Original research article

RP-HPLC Method Development for Estimation of Various Pharmacokinetic Parameters of Optimized Formulation of Ganciclovir

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Abstract

Aim: The aim of the present investigation is to study RP-HPLC Method Development for Estimation of Various Pharmacokinetic Parameters of Optimized Formulation of Ganciclovir.

Material & Methods: In the preformulation study & on the basis of previous formulation, solubility profile and release rate characteristics were found to be good in the formulations with PEG-8000, PVP-17, Poloxamer-188and Urea. So, solid dispersions were tried in these combinations. Healthy rabbits of either sex (weighing 1.5 – 2.5 kg) were fasted overnight. GCR and its solid dispersions were administered at dose equivalent to 2.6mg/kg of GCR. Each product was repeated 4 times (n = 4). The *in vivo* experiments were conducted as a crossover study. GCR in serum samples were estimated according to High Performance Liquid Chromatographic (HPLC) method.

Results: The best formulation (among F-1 to F-6) i.e. F-5 was exposed to *in vivo* examinations in rabbits. An overlay spectrum of GCR indicated λ_{max} at 250.2 nm. The simultaneous estimation of GCR was done by RP-HPLC. The retention time of GCR was found to be 2.578 min the asymmetric factor was within limits. After a single dose of formulation F-5 (GCR - 2.6mg/kg), the symmetrical mean C_{max} values of formulation F-5 (47.37±1.52 µg/mL for GCR), was obviously higher than that of pure drugs {GCR (P<0.05), which was 21.26±0.03 µg/mL. C_{max} of SDs (F-5) was 2.22 times more than GCR. The T_{max} values of the formulations F-5 was equivalent to the pure drug. The $AUC_{(0-8h)}$ values of the formulation (GCR 266.6±5.65 µg.h/mL) was obviously higher than those of the pure drugs (GCR 132.65±1.48 µg.h/mL).

Conclusion: The *in vitro* drug release from the best SD combination (F-5) was compared with marketed sample was revealed that the drug release from the SDs was on par equivalent with the marketed dosage form.

Keywords: RP-HPLC Method Development, Pharmacokinetic Parameters, Optimized Formulation of Ganciclovir, Peak concentration (C_{max}), Area under the curve (AUC), Elimination rate constant (Kel), Biological half-life (t½)

Introduction

The biopharmaceutical classification system (BCS) is the scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. The BCS was first devised in 1995, by Amidon et al. and has become a benchmark for regulating bioequivalence of oral drug products.[1] The BCS serves as a guiding tool to formulation scientists. It recommends strategies to improve the drug development process by proper selection of dosage form and bioequivalence tests. It also recommends a class of immediate release (IR) solid dosage forms, for which bioequivalence can be assessed by in vitro dissolution tests. The influence of each of the three factors, dissolution, solubility and intestinal permeability on the oral absorption of drugs can be assessed by BCS. [2,3] FDA has adopted it as a regulating tool in drug product development. The drug product dissolution standards can be set by BCS. This allows for in vivo in vitro correlation (IVIVC) and can significantly reduce in vivo studies. Thus, save time in product development. [4-7]

Rheumatoid arthritis (RA) typically exhibits diurnal variations and exacerbations in early 24 h wake and sleep cycle. These fluxes are due to daily swaying in concentrations of disease regulating cytokines, especially interleukin–6 which display vigorous oscillations and offers changes in disease symptoms options for RA are intensifying as research has provided an additional unambiguous understanding on the pathophysiology of disease.[8] Therefore, the research was focused on designing and evaluation of Microwave induced melted SDs of GCR for the management of RA.

Materials and Methods

Optimization Of Formulation

In the preformulation study & on the basis of previous formulation, solubility profile and release rate characteristics were found to be good in the formulations with PEG-8000, PVP-17, Poloxamer-188and Urea. So, solid dispersions were tried in these combinations. The formulae with drug: carrier ratios were shown in table 1 and 2.

Table 1: Optimized solid dispersions of Ganciclovir

Drug: Carrier	Ratio	Formulation code
GCR: carrier blend*	1:1 1:2	E-1 E-2
	1:3 1:4 1:5 1:6	E-3 E-4 E-5 E-6

GCR= Ganciclovir; Carrier blend* contains equal mixture of PEG 8000+PVP 25+P188+Urea

Table 2: Formulation of tablet containing solid dispersions(GCR)

Ingredients	Quantity per tablet (mg)
Solid dispersions equivalentto GCR	125
Lactose	50
Starch	15
Micro Crystalline Cellulose	50
Magnesium Stearate	5
Talc	5
Weight of the tablets	250

IN VIVO STUDIES

Calculation of Animal Equivalent Dose from Human Dose

To Calculate Animal Equivalent dose [9] (AED) from Human Dose by equation was employed.

$$0.33 \quad \text{AED} \quad \frac{\text{Human Dose (mg/kg)}}{\text{Animal weight (kg)}} \text{Human weight } (\mathbf{kg})$$

Using Above equation considering the average human weight as 70 kg, animal equivalent dose calculations were carried out.
Weight of rabbits in kg = 1.5- 2.5
Human Dose of Drugs in mg: Ganciclovir 60 mg.
Calculated Animal Equivalent Dose (AED): Ganciclovir = 2.6mg/kg

Treatment to the Animals

Healthy rabbits of either sex (weighing 1.5 – 2.5 kg) were fasted overnight. GCR and its solid dispersions were administered at dose equivalent to 2.6mg/kg of GCR. Each product was repeated 4 times (n = 4). The *in vivo* experiments were conducted as a crossover study. Blood samples (0.5mL) were collected from marginal ear vein of Rabbits. The blood samples were allowed to clot and centrifuged at 5000 rpm and the serum separated was collected into dry tubes.[10] All the samples were stored under refrigerated conditions prior to assay. Serum concentration of the drugs (GCR) was determined by the HPLC methods.

From the time vs. serum concentration data various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (t_{max}) area under the curve (AUC), elimination rate constant (Kel), biological half-life (t½), percent absorbed to various times and absorption rate constant (Ka) were calculated in each case.

Estimation of Ganciclovir in serum samples

GCR in serum samples were estimated according to High Performance Liquid Chromatographic (HPLC) method. [11]

Materials

1. Ganciclovir pure drug
2. Acetonitrile (HPLC grade)
3. *O* - Phosphoric acid (HPLC grade)
4. Distilled water (Triple glass distilled)

Instrument Conditions

The instrument (HPLC) conditions were shown in table 3.

Table 3: HPLC conditions for simultaneous estimation of drugs

Instrument	Shimadzu Software LC
Column	Phenomenex Luna C18 (250x4.6 mm; 5µ)
Mobile phase	Water (0.1% o-Phosphoric acid) and
Acetonitrile (70:30 v/v)	
Detector	UV-VIS Spectrophotometer
Wavelength (nm)	220
Flow rate (mL/min)	1.0
Injection volume	20 µl
Run time (min)	7.0

Preparation of standard solution and plotting calibration curves

The procedure adopted was as established by Sanjiv *et al*, 2011 [11] with little modification. Weighed accurately 60mg of GCR (for stock solution A) and transferred to a 100 mL volumetric flask, dissolve using mobile phase with the aid of sonication and final volume was made with mobile phase.

From these stock solutions working standard solutions were prepared with suitable dilution with mobile phase to get concentrations of 5-40 µg for GCR.

Preparation of spiked plasma sample

This was performed by using procedure explained by Babu *et al*, 2016.[12] 250µl of rabbit plasma, 50µl of internal standard, 10µl of GCR were pipetted into 10ml centrifuge tube and to this 2ml of Acetonitrile was added. 10 µl of the supernatant layer was collected (after centrifugation at 3200 rpm for 10min) and injected into HPLC.[12] A typical chromatogram is achieved from a sample solution.

Method development

The mobile phase used was a 70:30 (v/v) mixture of freshly prepared buffer 0.1% o-Phosphoric acid and Acetonitrile showed an effective mixture used for the separation. Then, the flow rate tested was 0.4, 0.8, 1.0, 1.2 and 1.5 mL/min. 1.0 mL/min was selected for the determination of GCR as it has better resolution of the peaks. The stated chromatographic situations were the best to deliver resolution between GCR in a reasonable time of 4.912 and 2.805 min respectively at the optimum wavelength for detection was 220 nm and no native nosey composites eluted at the retention times of GCR.

Determination of various pharmacokinetic parameters:

From the time versus serum concentration data various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peal occurred (t_{max}), area under the curve (AUC), elimination rate constant (Kel), biological half-life (t½) , percent absorbed to various times and absorption rate constant (Ka) were calculated in each case.

Determination of C_{max} and t_{max}

The peak serum concentration (C_{max}) and time at which peak attained (t_{max}) were determined with the help of calibration curves. [13]

Determination of Elimination Rate Constant (Kel) and Half- Life (t ½)

Time versus serum concentration data was plotted on a semi logarithmic graph paper. The elimination rate constant (Kel) was calculated from the slope of the linear line in the elimination

phase (the best fit linear regression line for the points in the elimination phase was drawn by the method of least squares). The corresponding biological half-life was calculated using the equation $t^{1/2} = 0.693/Kel$.

Determination of Percentage Absorbed and Absorption Rate Constant (Ka)
Percentage absorbed to various times and absorption rate constant (Ka) were calculated from serum concentration data by the method described by the Wagner and Nelson equation.

Estimation of Area under the Curve (AUC)
The AUC was determined by using trapezoidal rule. The remaining area from 8 h to ∞ time was calculated using the following eq. 11 and 12. [14-15]

$[AUC]_{8-\infty}$ = Concentration at 8^{th}h / Kel

$[AUC]_{0-\infty} = [AUC]_{0-8}\ h + [AUC]_{8-\infty}\ h$

RESULTS
***In vivo* Studies**

Results of calibraton curves and isobestic point

The standard calibration curve of GCR was represented in table 4 and shown in figure 1.

Table 4: Standard calibration data of GCR

Ganciclovir concentration (µg)	Peak area (mean ±SD)
10	242455±25.28
20	351388±29.31
30	474883±15.25
40	591380±13.92
50	719416±12.74

Values in mean ±SD; Number of trials (n=3)

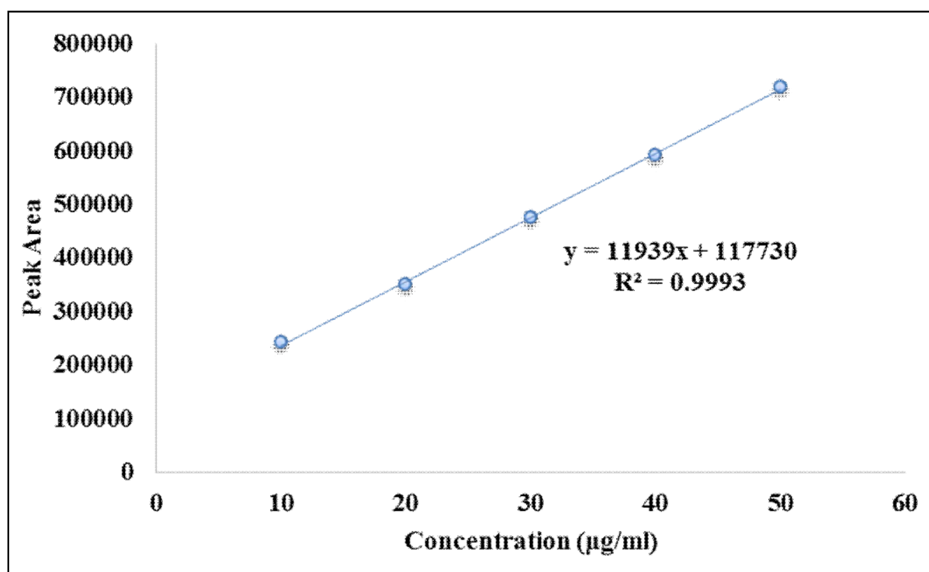

Fig.1.Calibration curve for the estimation of GCR in serum by HPLC

SIMULTANEOUS ESTIMATION OF GCR BY RP-HPLC

The HPLC conditions for simultaneous estimation of GCR was shown in table 5.

Table 5: Instrument (HPLC) Conditions for simultaneous estimation of GCR

Parameter	Chromatographic condition
Instrument	Shimadzu
Chemstation/ Software	LC Solutions
Column (250x4.6 mm; 5μ)	Phenomenex Luna C18
Mobile phase (70:30 v/v)	Water (0.1% o-Phosphoric acid) and Acetonitrile
Wavelength (nm)	250.2
Flow rate (mL/min)	9.0
Run time (min)	7.0

Table 6: Description of HPLC graph

Name	Retention Time (min)	Peak Area	Asymmetric factor
GCR	2.578	477568	1.12

Table 7: Statistical data of HPLC chromatogram

Parameters	GCR	LPR
Linearity (μg/mL)	10 – 50	1 – 5
Regression equation	y = 11939x + 117730	y = 115641x + 120220
Correlation coefficient	0.9993	0.9997
Slope	11939	115641
Intercept	117730	120220
Limit of Detection (μg/mL)	1.76	0.24
Limit of Quantification (μg/mL)	3.35	0.52

Values in mean ±SD; trials made (n=3)

The concentration of GCR in Serum with pure GCR and SDs (F-5) by after oral administration was represented in table 8.

Table 8: Concentration of GCR in Serum with pure GCR and SDs (F-5) by p.o

Concentration of GCR in Serum

Time (h)	Pure drug	SDs (F-5)
0.5	12.65±0.01	28.31±0.12
1.0	18.54±0.02	36.92±0.23
2.0	21.26±0.03	47.37±0.52
4.0	19.67±0.01	42.09±0.65
6.0	15.25±0.02	27.61±0.02
8.0	11.12±0.01	16.38±0.04

Values in mean ±SD; trials made (n=3)

The pharmacokinetic parameters estimated with GCR and its SDs (F-5) in serum after oral administration was shown in table 9.

Table 9: Pharmacokinetic parameters estimated with GCR and its SDs (F-5) in serum when administered orally

Pharmacokinetic parameter	Concentration of GCR in Pure drug	Serum SDs (F-5)
C_{max} (µg/mL)	21.26±0.03	47.37±1.52
T_{max} (h)	2.00±0.00	2.00±0.00
$K_{el}(h^{-1})$	0.126±0.01	0.205±0.01
$T^{1/2}$ (h)	4.46±0.05	2.93±0.09
$(AUC)^{0-8}$ (µg.h/mL)	132.65±1.48	266.6±5.65
$(AUC)^{0-\infty}$ (µg.h/mL)	211.4±2.95	237.5±6.59
$K_a(h^{-1})$	1.556±0.02	1.710±0.03
AUMC(µg.h/mL)	514.41±7.01	984.45±8.94
MRT (h^{-1})	8.027±0.07	5.4882±0.06

Values in mean ±SD; trials made (n=3)

Discussion

The best formulation (among F-1 to F-6) i.e. F-5 was exposed to *in vivo* examinations in rabbits. An overlay spectrum of GCR indicated λ_{max} at 250.2 nm. The simultaneous estimation of GCR was done by RP-HPLC. The retention time of GCR was found to be 2.578 min the asymmetric factor was within limits.

After a single dose of formulation F-5 (GCR -2.6mg/kg), the symmetrical mean C_{max} values of formulation F-5 (47.37±1.52 µg/mL for GCR), was obviously higher than that of pure drugs {GCR ($P<0.05$), which was 21.26±0.03 µg/mL. C_{max} of SDs (F-5) was 2.22 times more than GCR. The T_{max} values of the formulations F-5 was equivalent to the pure drug. The $AUC_{(0-8h)}$ values of the formulation (GCR 266.6±5.65 µg.h/mL) was obviously higher than those of the pure drugs (GCR 132.65±1.48 µg.h/mL). The $AUC_{(0-8h)}$ of SDs (F-5) was ~2 folds more than GCR. The $AUC_{(0-\infty)}$values of the formulation (GCR 237.5±6.59 µg.h/mL) were obviously higher than those of the pure drugs (GCR 211.4±2.95 µg.h/mL). The $AUC_{(0-\infty)}$ of SDs (F-5) was marginal increase i.e., 1.12 folds more than GCR and ~4 times (3.88 times). These fallouts suggest that the absorption rate and bioavailability of SD formulation F-5 are remarkably quicker and greater than that of pure drugs. *In vivo* animal trials in rabbits shown good levels of GCR in serum related to pure GCR.

Conclusion

The majority of drugs are of poor water soluble and such compounds may exhibit insufficient dissolution throughout the gastrointestinal tract results in failing to achieve systemic acquaintance after oral administration. Fading bioavailability is the major cause for leaving inventive oral dosage forms. The applicability of the solid dispersion technique as an approach

for improving the gastric absorption of drugs has been discovered in order to attain better dissolution characteristics and better bioavailability for poorly soluble drugs. The *in vitro* drug release from the best SD combination (F-5) was compared with marketed sample was revealed that the drug release from the SDs was on par equivalent with the marketed dosage form.

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