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Development of an in-vitro in-vivo correlation for Sitagliptin and Metformin prolonged release tablet formulations

Short Title: Development of an IVIVC for Sitagliptin and Metformin

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Abstract
Objectives: The objective of the investigation was to establish and validate an in-vitro in vivo-correlation. To investigate the safety of a fixed-dose combination (FDC) versus reference formulations (Januvia® 100 mg Filmtabletten co-administered with Glucophage® SR 1000 mg Prolonged-release Tablets), a bioequivalence study was conducted under fasted and fed state. The data generated in the bioequivalence study have been used for the establishment of the correlation.
Materials and Methods: The formulations used in bioequivalence study were FDC (Sitagliptin hydrochloride equivalent to 100 mg of Sitagliptin and Metformin hydrochloride 1000 mg prolonged release) and Januvia® 100 mg co-administered with Glucophage® SR

1000 mg. The plasma profiles from bioequivalence study and respective dissolution data was taken for the establishment of level 'A' IVIVC. The procedure comprises of pharmacokinetic modeling to derive the empirical constants and further to use for deconvolution and convolution procedures. Levy plots were constructed to understand the relationship between in-vitro and in-vivo properties. The internal and external predictabilities were evaluated by comparing the predicted pharmacokinetics with the observed values from the bioequivalence study.

Results: The formulations showed about 91-95% and 89-91% dissolution respectively in fasted and fed state dissolution media for Sitagliptin. Whereas the dissolution for Metformin was 96-98% and 89-95% respectively in fasted and fed state media. The regression coefficients of all the 'Levy' plots were more than 0.900 indicating a linear correlation exists between in vitro release and in vivo parameters. The prediction error value of internal and external predictabilities was below 10 and is meeting the FDA criteria. Therefore, the correlation models are validated and can be used for the predictions and in setting the dissolution specifications. The safety and tolerability of the FDC was found better than the safety and tolerability of the reference formulations as less adverse events occurred after administration of FDC.

Conclusions: The correlation models can be useful for the predictions of FDCs during the life cycle management of the product. The models can also serve as a surrogate for the in-vivo studies. The FDC was tolerable and the adverse events are similar to the reference products and are mild in nature, hence safe for the use in human subjects.

Key words: IVIVC, Level A correlation, Levy plot, Sitagliptin hydrochloride, and Metformin hydrochloride.

Introduction

Sitagliptin, a dipeptidyl peptidase 4 (DPP-4) inhibitor is indicated for hyperglycemia.[1] Sitagliptin exerts its action by prolonging the action of GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide) but facilitates the insulin production and reduces the secretion of glucagon, which in turns decreases hepatic glucose overproduction.[2] Food does not show a significant influence on the pharmacokinetics of Sitagliptin, hence Sitagliptin can be taken without regards to food. Metformin is prescribed as first line therapy in type-2 diabetes.[3] Metformin possess glucose lowering effect via inhibition of (i) gluconeogenesis in liver, (ii) delaying the glucagon actions, (iii) facilitating the actions of insulin and (iv) delay of glucose absorption from intestine.[4,5] As per biopharmaceutical classification system (BCS), both Sitagliptin and Metformin possess high solubility and poor permeability, therefore both drugs belonging to BCS class III.[6,7]

A fixed-dose combination (FDC) comprising of Sitagliptin hydrochloride equivalent to Sitagliptin 100 mg as an immediate release form and Metformin hydrochloride 1000 mg as prolonged release form was developed. FDCs offers numerous merits[8] in comparison to individual drug products which includes the simplicity of the dosage forms in terms of dosage schedule. This leads to improved patient compliance and results in overall better outcome of treatment. This aspect is especially important in elderly patients or patients suffering from multiple disorders. The *in vivo* behavior of Januvia®, Glucophage 100 mg SR and FDC were evaluated in a bioequivalence study including healthy subjects.[9] The FDC was developed in order to avoid consumption of two individual products for the routine treatment of type 2 diabetes mellitus. The formulations (FDC vs individual innovator products) as a part of development have been evaluated and proven for the safety, bioequivalence and tolerability in human volunteers under fasted and fed state.[9]

For the life-cycle management of formulations, always a need arises for the change of parameters like minor change of composition, process, equipment, scale-up or scale down or the manufacturing site. In certain instances, the post-approval changes may trigger conduct and demonstration of further bioequivalence for the modified and the marketed formulation.[10] Furthermore, the availability of an in-vitro in-vivo correlation (IVIVC) can simulate and predict the plasma profiles and can serve as a surrogate for the in-vivo studies. Therefore, an IVIVC was developed and validated for the predictability using the bioequivalence data under fasted and fed state. The present paper describes the aspects for the development of IVIVC and the aspects of internal and external validation. The correlation models can be useful for the predictions of FDCs during the life cycle management of the product.

Materials and Methods

Formulations tested

The formulations evaluated for bioequivalence was shown in Table-1. (FDC (composition is not disclosed) containing Sitagliptin Hydrochloride equivalent to Sitagliptin 100 mg and Metformin hydrochloride 1000 mg prolonged-release tablets (AET Laboratories Pvt. Ltd, India) was used as test formulation. The in-vivo behavior of test formulation as FDC was compared with individual reference formulations namely Januvia® 100 mg Filmtabletten (Lot No. 362117, marketed by Merck Sharp & Dohme Ltd., UK) and Glucophage® SR 1000 mg prolonged-release tablets (Lot No. GXC15222, marketed by Merck Serono Ltd., UK). In Cohort 1, the study has been done under fasting conditions and in cohort 2, the study has been done in fed state.[9]

Dissolution Method

The in-vitro release of Sitagliptin and Metformin from FDC, Januvia® 100 mg Filmtabletten and Glucophage® SR 1000 mg prolonged-release tablets were performed using USP I (basket) apparatus (Electrolab, Mumbai, India). The individual tablets were placed in dissolution vessels containing 900 mL of dissolution media. Phosphate buffer pH 6.8 and Acetate buffer pH 5.5 were selected as the dissolution media for fasted and fed state conditions, respectively. The pH of the dissolution media was selected based on the pH of gastrointestinal tract under fasted and fed states. The pre-prandial gastrointestinal pH is 1 to 7.5 and post prandial pH was 2.7-6.4 (stomach); 4-8 (intestine).[11-13] Due to high solubility and pH independent soluble nature of the molecules, aqueous buffers at one pH for each condition was selected. The study was conducted at a rotational speed of 100 rpm. The samples were collected until 60 min for Sitagliptin and 12 h for Metformin and replenished with fresh and respective media. The drug released from the samples was analyzed using high-performance liquid chromatography (HPLC) system (Waters, Singapore)equipped with quaternary pump, UV-Visible spectrophotometric detector (Perkin Elmer, Lambda 25, Massachusetts, USA), and using a C_8 column (100 x 2.1 mm, particle size of 1.7 µm). The mobile phase comprises of acetonitrile, 10 mM potassium dihydrogen phosphate buffer and 2 mM sodium hexane-1-sulfonate. The pH of the mobile phase was adjusted to 5.5 using phosphoric acid. The flow rate was 1 mL min^{-1} and the detection wavelength was 210 nm.[14] Required precision and accuracy of the chromatographic method were checked and were found to be within limits (percent coefficient of variation was less than 15%). The dissolution profiles were subjected for similarity assessment in accordance to the guidelines[10].

In vivo Characterization

Subjects and study approval

Mixed population comprising of twenty-four healthy subjects (including 9 male and 15 female subjects) after the screening were enrolled in the bioequivalence study (Table-1). The subjects were grouped in two cohorts, each consisted of twelve subjects. The subject inclusion was done in accordance to the guidelines,[15] as all the subjects with an age of 18 years and older and with a body mass index of 18.5-30 kg/m^2 were enrolled in the study. The study protocol was approved vide letter number 429 by National Ethics Committee for Drugs Clinical Trials and to the Medicines and Medical Devices Agency Chisinau, The Moldavian Republic. The study was conducted[9] in agreement with the Declaration of Helsinki (1964 and following amendments), ICH-GCP R2,[16] EEC rules and in accordance to the GCP (good clinical practice) for the conduct of clinical studies. The medical history of the subjects was recorded by the clinical investigator. A medical examination was conducted to record SAP (systolic arterial pressure), DAP (diastolic arterial pressure), heart rate (HR), ECG, body temperature and respiratory frequency. The biological samples (urine and blood) were collected for the analysis from clinical chemistry perspective.

Study design

The study has been performed as an open label, two-period, two-way cross-over, controlled randomized, single dose comparative bioequivalence study between FDC and reference formulations in healthy subjects with a wash-out period of 14 days between periods. The blood samples were collected at before the study drug administration and at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.33, 5.67, 6.00, 7.00, 8.00, 9.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00, 96.00, and 120.00 hours post-dose. The blood samples were centrifuged for 10 minutes at 4°C nominal with a force of 1500 (±5)-g. After centrifugation, plasma was separated into two aliquots and stored at -20° C until analysis of the samples.

Bioanalytical procedure-estimation of analytes from plasma

The sample analysis and processing were performed by Analytical Laboratory of 3S-Pharmacological Consultation & Research GmbH (Bucharest, Romania). The concentrations

of Sitagliptin and Metformin were measured by reversed phase HPLC coupled to a tandem mass spectrometry detector (LC/MS/MS). The separations were isocratically performed on a reversed-phase column (Agilent Zorbax 300-SCX, 2.1 x 50 mm, 5 µm) with a flow rate of 1.00 mL/min. The mobile phase comprises of methanol and 75 mM of ammonium acetate buffer (80:20). The internal standards used were Sitagliptin-D4 and Metformin-D6. The detection was carried out by MS/MS mass spectrometry, triple quadrupole, AB-Sciex model, API 5500 QTRAP, equipped with atmospheric pressure ionization interface (Model, Turbo Spray). The precursor and product ions used for detection were 408.123/235.100 for Sitagliptin, 412.088/239.100 for Sitagliptin-D4, 129.975/71.200 for Metformin and 136.026/ 60.000 for Metformin-D6.The analytical method was validated in accordance to GLP principles, FDA rules,[17] EMA guidelines[18] and the current Romanian GLP guidance. The method was developed and validated at a concentration range of 1.0–800 ng/mL and 5.0-4000 ng/mL respectively for Sitagliptin and Metformin. During validation, the stability of internal standard working solution (up to 16 hours at room temperature), system suitability test solution stability (up to 1 week when stored below -20°C), spiked plasma sample stability (up to 6 hours at room temperature, up to 1 week at -5ºC, up to 11.5 months below -20ºC, up to 1 week below -70ºC), freeze-thaw stability (up to 5 cycles), stability of spiked plasma sample extract (up to 48 hours at 10ºC) were evaluated.

Sample preparation

An aliquot of 0.150 mL of plasma sample was transferred to 2 mL multi-well plates. Fifty micro liter of working internal standard solution was added to plasma sample and mixed for 3 minutes followed by addition of 0.800 mL of acetonitrile. The contents were mixed for 5 minutes and centrifuged for 5 minutes at 4000 rpm (20ºC, nominal). The supernatant was separated and diluted and 20 µL was injected in to HPLC.

Pharmacokinetic variables

The pharmacokinetic parameters, C_{max} (peak drug concentration) and AUC_{0-t} (area under the curve from time zero to t) as primary parameters and AUC_{0-inf} (area under the curve from time zero to infinity) and T_{max} (time of the peak drug concentration) as secondary parameters were calculated.

Safety and tolerability

The clinical safety of the formulations was assessed via medical history, clinical examination (physical and systemic examination), 12-lead ECG and vital signs (blood pressure, heart rate, and respiratory rate and temperature) and biochemical parameters. The parameters were measured at the time of check-in to the study center, before treatment of each study period. Before dosing of each period, the subjects were asked for the health status and follow-up for the consumption of medications. The SAP, DAP, heart rate and body temperatures were measured before dosing and during the study for each period.

Development of In Vitro–In Vivo Correlation (IVIVC)

Phoenix® Version 8.1 software was used for the pharmacokinetic modelling, deconvolutions and convolutions procedures. The pharmacokinetic modeling was done in order to fit a best model by varying the parameters, e.g. with and without lag time and choosing one or two compartment models. Among the attempts, the one which yielded a high correlation coefficient was chosen for further consideration. Accordingly, the empirical constants (A and Alpha) were chosen for the deconvolution and convolution procedures. ‘A’ and ‘Alpha’ refer to the parameters of a poly exponential unit impulse response function of the form:

$$c(t) = \sum_{j=1}^{n} \mathrm{Aj}\, e^{-\alpha \mathrm{j}\, t}$$

Where, '*N' is* the number of exponential terms, 'Cδ' represents to concentration time course and 't' stands for time.
The *in-vivo* plasma concentrations versus time profiles were deconvolved to derive the fraction absorbed. Then the fraction of drug absorbed was correlated with the drug dissolved in order to construct a 'levy' plot.

Validation of the IVIVC

The empirical constants were chosen for the convolution of dissolution profiles in order to derive the plasma-concentration-time-profile. The simulated plasma profile was further subjected for the calculation of pharmacokinetics to compute C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}Inf}$. The prediction error (PE) was calculated for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}Inf}$ for each formulation and for each drug substance using below equation

PE= [(Observed-Predicted)/Observed]×100

The predictability of the correlation model was evaluated using the internal and external predictabilities as per FDA guidance.[19] For both internal and external validation, the mean PE was required and should be not more than 10% for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$.

Results

In-vitro release

Sitagliptin

The in-vitro drug-release profiles of FDC and Januvia® using the selected method (USP 1, 100 rpm at pH 6.8 and pH 5.5) are presented in Fig 1.The immediate-release form of Sitagliptin showed about 91-95% of dissolution in phosphate buffer pH 6.8 and 89-91% dissolution at acetate buffer pH 5.5. Despite of differences in salt from Januvia® and FDC, the dissolution appears to be complete and gradual. However, there exists differences in the dissolution pattern. To check the impact of difference in dissolutions between reference and test formulations, similarity factor (f_2) was assessed for the dissolution profiles. The f_2 values were found to be 54 for both fasted and fed state dissolutions. The f_2 values more than 50 is an indication of similarity[10].

Metformin

The in-vitro drug-release profiles of FDC and Glucophage® SR 1000 mg using the selected method (USP 1, 100 rpm at pH 6.8 and pH 5.5) are presented in Fig 2. The prolonged-release form of Metformin HCl showed about 96-98% dissolution at pH 6.8 and 89-95% dissolution at pH 5.5. The dissolution profiles appear to be gradual and complete. The f_2 values were found to be 65 (at pH 6.8) and 55 (at pH 5.5) respectively, also an indicating of similarity of dissolution profiles[10].

IVIVC Model

The plasma profiles of Sitagliptin (Fig 3A) and Metformin (Fig 3B) from the bioequivalence study were deconvoluted to derive the respective in vivo absorption profiles (Fig 4A-B). The levy plots were constructed to understand the relation between the in vitro Sitagliptin dissolved and in vivo Sitagliptin absorbed (Fig 5A-B). The regression coefficients of 0.952 and 0.976 for Januvia® and FDC indicates that there exists a good linear correlation between in-vitro and in-vivo parameters under fasting condition. Similarly, the correlation coefficients were 0.996 and 0.963 respectively for Januvia® and FDC tested under fed condition (Fig 5C-D). Despite of differences in the salts used in the formulations, the in-vitro tool showed good discrimination. Hence, the employed dissolution method can be used for the characterization of formulations containing either of the salt.

The levy plots of Metformin (Fig 6A-B) using the data obtained in fasted state study showed a regression coefficient of 0.971 and 0.937 respectively for Glucophage® SR 1000 mg and FDC. The regression coefficients of fed state data were 0.965 and 0.959 respectively for

Glucophage® SR 1000 mg and FDC (Fig 6C-D). The results indicating the chosen in-vitro conditions are appropriate and are mimicking the in-vivo environment.

Internal and External Validation

The internal and external predictability of the Sitagliptin for the C_{max}, AUC_{0-t} and AUC_{0-Inf} are presented in Table-2. The mean PE values are below 10 for all the parameters and for both the formulations under fasted and fed conditions. The internal and external prediction error values was even below 10% for Metformin for all the principle pharmacokinetics (Table-3).

Safety and tolerability

No serious adverse events (AEs) were reported in the bioequivalence study and the intensity of reported AEs were deemed by the principal Investigator as mild in severity. A total of five subjects showed eight AEs, among two subjects showed AEs for FDC and five showed AEs for reference formulation[9]. Despite of differences in salts of Sitagliptin from reference (Sitagliptin phosphate monohydrate) to test formulation (FDC comprises of Sitagliptin Hydrochloride), the number of AEs of test formulation was 'low' compared to reference formulation under both fast and fed conditions. The test and reference products were well tolerated considering the AEs observed in the study. Therefore, Sitagliptin Hydrochloride can be deemed to be safe and is behaving in line to the Sitagliptin phosphate monohydrate from the safety perspective.

Discussion

As per EMA[20] guidelines, a validated IVIVC serves as a surrogate for in vivo performance. The validation of IVIVC in terms of internal and external validations was proved in the current study. Therefore, the changes in manufacturing process, and some formulation modifications, including product strength using the same formulation, can be justified using IVIVC, without the need for additional bioavailability/bioequivalence studies. Therefore, in present study, a correlation was established between the in-vitro drug release at pH 6.8 (pre-prandial state) and pH 5.5 (post-prandial state) versus the fraction of respective drug absorption under fasted and fed state.

Sitagliptin

At pH 6.8, Sitagliptin release was initially slow from FDC compared to Januvia® and similar from 20 min onwards. The slow pattern of dissolution from FDC could be due to combination of immediate and prolonged-release formulation, where the Sitagliptin hydrochloride was present as an immediate-release form along with prolonged-release of Metformin HCl. Whereas the Januvia® is an immediate-release tablet, the dissolution progresses with the disintegration of tablets. A similar trend was also evident at pH 5.5. Despite of differences in the dissolutions, the Sitagliptin HCl from FDC showed bioequivalence with Januvia®,[9] indicating that both the salts demonstrated similar *in vivo* behavior which could be due to high solubility and high bioavailability of the Sitagliptin.[9],[21] The physiological conditions of GI tract, such as peristaltic motility,[22] appears to be resembling the in vitro conditions as it was evidenced from the similar in vivo performance. The in vitro difference during initial phase of dissolution does not impact the *in vivo* performance.

Metformin

The dissolution of FDC was slow at 0.5 h followed by similar release pattern in comparison to Glucophage® 1000 mg SR at pH 6.8. Similarly, the dissolution of FDC at pH 5.5 was slow by 5-11% until 10 hours. A similar trend was reflected *in-vivo*[9] indicating the discrimination ability of the dissolution method to mimic the *in-vivo* environment.

Furthermore, a levy plots were constructed to establish a mathematical relationship linking F_a (Fraction of absorbed) to F_d (Fraction of dissolved) for both Sitagliptin and Metformin. For establishment of such mathematical relationship, the respective *in-vivo* plasma concentration

profiles for sitagliptin and metformin were deconvoluted into the F_a and the F_d, which were derived from respective in-vitro dissolution profiles. The so derived least square regressions from levy plots are yielded essentially linear patterns (correlation coefficient>0.900) in both pre-prandial and post-prandial conditions, demonstrating that the proposed *in-vitro* biorelevant dissolutions can indeed explain the absorption of both Sitagliptin and Metformin satisfactorily. The constructed level 'A' levy plots demonstrated that the chosen *in-vitro* conditions are appropriate and are mimicking the *in-vivo* environment.
Further, the predictability of the developed IVIVC models were estimated in terms of PE values for the validation of the correlation models. An evaluation of internal and external predictability for the pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-Inf}) was below 10% and was demonstrating the predefined internal and external validation criteria[19]. Overall, the attained IVIVC models yielded the predicted C_{max} and AUC parameters below 10% of the observed values for both internal and external validations under both fasted and fed state. A proven IVIVC and a discriminatory *in-vitro* method can serve as a surrogate for *in-vivo* characterization as well as to select suitable formulations for *in-vivo* studies[23]. The bioequivalence results also substantiated the validity of developed IVIVC models for both Sitagliptin and Metformin, hence, the correlation models can be deemed to be robust, therefore can be considered for predicting the *in-vivo* performance.
The bioequivalence results reveal that FDC is well tolerated and safe for use in humans. From *in-vivo* behavior and safety perspective, the FDC product comprising of Sitagliptin HCl and Metformin HCl prolonged-release showed more or less similar *in-vivo* behavior in comparison to individual reference formulations. Therefore, FDC can enhance the patient compliance as it minimizes the consumption of individual products.

Conclusions

A robust in-vitro in-vivo correlation (level A) that meets the validation criteria for both internal and external predictability was established for Sitagliptin and Metformin prolonged-release formulations. Although the Sitagliptin salt form is different in the test and reference products (FDC contains Sitagliptin hydrochloride and Januvia® contains Sitagliptin Phosphate monohydrate), the developed IVIVC model exhibited good predictability. The correlation models can be used for the predictions of formulations containing immediate release Sitagliptin salts (as HCl or phosphate monohydrate as the salts) and prolonged-release Metformin HCl. The IVIVC can also be used as surrogate for bioequivalence studies in case of future formulation changes that are covered by the IVIVC release rates. The FDC comprising of Sitagliptin HCl and Metformin HCl is well tolerated in human volunteers and the rate of adverse events was similar to the AEs of reference products, hence FDC can be deemed to be safe for use in human subjects.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

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Table-1: Scheme of clinical study

Period	Cohort-1 (n=12)		Cohort-2 (n=12)	
	Group-1 (n=6)	Group-2 (n=6)	Group-1 (n=6)	Group-2 (n=6)
1	*FDC	#Januvia® 100 mg and Glucophage® SR 1000 mg	*FDC	#Januvia® 100 mg and Glucophage® SR 1000 mg
2	#Januvia® 100 mg and Glucophage® SR 1000 mg	*FDC	#Januvia® 100 mg and Glucophage® SR 1000 mg	*FDC

*FDC (Fixed dose combination) comprising of Sitagliptin Hydrochloride, equivalent to 100 mg Sitagliptin and Metformin hydrochloride 1000 mg and is manufactured by AET Laboratories Pvt Ltd., India
Originators comprises of Sitagliptin phosphate monohydrate, equivalent to 100 mg sitagliptin (Januvia® 100 mg) and Metformin hydrochloride 1000 mg as prolonged release (Glucophage® SR 1000 mg)

Table-2: Validation of the correlation model for Sitagliptin

Parameter	Januvia® 100 mg Internal Validation			FDC External Validation		
	Observed	**Predicted**	**PE**	**Observed**	**Predicted**	**PE**
Fasted state						
Cmax (ng/ml)	314.0	302.6	**3.64**	324.7	309.4	**4.69**
AUC_{0-t} (ng-h/ml)	3141.7	3024.8	**3.72**	2964.9	2931.0	**1.14**
AUC_{0-inf} (ng-h/ml)	3178.9	3044.1	**4.24**	2983.2	2951.2	**1.07**
Fed state						
C_{max} (ng/ml)	383.2	370.2	**3.40**	347.7	338.6	**2.62**
AUC_{0-t} (ng-h/ml)	3595.0	3395.0	**5.56**	3531.5	3355.0	**4.99**
AUC_{0-inf} (ng-h/ml)	3630.5	3400.6	**6.33**	3565.6	3362.0	**5.72**

Table-3: Validation of the correlation model for Metformin

Parameter	Glucophage® SR 1000 mg Internal Validation			FDC External Validation		
	Observed	**Predicted**	**PE**	**Observed**	**Predicted**	**PE**
Fasted state						
C_{max} (ng/ml)	946.7	869.9	**8.11**	991.8	924.9	**6.75**
AUC_{0-t} (ng-h/ml)	8785.2	8720.9	**0.73**	8802.8	9093.4	**-3.30**
AUC_{0-inf} (ng-h/ml)	9015.1	9057.6	**-0.47**	9003.8	9438.9	**-4.83**
Fed state						
C_{max} (ng/ml)	1255.9	1250.6	**0.42**	1375.68	1273.3	**7.44**
AUC_{0-t} (ng-h/ml)	15196.5	15549.6	**-2.32**	15215.12	16380.5	**-7.66**
AUC_{0-inf} (ng-h/ml)	16263.7	15815.8	**2.75**	15640.22	16653.2	**-6.48**

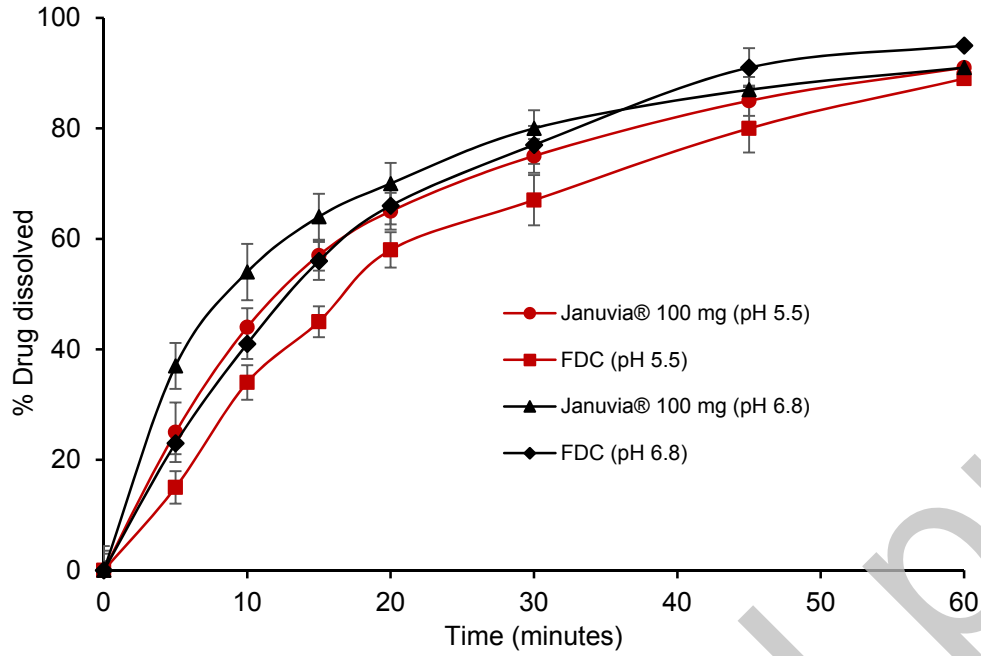


Fig-1: In vitro release of Sitagliptin from FDC and Januvia® 100 mg at pH 6.8 and pH 5.5. FDC contains Sitagliptin HCl and Januvia® 100 mg contains Sitagliptin Phosphate Monohydrate equivalent to 100 mg Sitagliptin respectively

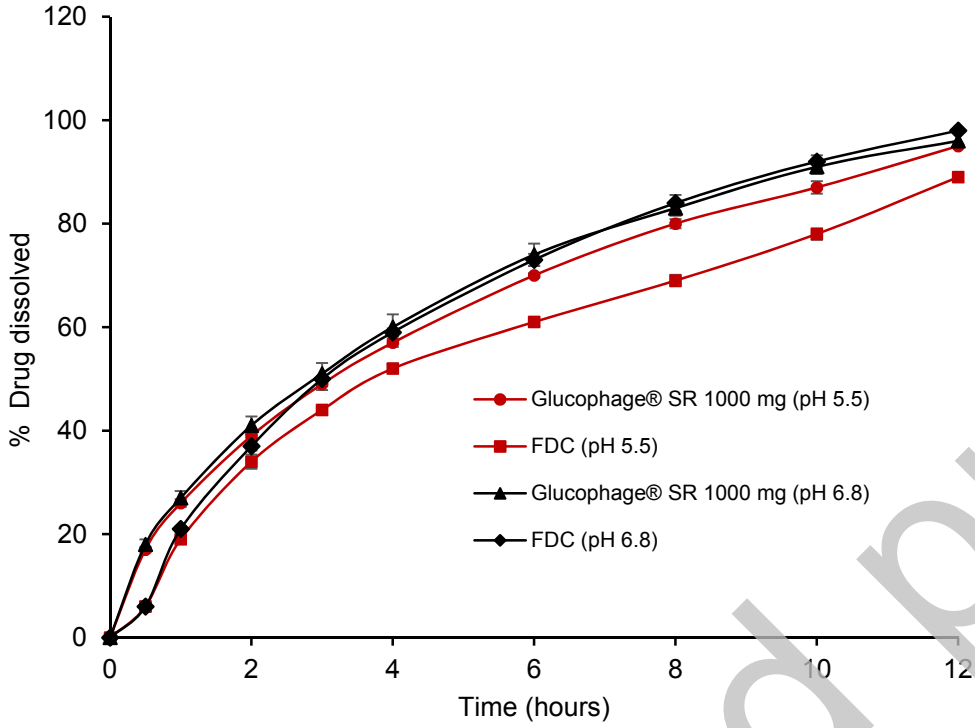
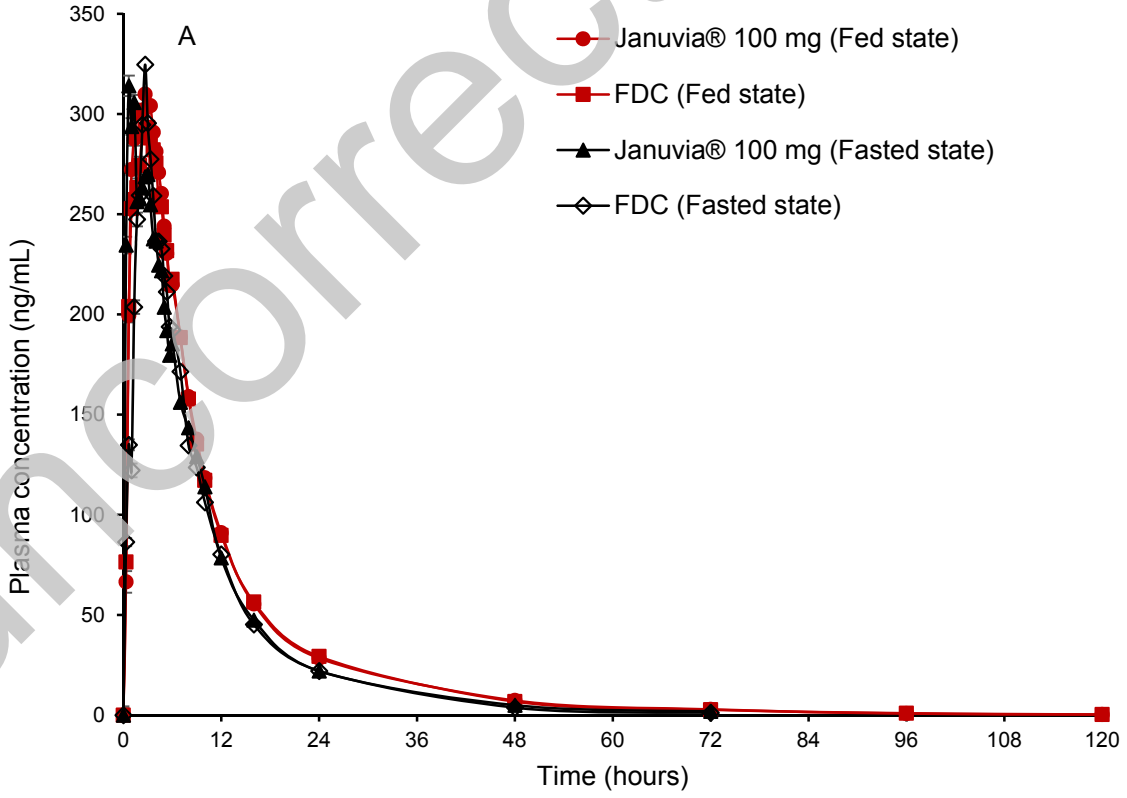


Fig-2: In vitro release of Metformin HCl from FDC and Glucophage® SR 1000 mg at pH 6.8 and pH 5.5. Both the formulations contain 1000 mg Metformin HCl in the form of prolonged release.



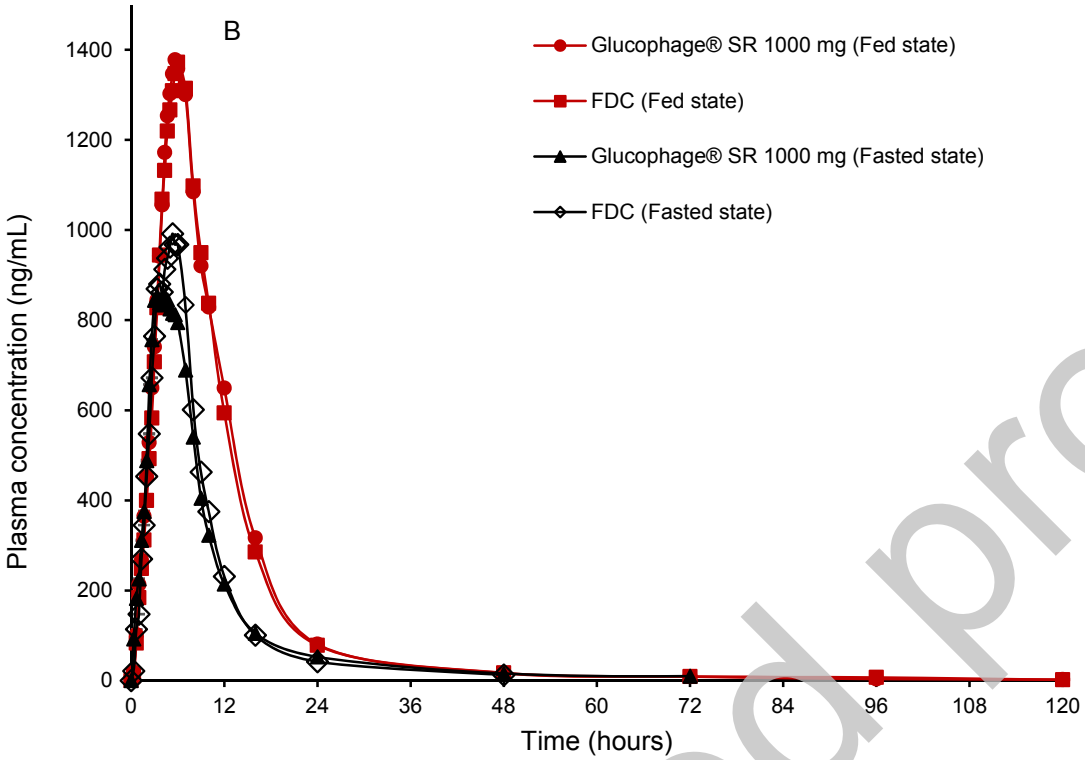
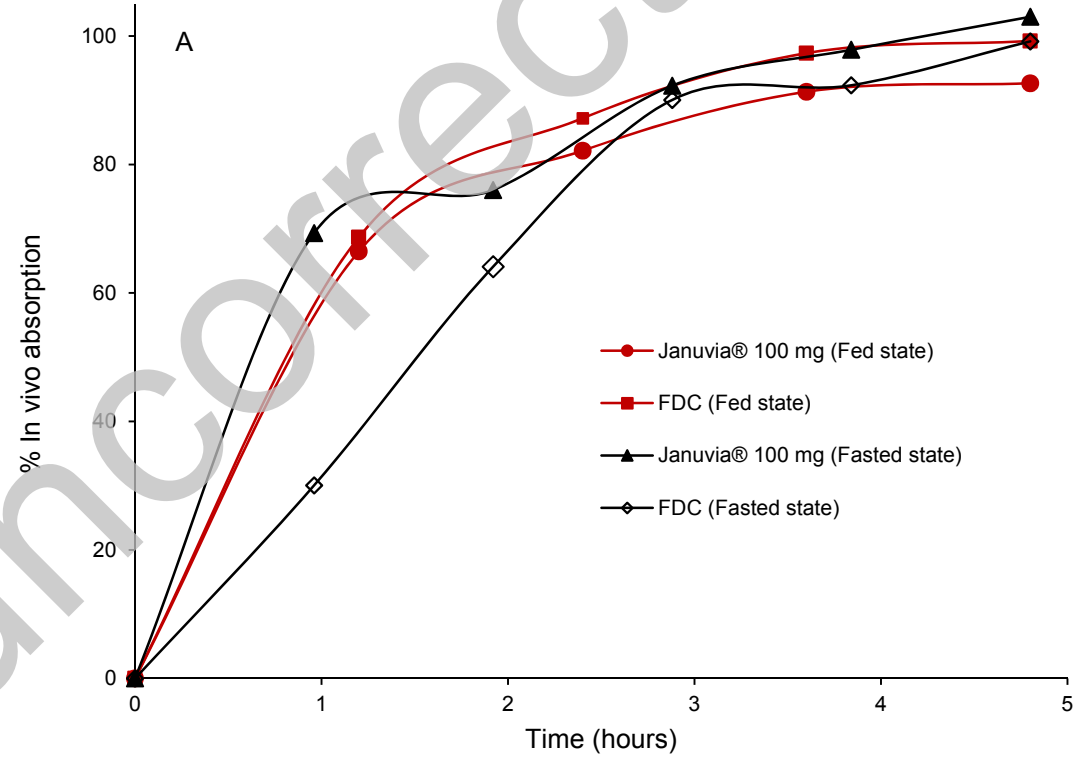


Fig-3: Mean plasma profile of Sitagliptin under fasted and fed states (A) and Metformin under fasted and fed states (B) for the formulations used in the study



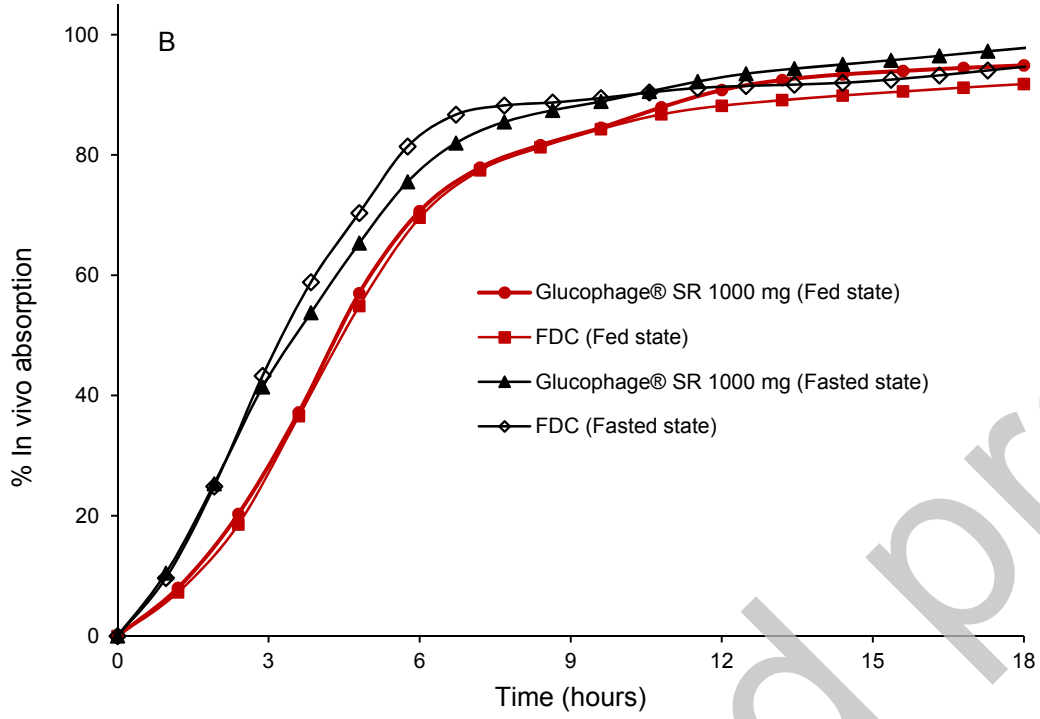
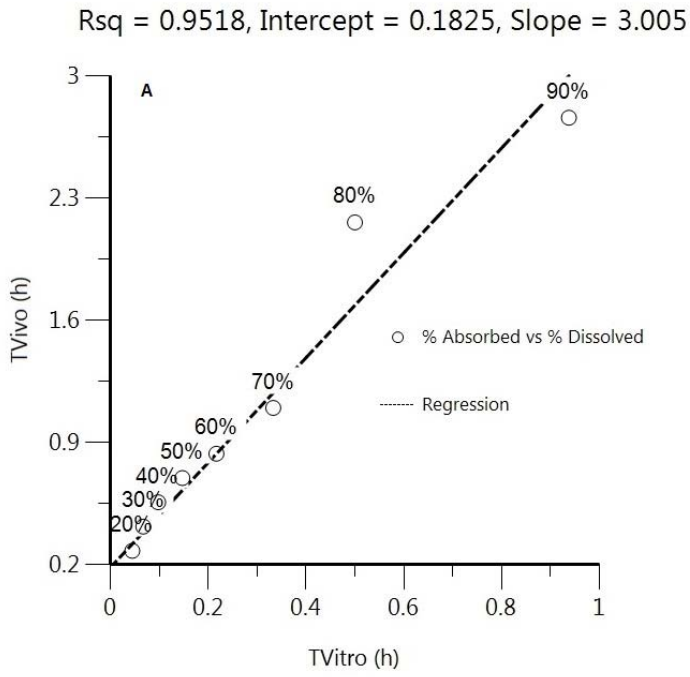
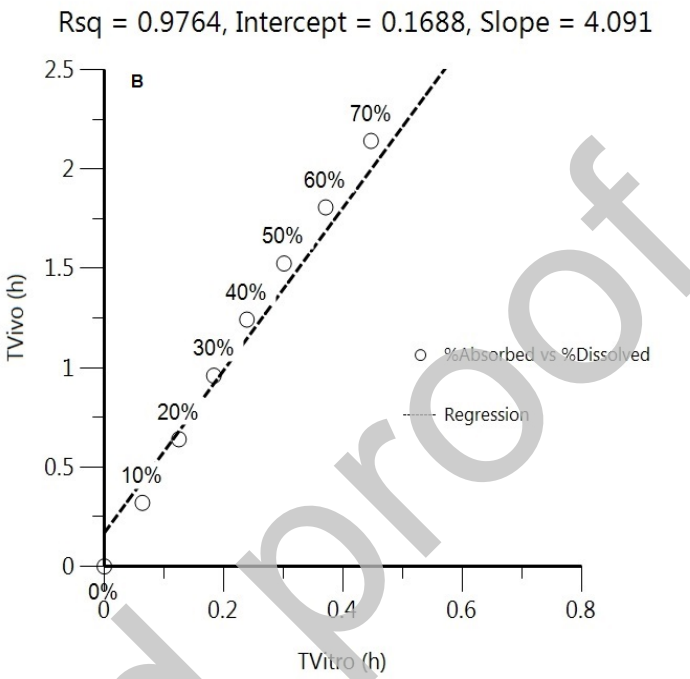
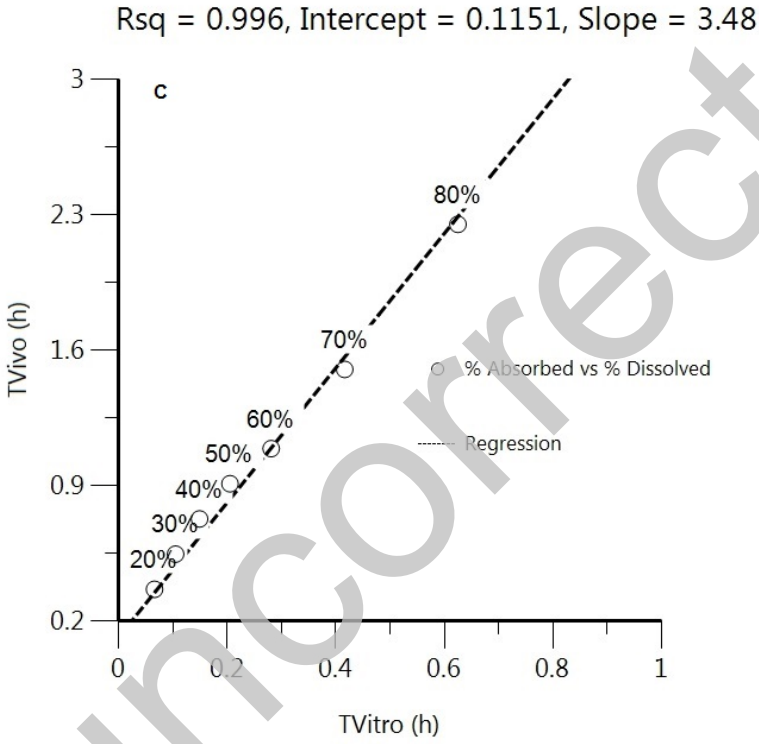


Fig-4: Mean in vivo absorption profiles for Sitagliptin fasted state and fed state (A) and Metformin fasted state and fed state (B) for reference and test formulations







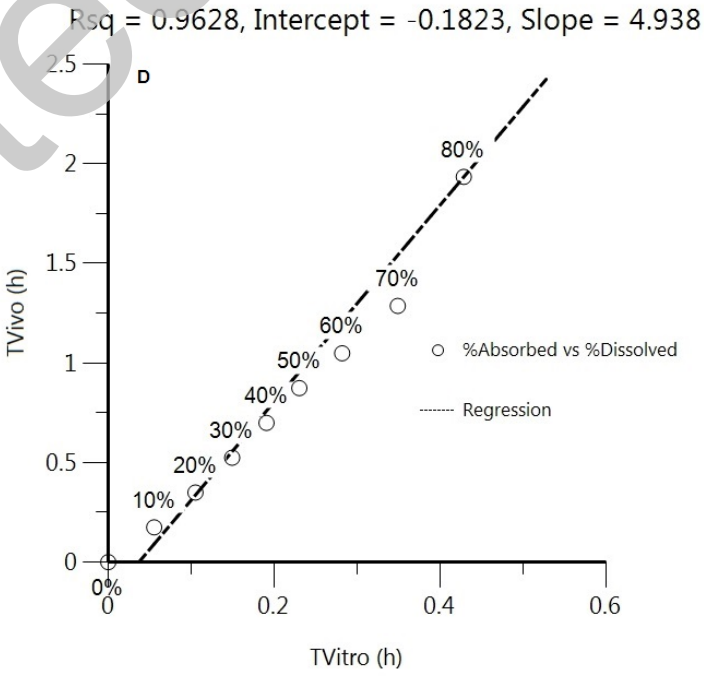


Fig-5: Levy plots for Sitagliptin constructed using the fasted state data (A and B) and fed state data (C and D). 'A and C' denotes for Januvia® and 'B and D' denotes for FDC

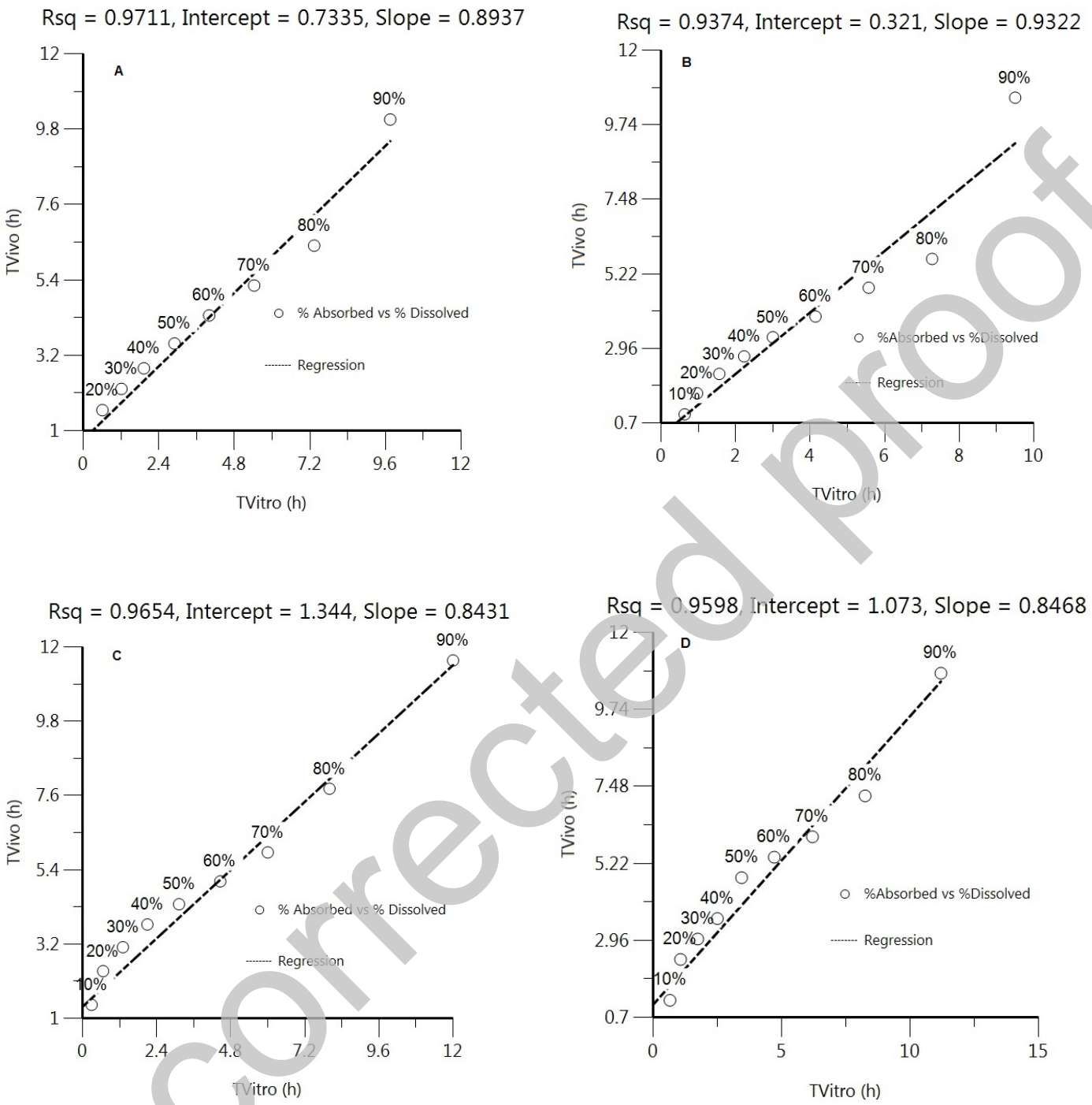


Fig-6: Levy plots for Metformin constructed using the fasted state data (A and B) and fed state data (C and D). 'A and C' denotes for Glucophage® SR 1000 mg and 'B and D' denotes for FDC