REVIEW ARTICLE

Accepted 25 November 2000
Revised 15 November 2000
Received 18 October 2000
Indian J. Pharm. Sci. 2001, 63(1) 1-9

Fixed Dose Combinations in the Treatment of Tuberculosis

R. PANCHAGNULA*, SHRUTI AGRAWAL AND C. L. KAUL
Department of Pharmaceutics
National Institute of Pharmaceutical Education and Research
Sector 67, S.A.S. Nagar-160 062, Punjab

Tuberculosis is a complex socio-economic problem that impedes human development. Tuberculosis exerts a toll of eight million new sufferers and two million deaths every year. Around one third of world population harbors *Mycobacterium tuberculosis* that is sensitive to rifampicin, isoniazid, pyrazinamide and ethambutol. As tuberculosis is contagious, it infects many others thereby necessitating early diagnosis and treatment. A standard 6 months treatment regimen formulated by World Health Organization (WHO), in its operational point of view is a complex procedure leading to treatment failure and resurgence of drug resistant strains. To increase the patient compliance, in its recently revised Tuberculosis Treatment Guidelines for National Tuberculosis Programs, WHO encourages the use of Fixed Dose Combination (FDC) tablets, which assures ingestion of all the components of tuberculosis treatment regimen. However, the major quality issue associated with FDCs is assuring the bioavailability of rifampicin. If not carefully manufactured, bioavailability of rifampicin is negatively affected which could directly lead to poor treatment outcome and may lead to drug resistance. In the light of above scenario, this review mainly focuses the potential advantages of FDC formulations with special emphasis on quality of FDC preparation to be used for tuberculosis control.

TUBERCULOSIS (TB) EPIDEMIC

Since time immemorial, TB has been a scourge to mankind. The earliest evidence of TB in man and animals is provided by fossil bones dating back to 8000 BC[1]. Even a century after Koch's discovery of tubercle bacillus and decades after the discovery of powerful anti-TB drugs to date, TB remains a leading cause of death due to infectious diseases in the developing countries. Taking into the consideration the severity and spread of the disease, in 1993 World Health Organization (WHO) declared TB as a 'global emergency' as more than 1900 million people are infected with this organism[2]. Each year 9-10 million people come in the grip of this disease worldwide and WHO projects that by 2020, another 200 million individuals to become sick, and 70 million to die from TB, most of them from the developing countries. On the other hand, about 50 million TB cases of multi-drug resistance will cost up to 100 times the available treatment cost of TB[3]. Furthermore, the impending HIV pandemic has increased morbidity and mortality due to TB[4-6]. By 2020, TB and HIV infection together are expected to account for 90% of adult deaths from infectious diseases[6]. HIV infection as a risk factor will account for 25% of the TB burden, as the lifetime risk of developing TB increases from 5-10% in HIV-positive individuals.

The problem in India is even greater as it is estimated that India accounts for one fourth of global TB burden India has an estimated 14 million TB cases to which about 2 million are added every year. Each year TB kills 5 lakh people in India – more than 1000 every day, nearly 1 every minute![7]

TREATMENT APPROACHES

Short Course Chemotherapy:

M. tuberculosis is a formidable organism having a

* For Correspondence
e-mail: panchagnula@yahoo.com
NIPER communication No. 79

TABLE 1: RECOMMENDED DOSES (mg/kg) FOR ESSENTIAL ANTI-TB DRUGS

Anti-TB drug (abbreviation)	Mode of action	Daily	Recommended dose (mg/kg) * Intermittent	
			3x per week	2x per week
Isoniazid (H)	Bactericidal	5 (4-6)	10 (8-12)	15 (13-17)
Rifampicin (R)	Bactericidal	10 (8-12)	10 (8-12)	10 (8-12)
Pyrazinamide (P)	Bactericidal	25 (20-30)	35 (30-40)	50 (40-60)
Ethambutol (E)	Bacteriostatic	15 (15-20)	30 (25-35)	45 (40-50)
Streptomycin (S)	Bactericidal	15 (12-18)	15 (12-18)	15 (12-18)
Thioacetazone (T)	Bacteriostatic	2.5	Not Applicable	

*(Dose ranges given in parentheses)

sturdy wall, which is impervious to most antibiotics. Because of the slow division of organism, no course of treatment of less than 6 months duration is effective in smear positive patients. Unfortunately, no drug that significantly improves treatment outcome or shortens the duration of therapy is likely to be available in the near future. Therefore, the only available option is to make use of existing drugs effectively and rationally. As treatment of TB always requires multi-drug therapy, to reduce the emergence of drug resistant strains, WHO has formulated a standardized Short Course Chemotherapy regimens consisting of essential first-line anti-TB drugs as shown in table 1. Treatment regimens have an initial (intensive) phase lasting for 2 months and a continuation phase for next four months. In the intensive phase, there is a rapid killing of tubercle bacilli; infectious patients become non-infectious within 2 weeks and symptoms improve. For sterilizing effects, in the continuation phase fewer drugs are necessary for a longer period of time[8].

Although, the term 'short course' is coined with standard anti-TB regimens, from patient's point of view, it is very difficult to adhere to the treatment for such a long period. To worsen the fact, patient has to consume large number of tablets and capsules every day, which is a common cause of patient noncompliance. As the dose given in the form of separate formulations is fixed, there exists an every possibility of overdosing or underdosing, as there is no flexibility in the calculation of dosage according to patient's requirement. Thus, these short course chemotherapeutic regimens lasting for 6 months are difficult to implement thereby resulting in patient noncompliance and emergence of drug resistance[9].

Directly Observed Treatment Shortcourse (DOTS); An Effective Tool for Management of TB:

The lack of organization of services to ensure widespread detection and cure of patients resulted in the unbelievable TB toll and surge in drug resistance. However, there is a proven, cost effective management package for the treatment of TB known as DOTS that ensures the effective delivery of health services to TB patients[10-11]. DOTS has five key components:

- Government commitment to sustained TB control activities.
- Case detection by sputum smear microscopy among symptomatic patients self-reporting to health services.
- Standardized treatment regimen of six to eight months for at least all confirmed sputum smear positive cases, with directly observed treatment for at least the initial two months.
- A regular, uninterrupted supply of all essential anti-TB drugs.
- A standardized recording and reporting system that allows assessment of treatment results for each patient and of the TB control program overall.

Although, yet DOTS is not widely implemented (20% case detection in 1998), with effective community participation WHO has targeted at least 70% detection of infectious cases and 85% cure rates by year 2005[12].

The rise in multi-drug resistant (MDR) TB has threatened the success of TB control, WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) have identified several geographical areas where MDR-TB's prevalence is greater than 3%. To effectively manage the problem of MDR-TB, WHO has formed a pilot project 'DOTS-plus', in the regions of high rates of MDR-TB also known as 'hot zones'. DOTS-plus concept includes the five tenets of DOTS strategy and additionally, takes into account specific issues that need to be addressed to control MDR-TB. The goal of DOTS-plus is to prevent further development and spread of MDR-TB[13-14].

Fixed dose combination (FDC) tablets in the management of TB:

The use of standardized regimen is the fundamental strategy of WHO and IUATLD. Deviations from such regimen result in increased cost and risks of side effects, or decreased chance of cure, or both. One of the best ways of ensuring compliance with such regimens is to physically combine the requisite drugs into one preparation – a fixed dose combination product. FDC formulation thus is a combination of two or more first line anti-TB drugs namely rifampicin, isoniazid, pyrazinamide and ethambutol in a fixed proportion in a single dosage form and this stemmed from the fact that TB always requires multi-drug therapy[15]. The potential advantages associated with the use of FDCs are:

- Reduced risk of emergence of dug resistant strains
- Less risk of medication errors
- Better patient compliance
- Reduced cost of treatment
- Dosages adjustment according to patient need
- Simplify drug supply management, shipping and distribution

Based on this rationale, WHO and IUATLD recommend the use of FDC tablets as a routine practice in the treatment of TB[16]. It would be pertinent to note here that the FDC is a part of WHO model list of essential drugs[17] as shown in Table 2.

RATIONALE FOR FDCS

FDC to prevent drug resistance:

The management and control of TB is complicated

TABLE 2: WHO MODEL LIST OF ESSENTIAL DRUGS (FROM DECEMBER 1997)

Drug	Forms	Strengths
Streptomycin	Powder for injection	S 1g (as sulphate) in vial
Rifampicin	Capsule or tablet	R 150 mg R 300 mg
Isoniazid	Tablet	H 100 mg H 300 mg
Pyrazinamide	Tablet	Z 400 mg
Ethambutol	Tablet	E 100 mg E 400 mg
Thioacetazone + isoniazid	Tablet	T 50 mg + H 100 mg T 150 mg + H 300 mg
Isoniazid + ethambutol	Tablet	H 150 mg + E 400 mg
Rifampicin + isoniazid	Tablet	R 150 mg + H 75 mg R 300 mg + H 150 mg R 150 mg + H 150 mg *
Rifampicin + isoniazid + pyrazinamide		R 150 mg + H 75 mg + Z 400 mg R 150 mg + H 150 mg + Z 500 mg *

* For intermittent use three times weekly

by the emergence of drug resistant strains. The results of a sentinel survey conducted in South Africa shows that the rate of MDR-TB among smear positive patients increased six folds from 1994 to 1996[18]. In some parts of the globe, the prevalence of acquired drug resistance (ADR) increased dramatically[19-21] and in some cases went as high as 51.5%[22]. ADR is mainly associated with monotherapy[23] suggesting a need for appropriate treatment regimen and increased supervision.

Although, multi-drug therapy is prescribed for TB, in clinical reality patients tend to take only one drug (monotherapy). The possible reasons for monotherapy include: a temporary lack of supply of one or more medications; mistakes in dispensing; patient's decision to purchase only one medication to save money and patient's deliberate decision to take only one drug because of perceived or real symptoms associated with other drugs[24]. Thus, poor compliance and multiple interruptions in the treatment are primary reasons of drug resistance.

To control the tide of emerging resistance, the usual measures recommended are: 1) increased supervision of TB treatment in the form of DOTS that eliminate or reduce the interruptions in the treatment and 2) ingestion of adequate number of drugs in the initial treatment of TB[15].

As FDC combines the most effective drugs in one formulation, use of these FDC formulations minimizes adaptation of monotherapy by patients. The spread of resistance can be controlled by FDCs, which afford protection against the potential selection of resistant strains. It is important that FDC tablets must be given under supervision of DOTS as FDC tablets if given unsupervised, interruptions in treatment may lead to drug resistance[25].

In the United Kingdom, which has low rates of drug resistance, 73% to 79% of rifampicin is sold as FDCs. While in the United States of America, which has a high rate of drug resistance, only 15 to 18% of rifampicin is utilized in the form of FDC. This data can be used as a basis to claim that the low use of FDCs in the United States of America is a reason for high rates of drug resistance[26]. This imperfect evidence and more importantly, the fact that treatment with combined preparations precludes monotherapy and its potential to create drug resistance, form a persuasive argument that FDCs should be used for treating TB whenever possible.

Simplified treatment:

Use of FDCs, as a routine therapeutic regimen, simplifies TB treatment thereby increasing patient's adherence to the therapy. This can be appreciated from Table 3, where patient is required to take only three FDC tablets in contrast to 9 to 16 tablets of separate formulations[15].

With the use of separate formulations, the apparent prescription errors can be anticipated, as number of formulations with varying dosage strengths is available in the market. In a survey conducted in the slums of Bombay, Uplekar found that 100 private doctors prescribed 80 different regimens; most of them were inappropriate and expensive[27]. Other reports available also convey the same message of inadequate knowledge and wrong practices of health providers in the treatment of TB[28-30]. Using FDCs, prescribing anti-TB medication is much simpler, thus minimizing prescription errors and increasing

TABLE 3: NUMBER OF TABLETS TO BE TAKEN DAILY IN THE INTENSIVE PHASE OF TB TREATMENT*

Single-drug tablets	Number of tablets	FDC tablets	Number of tablets
Rifampicin (R)150mg	3	RHZE	3
Isoniazid (H) 300 mg (100 mg)	1 (3)	(150mg + 75 mg	
Pyrazinamide (Z) 400 mg	3	+ 400mg	
Ethambutol (E) 400 mg		+ 275mg)	
(100 mg)	2(7)		
Total	9 (16)		3

* For a 50 kg patient. Figures in parentheses refer to alternative dose formulations and related number of tablets

patient compliance. Use of FDC formulations is particularly useful in the private sector where national guidelines are not readily available thereby preventing treatment with inadequate regimens[15].

The proof of increased patient compliance with FDCs is given by a study conducted in Hong Kong where, only 1% of patients had complaints about size, quantity or difficulties in swallowing in contrast to 5% of patients complaining about the compliance with separate formulations[31]. In general, FDCs prevent indiscriminate selection of drugs and limit the mistakes associated with choice and calculation of dosages.

Simplifying drug supply management:

With separate formulations, out of stock situations occur for three main reasons: no buffer stock, delays in receipt of orders, and expiry date reached without replacement stock being available. While in FDC tablets, there are fewer drug formulations to consider; thus making it easier to calculate the drug needs. Because of fewer drug formulations to order, ship and distribute; the result is less strain on staff in the national TB programs. In addition, FDC tablets minimize the risk of theft and misuse of rifampicin for conditions other than TB[15].

Dosage adjustment according to individual patient need:

In FDCs, it is possible to satisfy the dosage requirements in terms of mg/kg body weight for each drug. One such product, Rifater, has been developed from the basic principle starting with mg/kg doses for the patients in Asia. The fixed ratio of the different anti-TB drugs in this formulation is rifampicin 12 mg/kg, isoniazid 8 mg/kg, and pyrazinamide 25 mg/kg of body weight. To simplify the dosage adjustment, the tablet is designed to contain rifampicin 120 mg, isoniazid 80 mg, and pyrazinamide 250 mg, which ensures the simple dosage of one tablet for 10 kg of body weight.

By adopting this approach, the dosage of three essential drugs in the fixed combination can be given more accurately than has previously been the case with separate formulations. In case of separate formulations, the patients are given the same dose over a wide range of body weight. Thus, the patients with lower body weight receive more drugs than is required while; the higher body weight patients tend to be underdosed. Since most of the patients in Asia are of low body weight, there is a potential for serious large-scale overdoses with consequent adverse effects, and wastage of valuable drugs that could be saved for the treatment of other patients. FDCs, with its accuracy of dosages for all three or four active agents, overcome these problems[15].

Better management of DOTS using FDCs:

FDCs can simplify the procedures involved in the DOTS strategy to a great extent and thereby aid in its effective implementation. This can be appreciated from the fact that after diagnosis of TB, FDCs ensure that the drugs required are available in the right proportion in a single dosage form. In addition, FDCs at the same time reduce the burden on the health care professionals in terms of monitoring and analysis of DOTS strategy.

ISSUES ASSOCIATED WITH FDC

Although, the transition from single drug formulations to FDC tablets has been in process for many years, globally, only an estimated 23.8% of total number of notified cases are treated with two and /or three drug FDCs in public sector[32]. The problems associated with FDC formulations regarding quality, its registration and barriers in effective implementation into national program have limited the widespread use of FDCs.

Despite the potential advantages offered by FDC products over separate formulations, the successful treatment of TB is determined by quality of FDC formulation used. It has been shown that the bioavailability of rifampicin component when given as FDC tablets is adversely affected if good manufacturing procedures are not followed. The poor relative bioavailability of rifampicin from some of the FDC formulations is reported by Pillai *et al.*[33]. In addition, results of a series of studies have shown that while some FDC formulations had acceptable rifampicin bioavailability, others did not[9,34-43]. Moreover, a satisfactory *in vitro* dissolution test need not necessarily guarantee acceptable rifampicin bioavailability[40,44,45]. The bioavailability problems with isoniazid, pyrazinamide, and ethambutol components of FDC have not been encountered[41,46], presumably because of their much greater water solubilities and more rapid rates of absorption[15]. The major problem with FDCs is that when rifampicin is physically combined with other drugs, the crystalline structure of rifampicin can change, and the bioavailability of the drug can decrease dramatically[47]. Even very small changes in the manufacturing process, or a change in excipient can cause an unexpected alteration in bioavailability of rifampicin[48,49]. Thus, the bioavailability

of rifampicin is at risk if strict manufacturing procedures are not followed, or poor quality raw materials are used. Ultimately, using FDC tablets of poor rifampicin bioavailability could directly lead to treatment failure and may create instead of preventing drug resistance[25]. Further, the clinical and bacteriological investigations revealed that anti-TB activity of rifampicin is dose dependent[50]. Recently, its therapeutic ratio was shown to be only approximately four as compared with sixteen for isoniazid[51]. Its potency, therefore, will be severely affected by using formulations with impaired rifampicin absorption. Therefore, good quality FDC tablets with demonstrated bioavailability of rifampicin are an absolute requirement for successful treatment outcomes in programs utilizing FDC based regimens.

FDC formulations will be efficacious only if all the components of FDC are available at the tissue site in therapeutic effective concentration[52-54]. Against this background, WHO and IUATLD issued a joint statement in 1994 advising that only FDC tablets of good quality and proven bioavailability of rifampicin should be used in the treatment of TB[15]. Before 1996, when WHO published guidelines on registration requirements to establish the interchangeability of generic pharmaceutical formulations[55], the accurate assessment of rifampicin bioavailability was questionable, as there were no clear guidelines existing[56]. As involvement of human volunteers in bioavailability is cost prohibitive, WHO and IUATLD have developed a simplified and effective protocol, which is more convenient and cheaper. This protocol utilizes six blood sampling time points over eight-hour period compared with the conventional requirement of about thirteen samples over a period of twenty-four hour without affecting the precision of estimation[57,58]. In this context, it is pertinent to mention the studies conducted in India and South Africa, WHO has nominated National Institute of Pharmaceutical Education and Research (NIPER), India and Medical Research Centre/ University of Cape Town (MRC/UCT), South Africa as two reference centers for assessing the bioavailability of rifampicin containing FDC tablets[34,59-60]. At NIPER, the WHO/IUATLD recommended simplified protocol is further validated for all the types of FDCs (2, 3 or 4 drugs) available in the market[61].

The important hurdle in the development of FDCs is attributed to the fact that in most of the developing countries the regulatory authorities are prone to register, rather 'blindly', only those formulations, which have been already registered by European or American counterparts. Paradoxically, in Europe the time constraints and costs involved in further clinical trials to prove the efficiency of the FDCs in comparison to the separate formulations have restrained the manufacturers to produce FDCs. In addition, the concerns of manufacturers over the regulatory process arise from regulatory requirement paradox that the registration requirements vary across the countries. Some do not require bioequivalence testing for the registration of FDCs. In addition, number of human volunteers required is not uniform in national regulatory guidelines. A high registration fee in some of the countries is also a barrier for entry of FDC into market. Thus, the time constraints, costs of clinical trials and regulatory problems are the strong disincentives for the manufacturers to produce FDC tablets[15].

Further, the general concern about the quality of rifampicin in some FDC formulations has prevented their use on a wide scale. As they are not being widely purchased, there is a limited production, resulting in prices that many countries cannot afford. However, as the demand for FDCs increases, large-scale production and increased competition are expected to result in reduced prices[32].

On the contrary, the available FDC formulations in the market are characterized by large variety of different dosage ratios of the drugs. This plethora of different formulations and different dosages has created a considerable confusion impending the application of standardized therapeutic regimens. Hence, there is a need for uniformity of dosage on the part of manufactures to avoid confusion to prescribers.

In short, several factors that hinder the introduction of FDCs and the expansion of their use include higher prices, particularly for three and four drugs FDCs, lack of proof of bioavailability of rifampicin in some formulations, protectionist measures on the part of certain National Drug Regulatory Authorities, who favor locally produced single tablets versus imported FDCs. In addition, availability of inappropriate FDC formulations in the local or international market as compared to the international standardized regimens for TB is also another factor for slow progress in FDCs.

Role of reference laboratories in the quality assurance of FDCs:

Taking into consideration time and cost constraints

involved in the bioequivalence trials, WHO and IUATLD have developed a simplified screening protocol for evaluation of rifampicin bioequivalence[57]. WHO and IUATLD have also identified two reference laboratories, especially in high prevalence, low-income countries, to have assessed to FDC preparations which would be of good quality[59]. As mentioned earlier, in India, NIPER is the reference laboratory for the evaluation of FDCs and at present is actively involved in assessment of quality of FDCs to be registered for marketing. The objective of these quality control reference laboratories is to have a system in place that is efficient, yet simple to access and inexpensive to maintain. Optimal utilization and recognition of these laboratories would be essential to achieve the objective of providing good quality FDCs to National TB Program. Fourie and Spinaci have described the structure, management responsibilities of these reference laboratories for judging the standards of FDC preparations in detail[62]. The scope of activity of the surveillance laboratory in conjunction with the procurement office is as follows:

- To establish by testing, whether a given sample of drug, either locally manufactured or imported, confirms to required specifications and whether packaging is adequate.
- To examine anti-TB FDC preparations suspected to be of questionable efficacy or safety, and to demonstrate and document any evidence of deterioration, contamination, or adulteration.
- To check the stability of products under local conditions of storage

Blomberg *et al.* suggest that for prequalification of FDCs, a criteria similar to vaccine model should be described[63]. Assigning reference laboratories for quality evaluation of FDCs fulfills this requirement.

Future of FDCs:

The potential advantages inherent with FDC formulations are well known. It is expected that, a large global market of FDCs will encourage large-scale production and increased competition will result in affordable FDC prices. In addition, very soon, the conflict and hurdles associated with the FDC registration will be solved encouraging the widespread use of FDC in the control and management of TB. With the use of FDC as a routine therapeutic regimen in its revised national program known as DOTS, WHO can achieve its target of 85 % cure rate by 2005.

CONCLUSIONS

Worldwide, TB remains the most important communicable disease. Its control requires multi-drug therapy for at least six months that lead to patient non-compliance, failure of therapy and ultimately emergence of drug resistance. Anti-TB therapy using FDC tablets reduces the number of tablets to be consumed thereby increasing patient compliance and adherence to recommended treatment regimen. Thus, FDCs can be a primary way to prevent the emergence of drug resistance. However, not all the FDCs ensures the proper treatment as in some combinations the rifampicin bioavailability may be adversely affected due to lack of good manufacturing procedures. Hence, only FDCs with proven rifampicin bioavailability should be used in the treatment and control of TB.

ACKNOWLEDGEMENTS

The authors are thankful to WHO and Gordon Ellard for their constant support and encouragement to NIPER's FDC evaluation project. Our special thanks to Kanwal Jit Kaur and Inderjit Singh for their kind helps in conducting bioequivalence trials. Financial support from WHO, Lupin Laboratories Ltd.; Mumbai, Macleods Pharmaceuticals Ltd.; Mumbai and Maneesh Laboratories Pvt. Ltd. Mumbai is thankfully acknowledged. In addition, we are thankful to Sunil, Pradeep, Omathanu, Anand, Anurag, Shantaram, Pavan and Shradha for timely help in different parts of the project.

List of abbreviations used in the text:

ADR	:	Acquired Drug Resistance
DOTS	:	Directly Observed Treatment Short course
E	:	Ethambutol
FDC	:	Fixed Dose Combination
H	:	Isoniazid
IUATLD	:	International Union Against Tuberculosis and Lung Disease
MDR	:	Multi Drug Resistance
P	:	Pyrazinamide
R	:	Rifampicin
S	:	Streptomycin

TB : Tuberculosis

T : Thioacetazone

WHO : World Health organization

REFERENCES

1. Herzog, H., **Respiration**, 1998, 65, 5
2. Sinder, D.E.Jr. and Kochi, A., **J. Amer. Med. Assoc.**, 1995, 273, 220.
3. World Health Organization, **WHO/TB/97.224**, 1997
4. Punnotok, J.M. Shaffer, N., Naiwatanakul, T., Pumprueg, V., Subhannachart, P., Ittiravivongs, A., Chauchottthaworn, C., Ponglertnapagorn, P., Chantharojwong, N. andYoung, N.L., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 537.
5. Ferrazali, L., Palaci, M., Marques, L.R.M., Jamall, F., Afiune, J.B., Chimera, E., Martins, M.C., de Silva Telles, M.A., Olivera, C.A.F., Palhares, M.C., Spada, D.T.A. and Riley, L.W., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 18.
6. Baris, E., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 595.
7. Govt. of India, Ministry of Health and Family Welfare, **Times of India**, March 24, 1999.
8. World Health Organization, **WHO/TB/97.220**, 1997.
9. Acocella, G., Conti, R., Cuisetti, M. and Grassi, C., **Amer Rev. Resp. Dis.**, 1985, 132, 510.
10. World Health Organization, **WHO/CDS/CPC/TB/99.270**, 1999.
11. World Health Organization, **WHO/TB/95.183**, 1995.
12. World Health Organization, **WHO/CDS/STB/2000.6**, 2000.
13. World Health Organization, **WHO/CDS/TB/2000.279**, 2000.
14. Iseman, M.D., **Int. J.Tuberc. Lung Dis.**, 1998, 2, 867
15. World Health Organization, **WHO/CDS/CPC/TB/99.267**, 1999.
16. IUATLD/WHO, **Tubercle Lung Dis.**, 1994, 75, 180
17. World Health Organization, WHO Drug Information, 1998, 12, 22.
18. Davis, G.R., Pillai, M., Sturm, A.W. and Wilkinson, D., **Int. J.Tuberc. Lung Dis.**, 1999, 3, 799.
19. Laserson, K.F., Osorio, L., Sheppard, J.D., Hernandez, H., Benitez, A.M., Brim, S., Woodley, C.L., Hazbon, M.H, Villegas, M.V., Castano, M.C., Henriquez, N., Rodriquez, E., Metchock, B. and Binkin, N.J., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 673.
20. Antunes, M.L., Alexio-Dias, J., Antunes, A.F., Pereira, M.F., Raymundo, E. and Rodrigues, M.F., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 223.
21. Becerra, M.C., Bayona, J., Freeman, J., Farmel, P.E. and Kim. J.Y., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 387.
22. Kuaban, C., Bercion, R., Noeske, J., Cunin, P., Nkamsse, P. and Ngo Niobe S., **Int. J. Tuberc. Lung Dis.**, 2000, 4, 356.
23. Kuaban, C., Bercion, R., Jifon, G., Cunin, P. and Ngu Blackett, K., **Int. J.Tuberc. Lung Dis.**, 2000, 4, 427
24. Moulding, T., Dutt, A.K. and Reichman, L.B., **Ann. Intern. Med.**, 1995, 122, 951.
25. Mitchison, D.A., **Int. J.Tuberc. Lung Dis.**, 1998, 2, 10.
26. Davis, P.D., **Amer. Rev. Respr. Dis.**, 1993, 147, 497.
27. Uplekar, M.W. and Shepard, D.S., **Tubercle**, 1991, 72, 284.
28. Sumarojo, E., Hale, B. and Geiter, L., **Amer. Rev. Respr. Dis.**, 1993, 147, A702.
29. Uplekar, M.W. and Rangan, S., **Tubercle Lung Dis.**, 1993, 74, 332.
30. Uplekar, M., Juvekar, S., Morankar, S., Rangan, S. and Nunn, P., **Int. J.Tuberc. Lung Dis.**, 1998, 2, 324.
31. HongKong Chest service/British medical Research Council, **Amer. Rev. Respr. Dis.**, 1989, 140, 1618.
32. Norval, P.-Y., Blomberg B., Kitler, M.E., Dye, C. and Spinaci, S., **Int. J.Tuberc. Lung Dis.**, 1999, 3, S292.
33. Pillai, G., Forrie, P.B., Padayatchi, N., Smith, P.J., McIlleron, H., Smith, P.J. and Gabriels, G., **Int. J.Tuberc. Lung Dis.**, 1999, 3, S309.
34. Panchagnula, R., Kaur, K.J., Singh, I. and Kaul, C.L., **Int. J.Tuberc. Lung Dis.**, 1999, 3, S336.
35. Panchagnula, R., Kaur., K.J., Singh, I. and Kaul, C.L., **Meth. Find. Exp. Clin. Pharmacol.**, 1999, 21, 625.
36. Schall, R., Muller, F.O., Duursema, L., Hundt, H.K., Middle, M.V., Mogilnicka, E.M. and Swart, K.J., **Arzneimittelforschung**, 1995, 45, 1236.
37. Zwolska, Z., Niemirowska-Mikulska, H., Augustynowicz-Kopec, E., walkeiwicz, R., Stambrowska, H., Safianowska, A. and Grubek-Jaworska., **Int. J.Tuberc. Lung Dis.**, 1998, 2, 824.
38. Acocella, G., Nonis, A., Gialdroni-Grasse, G. and Grassi, C., **Amer. Rev. Respr. Dis.**, 1988, 138, 882.
39. Acocella, G., Nonis, A., Perna, G., Gialdroni-Grasse, G. and Grassi, C. **Amer. Rev. Respr. Dis.**, 1988, 138, 886.
40. Acocella G., Bull. Int. Tuberc. Lung. Dis., 1989, 41, 83.
41. Ellard, G.A., Ellard, D.R., Allen, B.W., Girling, D.J., Nunn, A.J., Teo, S.K., Ng, H.K. and Chan, S.L., **Amer. Rev. Respr. Dis.**, 1986, 133, 1076.
42. Fox, W., **Int. J.Tuberc.**, 1990, 37, 175.
43. Fox, W., **Tubercle**, 1990, 71, 241.
44. Buniva, G., Pagani, V. and Carozzi, A., **Int. J. Clin. Pharmcol. Therap. Toxicol.**, 1983, 21, 404.
45. Aspesi, F., Bull. **Int. Union. Tuberc. Lung. Dis.**, 1989, 64, 37.
46. Gelber, R., Jacobsen, P. and Levy, L., **Clin. Pharmacol. Therap.**, 1969, 10, 841.
47. Pelliza, G., Nebuloni, M., Ferrari, P. and Gallo, G.G., **Farmaco**, 1977, 32, 471.
48. Boman, G., Hanngren, A., Malmborg, A.S., Borga, O. and Sjoqvist, F. **Lancet**, 1971, 1, 800.
49. Boman, G., Lundgren, P. and Stjernstrom, G., **Eur. J. Clin. Pharmacol.**, 1975, 8, 293.
50. Long, M.W., Snider, D.E.Jr. and Farer, L.S., **Amer. Rev. Respr. Dis.**, 1979, 119, 879.
51. Sirgel, F.A., Botha, F.J., Parkin, D.P., Van De Wal, D.W., Donald, P.R., Clark, P.K. and Mitchison, D.A., **J. Antimicrob. Chemother.**, 1993, 32, 867.
52. Canetti, G., Le Lirzin, M., Porven, G., Rist, N. and Grumbach, F., **Tubercle**, 1968, 49, 367.

53. East African/ British Medical Research Councils, **Lancet,** 1974ii, 237.
54. Fox. W. and Mitchison, D.A., **Amer. Rev. Respr. Dis.,** 1975, **111,** 325.
55. WHO expert committee on specifications for pharmaceutical preparations, **WHO Technical Report Series.** No. 863, 1996.
56. IUATLD/WHO, **Int. J.Tuberc. Lung Dis.,** 1999, 3, S282
57. World Health Organization, **WHO/CDS/TB/99.274,** 1999.
58. Anonymous, **Int. J.Tuberc. Lung Dis.,** 1999, 3, S284.
59. Ellard, G.A., **Int. J.Tuberc. Lung Dis.,** 1999, 3, S322.
60. McIlleron, H., Gabriels, G., Smith, P.J., Fourie, P.B. and Ellard, G.A., **Int. J. Tuberc. Lung Dis.,** 1999, 3, S329.
61. Panchagnula, R., Agrawal, S., Kaur, K.J., Singh, I. and Kaul, C.L., **Int. J.Tuberc. Lung Dis.,** 2000, 4, 1169.
62. Fourie, P.B. and Spinaci, S., **Int. J.Tuberc. Lung Dis.,** 1999, 3, S368.
63. Blomberg, B., Kitler, M.E., Milstien, J. Dellepiane, N., Fanning, A., Norval, P.Y. and Spinaci, S., **Int. J.Tuberc. Lung Dis.,** 1999, 3, S371.