Fixed-dose combination (FDC) formulations of anti-tuberculosis (anti-TB) drugs have several distinct advantages over single drug formulations. Therefore, extensive efforts are being made to promote them in TB therapy\(^1\). However, serious concern has been raised on the utility of these products due to quality problems\(^2,3\). Over the years, two major problems have been identified, i.e., fall in bio-availability of Rifampicin when combined with other anti-TB drugs, and instability of the formulations. The stability related problems include changes in drug strength, increase in degradation product levels, alteration in dissolution profile, gain in moisture, etc.

The ‘bio-availability’ problem of FDCs was highlighted for the first time way back in 1989 by Acocella\(^4\), who observed that one out of three FDCs containing Rifampicin and Isoniazid, and all four FDCs containing Rifampicin, Isoniazid and Pyrazinamide had significant lower plasma concentrations of Rifampicin. Against it, the ‘stability’ problem is of relatively recent origin and was highlighted through publications by Keynon and Laserson et al\(^5,6\). These workers found that anti-TB FDC products with lower than required strength of Rifampicin were in wide circulation. The stability problem has been highlighted further in subsequent studies\(^7,8\) and has been found to be more acute with three or four-drug FDCs containing Rifampicin and Isoniazid, in comparison with formulations containing these two drugs\(^7,8\).

A lot of effort had been made by the World Health Organization (WHO) and other international agencies to address the bio-availability problem of FDCs\(^1,10\), from the time it was highlighted 16 years ago\(^4\). The steps taken include development of the bio-equivalence study protocol; identification of laboratories for carrying out bio-equivalence studies; addition of FDCs in the WHO Model List of Essential Drugs; training of the regulatory staff to review applications for registration of FDC tablets, and development of a system for pre-qualification of the products. The two approved laboratories, one in India and another in South Africa, have worked aggressively and carried out a number of bio-equivalence studies for the industry, clearing several FDC products, which are now being purchased by WHO and other international and national agencies.
However, an important issue is - whether the efforts made by WHO and other agencies over the years have really been successful to solve the bio-availability problem with FDCs? Is it that the bio-availability of Rifampicin from FDC products, procured and supplied by international agencies, is optimal and the problem has been eliminated? That may not be absolutely true, considering that WHO and International Union against Tuberculosis and Lung Diseases (IUATLD), while building up their basic strategy, may have been construed on two very important points.

The first relates to the absence of clear understanding on the fundamental reason for the bio-availability problem associated with FDCs. WHO/IUATLD framed their original strategy considering that the problem of reduced bio-availability of Rifampicin from FDCs was use of different crystal forms of Rifampicin by the manufacturers and non-conformance to GMP by them1,10. However, researchers in the field queried whether these may not be the complete or true reasons11,12, considering that the bio-availability problem was specific to FDCs, whereas both the reasons suggested by WHO and the IUATLD could also effect Rifampicin-alone formulations. It would appear that through investigations carried out in WHO’s own approved laboratories, both the proposed reasons are not the cause of the problem. That Rifampicin crystal structure has no clinical relevance was the conclusion of a recent study published by an approved centre in India13. The GMP reason stands negated from the fact that bio-equivalence was established in almost all pre-qualification studies involving freshly manufactured products carried out in the WHO approved centres14-22, despite the fact that FDCs included in the studies were produced by different manufacturers under a variety of GMP conditions.

The second is connected with the protocol laid down by WHO/IUATLD for bio-equivalence studies23, following which the manufacturers all over the world are getting their FDC products tested in approved laboratories as a pre-qualification requirement. The protocol suggests comparison of test FDC products against a reference comprising loose combination of individual drug formulations. This is opposite to the study by Acocella4, which involved Rifampicin-alone oral formulations as the reference. Therefore, the contentious issue is whether the WHO/IUATLD protocol in the present form assesses the real problem with anti-TB FDCs or yields routine information, as with any other pharmaceutical product.

Our assertion here has been that only bio-equivalence testing of FDCs against Rifampicin-alone oral dosage forms is expected to yield precise information on reduction in bio-availability of Rifampicin, which is specifically due to combining of the drug with other anti-TB agents11,12. The same was not possible with the WHO/IUATLD protocol, because inter-drug effects were expected to remain the same, whether the study is done on a fixed or a loose combination of individuals drugs. Also, in this case, the test and reference formulations would be anticipated to show the same bio-availability behaviour, unless variation is there in the formulation parameters (such as, high or low initial drug content, drug degradation on storage, failure in dissolution, etc.). These issues were raised by ourselves in 200111,12, but unfortunately no heed was paid and the status-quo remains the same today. However, the contention has proven true over the period, as evident from the results of several studies involving the two types of protocols that have appeared lately in the literature. At least three independent studies have been reported since 200124-26, where FDCs were tested against Rifampicin-alone formulations according to the Acocella’s approach. In all of them, an almost 30% fall in bio-availability of Rifampicin was observed. In comparison, bio-equivalence was established, as expected, in almost all the studies conducted on fresh pre-qualification products by the approved laboratories employing WHO/IUATLD protocol14-22. An exception is the study by Pillai et al27, in which authors used the latter protocol to test the products procured under global tuberculosis programmes. Bio-availability problems were observed in 7 out of 10 formulations, but these problems were attributed, not to inter-drug effects, rather to low content of Rifampicin in the formulations, as the products that failed in bio-availability studies also failed in colorimetric analysis of Rifampicin28. That clearly means the WHO/
IUATLD protocol does not detect a fall in the bio-availability of Rifampicin due to inter-drug reasons. Therefore, as the real objective is not being achieved, a logical question that arises is whether any purpose is being served by the effort of WHO and IUATLD in establishing the bio-equivalence protocol, identification of laboratories, and bio-availability studies carried out by them?

Intense research has been conducted by independent groups in India in recent years, and specific reasons that explain both the bio-availability and stability problems of FDCs have been proposed. In particular, the mystery of the drop of bio-availability of Rifampicin from FDC products has been solved by showing that the problem arises due to the inter-drug facile reaction of Rifampicin and Isoniazid under empty stomach conditions29-31, whereby significant loss of drug occurs before absorption. The mechanism has also been worked out, demonstrating that Rifampicin is first hydrolysed under acid conditions to 3-formylrifamycin, which reacts further with Isoniazid to form isonicotinyl hydrazone (HYD). The HYD converts back to Isoniazid and 3-formylrifamycin, resulting in recovery of Isoniazid, but eventually causing the loss of Rifampicin. This explains why the bio-availability problem is confined to Rifampicin alone and not Isoniazid. A further confirmation to the role of Rifampicin-Isoniazid interaction was provided when the extent of loss of the drug under in vitro fasting pH condition (pH 2)31 equalled the in vivo drop of bio-availability of Rifampicin, as reported in studies carried out using Acocella’s protocol24-26.

The chemical instability of FDCs is found to occur due to two reasons. One is direct interaction of Rifampicin with Isoniazid, the mechanism of which involves interaction of imine group of Rifampicin with amino of Isoniazid to yield HYD in the solid formulation environment32,35. The other reason is the creation of an acidic hydrolytic environment upon moisture gain by Ethambutol hydrochloride35,36. Here the problem is compounded as Pyrazinamide and Ethambutol hydrochloride, the two co-drugs present usually in FDCs, accelerate the reaction between Rifampicin and Isoniazid33,34. These explain the stronger physical and chemical changes in three- or four-drug FDCs, compared to that seen with those containing just the two drugs8,34-36.

The identification of precise reasons helped suggest solutions to both the problems with FDCs. For the bio-availability problem, the suggestion was to segregate the release of Rifampicin and Isoniazid in different parts of GIT tract, to prevent the two drugs to coming into contact with each other in the stomach31. To decide on what drug should be released in which part of GIT, a separate investigation was carried out, in which the sites of absorption of all the four drugs contained in anti-TB FDCs were determined37. The results indicated that Rifampicin, Pyrazinamide and Ethambutol could be released in stomach, while the optimal site for Isoniazid was the intestine. The modified product was expected to also solve even the chemical instability of FDCs by preventing and physical inter-action between Rifampicin with Isoniazid. Based on the concept, a modified tablet-in-tablet formulation has been developed38. A recent clinic study has confirmed that the product provides early symptomatic relief and radiological improvement39. The stability behaviour of this product is not yet public.

Other studies indicate that the assessment of Rifampicin loss after storage of FDCs at pH 2 (fasting state pH) for 1 hour at 37 degree C can act as a simple surrogate test to highlight, if a particular FDC formulation would have an associated bio-availability problem31. The other suggestion has been to include limits for HYD in the pharmacopoeial monographs on anti-TB FDCs containing Rifampicin along with Isoniazid40. This was recommended considering that if the limits for HYD are in place, the manufacturers of FDC products would require to modify their formulations to prevent inter-action between Rifampicin and Isoniazid and the formation of HYD. A very recent positive development is the inclusion of a limit for HYD in the draft monograph on four-drug anti-TB FDCs of the International Pharmacopoeia41. Of course, these discussed changes are not anticipated to tackle other stability problems such as alteration in dissolution profile, gain of moisture and increase in drug related impurities with time. These can be
handled easily at their level by the manufacturers, employing intensive pre-formulation studies and barrier packaging strategies.

Thus, new research has shown that the way forward to solve the twin problem of bioavailability and chemical instability of anti-TB FDCs is in modifying such formulations, and adding new tests and controls into the technical specification process. The products then can be administered in the usual way. Further more, it suggests that the problems of FDCs need to be tackled in an entirely different manner, than the approaches being recommended today by the WHO/IUATLD. Keeping in view that tuberculosis is a global emergency, both the drugs manufacturers and the world bodies may need to re-think their strategies and use the findings of new research to ensure that TB patients get the full dose of key anti-TB drugs from FDC products that they are prescribed.

REFERENCES

23. IUATLD/WHO. Quality assurance: protocol for assessing


