Abstract
ABSTRACT

The present work was undertaken to explore applicability of dissolution test in a) establishment of IVIVC for different MR formulations of theophylline (THP) and to explore the possibility of applicability of IVIVC across the THP formulations, irrespective of the composition and process of the formulation, and b) to study the relationship between effect isoniazid (INH) on stability and dissolution of rifampicin (RIF) in 0.1 N HCl and its effect on the bioavailability of RIF when used in the form of RIF-INH FDC formulation.

a) In vitro-in vivo correlation of modified release formulations of THP:
This part of the study describes the development of an experimental modified release capsule formulation, containing THP (200 mg) loaded microspheres (Formulation F4), its characterisation. The in vitro and in vivo performance of the developed THP MR capsule formulation was then compared with that of the three market MR formulations of THP (200 mg) - two tablets (Formulations F2 and F3) and one capsule (Formulation F1). All the four formulations were evaluated for in vitro THP release using different dissolution test conditions as per USP. Further, a highly sensitive HPLC method was developed and validated to monitor plasma levels of THP and used to perform pharmacokinetic evaluation of these formulations in six healthy volunteers. In vitro-in vivo correlations were established from the generated dissolution and bioavailability data. In vitro studies indicated that only one formulation (F1) showed pH-dependent drug release while the other three formulations, including experimental Formulation F4, showed almost condition independent dissolution behaviour. The bioavailability studies indicated that amongst the market formulations (F1, F2, F3), formulations F1 and F2 were bioequivalent but F3 failed to demonstrate acceptable dissolution and bioavailability. A good correlation ($R^2 = 0.9986$, slope = 1.0614 and intercept = 0.1824, supporting level A correlation) was observed for the experimental microsphere formulation, F4, under conditions of Test # 1. Although experimental formulation F4 showed acceptable dissolution behaviour and bioavailability, it did not, however, exhibit bioequivalence with the two other market samples, either F1 or F2. The study indicated that Formulation F4 needs further optimization so as to achieve in vivo performance comparable with formulation F1 and F2, which can be achieved by mixing THP-loaded microspheres of different drug:polymer ratio and particle size in a suitable proportions, so that the desired performance if attained. Dissolution conditions of Test # 1 (dissolution medium, pH 1.2 simulated gastric fluid without pepsin for 1 h followed by pH 6 phosphate buffer for rest
period) reflected highly significant correlation ($R^2 > 0.98$) with the corresponding bioavailability of THP for all the four THP MR formulations.

Further, it is demonstrated that compendial (USP) dissolution test conditions for ER formulations of THP and their specifications can be of immense use as the quality control test and can demonstrate their potential as surrogates to in vivo bioavailability studies provided they are supported by valid in vitro-in vivo correlation.

Using the in vitro and in vivo data generated from these four formulations it was attempted to generalise the IVIVC across these four MR formulations of THP in the form of an average IVIVC model. This model was developed using the average in vitro dissolution data and average in vivo data of three of the four formulations at a time. The predictability of this model - external and internal predictability – was determined.

The study indicated that applicability of IVIVC can be generalized across the formulations or technologies, provided proper selection of formulations, with respect to difference in release profiles, is made for establishing a generalized IVIVC. This was demonstrated by adapting an average IVIVC approach in case of formulation F1, F2 and F4 and by evaluating its internal predictability. But it did not work for the formulation F3 because of its poor dissolution pattern. The study indicated only a limited success in predicting the in vivo performance of THP MR formulations because of a small sample size (only 4 formulations). Thus, there is a need to generate a strong data base - in vitro dissolution and in vivo bioavailability data - by studying sufficiently large number of MR formulations to help develop a robust IVIVC model which will predict the in vivo performance of THP MR formulations from the in vitro dissolution data, irrespective of the type of formulation or the process of manufacturing.

b) Correlation between effect of INH on dissolution of RIF and its effect on bioavailability of RIF

Antitubercular Fixed Dose Combinations (FDCs) containing rifampicin (RIF) along with isoniazid (INH), and/or pyrazinamide (PZ) and/or ethambutol (ETB) ensure better patient compliance and help to minimize development of resistance to the drugs by *M. tuberculosis*. However, poor bioavailability of RIF in these FDC formulations is a subject of much concern for the last three decades. Poor bioavailability of RIF could lead to faulty treatment and to the drug resistance.

Since RIF hydrolyzes in acidic medium to form insoluble and poorly absorbed 3-Formyl rifamycin SV (3-FRSV), two specific and accurate methods - Dual Wavelength UV-Vis.
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spectrophotometry (DW spectrophotometry) and HPTLC - to determine 3-FRSV in presence of RIF in 0.1 N HCl - used as the dissolution medium. The proposed methods were successfully applied to determine the rate of degradation of RIF to 3-FRSV in dissolution medium (0.1 N HCl) and also in presence of INH. The rate of degradation of RIF in presence of INH was almost two times more than that of RIF alone. These methods were utilized to study the stability of RIF in market formulations of RIF and RIF with INH in dissolution medium. It was observed that degradation of RIF to 3-FRSV in acidic medium is accelerated in presence of INH. The stability study of RIF in presence and absence of INH in 0.1 N HCl, under dissolution test conditions (37±0.5°C, rotating paddle, 100 rpm), indicated that in absence of INH, RIF degrades to only about 8% in 45 min while the degradation in presence of INH increases to about 21% in 45 min (about 3 folds).

In vitro dissolution studies of RIF-INH FDC formulations vis-à-vis single component RIF formulations in 0.1 N HCl - both single-point and multi-point studies - indicated higher degradation of RIF from RIF-INH FDC formulations as compared to single component RIF formulations. However, single-point study could not discriminate between various formulations with respect to RIF release. On the other hand, multi-point dissolution studies could provide insight into formulation variability in release of RIF and hence, its degradation in 0.1 N HCl to 3-FRSV. It could provide the insight into 3-FRSV formed, based on the initial release rates of RIF from these formulations.

The role of INH in enhancing the degradation of RIF, from RIF-INH FDC formulations, in dissolution medium can be attributed to reversible formation of hydrazone of 3-FRSV with INH, with faster forward reaction or to the facilitated dissolution of RIF in presence of INH. At the same time, official dissolution test utilizes nonspecific method for analyzing the amount of RIF released in dissolution medium, which can not differentiate between RIF and its degradation product 3-FRSV, leading to overestimation of release of RIF from formulations containing RIF.

As envisaged from the in vitro dissolution studies, it was observed that bioavailability of RIF is significantly impaired when it is administered along with INH as a FDC (RIF-INH FDC), in comparison with administration of formulation containing only RIF (single component RIF formulation). Since, RIF from single component RIF formulation - the standard formulation for comparison with RIF-INH FDC - itself is bound to degrade in acidic environment of stomach, in absolute terms, the overall reduction of RIF from RIF-INH FDC formulation would be much higher than that observed by comparing with single component RIF formulation. The results of comparative bioavailability studies lend confirmation to the
observations from the in vitro studies indicating INH accelerates degradation of RIF into 3-FRSV in acidic environment of stomach. The present study underlines the fact that reduced bioavailability of RIF from RIF-INH FDC formulations may be one of the factors responsible for development of resistance to RIF.

In view of the considerable decomposition of RIF in presence of INH, it might be more meaningful to conduct bioavailability or bioequivalence studies on these FDCs by comparing the test FDC product with a standard formulation containing RIF alone.

Finally, the correlation between in vitro dissolution and bioavailability data (in vitro-in vivo correlation) underlines the fact that it is the rate of dissolution of RIF in acidic environment of stomach (0.1 N HCl as dissolution medium) that decides the course of RIF degradation and its bioavailability in presence of INH. Hence there is a need to modify the dissolution specifications from a single-point evaluation to a multi-point dissolution profile studies, which can help to assure the in vivo performance of RIF formulations from batch-to-batch.

The present study has lead to a very important conclusion that RIF and INH are incompatible in their present form of FDC oral dosage form, are incompatible. Therefore, a new FDC formulation of RIF and INH needs to be prepared so that RIF and INH are not released simultaneously into the acidic environment of stomach.