, 1, and 2 mg, respectively. Whereas, all nateglinide formulations contain 180 mg of nateglinide, 2 mg of aerosil, and 4 mg of magnesium stearate.

The tablets were evaluated for different physico-chemical parameters such as appearance, weight variation, thickness, hardness, drug content, and in vitro drug release. For each batch, 10 randomly taken tablets were checked for physical testing of weight variation using an electronic balance (Mettler AE-50 electronic balance, Greifensee, Switzerland) in order to verify the uniformity of the tablets within each batch. The weight variation of dosage units was measured as described (USP 29 - NF 24, 2006). Tablet diameter (D) and thickness (T) were measured with a digital vernier caliper (Model no. 111-312, Forbes gokak Ltd, Mumbai), and a hardness tester (Model no. 1392, Yuyutsu, SQC & Inspection instruments, Mumbai) was used to measure the hardness / crushing strength (F) of tablets and it was expressed in kg/cm^2. Means and relative standard deviations were calculated.

4.3.4. GLIPIZIDE MATRICES

Preliminary experiments were carried out by preparing different matrix tablets by direct compression of several homogenous blends of different polymers and excipients in different ratios in order to achieve desired drug release profile. Tablets were divided in sub groups depending on the formulation composition.

The excipients were included in the formulations not only to overcome the capping or compression related formulation problems but also to regulate the release rate of the drug. Lactose (freely water-soluble), dicalcium phosphate (water-insoluble) and microcrystalline cellulose (water-insoluble), eudragit, starch, inorganic salts etc. were used as shown in the following sections.

Table 4.1. The preliminary experimental plan used to investigate the influence of MCC, HPMC, starch and lactose on the release profile of glipizide.*

Batch No.	MCC PH 301	MCC PH 302	HPMC K100LV	HPMC K4M	HPMC K15M	HPMC K100M	Starch 1500	Lactose
M-1	100	-	-	0	-	-	-	-
M-2	85	-	-	15	-	-	-	-
M-3	**75**	**-**	**-**	**25**	**-**	**-**	**-**	**-**
M-4	65	-	-	35	-	-	-	-
M-5	50	-	-	50	-	-	-	-
M-6	25	-	-	75	-	-	-	-
M-7	-	100	-	0	-	-	-	-
M-8	-	80	-	20	-	-	-	-
M-9	-	75	-	25	-	-	-	-
M-10	-	50	-	50	-	-	-	-
M-11	-	25	-	75	-	-	-	-
M-12	75	-	25	-	-	-	-	-
M-13	75	-	-	-	25	-	-	-
M-14	75	-	-	-	-	25	-	-
M-15	75	-	-	25	-	-	-	-
M-16	75	-	-	25	-	-	-	-
M-17	75	-	-	25	-	-	-	-
M-18	75	-	20	-	-	5	-	-
M-19	75	-	15	-	10	-	-	-
M-20	75	-	15	10	-	-	-	-
M-21	60	-	30	10	-	-	-	-
M-22	75	-	-	20	-	-	5	-
M-23	50	-	-	25	-	-	25	-
M-24	37.5	-	-	25	-	-	37.5	-
M-25	**30**	**-**	**-**	**25**	**-**	**-**	**45**	**-**
M-26	25	-	-	25	-	-	50	-
M-27	75	-	-	20	-	-	-	5
M-28	-	-	-	25	-	-	75	-
M-29	-	-	-	30	-	-	70	-
M-30	-	-	-	40	-	-	60	-
M-31	-	-	-	50	-	-	50	-
M-32	-	-	-	75	-	-	25	-
M-33	0	-	-	-	-	-	100	-
M-34	50	-	-	-	-	-	50	-
M-35	75	-	-	-	-	-	25	-

* Values in table show weight of respective excipient in mg. The amount of glipizide, silicon dioxide and magnesium stearate was fixed at 10, 1 and 2 mg, respectively (not shown in table). The total weight of tablet was fixed at 113 mg. Matrices composition in bold figures resulted in drug release within the constraints predetermined.

4.3.4.1. HPMC-MCC-Starch Matrices

Directly compressed monolithic matrices of homogenous blends of cellulose derivatives (MCC, and HPMC) and fillers (starch, lactose) at different proportions were prepared as shown in Table 4.1. Three MCC-Starch 1500 matrices were also prepared to evaluate the functionality of starch to prepare the gel and its contribution in controlling the drug release (M-33 to M-35). Desired release profile was obtained with the proper composition of MCC PH 301 and HPMC K4M; hence, it was further studied using 3^2 full factorial experimental design with two formulation variables at three different levels.

4.3.4.1.1. 3^2 Full Factorial Experimental Design & Response Surface

In order to easily optimize the formulation and evaluation of the influence of each additive on the release profile, 3^2 full factorial experimental design was used to prepare systematic model formulations which were composed of two formulation variables: the MCC: HPMC composition and tablet hardness (Table 4.2). Measured responses were cumulative % glipizide dissolved at 2, 4, 6, 8, 10 and 12 hr (Q_t). The quadratic model was derived by stepwise multiple linear regression for each response using Microsoft Excel® 2003. The response surfaces were generated from the derived quadratic equations using SPSS® SigmaPlot® v 8.02.

Table 4.2. Variables used for 3^2 full factorial experimental design with constraints.

Formulation variables	Low level (-1)	Middle level (0)	High level (1)
A: MCC: HPMC composition	65:35	75:25	85:15
B: Tablet hardness (kg cm^{-2})	4	5.5	7
Response variables		**Constraints**	
$Q_{(2)}$= percent dissolved in 2 h		$15\% \le Q_{(2)} \le 30\%$	
$Q_{(4)}$= percent dissolved in 4 h		$40\% \le Q_{(4)} \le 55\%$	
$Q_{(6)}$= percent dissolved in 6 h		$55\% \le Q_{(6)} \le 70\%$	
$Q_{(8)}$= percent dissolved in 8 h		$70\% \le Q_{(8)} \le 85\%$	
$Q_{(10)}$= percent dissolved in 10 h		$80\% \le Q_{(10)} \le 95\%$	
$Q_{(12)}$= percent dissolved in 12 h		$95\% \le Q_{(12)} \le 110\%$	

4.3.4.2. MCC-Alginate & MCC-Glyceryl Behenate Matrices

Sodium alginate is also known to form a physical gel by hydrogen bonding at low pH (acid gel) and by ionic interactions with polyvalent cations such as calcium, the cation acting as a crosslinker between the polymer chains (King, 1983). Hence, sodium alginate matrices containing MCC and different cations (calcium dibasic phosphate, calcium gluconate and Disodium hydrogen phosphate) at different ratios (M-36 to M-47, Table 4.3) were prepared to check the performance of alginate as a hydrogel matrix former. Glyceryl behenate and MCC tablets at different ratio were prepared and are shown in Table 4.3 (M-49 to M-51).

Table 4.3. Formulation design of MCC-Alginate, guar gum and glyceryl behenate containing matrices to investigate the effects of different gel promoting salts on their in vitro release profiles along with guar gum and glyceryl behenate based matrices

Batch No.	MCC PH 102	MCC PH 301	Starch 1500	Chito-san	Sodium Alginate (150 #)	Protonal LF 120M	Calcium Dibasic Phosphate	Ca. Gluco-nate	Disodium Hydrogen Phosphate	Guar Gum	Glycerayl Behenate
M-36	-	50	-	-	50	-	-	-	-	-	-
M-37	-	25	-	-	75	-	-	-	-	-	-
M-38	-	20	-	-	50	-	35	-	-	-	-
M-39	-	35	-	-	30	-	35	-	-	-	-
M-40	-	50	-	-	15	-	35	-	-	-	-
M-41	-	40	-	-	50	-	-	-	10	-	-
M-42	-	45	-	-	35	-	-	-	25	-	-
M-43	-	0	-	-	60	-	35	-	10	-	-
M-44	25	-	-	-	-	37.5	-	37.5	-	-	-
M-45	25	-	-	-	-	50	-	25	-	-	-
M-46	-	40	-	30	-	30	-	-	-	-	-
M-47	-	60	-	20	-	20	-	-	-	-	-
M-48	-	30	-	-	-	-	-	-	-	80	-
M-49	-	-	-	-	-	-	-	-	-	-	100
M-50	-	25	-	-	-	-	-	-	-	-	75
M-51	-	50	-	-	-	-	-	-	-	-	50

4.3.4.3. EC-HPMC, Guar Gum & Xanthan Gum Matrices

The ability of the EC, guar gum and xanthan gum as a drug release retardant in the direct compression formulations and their performance mechanism were evaluated. The matrix code and unit formula are listed in Table 4.4 (M-52 to M-76).

Table 4.4. Composition of ethyl cellulose, guar gum and xanthan gum matrices.*

Batch No.	EC Std 7 FP Premium	MCC PH 301	HPMC K4M	HPMC K15M	HPMC K100M	Starch 1500	Lact-ose	Xanthan Gum	Guar Gum
M-52	75	-	25	-	-	-	-	-	-
M-53	70	-	30	-	-	-	-	-	-
M-54	50	-	50	-	-	-	-	-	-
M-55	25	-	75	-	-	-	-	-	-
M-56	75	-	-	25	-	-	-	-	-
M-57	50	-	-	50	-	-	-	-	-
M-58	25	-	-	75	-	-	-	-	-
M-59	75	-	-	-	25	-	-	-	-
M-60	50	-	-	-	50	-	-	-	-
M-61	25	-	-	-	75	-	-	-	-
M-62	50	50	-	-	-	0	-	-	-
M-63	25	50	-	-	-	25	-	-	-
M-64	25	25	-	-	-	50	-	-	-
M-65	-	-	-	-	-	-	-	-	110
M-66	-	30	-	-	-	-	-	-	80
M-67	-	-	30	-	-	-	-	-	80
M-68	-	-	-	-	-	-	-	110	-
M-69	-	-	-	-	-	-	-	130	-
M-70	**-**	**40**	**-**	**-**	**-**	**-**	**-**	**70**	**-**
M-71	-	30	-	-	-	-	-	80	-
M-72	-	-	30	-	-	-	-	80	-
M-73	-	15	15	-	-	-	-	80	-
M-74	-	20	-	-	-	20	-	70	-
M-75	**-**	**-**	**25**	**-**	**-**	**15**	**-**	**70**	**-**
M-76	-	-	25	-	-	-	5	80	-

* Matrices composition in bold figures resulted in drug release within the constraints predetermined.

4.3.4.4. Carbopol Matrices

Matrices of different grades of anionic carbopol polymers were studied. Moreover, the change in the drug release kinetics by the presence of water soluble or insoluble fillers and other matrix former were also checked as per the matrix composition strategy shown in Table 4.5.

Table 4.5. Composition of Carbopol containing matrices.*

Batch No.	MCC PH 301	HPMC K4M	Lactose	Carbopol 931P	Carbopol 941P	Carbopol 971P NF	Carbopol 974P	Tulsion 344
M-77	-	-	0	90	-	-	-	-
M-78	-	-	30	60	-	-	-	-
M-79	-	-	60	30	-	-	-	-
M-80	0	-	-	30	-	-	-	80
M-81	20	-	-	30	-	-	-	60
M-82	40	-	-	30	-	-	-	40
M-83	10	-	-	90	-	-	-	-
M-84	**30**	**-**	**-**	**70**	**-**	**-**	**-**	**-**
M-85	40	-	-	60	-	-	-	-
M-86	50	-	-	50	-	-	-	-
M-87	60	-	-	40	-	-	-	-
M-88	30	-	-	-	70	-	-	-
M-89	10	-	-	-	-	90	-	-
M-90	30	-	-	-	-	70	-	-
M-91	50	-	-	-	-	50	-	-
M-92	25	-	-	-	-	-	75	-
M-93	50	-	-	-	-	-	50	-
M-94	75	-	-	-	-	-	25	-
M-95	-	0	-	-	-	-	140	-
M-96	-	25	-	-	-	-	100	-
M-97	-	25	-	-	-	-	75	-

* Matrix composition in bold figures resulted in drug release within the constraints predetermined.

4.3.4.5. Carrageenan Matrices

Kappa (κ) and iota (ι) carrageenans gelation with inorganic salt KCl as well as positively charged chitosan to retard the glipizide release was studied. In addition, the contribution of the other cellulose derivatives in the performance of the carrageenan as a matrix former was also investigated to achieve the drug release within the defined constraints. The quantitative formula for each carrageenan matrices is shown in Table 4.6.

Table 4.6. Composition of carrageenan containing matrices.

Batch No.	MCC PH 102	MCC PH 301	HPMC K4M	Starch 1500	Chitosan	Carra-geenan	Gelcarin GP-379	Gelcarin GP-911	KCl
M-98	-	-	-	-	-	100	-	-	-
M-99	-	25	-	-	-	75	-	-	-
M-100	-	-	25	-	-	75	-	-	-
M-101	-	-	-	-	-	-	100	-	-
M-102	25	-	-	-	-	-	75	-	-
M-103	50	-	-	-	-	-	50	-	-
M-104	-	-	-	-	-	-	-	100	-
M-105	25	-	-	-	-	-	-	75	-
M-106	50	-	-	-	-	-	-	50	-
M-107	-	-	10	-	-	-	100	-	-
M-108	-	-	15	-	-	-	100	-	-
M-109	-	-	30	-	-	-	80	-	-
M-110	-	-	5	-	-	-	80	-	30
M-111	-	-	10	-	50	-	50	-	-
M-112	-	-	20	40	-	-	40	-	-
M-113	-	-	-	-	-	-	70	-	40
M-114	-	-	-	-	-	-	80	-	30
M-115	-	-	-	-	-	-	95	-	55
M-116	-	-	-	-	-	-	120	-	70
M-117	-	-	-	-	-	-	153	-	87

4.3.4.6. Eudragit Matrices

The composition of the hydrophilic matrix tablets containing eudragit polymers at different ratios is depicted in Table 4.7.

Table 4.7. Composition of eudragit containing matrices.*

Batch No.	MCC PH 301	HPMC K4M	Starch 1500	Lactose	Eudragit L100	Eudragit S100	Chitosan	Protonal LF 120M
M-118	-	-	-	-	100	-	-	-
M-119	25	-	-	-	75	-	-	-
M-120	**-**	**25**	**-**	**-**	**75**	**-**	**-**	**-**
M-121	-	-	-	-	-	100	-	-
M-122	25	-	-	-	-	75	-	-
M-123	-	25	-	-	-	75	-	-
M-124	-	40	-	10	50	-	-	-
M-125	-	40	-	20	40	-	-	-
M-126	**-**	**40**	**10**	**-**	**50**	**-**	**-**	**-**
M-127	-	40	20	-	40	-	-	-
M-128	-	-	40	-	40	-	20	-
M-129	-	-	40	-	40	-	-	20

* Matrices composition in bold figures resulted in drug release within the constraints predetermined.

4.3.4.7. Sodium Alginate Matrices

The influence of the different inorganic cations, chitosan and other matrix former on the glipizide release profiles from alginate matrices were studied. The quantitative proportions of the formulation variables are enumerated in Table 4.8.

Table 4.8. Composition of sodium alginate containing matrices.

Batch No.	HPMC K4M	Starch 1500	Carbopol 931P	Carbopol 934P	Chito-san	Sodium Alginate (150 #)	Protonal LF 120M	Calcium Dibasic Phosphate	Calcium Gluco-nate	Disodium Hydrogen Phosphate
M-130	-	-	50	-	-	50	-	-	-	-
M-131	-	-	75	-	-	25	-	-	-	-
M-132	-	-	-	25	-	60	-	-	-	15
M-133	25	-	-	-	-	75	-	-	-	-
M-134	75	-	-	-	-	25	-	-	-	-
M-135	25	-	-	-	-	60	-	-	-	15
M-136	0	-	-	-	-	-	100	-	-	-
M-137	10	-	-	-	-	-	110	-	-	-
M-138	20	-	-	-	-	-	100	-	-	-
M-139	-	-	-	-	-	-	50	50	-	-
M-140	-	-	-	-	-	-	75	-	25	-
M-141	-	-	-	-	-	-	50	-	50	-
M-142	-	-	-	-	-	-	25	-	75	-
M-143	10	-	-	-	50	-	50	-	-	-
M-144	-	30	-	-	40	-	40	-	-	-

4.3.5. NATEGLINIDE MATRICES

Different drug release retardant polymers along with other auxiliary excipients were combined at different ratios to prepare the nateglinide sustained release matrices that give neither lag phase or burst effect during their in vitro release studies and ultimately provide drug release within the defined constraints. Depending on the composition, total number of experimental batches are grouped as shown in following sections.

4.3.5.1. HPMC-MCC-Starch Matrices

The formulation variables such as – polymer ratio, total polymer wt in the matrix, combination of polymers, fillers and/or other excipients is given in Table 4.9.

Table 4.9. Composition of HPMC-MCC matrices.*

Batch No.	MCC PH301	HPMC K4M	HPMC K15M	HPMC K100M	HPMC K4M CR	HPMC K15M CR	Starch 1500	HPMC/MCC ratio	Total poly. wt.
J-1	225	-	-	75	-	-	-	25/75	300
J-2	225	-	75	-	-	-	-	25/75	300
J-3	225	-	75	-	-	-	-	25/75	300
J-4	225	75	-	-	-	-	-	25/75	300
J-5	225	75	-	-	-	-	-	25/75	300
J-6	225	75	-	-	-	-	-	25/75	300
J-7	225	-	-	-	75	-	-	25/75	300
J-8	225	-	-	-	-	75	-	25/75	300
J-9	240	60	-	-	-	-	-	20/80	300
J-10	240	60	-	-	-	-	-	20/80	300
J-11	**187.5**	**62.5**	**-**	**-**	**-**	**-**	**-**	**25/75**	**250**
J-12	200	50	-	-	-	-	-	20/80	250
J-13	150	50	-	-	-	-	-	25/75	200
J-14	160	40	-	-	-	-	-	20/80	200
J-15	112.5	37.5	-	-	-	-	-	25/75	150
J-16	**60**	**50**	**-**	**-**	**-**	**-**	**90**	**25/30/45**	**200**

* Values in table show weight of respective excipient in mg. The amount of nateglinide, silicon dioxide and magnesium stearate was fixed at 180, 2 and 4 mg, respectively (not shown in table). Matrices composition in bold figures resulted in drug release within the constraints predetermined.

4.3.5.2. Ethyl Cellulose & Carbopol Matrices

The formulation composition of the ethylcellulose-MCC matrices and Carbopol-eudragit matrices is shown in Table 4.10.

Table 4.10. Composition of ethyl cellulose and carbopol matrices.*

Batch No.	EC Std 7 FP Premium	MCC PH 301	Starch 1500	Eudragit L100	Carbopol 934P	EC/MCC or Eu./Carbopol	Total poly. wt.
J-17	150	100	-	-	-	60/40	250
J-18	120	80	-	-	-	60/40	200
J-19	105	70	-	-	-	60/40	175
J-20	**100**	**100**	-	-	-	**50/50**	**200**
J-21	-	-	-	**130**	**70**	**65/35**	**200**
J-22	-	-	-	80	120	40/60	200
J-23	-	-	**40**	**80**	**80**	**40/40/20**	**200**
J-24	-	40	-	-	160	0/80/20	200

* Matrices composition in bold figures resulted in drug release within the constraints predetermined.

4.3.5.3. Xanthan Gum Matrices

The effects of different cellulose derivatives and eudragits on the nateglinide release from the xanthan gum matrices were studied and its composition is given in Table 4.11.

Table 4.11. Composition of xanthan gum matrices.*

Batch No.	EC Std 7 FP Premium	MCC PH 301	HPMC K4M	Starch 1500	Lactose	Eudragit L100	Xanthan Gum	Total poly. wt.
J-25	-	-	58	-	12	-	180	250
J-26	-	-	30	-	15	-	180	225
J-27	-	-	30	90	-	-	80	200
J-28	-	-	22.5	90	-	-	37.5	150
J-29	-	-	30	-	-	45	75	150
J-30	-	-	-	-	-	100	150	250
J-31	-	-	-	-	-	90	110	200
J-32	-	-	-	40	-	60	100	200
J-33	-	-	-	70	-	30	100	200
J-34	**30**	-	-	-	-	**45**	**75**	**150**
J-35	-	70	-	-	-	-	180	250

* Matrix composition in bold figures resulted in drug release within the constraints predetermined.

4.3.5.4. Alginate-Chitosan & Eudragit-HPMC Matrices

The strategy of the combination of the sodium alginate and eudragit with other excipients and polymers are depicted in Table 4.12.

Table 4.12. Composition of sodium alginate and eudragit-HPMC matrices.*

Batch No.	MCC PH 301	HPMC K4M	Starch 1500	Eudragit L100	Chitosan	Protonal LF 120M	Total poly. wt.
J-36	150	-	-	-	50	50	250
J-37	160	-	-	-	20	20	200
J-38	-	-	80	80	20	20	200
J-39	-	62	-	188	-	-	250
J-40	-	75	-	175	-	-	250
J-41	-	87.5	-	162.5	-	-	250
J-42	**-**	**61**	**-**	**114**	**-**	**-**	**175**
J-43	-	50	90	60	-	-	200
J-44	**-**	**40**	**80**	**80**	**-**	**-**	**200**

* Matrices composition in bold figures resulted in drug release within the constraints predetermined.

For in vivo study, 50 matrix tablets weighing 21 mg (contains 1.87 mg glipizide per tablet) were prepared separately using 3 mm × 4 hole die and 4 flat tip upper–lower punch set (Jyoti Mech Industries, Navsari, Gujarat, India) on above mentioned tablet machine. Simillarly, matrix tablets weighing 81 mg (containing 33.6 mg of nateglinide per tablet) were compressed using a set of die-punch having diameter of 5 mm. The composition of the small tablet was kept proportionally similar to that of the optimized batch.

4.4. In Vitro Study

4.4.1. ASSAY OF GLIPIZIDE CONTENT OF TABLET

Five matrix tablets were pulverized and powder equivalent to 10 mg of glipizide was weighed accurately and transferred into a 250 ml volumetric flask, suspended in 100 ml of phosphate buffer pH 6.8 and sonicated in the ultrasonic bath for 10 min. The volume of the suspension was adjusted with the same buffer and then filtered through whatman® filter paper no. 42 and was assayed spectroscopically as mentioned above in section 4.2.1. Preliminary studies have shown that the presence of the tablet ingredients has no interference with the spectroscopic method. The glipizide contents of tablet was estimated by using standard curve obtained by spectroscopic method. Each data represents the mean of three different samples for each lot (SD<3%).

4.4.2. DRUG RELEASE STUDIES

For routine quality control analysis, the dissolution of the developed matrix formulations were carried out in phosphate buffer pH 6.8 (prepared as per IP 1996 using KH_2PO_4-NaOH) at the paddle speed of 100 rpm and a temperature of 37±0.5°C up to 12 h. Twelve-hour dissolution test was conducted in USP dissolution apparatus (TDT-60T, Electrolab, Mumbai, India) type II (paddle) at 100 rpm and a temperature of 37±0.5°C. Initial drug release studies were conducted in 750 ml of 0.1N HCl for 2 h, followed by addition of 250 ml of 0.2 M trisodium phosphate to the dissolution media and the pH adjusted to 6.8. The dissolution was then continued in this medium for additional 10 h. At the scheduled time (0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 h), 2 ml of the dissolution media was withdrawn with replacement of same volume of dissolution media. These were filtered, and analyzed spectroscopically as mentioned above. Each release determination was carried out in six replicates and the mean values were plotted versus time with a standard deviation of <3 of the percentage released which indicated the reproducibility of the results. It is worth noting that none of the additives used in the matrices interfered with the assay.

In general, an optimal extended-release dosage form must have a minimal burst effect with almost complete drug being released in a specific time period. The USP monographs for extended release dosage forms specify the percent of drug released after more than one time point. Therefore, the percent of drug release after 2, 4, 6, 8, 10 and 12 hr ($Q_{(t)}$) with constraint were selected as the response variables (shown in Table 4.2).

Drug release parameter like mean dissolution time (MDT) was determined from the amount of glipizide actually detected during the dissolution test. $MDT_{50\%}$ and $MDT_{80\%}$ represent the mean time needed to release 50% or 80% of drug dose, respectively and were calculated by the following expression:

$$MDT = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \qquad 4.11$$

where j is the sample number, n is the number of dissolution sample times, $\hat{t}_j$ is the time at midpoint between t_j and t_{j-1} (easily calculated with the expression $(t_j + t_{j-1})/2$) and ΔM_j is the additional amount of drug dissolved between t_j and t_{j-1}.

4.4.3. CURVE FITTING AND RELEASE KINETICS

In order to propose the possible release mechanism, the release pattern was evaluated to check the goodness of fit for zero-order release kinetic (Eq. 4.12), Higuchi's square root of time equation (Higuchi, 1961; 1963) (Eq. 4.13), Korsmeyer-Peppas' power law equation (Korsmeyer et al., 1983; Peppas, 1985; Spiepmann and Peppas, 2001) (Eq. 4.14) and Hixson-Crowell's cube root of time equation (Hixson and Crowell, 1931) (Eq. 4.15). The goodness of fit was evaluated by *r* (correlation coefficient) values.

$$Q_t = Q_0 + K_0 t \qquad 4.12$$

where Q_t is the amount of drug dissolved in time *t*, Q_0 is the initial amount of drug in the solution (most times, Q_0=0), K_0 is the zero order release constant and *t* is release time.

$$Q_t = K_H \sqrt{t} \qquad 4.13$$

where Q_t is the amount of drug dissolved in time *t*, K_H is the Higuchi dissolution constant and *t* is release time.

$$Q_t / Q_\infty = K_k t^n \qquad 4.14$$

where Q_t is the amount of drug dissolved in time *t*, Q_∞ is the amount of drug dissolved in ∞ time (the drug loaded in the formulation), Q_t / Q_∞ is the fractional release of the drug in time *t*, K_k is a constant incorporating structural and geometric characteristic of drug dosage form, *n* is the release (diffusional) exponent that depends on the release mechanism and the shape of the matrix tested (Ritger and Peppas, 1987) and *t* is release time. Interpretation of diffusional exponent is given in Table 4.13.

Table 4.13. Interpretation of diffusional release mechanisms.

Release exponent (*n*)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<*n*<1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero order release
Higher than 1.0	Super Case-II transport	t^{n-1}

$$Q_0^{1/3} - Q_t^{1/3} = K_s t \qquad 4.15$$

where Q_0 is the initial amount of drug in the pharmaceutical dosage form, Q_t is the remaining amount of drug in pharmaceutical dosage form at time *t*, K_s is a constant incorporating the surface-volume relation and *t* is release time.

In order to understand the release mechanism, the release data of the optimized batch (M-6) was fitted to empirical equations proposed by Kopcha (Kopcha et al., 1991) (Eq. 4.16),

$$M = At^{1/2} + Bt \qquad 4.16$$

In the above equations, M (≤ 70%) is the percentage of drug released at time t, while A and B are, respectively, diffusion and erosion terms. According to this equation, if diffusion and erosion ratio, A/B=1, then the release mechanism includes both diffusion and erosion equally. If A/B>1, then diffusion prevails, while for A/B<1, erosion predominates (Ratsimbazafy et al., 1996).

The release profile of optimized batch was compared with that of the commercial formulation using the model independent pair-wise approach, the similarity factor (f_2). The FDA has included the use of this fit factor in recent guidelines (US FDA CDER Guidlines, 1997). This parameter allows comparing and establishing similarities or differences between two dissolution curves obtained from experiments, through a mathematical approach. The similarity factor is a logarithmic transformation of the sum-squared error of differences between the test T_j and reference products R_j over all time points, m, where m is not more than one measurement after 85% of drug released (Moore and Flanner, 1996).

$$f_2 = 50\log\left\{\left[1+\left(\frac{1}{m}\right)\sum_{j=1}^{m} w_j\left|R_j - T_j\right|^2\right]^{-0.5} \times 100\right\} \qquad 4.17$$

where w_j is an optional weight factor. The similarity factor fits the result between 0 and 100. It is 100 when the test and reference profiles are identical and approaches 0 as the dissimilarity increases. This method is more adequate to compare dissolution profile when more than three or four dissolution times points are available. In this approach, similarity between two drug products is defined by an f_2 value between 50 and 100. An average difference of 10% at all measured time points results in an f_2 value of 50. The f_2 value of 100 suggests that the test and reference profiles are identical and as the value becomes smaller, the dissimilarity between releases profiles increases.

4.4.4. SWELLING STUDY

Measurement of swelling/hydration rates of different matrices were carried out to gain insight in to the observed phenomena of drug release with the rates of polymer hydration and to evaluate the extent of water penetration into the tablets. Studies were performed using a modification of a previously described method (Ebube et al., 1997).

Briefly, the selected matrices were subjected to swelling studies at a temperature of 37±0.5°C and at a pH of 6.8, using the same buffer that was used for dissolution studies. Diameter, height and weight of individual matrices (S_0) were measured, and gently placed in a beaker containing about 250 ml dissolution medium. At predetermined time intervals, swollen/hydrated tablets were withdrawn from the medium, extra buffer present on the matrix surface was gently wiped with the soft tissue. Individual diameter, height and weight (S_1) of these tablets were measured and returned to the medium. Percent of the Radial (diameter) and axial (height) swelling of tablet and percent water uptake was calculated according to the following formula:

$$\%\ \text{of swelling/hydration}\ (S_W) = \left(\frac{S_1 - S_0}{S_0}\right) \times 100 \qquad 4.18$$

where S_1 and S_0 are the diameter or height of swollen and dried tablets, respectively. The percentage swelling of the original tablet was calculated and plotted versus time to reflect the rate of hydration (results are an average of three experiments).

4.4.5. SCANNING ELECTRON MICROSCOPY (SEM)

The surface morphology of the optimized batch before and after in vitro dissolution at different time intervals was analyzed by SEM. The tablet was mounted on brass stubs using carbon paste. SEM photographs were taken using a scanning electron microscope (JSM-5610LV; Jeol Ltd., Tokyo, Japan) at the required magnification at room temperature. A working distance of 39 mm was maintained, and the acceleration voltage used was 10 kV, with the secondary electron image (SEI) as the detector.

4.4.6. STABILITY STUDY

All optimized formulations of glipizide and nateglinide were strip packed (Al–Al strip, 0.04 mm) and subjected to accelerated stability studies as per ICH guidelines (40°C ± 2°C/75% RH ± 5% RH). The samples were withdrawn periodically (0, 15, 30, 60, 90, and 180 days) and evaluated for the different physico-chemical parameters viz. appearance, weight variation, thickness, hardness, drug content, and in vitro release studies.

4.5. In Vivo Study

4.5.1. DRUG ABSORPTION STUDY IN RABBITS

4.5.1.1. Animal Housing and Handling

Three white albino rabbits per groups, weighing 3.8-4.0 kg, were used in the study in accordance with a protocol approved by the Institutional ethical committee at the M. S. University of Baroda at Vadodara, India. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. All rabbits were housed individually in cages under environmentally controlled conditions (23±2°C; 55±5% relative humidity, 12 h light/dark cycle). All rabbits were housed with free access to food and water; except for the final 24 h before experimentation. Blood samples (2.0 ml) were collected from the marginal ear vein at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h time-points into heparinized collection tubes. The blood samples were immediately centrifuged (1900 *g*) for 10 min at an ambient temperature. The supernatant plasma layer was separated and kept frozen at –20°C pending analysis. The plasma samples were analyzed for glipizide or nateglinide concentrations as described above by HPLC.

4.5.1.2. IVIVC Development using Slow, Medium, & Fast Release Rate Formulations of HPMC-MCC Matrix

As compared to initial IVIVC development and evaluation of predictability using single batch, more additional data may be needed to define the IVIVC's predictability completely. Some combination of three or more formulations with adequate different (e.g. by 10%) release rates is considered optimal (US FDA CDER Guidance for the Industry, 1997). Out of the 9 batches (A-1 to A-9) of HPMC:MCC system studied using 3^2 full factorial design, three batches namely A-3, A-6 (=M-3), and A-4 exhibited the slow, medium, and fast release profile, respectively. Hence, these three batches were selected for establishment of IVIVC according to US FDA guidelines. Four groups (n=3) of white albino rabbits were used. All rabbits were housed with free access to food and water; except for the final 24 h before experimentation (only water was allowed for group 2, 3, and 4 rabbits). The group 1 rabbits were administered glipizide oral solution (1.87 mg in 20 ml aqueous solution) by oral-gastric intubation. Group 2, 3, and 4 were administered slow (M-3), medium (M-6) and fast (M-4) release rate formulations (contains 1.87 mg glipizide per tablet), respectively.

4.5.1.3. Establishment of IVIVC for Glytop® 2.5 SR, M-25, M-75, M-120, Natelide® 60 (Immediate Release Tablet), J-11, and J-21

For establishment of IVIVC of marketed formulation Glytop® 2.5 SR, M-25 (HPMC K4M:MCC PH301:Starch 1500 at 25:30:45), M-75 (xanthan gum:HPMC K4M:Starch 1500 at 70:25:15), M-120 (Eudragit L100:HPMC K4M at 75:25), Natelide® 60, J-11 (HPMC K4M:MCC PH301 at 62.5:187.5), and J-21 (Carbopol 934P:Eudragit L100 at 70:130) formulations, six groups (n=3) of white albino rabbits were used. All rabbits were housed with free access to food and water; except for the final 24 h before experimentation. All groups were administered with the above mentioned formulations containing 1.87 mg glipizide or 33.6 mg nateglinide per tablet orally.

4.5.2. PHARMACOKINETIC ANALYSIS

The measured plasma concentrations were used to calculate the area under the plasma concentration-time profile from time zero to the last measurable concentration time point (AUC_{0-t}) using the trapezoidal rule. The first order elimination rate constant (k_{el}) was estimated by fitting the logarithm of the concentrations versus time to a straight line over the observed exponential decline. The area was extrapolated to infinity ($AUC_{0-\infty}$) by addition of C_t/k_{el} to AUC_{0-t}, where C_t is the last detectable drug concentration. A $T_{½}$ was derived from k_{el} ($T_{½}$=ln 2/ k_{el}, where ln is the natural logarithm). The absorption rate constant (k_a) was determined by residual method (Bourne, 2002). The maximum observed glipizide or nateglinide concentration (C_{max}) and the time at which C_{max} was observed (T_{max}) were reported directly from the profile. The clearance (Cl) was calculated as dose divided by AUC with extrapolation to infinity ($AUC_{0-\infty}$). The AUMC is the area under the plot of time vs. the product of time and concentration extrapolated to infinity and was calculated by trapezoidal rule. The mean residence time (MRT) was determined by $AUMC$ divided by AUC. All the pharmacokinetic parameters were calculated using Microsoft® Office Excel 2003 (Microsoft Corporation, USA) software application. The Wagner–Nelson method (Wagner, 1971) was applied to deconvolute the percentage of the glipizide or nateglinide absorbed using the eq. 4.19.

$$F_{(t)} = C_{(t)} + k_{el} AUC_{(0-t)} \qquad 4.19$$

where, $F_{(t)}$ is the amount of drug absorbed. The fraction absorbed (FRA) was calculated using eq. 4.20 and was plotted against the fraction of drug dissolved

(FRD) at the same time and the linear regression analysis was used to examine the in vitro-in vivo relationship.

$$\text{Fraction absorbed (FRA)} = \frac{C_t + k_{el} AUC_{(0-t)}}{k_{el} AUC_{(0-\infty)}} \qquad 4.20$$

4.5.3. ESTABLISHMENT OF IN VITRO-IN VIVO CORRELATION (IVIVC)

An IVIVC refers to the predictive mathematical model describing the relationship between in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, such as plasma drug concentration or amount of drug absorbed. A level A correlation is usually estimated by a two-stage procedure: deconvolution followed by comparison of the fraction of drug absorbed (FRA) to the fraction of drug dissolved (FRD). A correlation of this type represents a point-to-point relationship between in vitro dissolution and the in vivo input rate (Guidance for the Industry, 2000).

Accordingly, the deconvolution procedure was done on optimized formulation using mean glipizide or nateglinide plasma concentration vs. time profile using the method of Wagner-Nelson. The data of FRA (obtained by deconvolution) and FRD for each formulation were then plotted to develop an IVIVC model. The linear, quadratic, and cubic models (Eq. 4.21) bearing the following forms were tested to obtain the best fit:

$$Y = a + bX$$
$$Y = a + bX + cX^2 \qquad 4.21$$
$$Y = a + bX + cX^2 + dX^3$$

where *a, b, c, d* represent regression parameters associated with each function, *Y* is the FRA in vivo and *X* is the FRD in vitro. The coefficients of determination (r^2) for all developed models were determined. The highest r^2 value equation was considered as the best fitting model.

4.5.3.1. Internal Validation of IVIVC

The reliability of the developed IVIVC model was evaluated by using the internal predictability as described in relevant regulatory guidance (US FDA CDER Guidance for the Industry, 1997). One recommended approach is based on a convolution procedure that models the relationship between in vitro dissolution and plasma concentration in a single step. Plasma concentrations predicted from the model and those observed are compared directly (Guidance for the Industry, 2000). For this method, a reference treatment using bolus input is desirable. An aqueous oral solution can be the bolus input for sustained-release formulation by gastrointestinal route (Langenbucher, 2003).

The predictability was tested as follows: The in vitro release data of each formulation was transformed into its in vivo release data by using the IVIVC equation. The plasma concentration profiles were calculated by numerical convolution (Eq. 4.22) of the in vivo release data with the help of the weighting function.

$$c(t) = \int_0^t c_\delta(t-u) \cdot r_{abs}(u) \cdot du \qquad 4.22$$

Where c_δ represents the concentration time profile resulting from an instantaneous absorption of a unit amount of drug, which is typically from bolus intravenous injection or reference oral solution data, $c(t)$ is the plasma concentration vs. time profiles of the tested formulations, r_{abs} is the input rate of the oral solid dosage form and u is the variable of integration.

The predicted plasma concentrations were compared with the observed plasma data and the percentage prediction error (%PE, eq. 4.23) of the IVIVC model was calculated with regard to C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$. According to the relevant FDA regulatory guidance (Guidance for the Industry, 2000) the permissible %PE values for C_{max} and AUC should be less than ± 15% for each product and less than 10% for the average.

$$\%\ \text{Prediction error (PE)} = \frac{\text{observed value} - \text{predicted value}}{\text{observed value}} \times 100 \qquad 4.23$$

4.6. References

BOURNE, D. W. A., **2002**. Pharmacokinetics. in: BANKER, G. S. and RHODES, C. T. (Eds.), Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, 4th Ed., Vol. 121, Marcel Dekker, Inc., New York and Basel, pp. 67-92.

CARR, G. P. R., WAHLICH, J. C. and TANNER, R. J. N., **1994**. Analytical Validation. in: CARTWRIGHT, A. C. and MATTHEWS, B. R. (Eds.), International Pharmaceutical Product Registration - Aspects of Quality, Safety and Efficacy, 1st Ed., Taylor & Francis Ltd., Bristol, PA, pp. 246-304.

CARR, R. L., **1965**. Evaluating flow properties of solids. *Chemical Engineering* 72, 163-168.

CURRIE, L. A., **1995**. *Pure Appl. Chem.* 67, 1699.

EBUBE, N. K., HIKAL, A. H., WYANDT, C. M., BEER, D. C., MILLER, L. G. and JONES, A. B., **1997**. Sustained release of acetaminophen from heterogeneous matrix tablets: influence of polymer ratio, polymer loading, and co-active on drug release. *Pharmaceutical Development and Technology* 2, 161-170.

Guidance for the Industry, **2000**. Extended Release Oral Dosage Forms : Development, Evaluation and Application of In Vitro/In Vivo Correlations, U.S. Department of Health and Human Services, US FDA, CDER, Rockville, MD.

HAUSNER, H., **1972**. The role of interparticle friction in powder technology, in GOELDBERG, A. S. (Ed.) 1st Int. Conf. on the Compaction and Consolidation of Particulate Matter, Brighton, 7-12.

HIGUCHI, T., **1961**. Rate of release of medicaments from ointment bases containing drugs in suspension. *J. Pharm. Sci.* 50, 874-875.

HIGUCHI, T., **1963**. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.* 52, 1145-1149.

HIXSON, A. W. and CROWELL, J. H., **1931**. Dependence of reaction velocity upon surface and agitation. *Ind. Eng. Chem.* 23, 923-931.

HUBERT, P. H., CHIAP, P., CROMMEN, J., BOULANGER, B., CHAPUZET, E., MERCIER, N., BERVOAS-MARTIN, S., GRANDJEAN, D., LAGORCE, P., LALLIER, M., LAPARRA, M. C., LAURENTIE, M. and NIVET, J. C., **2003**. Validation of the quantitative analytical procedures. Harmonization of the steps. *S.T.P. Pharma Practise* 13 (101-138).

HUBERT, P. H., CHIAP, P., CROMMEN, J., BOULANGER, B., CHAPUZET, E., MERCIER, N., BERVOAS-MARTIN, S., CHEVALIER, P., GRANDJEAN, D., LAGORCE, P., LALLIER, M., LAPARRA, M. C., LAURENTIE, M. and NIVET, J. C., **1999**. The SFSTP Guide on the validation of chromatographic methods for drug bioanalysis : from the Washington Conference to the laboratory. *Anal. Chim. Acta* 391, 135-148.

ISO Standard 8466-2, **1994**. Calibration strategy for non-linear second order calibration functions, Part 2, Water quality - Calibration and evaluation of analytical methods and estimation of performance characteristics.

KANJI, M. and SHIGEYA, T., **1998**. Effect of the Type of Organic Modifiers on Mass Transfer in Reversed-Phase Liquid Chromatography. *Anal. Sci.* 14, 361-368.

KING, A. H., **1983**. Brown seaweed extracts (Alginates). in: GLICKSHAM, M. (Ed.) Food Hydrocolloids, Vol. 2, CRC Press, Boca Raton, FL, pp. 116-154.

KOPCHA, M., LORDI, N. and TOJO, K. J., **1991**. Evaluation of release from selected thermosoftening vehicles. *J. Pharm. Pharmacol.* 43, 382-387.

KORSMEYER, R. W., GURNY, R., DOELKER, E. M., BURI, P. and PEPPAS, N. A., **1983**. Mecanism of solute release from porous hydrophilic polymers. *Int. J. Pharm.* 15, 25-35.

LANGENBUCHER, F., **2003**. Handling of computational in vitro/in vivo correlation problems by Microsoft Excel: III. Convolution and deconvolution. *Eur. J. Pharm. Biopharm.* 56, 429-437.

MILENA, G., RAMIREZ, G., VELASQUEZ, O., PEREZ, M. and RESTREPO, P., **2005**. Validation of analytical method for the determination of glimepiride in human plasma by HPLC/UV with addition standard using glibenclamide as an internal standard. *ARS Pharma.* 46 (4), 411-427.

MONEGHINI, M., CARCANO, A., PERISSUTTI, B. and RUBESSA, F., **2000**. Formulation design studies of atenolol tablets. *Pharmaceutical Development and Technology* 5, 297-301.

MOORE, J. W. and FLANNER, H. H., **1996**. Mathematical comparison of dissolution profiles. *Pharm. Tech.* 20, 64-74.

PEPPAS, N. A., **1985**. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta.Helv.* 60, 110-111.

RATSIMBAZAFY, V., BOURRET, E. and BROSSARD, C., **1996**. Drug release from matrix tablets and minitablets containing glycerides. *Pharm. Ind.* 58, 442-446.

RITGER, P. L. and PEPPAS, N. A., **1987**. A simple equation for description of solute release II. Fickian and anomalous releaase from swellable devices. *J. Controll. Rel.* 5, 37-42.

SPIEPMANN, J. and PEPPAS, N. A., **2001**. Modeling of drug release from delivery system based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug. Deliv. Rev.* 48, 139-157.

TRAIN, D., **1958**. Some aspects of the property of angle of repose of powders. *Journal of Pharm. Pharmacol* 10, 127T-134T.

US FDA CDER Guidance for the Industry, **1997**. Extended Release Solid Oral Dosage Forms: Development, Evaluation and Applicatin of In Vitro/In Vivo Correlations, US Department of Health, Food and Drug Administration, Centre for Drug Elaluation and Research (CDER), Rocville, MD.

US FDA CDER Guidance for the Industry, **2001**. Bioanalytical Method Validation (http://www.fda.gov/cder/guidance/4252fnl.pdf), US Department of Health, Food and Drug Administration, Centre for Drug Elaluation and Research (CDER), Rockville, MD.

US FDA CDER Guidlines, **1997**. Modified Release Solid Oral Dosage Forms Guidance: Scale-Up and Postapproval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing and In Vivo Bioequivalance Documentation, US Department of Health, Food and Drug Administration, Centre for Drug Elaluation and Research (CDER), Rocville, MD.

USP 29 - NF 24, **2006**. U. S. Pharmacopeial Convention, Rockville, MD.

WAGNER, J. G., **1971**. Biopharmaceutics and Relevant Pharmacokinetics, 1st Ed., Drug Intelligence Publishers, Illinois.