WHO Drug Information

Contents

Current Topics

Pharmacogenetics: towards improving treatment with medicines

Increasingly, sponsors of new drugs are integrating pharmacogenetics into their drug development programmes. The outcome of this integration will present challenges to the traditional paradigms for drug development, regulatory evaluation of safety and efficacy and clinical use of drugs. Pharmacogenetics is still an evolving discipline and a very active area of research. It promises to revolutionize therapeutics through 'individually targeted therapy'. In principle, genotype-based individually targeted prescribing ought to be more effective at improving response rates and decreasing the burden of adverse drug reactions.

The extent to which this promise of pharmacogenetics is fulfilled remains to be seen. The experience to date is mixed with a few successes but many frustrations. Discovering highly predictive associations during drug development and demonstrating their clinical validity and utility in clinical trials will no doubt better define the role of pharmacogenetics in future clinical practice.

The Council for International Organizations of Medical Sciences (CIOMS) and its Working Group on Pharmacogenetics have recently published a report entitled *Pharmacogenetics: towards improving treatment with medicines.* This is the outcome of discussions among a number of senior scientists from drug regulatory authorities, pharmaceutical companies and academia. It reflects their current views and visions and expectations for the future. The article below is drawn from the report, which is available from CIOMS. Details can be found on http://www.cioms.ch

Abnormal drug response: opportunities for risk reduction through pharmacogenetics

An adverse drug reaction (ADR) can result from a variety of risk factors including variability in pharmacokinetics and pharmacodynamics of a drug due to the genetic make-up of an individual. Other important influences are external factors such as co-medications and co-morbidities, which give rise to drug-drug or drug-disease interactions. The net effect of these interactions is that the prescribed dose of a drug is an inappropriate one. Usually, clinically relevant drug interactions result when the plasma concentration of one of the interacting drugs increases to toxic levels.

With careful attention to prescribing information regarding dose, age-related adjustments and populations at risk for drug-drug and drug-disease interactions, the impact of ADRs can be greatly minimized. However, it is unlikely that any single approach will completely eliminate all ADRs. With available data suggesting that some ADRs might have a monogeneic or polygeneic basis, the application of pharmacogenetics provides an opportunity for further reductions in both the incidence and severity of ADRs.

The article below reviews some of the data on abnormal drug response related to polymorphisms in drug metabolizing enzymes, pharmacological targets and drug transporters. It illustrates how, at least in some areas, pharmacogenetics may offer the prospects of minimizing the risks of drug toxicity and therapeutic failures.

Pharmacogenetics and drug metabolizing enzymes

A number of drug metabolizing enzymes displays genetic polymorphisms. Candidate gene association studies, investigating the role of these polymorphic drug metabolizing enzymes such as CYP2D6, CYP2C9, CYP2C19, *N*-acetyltransferase (NAT2), thiopurine *S*-methyltransferase (TPMT), UDP-glucuronosyltransferases (UGTs)

and dihydropyrimidine dehydrogenase (DPD) have already shown that there is a genetic predisposition to a number of ADRs.

It is now generally assumed that because of this genetic predisposition, there may be a great potential for preventing ADRs and improving the safe and effective use of medicines through the increasing knowledge of genetic factors that determine drug response. Polymorphic genes and products of gene expression have been considered as markers for optimization of drug therapy, most especially in the field of oncology.

Polymorphic variation in CYP2D6

Studies over the last two decades have shown that any given population may be divided into two phenotypes – extensive metabolizers (EMs) or poor metabolizers (PMs) – depending on their ability to mediate CYP2D6-dependent

Table 1. Clinical consequences for PM and ultrarapid EM phenotypes of CYP2D6

Clinical Consequences for the Poor Metabolizer	
Increased risk of toxicity	
Debrisoquine	Postural hypotension and physical collapse
Sparteine	Oxytocic effects
Perphenazine	Extrapyramidal symptoms
Flecainide	Ventricular tachyarrhythmias
Perhexiline	Neuropathy and hepatotoxicity
Phenformin	Lactic acidosis
Propafenone	CNS toxicity and bronchoconstriction
Metoprolol	Loss of cardioselectivity
Nortriptyline	Hypotension and confusion
Terikalant	Excessive prolongation in QT interval
Dexfenfluramine	Nausea, vomiting and headache
L-tryptophan	Eosinophilia-myalgia syndrome
Indoramin	Sedation
Thioridazine	Excessive prolongation in QT interval
Failure to respond	
Codeine	Poor analgesic efficacy
Tramadol	Poor analgesic efficacy
Opiates	Protection from oral opiate dependence
Clinical Consequences for the Ultrarapid Metabolizer	
Increased risk of toxicity	
Encainide	Proarrhythmias
Codeine	Morphine toxicity
Failure to respond	
Nortriptyline	Poor efficacy at normal doses
Propafenone	Poor efficacy at normal doses
Tropisetron	Poor efficacy at normal doses
Ondansetron	Poor efficacy at normal doses

hydroxylation of the antihypertensive drug debrisoquine. Among the EM phenotype, there are two subgroups of particular interest at either extreme of the EM population distribution. One subgroup, termed the ultrarapid metabolizers (UMs), is comprised of individuals possessing multiple copies of the gene for normal metabolic capacity and the other group, termed the intermediate metabolizers (IMs), is comprised of a heterozygous genotype ("gene-dose effect"). UMs metabolize drugs so avidly that they attain very low concentrations of the parent drug and high concentrations of rapidly accumulating metabolites while IMs display a modest impairment in drug metabolizing capacity.

CYP2D6 is responsible for the metabolism of well over 60 drugs that include antiarrhythmics, b-adrenoreceptor antagonists, antihypertensives, antianginals, neuroleptics, antidepressants, analgesics as well as a number of other miscellaneous drugs. Candidate gene association studies have shown that a number of ADRs to CYP2D6 substrates are related to CYP2D6 genotype (Table 1).

One of the first reports on the clinical significance of CYP2D6 polymorphism and its association with serious toxicity was perhexiline-induced neuropathy in patients with impaired CYP2D6 metabolism. Although the recommended dose of perhexiline was 100 mg three times daily, a recent study of 23 patients has shown that to maintain the plasma concentrations of perhexiline within the therapeutic and non-toxic range, PMs required a dose of 10-25 mg/day while EM and ultrarapid EM required 100-250 and 300-500 mg/day respectively [1]. Other clinical consequences for individuals with the PM or ultrarapid phenotypes of CYP2D6 are also shown in Table 1.

Application of pharmacogenetic principles may also improve efficacy. There are several examples where subjects carrying certain alleles suffer from a lack of drug efficacy because of ultrarapid metabolism caused by multiple genes or by induction of gene expression. As with perhexiline, some patients who are ultrarapid metabolizers fail to respond to conventional doses of nortriptyline and require 'megadoses' of this antidepressant. Similarly, poor metabolizers fail to respond to therapeutic effects mediated by metabolites. This is illustrated by the relative loss in PMs of analgesic effects following administration of codeine or tramadol or the loss of antiarrhythmic effects of encainide.

Polymorphic variation in CYP2C9

Retrospective case studies have shown that the presence of mutant CYP2C9 allele (especially CYP2C9*3 allele) confers a significantly increased risk of bleeding following treatment with warfarin. Available data, however, indicate that although the CYP2C9*3/ CYP2C9*3 genotype is associated with dramatic over anticoagulation soon after the introduction of oral anticoagulants, overdose during the maintenance period is mostly related to environmental factors [2, 3]. It is also recognised that inter-individual variability in warfarin sensitivity also originates from environmental factors. In one study, age and CYP2C9 genotype accounted for 12% and 10% of the variation in warfarin dose requirements, respectively [4]. Clearly, other pharmacodynamic (such as to an abnormality in the target enzyme vitamin K epoxide reductase) and dietary factors also play an important role. In a retrospective cohort study of patients on long-term warfarin, it was found that the mean maintenance dose varied significantly among the six genotypes of CYP2C9. Compared to patients with the wild type genotype, patients with at least one variant allele required longer time to achieve stable dosing and had a significantly increased risk of a serious or life-threatening bleeding event, although patient numbers were small for some genotypes in this study [5].

Similarly, to achieve a therapeutic serum concentration of phenytoin, patients carrying at least one mutant CYP2C9 allele required a mean phenytoin dose that was about 37% lower than that in patients with wild type genotype (199 mg/day versus 314 mg/day) [6]. Since phenytoin has a narrow therapeutic index and genotyping may be carried out rapidly and at a relatively low cost, dosage adjustment based on CYP2C9 genotype, especially at the induction of therapy, would be of value in order to lower the risk of concentration dependent phenytoin toxicity in the carriers of mutant alleles.

Polymorphic variation in CYP2C19

CYP2C19 mediates the major pathway responsible for metabolic elimination of proton pump inhibitors. Since therapeutic activity correlates with exposure to the parent compound, it is not surprising that a number of studies have shown that PMs of CYP2C19 respond better to *H. pylori* eradication therapy. These preliminary findings need to be confirmed in large prospective studies [7]. EMs of CYP2C19 require higher doses of these drugs.

Polymorphic variation in thiopurine S-methyltransferase

Azathioprine and 6-mercaptopurine are metabolised by thiopurine S-methyltransferase (TPMT). The activity of TPMT is inversely related to the risk of developing acute leucopenia associated with the use of these drugs. A number of studies have shown that the risk of azathioprine-induced acute leucopenia can be greatly reduced by basing the initial azathioprine dose on TPMT genotype or phenotype [8, 9]. Of course, not all azathioprine-induced toxicities have a genetic basis. In one study of 93 patients, it was noted that azathioprine-related gastrointestinal side effects are independent of TPMT polymorphism [10]. The value of genotyping for TPMT is illustrated by a report from Murphy and Atherton [11] that by initiating therapy at dose levels of 2.5-3.5 mg/kg in atopic eczema patients with a normal TPMT level, they felt confident in reducing the frequency with which tests of bone marrow and liver function had to be undertaken.

Polymorphic variation in UDP-glucuronosyltransferases

Conjugation reactions such as glucuronidation mediated by UDP-glucuronosyltransferases (UGTs) are now also attracting increasing attention, especially in the field of oncology. Glucuronidation is by far the most important conjugation pathway in man. A multigene family encodes the UGTs and a relatively small number of human UGT enzymes catalyse the glucuronidation of a wide range of structurally diverse endogenous (bilirubin, steroid hormones and biliary acids) and exogenous chemicals. Genetic variations and single nucleotide polymorphisms (SNPs) within the UGT genes are remarkably common, and lead to genetic polymorphisms [12, 13]. Some polymorphic UGTs have demonstrated a significant pharmacological impact in addition to being relevant to drug-induced ADRs. Two major isoforms of UDP-glucuronosyltransferase, UGT1A1 and UGT1A9, have been shown to display genetically determined wide inter-individual variability in their activities. Studies investigating the role of UGT1A isoforms in the metabolism of drugs such as irinotecan [14, 15], flavopiridol [16, 17], tranilast [18] and atazanavir [19] have been most valuable in explaining the safety issues (myelosuppression, diarrhoea or hyperbilirubinaemia) associated with the use of these drugs.

A meta-analysis by Phillips et al [20] identified 131 specific drugs, 55 drug classes, and 19 therapeutic drug categories as being associated with ADRs. All except three of these drugs were included among the top 200 selling drugs in the United States. The therapeutic categories associated with the most common ADRs were cardiovascular, analgesics, psychoactive drugs and antibiotics. This meta-analysis included 18 of 333 ADR studies and 22 of 61 variant allele review articles. It identified 27 drugs frequently cited in ADR studies. Among these drugs, 59% were metabolised by at least one enzyme with a variant allele known to cause poor metabolism. In contrast, only 7% to 22% of randomly selected drugs were metabolised by enzymes displaying genetic polymorphism ($p = 0.006 - < 0.001$). These data suggest that drug therapy based on the genotype of individual patients may result in a clinically important reduction in adverse outcomes.

Pharmacogenetics and transporters

For the vast majority of drugs, however, the reason for individual susceptibility to ADRs has remained unknown and there are hardly any data on genetic susceptibility. However, recent studies have shown that organ-specific organic anion and cation transporters play an important role in the transport of drugs into the cells. These transporters may account for drug-induced toxicity, hitherto termed “idiosyncratic”.

Molecular studies have found evidence of genetic polymorphisms of these transporters in hepatocytes [21, 22]. Mutations in the genes coding for these transporters may lead to dysfunctional polypeptides, which affect not only the pharmacokinetics of the drugs concerned but also the potential hepatotoxic effects of some of these drugs [23, 24]. Furthermore, the variant alleles show inter-ethnic differences [22, 25] that may possibly explain inter-ethnic differences in the hepatotoxic potential of a drug (such as ibufenac). Studies investigating these transporters in patients with hepatotoxicity offer exciting prospects for exploring the potential role of pharmacogenetics in drug-induced hepatotoxicity.

These transporters and P-glycoproteins co-localize in organs of importance to drug disposition (intestine, liver and kidney). The expression of P-glycoprotein activity is under the control of the MDR1 gene [26] and is an important factor in the disposition of many drugs. In multidrug resistance (MDR), the processes involved show considerable interindividual and inter-ethnic variability. For example, a variant allele recently

designated as MDR1*2 (resulting from three linked SNPs) occurred in 62% of European Americans and only 13% of African Americans [27].

The MDR1 gene and its variants have significant implications in terms of efficacy or development of resistance to anticonvulsants, antineoplastic therapy and anti-HIV drugs [28, 29].

Pharmacogenetics and pharmacological targets

In addition to pharmacogenetic effects on drug metabolism, therapeutically promising examples of genetic variations in pharmacological targets are also beginning to emerge. These targets include receptors, transporters, enzymes, channels and intracellular coupling processes that modulate pharmacodynamic responses. Among the most widely studied are the pharmacological targets related to cardiac arrhythmias, asthma, depression and the HLA antigen genotype in hypersensitivity reactions.

To date, the focus of pharmacogenetic studies in the context of ADRs has been on drug metabolising enzymes. It is now becoming evident that polymorphisms of pharmacological targets (pharmacodynamic polymorphisms) may in fact be even more important. In one study of 270 cancer patients given antiemetic therapy with 5-HTR_{3B} receptor antagonists, approximately 30% suffered from nausea or vomiting despite these drugs. Ultrarapid metabolism of tropisetron (and to a lesser extent for ondansetron) was shown to predispose patients to poor efficacy [30]. In another study by the same group of investigators, patients homozygous for a deletion variant of the promotor region of 5-HTR_{3B} gene were shown to experience vomiting more frequently than did all the other patients [31]. In a pharmacogenetic study that compared paroxetine and mirtazapine in 246 elderly patients with major depression, discontinuations due to paroxetine-induced side effects were strongly associated with the 5-HTR_{2A} C/C, rather than CYP2D6, genotype. There was a significant linear relationship between the number of C alleles and the probability of discontinuation. The severity of side effects in paroxetine-treated patients with the C/C genotype was also greater [32]. Thus, although paroxetine is metabolised by CYP2D6, polymorphism of 5-HTR_{2A} is a more important determinant of paroxetine-induced ADRs.

Polymorphisms of cardiac potassium channels

Drugs prolonging the QT interval of the surface electrocardiogram (ECG) have attracted considerable attention recently. Excessive prolongation of the QT interval, in the right setting, predisposes to torsade de pointes (TdP), a potentially fatal ventricular tachyarrhythmia [33]. The duration of this interval reflects the duration of ventricular action potential. The major determinant of the action potential duration is the potassium current mediated by the rapid component of the delayed rectifier potassium channels (IKr). Many drugs have been withdrawn as a result of their potential to prolong the QT interval and induce TdP.

Following advances in molecular biology, genetics and pharmacology of ion channels, it has become evident that there is a great diversity of genes that control the expression of these potassium channels. Mutations of the subunits that form these channels, including IKr, are common and give rise to congenital long QT syndromes.

Relatively large numbers of individuals carry variants of long QT syndrome genes that are clinically silent. While these individuals have a normal ECG phenotype, they nevertheless have a diminished repolarization reserve and are highly susceptible to drug-induced QT interval prolongation and/or TdP following normal therapeutic doses of drugs (such as cisapride, astemizole, terfenadine and halofantrine among others) even in the absence of inhibitors of their metabolism [34]. In an analysis of 341 reports of cisapride-induced ventricular arrhythmias, there were 38 (11%) cases in whom there were no identifiable risk factors or contraindications [35]. These individuals may well represent a population with a concealed genetic defect of their potassium channels.

Polymorphisms of b2-adrenoceptors and ALOX-5

Individuals who carry Arg16/Gly16 or Gly16/Gly16 mutations of b2-adrenoceptors have been shown to respond much less favourably to salbutamol-induced bronchodilatation, in contrast to those with wild type receptor characterised by Arg16/Arg16 genotype – the difference in FEV1 response between Gly16/Gly16 and Arg16/Arg16 genotypes is 6.5-fold [36]. Similarly, asthmatic patients who carry mutations of the core promoter of 5-lipoxygenase (ALOX-5) respond poorly to ALOX-5 inhibitors such as zileuton [37].

Kaye et al [38] have recently shown that in individuals with cardiac failure, patients who were homozygous for the Gln27 allele of b2-adrenoceptor displayed a significantly lower proportion of good responders to carvedilol than did patients who were homozygous or heterozygous for the Glu27 polymorphism (26% versus 63%, p=0.003).

Polymorphisms of the serotonin transporter

Genetic polymorphism in the promoter region of the serotonin transporter (5-HTT) gene is reportedly a determinant of response to fluvoxamine, a selective serotonin re-uptake inhibitor. The insertion variant of this polymorphism (long allele) is associated with higher expression of brain 5-HTT compared to the deletion variant (short allele) [39]. Patients who have one or two copies of the long variant (homozygous l/l or heterozygous l/s) may show a better therapeutic response than patients who are homozygous for the short variant (s/s). The efficacy of fluvoxamine in the treatment of delusional depression has been shown to correlate with 5-HTT genotypes [40].

Abacavir-induced hypersensitivity reactions and HLA genotype

Hypersensitivity reactions (HSR) to abacavir occur in about 5% of patients who receive the drug for HIV-1 infection. Three independent research groups have identified an association between HLA-B*5701 and hypersensitivity to abacavir in patients of Caucasian ancestry [41-44]; the sensitivity of HLA-B*5701 ranged from 46-94%. While two groups suggest that there may be clinical value in prospectively screening Caucasian patients for HLA-B*5701 prior to the use of abacavir [43, 44], in the largest, and most ethnically diverse study, the association between HLA-B*5701 and hypersensitivity was much weaker in Hispanic patients and was absent in Black patients [45]. While this is an interesting example of the potential of pharmacogenetics, there is legitimate risk that HLA-B*5701 screening could unintentionally compromise the highly successful risk management programme established for abacavir hypersensitivity. Specifically, physician vigilance might be reduced in patients who do not carry markers associated with hypersensitivity and marker-negative patients might be at increased risk for experiencing serious and/or life-threatening hypersensitivity reactions because symptoms associated with abacavir hypersensitivity are not promptly recognised and abacavir discontinued. Efforts to analyse thousands of SNPs across the genome for association to HSR are under way to identify additional genetic markers with sufficient predictive value to be clinically useful [46].

Pharmacogenetics and hepatotoxicity

Hepatotoxicity is of serious concern not only because of the morbidity and mortality associated with it but also because it is the leading reason for withdrawal of drugs from the market [47]. Apart from the role of transporters at the hepatocytes-biliary canalicular interface, there is conclusive evidence for the role of polymorphic drug metabolism in hepatotoxicity associated with some drugs.

For isoniazid, the genetic basis for this toxicity is well known. Individuals who have a low activity of N-acetyltransferase (NAT2 slow acetylators) are at a much greater risk of developing isoniazid-induced hepatotoxicity. Slow acetylators produce a low level of an intermediate metabolite that is also eliminated by acetylation. Failure to eliminate this effectively results in production of an alternative metabolite that is hepatotoxic [48, 49].

Perhexiline-induced hepatotoxicity, a major factor in the drug's withdrawal from the market, is associated with impaired CYP2D6 status [50]. The involvement of genetic factors in drug-induced hepatotoxicity generally is strongly suggested by the susceptibility of the female gender. In addition, there are reports of familial or ethnic susceptibility to hepatotoxicity associated with some drugs such as phenytoin [51] or ibufenac [52] respectively.

Pharmacogenetics and drug interactions

Drug-drug interactions can be dramatically influenced by genotypic differences. A number of studies have shown that CYP2D6 PMs (with alleles expressing no functional enzyme) do not show the drug-drug interactions predicted from *in vitro* studies. This is hardly surprising since there is no functional CYP2D6 activity to inhibit or induce. Likewise, UMs too may fail to exhibit the expected drug-drug interaction unless the dose of the inhibitor is (toxic) high enough. The individuals most likely to display a drug interaction are those who have an intermediate drug metabolising capacity or those who have inherited CYP2D6 alleles with reduced or altered affinity for CYP2D6 substrates. At the level of CYP2D6, the dependence of drug interactions on the metabolic

phenotype has already been shown for a number of its substrates, for example codeine [53], propafenone [54, 55], mexiletine [56], encainide [57], metoprolol [58] and desipramine [59]. The organic ion transporters and P-glycoproteins referred to earlier are additional sites of important drug interactions and pharmacogenetic factors are also likely to be important here.

Predictive genotyping: improving drug response and minimizing ADRs

It has been estimated that predictive genotyping (for candidate genes) will lead to benefit in 10–20% of drug treatment by allowing prevention of ADRs [60, 61].

If genetic markers of a greater number of ADRs (candidate genes, SNPs or haplotypes) can be identified and if cheap and rapid genotyping of patients can be done routinely, then the impact of ADRs on morbidity and mortality can be considerably reduced. Veenstra et al [62] have reviewed cost-effectiveness of genetic tests and have identified five primary characteristics that will enhance the cost-effectiveness of the application of pharmacogenetics. These are:

1. A well-established association between the genotype and drug response.

2. The variant gene is relatively common.

3. Relatively cheap and rapid genetic test.

4. Difficulties in monitoring drug response.

5. Severe clinical or economic consequences from not using the pharmacogenetic information.

Similar conclusions have been reached by Rioux [63] who has also emphasised the importance of the frequency of the variant allele in determining the cost-effectiveness of the application of pharmacogenetics in therapeutics.

Other workers who have evaluated the potential impact of pharmacogenetics have concluded that its application in therapeutics will be cost-effective “sometimes” and that it would be effective primarily for chronic diseases where unnecessary long-term therapy with an ineffective drug for many years could be avoided in some patients [64].

Limitations

It is not intended to suggest that the application of pharmacogenetics will totally eliminate the problems of ADRs. Recently, Kirchheiner et al have provided a preliminary guidance for a number of drugs metabolised by CYP2D6 and CYP2C19 with a view to introducing genotype/phenotype-specific dose schedules [65]. Recommending inappropriately high dose can easily offset the potential benefits of pharmacogenetics. Co-administration of a metabolic inhibitor converts an extensive metabolizer into a poor metabolizer. It is therefore not surprising that drug interactions feature prominently among the causes that lead to withdrawal of drugs from the market.

One unpublished report analysed 17 studies (with a total of about 1,350 patients) published between 1995-2000 on antipsychotic drug therapy, investigating an association between CYP2D6 genotype and both plasma levels of the drug(s) and response to these drugs [66]. There was a relationship between genotype and plasma concentrations of drugs that were predominantly metabolised by CYP2D6 but a large intra-genotypic variability obscured clinical utility of concentration measurements.

However, there was no relationship evident between genotype and drug response (i.e. failure to respond beneficially). There was only a modest positive trend between the genotype, especially the presence of CYP2D6*10 allele in the Japanese, and severity of tardive dyskinesia and extrapyramidal syndrome. This may not altogether be surprising since many neuroleptics have active metabolites. When applying pharmacogenetic testing in routine clinical practice, it is important to take note of the pharmacology of the metabolites relative to that of the parent drug, the fraction of the drug cleared by the polymorphic pathway and the therapeutic index of the drug concerned [67].

In humans, diclofenac is metabolised to 4'-hydroxy (OH), 3'-OH and 5-OH metabolites. The polymorphic CYP2C9 is involved in the metabolism of diclofenac to 4'-OH diclofenac and 3'-OH diclofenac. However, the CYP2C9 genotype does not correlate with diclofenac-induced hepatotoxicity or COX-1 and COX-2 inhibition [68, 69]. Similarly, in asthma, patients who are deficient in 5-lipoxygenase due to a genotypic variant in the ALOX-5 gene are non-responsive to 5-lipoxygenase inhibitors. However, most of the 5-lipoxygenase inhibitor non-responders have

normal ALOX-5 genes, and the basis of their non-responsiveness lies in other factors, probably related to the nature of their asthma.

However, if a genotype/phenotype relationship can be shown, pharmacogenetics offers another important strategy by which to reduce ADRs. The dose schedules recommended need to be carefully chosen and the clinical awareness of the consequences of co-administration of interacting drugs need to be heightened. Prior genotyping of patients can be used to improve safe and more effective use of specific and carefully chosen medicines by identifying patients most likely to respond beneficially and those most likely to develop an ADR. This strategy would immediately translate into great reductions in healthcare and economic resources that are currently expended in managing the consequences of ADRs.

Even if a correlation between genotype and phenotype can be established, it is worth remembering that drug-related problem(s) may not be completely eliminated. This is because a number of non-genetic external factors interact with genotype or modulate the response to a drug. In addition, there are a number of other factors that complicate what may appear to be a simple relationship.

Conclusions

This article highlights the potential contribution of pharmacogenetics in reducing the incidence of dose-related and idiosyncratic ADRs. In relation to ADRs, the research aim of pharmacogenetics is to identify a genetic profile that characterises patients who are more likely to suffer an ADR compared with those in whom the risk is unlikely. Using this knowledge in the clinic, the choice of medicine and dose can be targeted for an individual and the overall result may be an improvement in the safety profile of the drug. Moreover, as a result of improved safety following application of pharmacogenetic principles, improved efficacy may also accrue. Many dosing schedules are limited by appearance of side effects. By eliminating the use of high doses in those genotypes most at risk, it may become possible to evaluate the additional benefits of higher doses in the remaining genotypes.

Advances in biotechnology promise the prospects of characterising genetic variations in individual patients rapidly and cheaply with a view to individualisation of therapy. Exploration of the role of pharmacogenetics should be undertaken during drug development and continued well into the post-marketing period to include the study of rare and delayed adverse reactions. This will make the application of pharmacogenetics in minimising morbidity and mortality from ADRs a realistic and worthwhile proposition.

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Safety and Efficacy Issues

The role of pharmacovigilance in medicines safety

Modern medicines have changed the way in which diseases are managed and administered. Despite obvious benefits, evidence continues to accrue that adverse reactions to medicines are a common — and often preventable — cause of illness, disability and death. In some countries, adverse drug reactions (ADRs) rank among the top ten leading causes of mortality.

In order to prevent or reduce harm to patients and thus improve public health, mechanisms for evaluating and monitoring the safety of medicines in clinical use must be in place. In practice, this means having a well-organized pharmacovigilance system. WHO defines pharmacovigilance as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem.

Pharmacovigilance is a dynamic clinical and scientific discipline. It plays a crucial role in meeting the challenges posed by the ever increasing range and potency of medicines, all of which carry an inevitable and sometimes unpredictable potential for harm. When adverse effects and toxicity do appear – especially if previously unknown – it is essential that these are reported, analysed and their significance communicated effectively to an audience that has the knowledge to interpret the information.

For all medicines there is a trade-off between the benefits and the potential for harm which can be minimized by ensuring that medicines of good quality, safety and efficacy are used rationally, and that the expectations and concerns of the patient are taken into account when therapeutic decisions are made.

The aims of pharmacovigilance

Nearly 40 years of worldwide pharmacovigilance practices have highlighted the need for effective drug monitoring systems. The aims of pharmacovigilance programmes are to:

- improve patient care and safety in relation to the use of medicines, and all medical and paramedical interventions;
- improve public health and safety in relation to the use of medicines;
- contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use. Risk management demands close and effective collaboration between a host of key players.
- promote understanding, education and clinical training in pharmacovigilance and effective communication to health professionals and the public.

With the increasing use of medicines within the last decade, the scope of pharmacovigilance has extended beyond the strict confines of detecting new signals of safety concerns. Globalization, consumerism, the resulting explosion in free trade and communication across borders, and use of the Internet have all contributed to a change in the way people use medicinal products and information about them. These changing patterns require a shift in the approach to pharmacovigilance, more specifically, towards one that is more closely linked and responsive to the prevailing patterns of medicines use within society.

Key elements of pharmacovigilance:

- Establishment of national pharmacovigilance systems especially for spontaneous reporting of adverse events, including national pharmacovigilance centres, and, if appropriate, regional centres.
- Development of necessary legislation/regulation for medicine monitoring.
- Development of national policy and plans of action (to include costing, budgeting and financing).
- Undergraduate and continuing education of health-care providers on safe and effective pharmacotherapy.

- Continuously providing information on adverse reactions to professionals and consumers.

- Monitoring of the impact through process indicators and outcome.

The provision of good quality, safe and effective medicines and their appropriate use is the responsibility of national governments. The achievement of these goals requires the establishment of a national regulatory agency and a designated centre for the monitoring and study of adverse reactions. Multidisciplinary collaboration is of great importance within and among governments and with other stakeholders, including the pharmaceutical industry, universities, nongovernmental organizations (NGOs) and professional associations having responsibility for education on rational use of medicines and pharmacotherapy.

Reference: World Health Organization. Pharmacovigilance: ensuring the safe use of medicines. *WHO Policy Perspectives on Medicines*. October 2004.

WHO Programme for International Drug Monitoring

The WHO Programme for International Drug Monitoring was launched in 1968 as a pilot project of ten countries with established national reporting systems for ADRs. Since then, many more countries worldwide have developed national pharmacovigilance centres for the recording of ADRs and the reporting network has expanded significantly. Currently, a total of 86 countries participate in the International Drug Monitoring Programme. The Programme depends on a WHO Collaborating Centre based in Sweden (Uppsala Monitoring Centre), which is responsible for maintaining the global ADR database. VIGIBASE now contains more than three million ADR reports.

As part of its work, the WHO Collaborating Centre analyses the reports in the database to :

- identify early warning signals of serious adverse reactions to medicines;

- evaluate the hazard; and

- undertake research into the mechanisms of action to aid the development of safer and more effective medicines.

WHO also plays an important role in the provision of expert advice through an advisory committee, on all matters of safety of medicines. Its aim is to facilitate consistent policies and action among member countries and to advise those who may be concerned about action taken in another country.

The success of the WHO International Drug Monitoring Programme is entirely dependent on collaboration with national pharmacovigilance centres. Such centres provide an essential pool of

Structure of the WHO Programme for International Drug Monitoring

Partner	Role	Responsibility
World Health Organization	Policy and oversight Coordination Communication	Exchange of Information Advisory Committee on Safety of Medicinal Products Guideline development International training courses
WHO Collaborating Centre for International Drug Monitoring	Operational management Communication	Global ADR data base Guideline development Signal detection Review Panel for Signal Detection International training courses
National Centres	Reporting network Communication	Annual meetings Advisory Committee for Pharmacovigilance National data base Signal detection National training courses

experience and competence which has been instrumental in the continuous development of the WHO programme. Ideally every country should have a national pharmacovigilance system.

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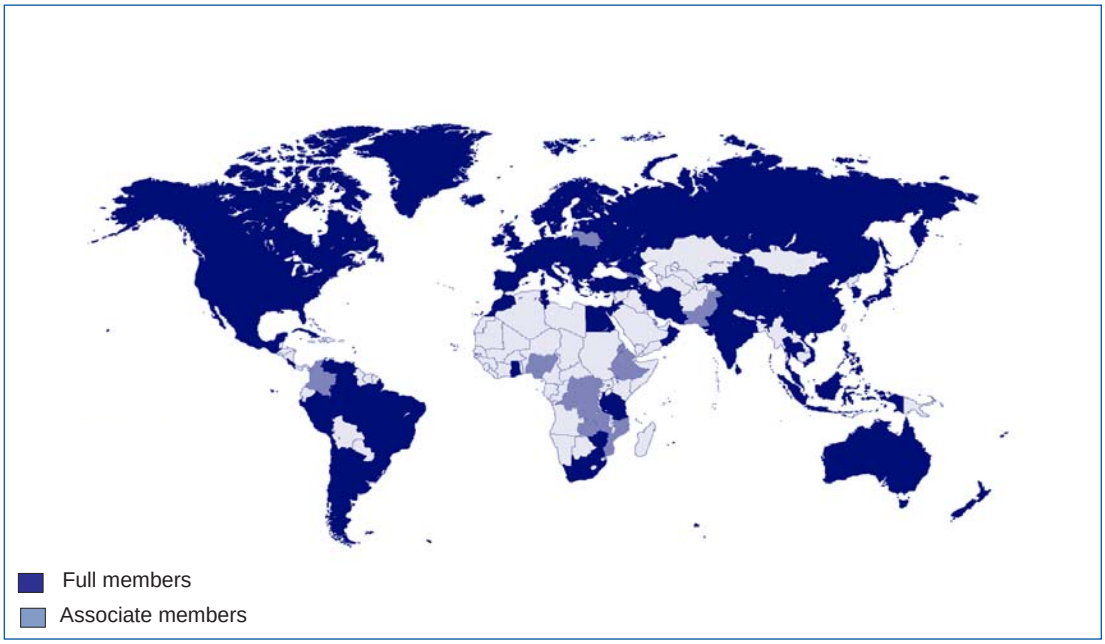
WHO International Drug Monitoring Programme: membership



National Centres discuss new safety monitoring methods

The Twenty-seventh meeting of National Centres participating in the WHO Programme for International Drug Monitoring took place in Dublin, Ireland, in October 2004. The meeting focused on identification of new methods of pharmacovigilance, drug monitoring and reporting with particular attention to facilitating regulatory action within the constraints of individual country settings.

Focused surveillance methods (FSMs) provide information concerning medicines use and adverse drug reactions (ADRs) but are not commonly employed in developing countries beyond the existing spontaneous reporting systems. However, FSMs have numerous advantages since they provide specific types of data (population-specific, gender-specific, etc.) and identify specific factors needing long-term follow-up. Additionally, non-serious or rare ADRs can be captured. The disadvantage of FSMs is that they tend to be resource intensive and time consuming. The type of FSM that will work best will therefore depend on country needs, financial resources, support systems, and available knowledge and expertise. Also of relevance would be the deployment of mass treatment and immunization programmes, local problems of counterfeiting, or populations with special characteristics such as rare and neglected diseases.

Reporting data

During the meeting, it was proposed that the WHO Collaborating Centre for International Drug Monitoring should develop a user friendly template for reporting data from focused surveillance studies which is flexible and adaptable to each country's needs. Additionally, a registry of all FSM studies carried out by different countries should be maintained and the information made available to all national centres. Questions of data ownership and confidentiality were also discussed and it was agreed that provision and use should follow clearly defined rules, in particular concerning data ownership and commercial use.

The importance of registry creation

A registry is defined as a list of patients sharing the same characteristics. These characteristics could be a disease (disease registry) or a specific exposure (drug registry). A registry should involve a systematic collection of defined events in a defined population over a defined period of time. They should include sufficient information to enable a search of patient medical records or linkages with other databases. Different types of registries exist and may be useful in pharmacoepidemiological studies, data linkage analyses, calculation of incidence rates, and to obtain information on disease prevalence, drug usage, off label usages and outcomes in general.

Registries can be developed on vaccine use, prescription drug utilization data, large public health programmes, pregnancy exposure and outcomes including birth defects. Setting up a registry can be resource intensive and expensive to maintain so that countries need to be clear about the objectives and scope of a registry before launching, including definition of the population to be covered and variables to be recorded. It was suggested that a need exists for developing a master registry as a first step in bringing together all available registries in different countries.

Reference: World Health Organization. *Report of the Twenty-seventh Annual Meeting of Representatives of the National Centres*. EDM/QSM/2004.3 available from http:www.who.int/medicines/

Signalling and safety problems

One of the most important tasks of the WHO Programme for International Drug Monitoring is to identify signals of drug safety problems as early as possible. The WHO database, which is maintained by the WHO Collaborating Centre for International Drug Monitoring in Uppsala, Sweden holds over 3.1 million case reports making it the largest database of spontaneous ADR reports from health professionals in the world.

Since 1998, the Collaborating Centre has been using the Bayesian Confidence Propagation Neural Network (BCPNN) methodology to identify unexpectedly strong quantitative associations between drugs and adverse reactions. The BCPNN uses a logarithmic measure of disproportionality called the information component (IC) which is based on observed to expected ratios for the co-occurrence of a drug and ADR in the database. Using this methodology, the complete WHO database is screened quarterly generating a computerized table known as the combinations database of more than 50 000 drug-ADR combinations reported the last quarter (approximately 2000 associations). This comprehensive listing of drug-ADR combinations contains no review, filtering or analysis. It is sent

every quarter to the national centres (NCs) who review its international contents for issues of relevance to their own countries. This signal detection work by the Collaborating Centre and its review team is complementary to the work performed by national centres and not a substitute for local evaluation and decision-making.

Since 2001 these associations have been filtered further using the following algorithms:

- Rapid reporting increase. The drug-ADR combination is a new association and the IC has increased by a set value, for example two or more, since the previous quarter
- Serious reactions and new drugs. The drug-ADR combination is a new association and the drug was first entered into the database in the last one or two years and the ADR is a WHO-ART critical term and there are few reports on the association and there was a fatality in at least one case.
- Reactions of special interest. Special attention will be put on important typically drug related reactions such as: Stevens-Johnson or Lyells syndrome, agranulocytosis. or rhabdomyolysis. The filter is IC- independent.

In addition to these algorithms other factors can be focused on, such as multinational reporting, since a signal is more credible if the drug-ADR is reported from several countries. Quality criteria, such as positive re-challenge cases, can also be added to the algorithms.

A signal is defined as: Reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously. Usually more than a single report is required to generate a signal, depending upon the seriousness of the event and the quality of the information.

The filtering process known as the triage is performed by the staff at the WHO Collaborating Centre for International Drug Monitoring. However, the use of sophisticated computer systems and algorithms cannot replace the unique human qualities necessary for clinical review. The next stage in the triage process for the retrieved drug–ADR combinations is to check for its occurrence in the available sources of published product information (Martindale, Physician's Desk Reference, Drug Dex and summaries of product characteristics), to see what is already known and if the combination should be passed to reviewers for in-depth study. For drugs where the reaction is not found in the literature or fully described, complete case reports are retrieved from the WHO database.

The different topics are divided amongst the members of the international expert review panel, which consists of 36 consultants from 20 countries. The reviewers are asked to assess the case reports using their clinical experience and pharmacological knowledge. Analysis of potential signals includes checking the available case data and making literature searches. After assessing the cases, including judgment on the causal strength of the drug-ADR association, the reviewer drafts a short report on the assessment indicating if it is a signal or not and if the issue is worth notifying to national centres. After review within the Collaborating Centre, the text of this report may be included in the publication, SIGNAL, for distribution to national centres. It is the responsibility of the national centre to take action on the signal raised.

Although whole populations can be covered, most often drug usage data is collected using sampling methods and, therefore, in addition to the uncertainties affecting the numerator, generalizability also depends on the validity of the methods used in obtaining the denominator information. There are other reasons why results must be interpreted cautiously, including the following:

- the numerator and denominator information is obtained from separate sources, therefore different biases may apply
- correlational studies refer to populations rather than individuals, thus it is not possible to link an exposure to occurrence of an outcome in the same person.

A cautionary statement is included in a caveat document which accompanies data released from the WHO Collaborating Centre.

Third meeting of the Signal Review Panel

On 13 and 14 December 2004, 25 members of the Signal Review Panel met together in Uppsala, Sweden in order to exchange experiences on their work and to learn how to make best use of the information in the WHO database. Discussions focused on the balance needed for quickly finding early signals and having enough quality data to meet the criteria to determine that the signal is important for public health.

Quality of reports

The low quality of a large number of reports in the WHO database is a problem. The BCPNN, however, is not dependent on the quality of reports to be able to highlight associations standing out from the background of the database. Since 2002, the WHO database has been capable of linking to narratives, but not all national centres provide such information. The Collaborating Centre can always ask national centres for additional information but this may take time and some centres do not respond. Concerning the question of whether original reports should be requested before each signal, some reviewers think that each case report summary in the WHO database is a concern and getting external data is a waste of time since it is the national centre's responsibility to investigate the possible drug safety problem presented in the signal texts. Others think that one should always go back and ask for original data as the hypothesis may otherwise be based on faulty data.

Reporting from national centres

The WHO database contains different types of reports. Approximately 50 000 new reports are entered each quarter. The Collaborating Centre encourages regular reporting, but less than 10 countries send reports once per month; some countries do not send any reports at all; some countries send reports in large batches and nothing in between for long periods. Case reports can be valued according to whom the sender is; a report from a specialist is regarded differently to that of a report from a general practitioner or a pharmacist. Every report counts, but the reviewer should weigh/judge them differently. Consumer reports may be very valuable for certain types of reactions. At the moment, consumer reports are part of the background for BCPNN runs and approximately 20% of all US reports are consumer reports. The Collaborating Centre is currently investigating the effects of this.

Collaboration

In order for the Collaborating Centre to obtain high quality narratives there is a need to increase the collaboration with both national centres and industry. The work should be complementary to the national centres work and therefore the reviewers want to collaborate/work more closely with national centres. The Collaborating Centre should aim to involve national centres more and encourage them to announce when they are investigating a problem or have discovered a signal. The closeness of the relationship/collaboration regarding signal detection with national centres varies very much. The contact should be on a personal level, but workload and time are limiting factors at the national centres.

Education

The pharmacovigilance culture is weak in many countries. Medical schools should be made aware of the importance of pharmacovigilance. In some countries, the deans of medicine do not discuss drug safety problems in their country. Drugs banned from the market are still available over-the-counter. In order to promulgate pharmacovigilance, education could be added to the agenda of meetings of different groups such as EACPT and IUPHAR. A clinical harm registry could be developed in order for the world to know which different drug safety harms people are working on.

National problems with banned drugs should always be brought to the attention of WHO. There should be increased collaboration with universities, medical schools, health departments, pharmacoepidemiological groups and acadaemia.

Improving signals

There is no general solution as to how a signal should be presented. Signals have a different character, different amount of information and level of evidence but all are equally important. It is also important to describe the case reports and determine why the finder thinks the selected drug-ADR association should be investigated further. Even an experienced reviewer can feel uncertain, therefore feedback from the Collaborating Centre on the assessments was asked for. The reviewers were asked to always inform the Collaborating Centre in each assessment about the level of evidence used, and motivation for deciding if a combination/topic is a signal or not or if it should be included for follow-up. By always writing down and adding the logic, the conclusion of the reviewer will become clear and will facilitate the work of the Collaborating Centre. Reviewers should always inform the Collaborating Centre about any problems they may have and if their current workload prevents them from performing the review task satisfactorily. If a combination needs a major investigation, this should be left to the national centres.

Signals with no change in the literature but increasing report numbers should be communicated via publications, directly with the national centres or included in *WHO Pharmaceutical Newsletter*. Single country signals are sometimes important to include in the SIGNAL document

since other countries need to know of the problem. For high reporting rates the responsible national centre should be requested at an early stage prior to signalling for a possible reason behind increased reporting. Ideally, original case reports should be requested before sending any cases to reviewers but this would be too time consuming. Therefore, it was suggested that original data could be requested at least for potential signals when reviewer has made first assessment. All kinds of signals are allowed. For brief and quick signals the importance of the earliness due to the topic should be clearly stated.

In conclusion, the Collaborating Centre will increase the feedback to reviewers on the written assessments. Since signals have different character, different amount of information and level of evidence but all are equally important, all kinds of signals will be allowed. The Collaborating Centre should always be informed about the level of evidence used in the assessment.

Wider audience of signals

Formally, the task of the Collaborating Centre and reviewer stops when the signal reaches the national centre, but the SIGNAL document should not be the end of a combination. National centres take over in a sense, but the continuation of an investigation of a possible drug safety hazard is important. It is the right of each national centre to use the information in any way they see fit. National centres could publish translated signals in national drug bulletins. Reviewers could help by informing the Collaborating Centre when they think some signal is worth publishing externally. WHO encourages the publication of signals in order to reach a larger audience.

It is the right of each national centre to use the information generated within the WHO International Drug Monitoring Programme in any way they see fit. A larger audience could be reached by publishing more signals. It was re-emphasized that the three statements of the caveat document always must be included in all external publications, i.e. the source of the information; that the information is not homogenous, at least with respect to origin or likelihood that the pharmaceuticala product caused the adverse reaction; and that the information does not represent the opinion of the World health organization.

Re-signalling

The panel discussed the importance of following up signals after they have been presented in SIGNAL as well as the importance of follow-up of associations of possible interest having, at the time, too small amount of data to assess. It was agreed that a follow-up of all previous signals should be conducted by checking the changes in reporting and IC to determine developments, but literature should only be checked when there is an increase in reporting. When re-signalling, the reporting should ideally be linked to the usage of the drug. All methods of promoting and publishing signals should be explored.

COX–2 inhibitors: overview

On 30 September 2004, Merck & Co., Inc. announced a voluntary withdrawal of rofecoxib (Vioxx®) from the worldwide market due to safety concerns of an increased risk of cardiovascular events (including heart attack and stroke) in patients on rofecoxib. Rofecoxib is a prescription cyclooxygenase-2 (COX-2) selective, nonsteroidal anti-inflammatory drug (NSAID) approved by the US Food and Drug Administration (FDA) in May 1999 for the relief of the signs and symptoms of osteoarthritis, for the management of acute pain in adults, and for the treatment of menstrual symptoms. It was later approved for the relief of the signs and symptoms of rheumatoid arthritis in adults and children.

The manufacturer withdrew rofecoxib (Vioxx®) from the market following recommendations of the data safety monitoring board overseeing a long-term study in patients at risk of developing recurrent colon polyps (1). This study was halted following an increased risk of serious cardiovascular events, including heart attacks and strokes, among study patients taking rofecoxib compared with patients receiving placebo.

Market withdrawal of rofecoxib comes more than five years after its launch. More than 80 million patients have used this medicine, with annual sales topping US$ 2.5 billion.

The first trial to show an association of COX-2 inhibitors with cardiovascular events was the Vioxx Gastrointestinal Outcomes Research (VIGOR) study in 2000 in which 0.4% of the rofecoxib group and 0.1% of the naproxen group developed myocardial infarction (2). This result was extended by a between-study comparison (3). The comparison, which included celecoxib and rofecoxib, implicated both medicines as being associated with a significantly higher rate of myocardial infarction than placebo. The authors postulated that COX-2 inhibitors may have a prothrombotic effect through inhibition of prosta-

cyclin and concluded that 'it is mandatory to conduct a trial specifically assessing cardiovascular risk and benefit of these agents'.

Such a specific trial, however, was never conducted. While the world debates whether the rofecoxib story deserves a full congressional review, it is timely to focus on the importance of post-marketing adverse drug reaction (ADR) reporting and to evaluate the 'signal' generating capabilities of the WHO global ADR database which is accessible to 86 member countries (See pages 13–18).

The risk of cardiovascular adverse reactions and rofecoxib was discussed in early November 2000, at the 23rd Annual Meeting of National Centres in the WHO Programme for International Drug Monitoring. It was pointed out that within 10 months of its introduction in 2000, there had been eight reports of cardiovascular problems with four fatalities associated with rofecoxib use in the Netherlands; all four cases occurred within four days of commencing therapy and one case occurred two hours after taking the first tablet. It also came to light that there had been one report of a fatality in Malaysia, three reports of cardiac failure in Australia and a total of five reports with various cardiovascular events in Portugal.

COX-2 inhibitors and cardiovascular events were also discussed during the 25th and 27th Annual National Centre Meetings. Data from the New Zealand Intensive Medicines Monitoring Programme (IMMP) database demonstrated a higher proportion of prothrombotic events and a shorter time to onset of death associated with the use of COX-2 inhibitors than with comparator drugs (that is, all other drugs in the IMMP cohorts for the proportion of prothrombotic events and versus omeprazole in the survival analysis). The only identifiable difference to explain the shorter survival was the higher rate of myocardial infarction and stroke. A cohort of 32 630 patients on celecoxib (mean age 63 years) and 26 666 on rofecoxib (mean age 58 years) was reviewed; ischaemic heart disease was the fourth most common type of event reported for celecoxib and rofecoxib. Of note, there was no difference in rate between the two but celecoxib had twice the rate of cardiac dysrhythmias. Deaths were most commonly represented in the cardiovascular system organ classification for celecoxib and the second most common for rofecoxib.

The WHO Collaborating Centre for International Drug Monitoring uses the BCPNN (Bayesian Confidence Propagation Neural Network) data mining method, to assess disproportionality of a specific ADR-drug combination against the ADR distribution for all drugs in the global ADR database. Of interest is an analysis (4) using the BCPNN method comparing the renal-related adverse drug reactions between rofecoxib and celecoxib, based on ADR reports in the WHO global database at the end of the second quarter of 2000. They concluded that rofecoxib had greater renal toxicity than celecoxib and other traditional NSAIDs; and that this negative renal impact may have the potential to increase the risk for serious cardiac and/or cerebrovascular events.

A similar analysis of the WHO global database for celecoxib has also shown an association of myocardial infarction with celecoxib use (5). Results of analyses are transmitted to the parties concerned for feedback. Sulfonamide reaction terms were reported significantly more frequently with celecoxib compared to rofecoxib in the WHO database (overall sulfonamide relative reporting rate 1.8, 95% CI 1.6-1.9) (6). Amongst these type of reactions, fatal reactions were reported 80% more often (relative reporting rate 1.8, 95% CI 0.9-4.0). These observations, as well as the recent experience with rofecoxib should caution us against dismissing the findings with celecoxib, particularly amidst concerns that the cardiovascular effects of rofecoxib may be a class effect, applicable to all COX-2 selective inhibitors (6, 7).

Extracted from Drugs of Current Interest, WHO Pharmaceutical Newsletter, Number 5, 2004 on http://www.who.int/medicines/

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Telithromycin and warfarin: suspected interaction

Telithromycin (Ketek®) is a novel antimicrobial that belongs to a new chemical family, the ketolides (1). Ketolides are recent additions to the macrolide-lincosamide streptogramin class and are designed to treat macrolide-resistant respiratory tract pathogens (2, 3). The Ketek® product monograph states that, in a study involving healthy volunteers, there were no pharmacodynamic or pharmacokinetic effects on racemic warfarin (1).

From 29 May 2003 (the date of marketing in Canada) to 15 September 2004, Health Canada received 25 reports of suspected adverse reactions involving telithromycin. Telithromycin is metabolized by cytochrome P450 3A4 (CYP3A4) and to a lesser extent by cytochrome P450 1A (CYP1A) (1). Warfarin exists as a racemic mixture of R- and S-warfarin. The S-isomer, metabolized by CYP2C9, is primarily responsible for the hypoprothrombinaemic activity. The R-isomer, metabolized by CYP1A2 or CYP3A4, is less pharmacologically active than the S-isomer, but significant drug interactions have resulted from inhibition of its metabolism (2, 4). Telithromycin is a substrate and inhibitor of CYP3A4. Its concentrations may be increased with concomitant administration of CYP3A4 inhibitors (e.g., ketoconazole), and telithromycin will increase the concentrations of other drugs metabolized by CYP3A4 (5). Antibiotics have been reported to decrease the intestinal flora that produce vitamin K, reduced concentrations of which impair prothrombin production. Also, genetics, age, diet (e.g., vegetables rich in vitamin K), fever, stress and concomitant medication could modify the metabolism of warfarin and affect the intensity of the resulting interaction (6).

Although it has been stated that telithromycin does not interact with warfarin, (1, 7) the prothrombin time and INR should be monitored closely, (2) especially in elderly patients, as should be the case whenever a new drug is started in a patient taking warfarin.

Extracted from Canadian Adverse Reaction Newsletter, Volume 15, Issue 1, January 2005.

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Penicillin: information strengthened

The manufacturer of penicillin G benzathine and penicillin G procaine (Bicillin®) products has advised of important labelling changes to ensure that these products are used and administered appropriately.

Penicillin G benzathine injectable suspension is the only currently approved penicillin G benzathine product indicated for use in sexually transmitted infections, including syphilis. Conversely, penicillin G benzathine and penicillin G procaine injectable suspension, which consists of a combination of penicillin G benzathine and penicillin G procaine in an injectable suspension, should not be used for the treatment of syphilis.

The Centers for Disease Control (CDC) 2002 Sexually Transmitted Diseases Treatment

Guidelines (1) recommend penicillin G benzathine for the treatment of syphilis infection. However, postmarketing reports from multiple STD clinics in the US show that Bicillin® C-R has been inappropriately used to treat patients infected with syphilis. Bicillin® L-A is the only currently approved penicillin G benzathine product indicated for the treatment of syphilis

Reference: Communication from King Pharmaceuticals, Inc. December 10, 2004 available at http://www.fda.gov/medwatch

Linezolid and neuropathy

Linezolid (Zyvoxam®), a synthetic antibacterial agent in a new class of antibiotics, the oxazolidinones, has been marketed in Canada since 2001 (1). Linezolid is active against methicillin- and vancomycin-resistant Gram-positive microorganisms (2).

The safety and efficacy of linezolid given for longer than 28 days has not been evaluated in controlled clinical trials (1). Dosage and administration guidelines recommend that treatment lasts no more than 28 consecutive days (1) but in view of its activity against resistant organisms, linezolid has been used in clinical practice for longer than the recommended treatment course (2). The long-term use of linezolid has been associated with severe peripheral and optic neuropathy (2–4). In most cases, the optic neuropathy resolved after stopping the drug, but the peripheral neuropathy did not (4).

Neuropathy (peripheral or optic) has rarely been reported in patients treated with linezolid and has primarily occurred in patients treated for more than the maximum recommended duration of 28 days. Myelosuppression including anaemia is listed in the product monograph under warnings and postmarketing experience (1). Pure red-cell aplasia is not listed in the product monograph. Health care professionals should be aware of the potential for adverse reactions when linezolid is used beyond its recommended duration (2).

Extracted from Canadian Adverse Reaction Newsletter, Volume 15, Issue 1, January 2005.

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Ceftriaxone and immune haemolytic anaemia in children

Ceftriaxone (Rocephin®), marketed in Canada since 31 December 1987, is a third-generation cefalosporin indicated for the treatment of susceptible strains of bacteria, as well as for prophylaxis against infections in patients undergoing hysterectomy, coronary artery bypass surgery or biliary tract surgery (1). Immune haemolytic anaemia (IHA) is a hypersensitivity adverse reaction (AR) known to occur in adults and children. The Rocephin® product monograph describes autoimmune haemolytic anaemia as a rare AR (< 0.1% of cases), (1) but does not mention IHA.

Ceftriaxone antibodies appear to be induced by an immune complex mechanism during a sensitization phase after initial exposure to the drug (2). Intravascular haemolysis may be triggered after subsequent re-exposure. The signs and symptoms of drug-induced IHA include severe haemolytic anaemia, haemoglobinuria, hypotension, acute renal failure, fever and back pain (3).

Health Canada has received 1 report of acute haemolysis suspected of being associated with ceftriaxone. A young child with sickle cell disease had been given a single dose of ceftriaxone (80 mg/kg body weight) intravenously for fever and cough, and within 30 minutes developed a rash, pallor and decreased level of consciousness. Laboratory examination showed a positive direct Coomb's test result, a haemoglobin level of 7 g/L (the pre-infusion level was 110 g/L) and haemolysed red blood cells. The following day, the patient died despite resuscitation attempts. The only concomitant medication was a single oral dose of erythromycin. The patient had been exposed to ceftriaxone in the past.

Nine paediatric cases of IHA associated with exposure to ceftriaxone were identified in the literature, 6 of which were fatal (4–12). One child with sickle cell anaemia received ceftriaxone on several occasions and experienced 6 episodes of unexplained transient haemoglobinuria before the onset of the IHA (10).

Drug-induced IHA is associated with a high mortality rate (3). Other than supportive care and red blood cell transfusion, there are few effective treatment options. Reintroduction of the drug is contraindicated because of the high risk of recurrence of haemolysis, which is often more severe (3).

IHA associated with ceftriaxone is rare and has been reported to occur with repetitive, intermittent use of this drug. Children with underlying conditions such as haemoglobinopathies and immunodeficiencies are likely to require frequent treatment or prophylaxis with ceftriaxone, which may place them at increased risk of IHA. The development of signs and symptoms of IHA, including haemoglobinuria or unexplained anaemia, should prompt health care professionals to consider this diagnosis and the discontinuation of the suspect drug (3).

Extracted from Canadian Adverse Reaction Newsletter, Volume 15, Issue 1, January 2005.

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Ethinylestradiol/cyproterone: increased risk of thrombosis

The Norwegian Medicines Control Agency (NMCA) has issued a reminder that ethinylestradiol/cyproterone (Diane®) is associated with an increased risk of thrombosis. The NMCA has received 26 reports of adverse reactions associated with ethinylestradiol/ cyproterone, including 15 of venous thrombosis, two of which were fatal. They note that ethinylestradiol/ cyproterone should be used with the same caution as oral contraceptives, and should not be used in women with known risk factors for thrombosis, such as smoking, obesity and a personal or family history of thrombosis.

Reference: *Nytt om Legemidler*, 11 August 2004. Available at http://www. legemiddelverket.no

Influenza virus vaccine: interactions

Prescribers are advised to be alert for signs of toxicity following influenza vaccination in patients receiving anti-epileptic drugs or warfarin. Medsafe, New Zealand, notes that, in addition to published reports of warfarin, phenytoin and theophylline toxicity following influenza vaccination, a report of carbamazepine toxicity has been received by the Australian Adverse Drug Reactions Advisory Committee, and a report of elevated international normalized ratio (INR) in a patient receiving warfarin has been received by the Centre for Adverse Reactions Monitoring, both following influenza vaccination. It is sug-

gested that inhibition of cytochrome P450 3A4 may be involved in these interactions, and prescribers are advised to watch for toxicity in patients receiving drugs metabolized by this enzyme.

Reference: *Prescriber Update* **25**(2), November 2004.

Rosiglitazone and pioglitazone: dangers of off–label use

Rosiglitazone and pioglitazone are contraindicated in combination with insulin and in patients with cardiac failure or a history of cardiac failure Evidence from United Kingdom reports and usage data indicate that rosiglitazone and pioglitazone are being prescribed in combination with insulin, despite this being a contraindication for both products.

These data also indicate that thiazolidinediones are being prescribed in patients with cardiac failure, which is a contraindication with rosiglitazone and pioglitazone. This off-label use of glitazones may be causing or aggravating cardiac failure.

Diabetes is in itself a strong risk factor for congestive heart failure (CHF) (1). Since 2000, approximately 120 000 patients have received a prescription for rosiglitazone and 33 000 patients for pioglitazone in the United Kingdom (2). Seven spontaneous reports of cardiac failure or oedema or both in patients receiving either rosiglitazone or pioglitazone in combination with insulin have been received. In addition, 12 reports of aggravated cardiac failure in association with the use of these agents have been received. Due to higher levels of patient exposure, rosiglitazone has been associated with the greatest number of spontaneous reports.

Prescribers are reminded that rosiglitazone and pioglitazone should not be used:

- In patients with cardiac failure or a history of cardiac failure (NYHA stages I to IV).
- In combination with insulin.

Extracted from: Committee on Safety of Medicines, Current Problems in Pharmacovigilance, Volume 30, October 2004.

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Naproxen and celecoxib suspended in Alzheimer prevention trial

The National Institutes of Health (NIH) has announced that investigators have suspended the use of two drugs, naproxen (220 mg twice a day) and celecoxib (200 mg twice a day), in a large, three-arm, national Alzheimer disease prevention trial sponsored by the National Institute on Aging (NIA), a part of the NIH. The trial, called the Alzheimer Disease Anti-Inflammatory Prevention Trial (or ADAPT) was designed to assess the potential benefit of long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) — naproxen (Aleve®) and the COX-2 inhibitor celecoxib (Celebrex®) in decreasing the risk of developing Alzheimer disease in people 70 years of age or older who were considered to be at increased risk because of family history, but did not have symptoms of the disease.

Approximately 2400 volunteer participants were randomly assigned to receive naproxen, celecoxib, or placebo for periods of time up to three years. Although no significant increase in risk for celecoxib was found in this trial, the use of these drugs in the study was suspended in part because of findings reported last week from a National Cancer Institute (NCI) trial to test the effectiveness of celecoxib in preventing colon cancer. In addition, however, data from the ADAPT trial indicated an apparent increase in cardiovascular and cerebrovascular events among the participants taking naproxen when compared with those on placebo.

The ADAPT trial began in 2001 and was conducted at six sites across the USA. Investigators and NIH scientists will continue to review this and other studies. The cancer prevention trials and the ADAPT study are among the first long-term, clinical trials to test these classes of drugs. These studies are examining these compounds for uses very different from the uses for which these medications are currently approved.

Reference: NIH News, 20 December 2004. http://www.nih.gov

Heparin contraindicated in severe renal impairment

Monitoring anti-factor Xa activity may be helpful in patients receiving low molecular weight heparins (LMWH) who are at risk of bleeding or are actively bleeding.

Bemiparin, certoparin, dalteparin, enoxaparin, reviparin and tinzaparin are LMWHs. Some are indicated for the prevention of venous thromboembolism and some are indicated for the treatment of deep venous thrombosis, pulmonary embolism, unstable coronary artery disease and for the prevention of clotting in extracorporeal circuits.

Prescribers are reminded of the need to refer to individual product information regarding indications, cautions, appropriate dose-adjustment and contraindications in patients with risk factors for bleeding, such as renal or hepatic impairment. Severe renal impairment is a contraindication for use of reviparin and certoparin, whereas caution is advised for dalteparin and bemiparin and dose reduction should be considered for tinzaparin.

The Marketing Authorization holder for enoxaparin has issued new prescribing advice on dose reduction in severe renal failure. Careful clinical monitoring is advised for mild/moderate renal impairment. Anti-Factor Xa monitoring is not normally required, but may be considered in those patients treated with LMWH who also have either an increased risk of bleeding (such as those with renal impairment, elderly and extremes of weight) or are actively bleeding.

Reference: Committee on Safety of Medicines. *Current Problems in Pharmacovigilance,* **30**. 10 (2004).

Flucloxacillin: serious hepatic disorders

Flucloxacillin treatment is very rarely associated with an increased risk of hepatic disorders, namely, hepatitis and cholestatic jaundice. In some patients, almost always those with serious underlying disease, these adverse reactions have been fatal.

The United Kingdom Committee on Safety of Medicines (CSM) has previously advised that:

- The onset of hepatic reactions may be delayed for several weeks (up to 2 months) after treatment with flucloxacillin has stopped.
- These reactions are related neither to the dose nor to the route of administration of flucloxacillin.
- Risk factors include treatment for more than two weeks and increasing age.

Prescribers are reminded that:

- Flucloxacillin should not be used in patients with a history of flucloxacillin-associated jaundice or hepatic dysfunction.
- Flucloxacillin should be used with caution in patients with evidence of hepatic dysfunction.
- Careful enquiry should be made concerning previous hypersensitivity reactions to ß-lactams.

Extracted from: Committee on Safety of Medicines, Current Problems in Pharmacovigilance, Volume 30, October 2004.

Reference

1. Committee on Safety of Medicines *Current Problems in Pharmacovigilance,* **35**: 2. (1992).

Bevacizumab and arterial thromboembolic events

The manufacturer of bevacizumab (Avastin®) has drawn attention to an increased risk of arterial thromboembolic events associated with the use of bevacizumab in combination with chemotherapy. These events included cerebral infarction, transient ischemic attacks (TIAs), myocardial infarctions (MI), angina, and a variety of other arterial thromboembolic events. Some of these events were fatal.

The risk of these events should be viewed in the context of bevacizumab's ability to improve overall survival in patients with metastatic colorectal cancer (median survival 20.3 vs.15.6 months). Bevacizumab should be discontinued in patients developing severe arterial thromboembolic events during treatment.

In randomized, active-controlled studies, the overall incidence of arterial thromboembolic events was increased with the use of bevacizumab in combination with chemotherapy. The incidences of both cerebrovascular arterial events and cardiovascular arterial events were increased. In addition, there was a correlation between age (65 years and over) and the increase in risk of thromboembolic events.

The clinical benefit of bevacizumab, as measured by survival in the two principal arms, was seen in all subgroups tested. The subgroups examined were based on age, sex, race, ECOG performance status, location of primary tumour, prior adjuvant therapy, number of metastatic sites, and tumour burden.

Reference: Communication from Genentech on http://www..fda.gov/medwatch/ 5 January 2005.

Amiodarone toxicity concerns

The US Food and Drug Administration (FDA) has required pharmacists and other health care professionals who dispense medication to distribute Medication Guides to patients for certain products, including amiodarone tablets.

Amiodarone HCl (Cordarone®) is intended for use only in patients with indicated life-threatening arrhythmias because its use is accompanied by substantial toxicity. Amiodarone has several potentially fatal toxicities, the most important of which is pulmonary toxicity (hypersensitivity pneumonitis or interstitial/alveolar pneumonitis) that has resulted in clinically manifest disease at rates as high as 10 to 17% in some series of patients with ventricular arrhythmias given doses around 400 mg/day, and as abnormal diffusion capacity without symptoms in a much higher percentage of patients. Pulmonary toxicity has been fatal about 10% of the time.

Liver injury is common with amiodarone, but is usually mild and evidenced only by abnormal liver enzymes. Overt liver disease can occur, however, and has been fatal in a few cases. Like other antiarrhythmics, amiodarone can exacerbate the arrhythmia. Although the frequency of such proarrhythmic events does not appear greater than with many other agents used in this population, the effects are prolonged when they occur. Even in patients at high risk of arrhythmic death, in whom the toxicity of amiodarone is an acceptable risk, amiodarone poses major management problems that could be life-threatening in a population at risk.

Patients with the indicated arrhythmias must be hospitalized while the loading dose of amiodarone is given, and a response generally requires at least one week, usually two or more. Because absorption and elimination are variable, maintenance-dose selection is difficult, and it is not unusual to require dosage decrease or discontinuation of treatment. Attempts to substitute other antiarrhythmic agents when amiodarone must be stopped will be made difficult by the gradually, but unpredictably, changing amiodarone body burden. A similar problem exists when amiodarone is not effective; it still poses the risk of an interaction with whatever subsequent treatment is tried.

Reference: Communication from Wyeth dated 30 December 2004 on http://www.wyeth.com. and http://www..fda.gov/medwatch/

Darbepoetin alfa: adverse outcomes

Darbepoetin alfa (Aranesp®) is indicated for the treatment of chemotherapy-induced anaemia in patients with nonmyeloid malignancies. The manufacturer has updated the safety information to reflect results from two recent investigational studies with other erythropoietic products, epoetin alfa and epoetin beta conducted outside the USA, where patients with cancer were treated to higher haemoglobin target levels beyond the correction of anaemia in those patients. These studies permitted or required dosing to achieve haemoglobin levels of greater than 12 grams per deciliter. An increased frequency of adverse patient outcomes, including increased mortality and thrombotic vascular events were reported in these studies.

The prescribing information of darbepoetin alfa has been revised to include a warning for thrombotic events and increased mortality and the precautions to include tumour growth factor potential. The manufacturer continues to recommend that target haemoglobin should not exceed 12 grams per deciliter in men or women as indicated in the prescribing information.

Reference: Communication from Amgen on http://www.amgen.com and the Medwatch website at http://www.fda.gov/medwatch. 11 January 2005.

Spontaneous monitoring systems are useful in detecting signals of relatively rare, serious and unexpected adverse drug reactions. A signal is defined as "reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously. Usually, more than a single report is required to generate a signal, depending upon the seriousness of the event and the quality of the information". All signals must be validated before any regulatory decision can be made.

Vaccines and Biomedicines

Latest developments in biological standardization

The WHO Expert Committee on Biological Standardization met in Geneva from 15 to 18 November 2004 to establish biological reference standards (International Standards), guidance on production and quality control of biological products, and to develop international scientific consensus on quality, safety and efficacy issues.

The following summary provides an overview of the decisions made by the Committee. A full report of the meeting is in preparation and will be published in the WHO Technical Report Series. In the meantime, pre-publication versions of the documents have been posted onto the WHO website to provide early notice of their content (www.who.int/biologicals).

International Reference Standards

A list of new or replacement reference standards is given in Table 1 overleaf.

Recommendations and guidelines

The Committee recommended establishment of the following two documents:

Guidelines on the production and quality control of candidate live tetravalent attenuated dengue vaccines

The guidelines were developed in response to interest from many countries. The scope of the guideline covers candidate live attenuated tetravalent dengue vaccines with active clinical trials in progress. Extensive testing has been conducted to define the attenuation phenotype for each of the vaccine candidates. Vaccine formulations are being developed based on the optimal degree of attenuation and immunogenicity. Another type of vaccine candidate has been generated from molecular clones of each of the four dengue virus serotypes. In addition, chimeric vaccine candidates are being prepared for clinical development. A combination tetravalent vaccine is currently under development which will have all four dengue serotypes, represented as chimeric dengue-yellow fever vaccines.

Issues that were considered included specifications for:

- Vaccines developed in Vero cells, in primary dog kidney cells and in fetal rhesus lung diploid cells.
- Plaque or focus-forming assay specified for infectivity determinations in a tetravalent mixture. Since candidate titration standards do not exist at present, WHO was advised to consider developing such reagents and their subsequent characterization by international collaborative study.
- Stability study programmes to determine the thermal stability of the tetravalent final freeze-dried product and the stability of the liquid vaccine after reconstitution. It was noted that in some countries stability testing of intermediates is required. WHO was requested to develop further guidance on this issue.
- An accelerated degradation test to each new batch of vaccine to show the consistency of manufacture of the final stabilized formulation.
- Vectored vaccines where reference to general WHO principles for vaccines for human use derived by molecular methods, that are under development, should be considered.
- Nonclinical testing of candidate dengue vaccines where in addition to the conventional procedures the issue of antibody-mediated disease enhancement should be addressed.

During discussion, the Committee emphasized that these guidelines cover only candidate vaccines since, so far, no vaccine has been licensed.

Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revision)

Biological reference standards form the basis of regulation and clinical dosing for biological medicines, and also for regulation of *in vitro* diagnostic devices. The process whereby such

international biological reference standards are established and the technical specifications to which they comply are set out in a written standard, which is intended to be scientific and advisory in nature.

WHO has worked with the scientific community, national regulatory authorities, other standards setting bodies and users through a series of consultations to review the scientific basis of characterization of biological reference materials. As a result, the concepts used by WHO for biological standardization have been re-affirmed as appropriate to ensure the continued usefulness of this class of reference materials. During the consultation process it was recognized that improved clarity in explaining the rationale for the principles used by WHO in biological standardiza-

Table 1. New or Replacement Standards established by the Committee.

Additions		
Antigens and related substances		
Poliomyelitis vaccine, oral	7.51 $\log_{10}$ $TCID_{50}$/ml poliovirus type 1 6.51 $\log_{10}$ $TCID_{50}$/ml poliovirus type 2 6.87 $\log_{10}$ $TCID_{50}$/ml poliovirus type 3 7.66 $\log_{10}$ $TCID_{50}$/ml total poliovirus content	Second International Standard 2004
Pertussis serotype 2, typing serum	No assigned value	First Reference Reagent 2004
Pertussis serotype 3, typing serum	No assigned value	First Reference Reagent 2004
Blood products and related substances		
Anti-D blood grouping reagents (monoclonal)	No assigned value	First International Standard 2004
Factor V Leiden, human	No assigned value	First International Genetic Reference Panel 2004
Factor XIII, plasma, human	0.91 IU/ampoule	First International Standard 2004
Immunoglobulin, intravenous:		
anti-D positive control	No assigned value	First Reference Reagent 2004
anti-D negative control	No assigned value	First Reference Reagent 2004
These substances are held and distributed by the International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Herts. EN6 3QG, England		
Disestablishment		
Antigens and related substances		
Hepatitis B vaccine, plasma derived	No assigned value	First International Reference Reagent

tion would be of benefit. Accordingly a revised version of the 1990 WHO guidance was prepared.

In addition the consultative process has revealed a need for continued scientific and capacity building work in the area of biological standards. Thus the Committee also recommended that WHO considers starting or continuing work specifically on:

- predicting and monitoring the stability of biologicals;
- developing specific training modules for biological standardization, with the collaboration of the WHO Global Training Network; and
- developing a manual to describe in detail calibration procedures for secondary standards.

Consolidated list of WHO Recommendations and Guidelines

Recommendations and guidelines published by WHO are scientific and advisory in nature, but they may be adopted by a national regulatory authority as national requirements, and are also intended to provide guidance to those responsible for the production of biologicals or those who may have to decide upon appropriate methods of testing, assay and control in order to ensure the quality, safety and efficacy of products.

A consolidated list of WHO Recommendations and Guidelines, together with a list of a variety of other documents produced by the WHO biological standardization programme has now been reviewed and is available on the WHO website at: www.who.int/biologicals

Regulatory Action

Northern hemisphere influenza vaccine composition 2005/2006

World Health Organization — The following influenza virus vaccine composition has been recommended for the forthcoming winter in the northern hemisphere.

- an A/New Caledonia/20/99(H1N1)-like virus;
- an A/California/7/2004(H3N2)-like virus;
- a B/Shanghai/361/2002-like virus (currently used vaccine viruses include B/Shanghai/361/2002, B/Jilin/20/2003 and B/Jiangsu/10/2003).

As in previous years, the national control authorities should approve the specific vaccine viruses used in each country. National public health authorities are responsible for recommendations regarding the use of the vaccine.

Reference: *Weekly Epidemiological Record*, **80**: 71–75 (2005).

Valdecoxib: severe skin reactions and cardiovascular risk

United States of America — The Food and Drug Administration (FDA) has announced important new information on side effects associated with the use of valdecoxib (Bextra®), a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID) which is indicated for the treatment of osteoarthritis, rheumatoid arthritis and dysmenorrhoea (menstrual pain). A boxed warning, strengthening previous warnings about the risk of life-threatening skin reactions and a new bolded warning contraindicating the use of valdecoxib in patients undergoing coronary artery bypass graft (CABG) surgery will be added to the label.

Serious skin reactions
Patients taking valdecoxib have reported serious, potentially fatal skin reactions, including Steven-Johnson Syndrome and toxic epidermal necrolysis. These skin reactions are most likely to occur in the first 2 weeks of treatment, but can occur any time during therapy. In a few cases, these reactions have resulted in death. The labelling advises that valdecoxib should be discontinued at the first appearance of a skin rash, mucosal lesions (such as sores on the inside of the mouth), or any other sign of allergic reactions. Valdecoxib contains sulfa, and patients with a history of allergic reactions to sulfa may be at a greater risk of skin reactions.

As of November 2004, FDA has received reports of a total of 87 cases in the USA of severe skin reactions in association with valdecoxib.

Cardiovascular risks
In addition to highlighting serious skin reactions, the strengthened label warnings also highlight new data about cardiovascular risks. A recently-completed study conducted by the manufacturer, which included over 1500 patients treated after CABG, showed an increased cardiovascular risk in patients treated with valdecoxib compared to placebo.

Reference: *FDA Talk Paper*, T04-56 . 9 December 2004 http://www.fda.gov/medwatch/index.html .

COX– 2 inhibitors: review plans

European Union — Following the worldwide withdrawal of rofecoxib (Vioxx), the European Medicines Agency (EMEA) has been asked by the European Commission to conduct a review of all cyclo-oxygenase-2 (COX-2) inhibitor medicines. The Agency's scientific committee responsible for human medicines (CHMP) will look at newly available data on all aspects of cardiovascular safety of the COX-2 inhibitors celecoxib, etoricoxib, lumiracoxib, parecoxib and valdecoxib, including thrombotic events (heart attack and stroke) and cardiorenal events (e.g. hypertension, oedema and cardiac failure).

The objective of this review is to assess whether there is a need to make changes to existing marketing authorizations including labelling throughout the whole of the European Union and whether additional studies are needed. When completed, the outcome of the review will be posted on the Agency's website. In the meantime the agency

reminds that the earlier warnings, issued on 6 October 2004, remain valid:

- Rofecoxib has been withdrawn due to serious thrombotic events. Patients on rofecoxib should be reviewed and alternative treatment considered. When considering treatment with other COX- 2 inhibitors, prescribers should consult the latest version of the summary of product characteristics, particularly for cardiovascular events.

- Patients who were taking rofecoxib should consult their doctor at the next available opportunity to discuss their treatment options.

Reference: *EMEA Press Release*, EMEA/117908/2004. Available at: http://www.emea.eu.int

Co–proxamol products withdrawn

United Kingdom — The Committee on Safety of Medicines (CSM) has recently reviewed the risks and benefits of co-proxamol (paracetamol/ dextropropoxyphene). The efficacy of co-proxamol is poorly established and the risk of toxicity in overdose, both accidental and deliberate, is now considered to be unacceptable. Co-proxamol contains a dose of paracetamol (325 mg) that would, on its own, be considered sub-therapeutic, and dextropropoxyphene (32.5 mg) is a weak opioid analgesic that is known to be toxic in overdose. Each year there are 300–400 fatalities following deliberate or accidental drug overdose involving co-proxamol in England and Wales alone.

CSM has therefore advised that:

- In relation to safety, there is evidence that fatal toxicity may occur with a small multiple of the normal therapeutic dose and a proportion of fatalities are caused by inadvertent overdose. Pharmacokinetic and pharmacodynamic interactions with alcohol further reduce the threshold for fatal toxicity.

- There is no robust evidence that efficacy of this combination product is superior to full strength paracetamol alone in either acute or chronic use.

- It has not been possible to identify any patient group in whom the risk-benefit may be positive.

- Co-proxamol products should be withdrawn altogether over the next 6–12 months.

- During the withdrawal phase, interim restrictions and warnings regarding the use of co-proxamol should be introduced to the product information.

Reference: Committee on Safety of Medicines. CEM/ CMO/2005/2, 31 January 2005 at http://medicines.mhra.gov.uk/aboutagency/regframework/csm/csmhome.htm

Cisapride licences voluntarily cancelled

United Kingdom — The marketing authorizations for cisapride-containing products were suspended in July 2000, following advice from the Committee on Safety of Medicines (CSM), due to the risk of cardiac side effects. CSM advised that the balance of risks and benefits was no longer favourable. This advice was communicated to UK health professionals (1).

Following this suspension, a Europe-wide review of the risks and benefits of cisapride took place under the auspices of the Committee for Proprietary Medicinal Products (CPMP). On the advice of the CPMP, the European Commission decided that cisapride-containing products should be maintained within Europe but with restricted indications in adults and children after failure of other treatment options. A condition was that all patients treated with cisapride should be enrolled in either a clinical safety study/registry or a clinical trial to evaluate efficacy.

The UK marketing authorizations remained suspended until October 2003, when the licence holder voluntarily decided not to implement the EU decision in the UK. Instead they have decided to cancel all existing UK marketing authorizations.

References

1. Committee on Safety of Medicines. *Current Problems in Pharmacovigilance,* **26**: 9 (2000).

2. Committee on Safety of Medicines, *Current Problems in Pharmacovigilance*, **30**: (2004).

Tentative approval for generic co–packaged antiretrovirals

United States of America — The Food and Drug Administration (FDA) has announced the tentative approval of a co-packaged antiretroviral drug regimen manufactured in South Africa for the treatment of HIV-1 infection in adults. The agency's tentative approval means that although

existing patents and/or exclusivity prevent US marketing of this product, it meets FDA's quality, safety and efficacy standards for US marketing.

This action is the first tentative approval of an HIV drug regimen manufactured by a non-US-based generic pharmaceutical company. Once the marketing application was made on 13 January 2005, FDA completed its review within two weeks.

The tentatively approved regimen consists of co-packaged lamivudine/zidovudine fixed-dose combination tablets and nevirapine tablets. Lamivudine/zidovudine fixed dose combination tablets are a version of the already approved Combivir® tablets and Viramune® tablets. The new co-packaged product consists of two tablets (lamivudine/zidovudine and nevirapine) each to be taken twice daily, after the initial two-week initiation phase of a nevirapine regimen.

More information on HIV and AIDS is available online at FDA's website: http://www.fda.gov/oashi/aids/hiv.html.

Reference: *FDA News,* P05-02. 25 January 2005

Natalizumab approved for multiple sclerosis

United States of America — The Food and Drug Administration (FDA) has licensed a new biologic approach to treat patients with relapsing forms of multiple sclerosis (MS) to reduce the frequency of symptom flare-ups or exacerbations of the disease. Natalizumab (Tysabri®) is a monoclonal antibody bioengineered from part of a mouse antibody to closely resemble a human antibody.

Although the cause of MS is unknown, it is widely considered to be an autoimmune disease in which the person's immune system attacks the brain and/or spinal cord. Natalizumab appears to work by binding to these immune system cells, thus preventing them from travelling to the brain where they can cause damage.

The approval is based on positive results seen in patients after one year of treatment. This product received accelerated approval because it appears to provide substantial benefit for patients with a serious disease. As part of that approval, the manufacturer has committed to continuing its trials of this product for another year.

The most frequently reported serious adverse reactions in clinical trials were infections, including pneumonia, temporary hypersensitivity reactions (such as rash, fever, low blood pressure, and chest pain), depression, and gallstones. These serious adverse reactions were uncommon. Common adverse reactions were generally mild and included non-serious infections (such as urinary tract, lower respiratory tract, GI system, and vaginal infections), headache, depression, joint pains, and menstrual disorders.

Reference: *FDA News,* P04-107. November 2004

Pegaptanib for age-related macular degeneration

United States of America — The Food and Drug Administration (FDA) has approved pegaptanib sodium injection (Macugen®), a new therapy to slow vision loss in people with the eye disease neovascular (wet) age-related macular degeneration (AMD). Pegaptanib is a selective vascular endothelial growth factor (VEGF) antagonist.

AMD, a retinal disease causing severe and irreversible vision loss, is a major cause of blindness in individuals older than 55 years. Untreated, the majority of eyes affected with wet AMD may become functionally blind. Wet AMD, which makes up approximately 10 percent of AMD, is caused by the growth of abnormal leaky blood vessels that eventually damage the area of the eye responsible for central vision, which is essential for most fine detail visual activities, including reading, driving, and recognizing faces.

The safety and efficacy of pegaptanib was studied in two trials in patients with wet AMD for two years. Patients exhibited a significant decrease in vision loss in both trials. Serious adverse events related to the injection procedure included infections, retinal detachment, and traumatic cataract. Other frequently reported adverse events in patients treated with pegaptanib were eye irritation, eye pain, haemorrhage under the outer membrane of the eye (conjunctiva), and blurred vision.

Reference: *FDA News*, P04-110. 20 December 2004

Amphetamine salts suspended

Canada — Health Canada has suspended marketing of amphetamine salts (Adderall XR®) for attention deficit hyperactivity disorder (ADHD) with effect 9 February 2005.

The decision to withdraw amphetamine salts is founded on very rare, international, spontaneous reports of sudden deaths in paediatric and adult patients. Reports for death include those for patients taking usual recommended doses, including recommended starting doses. In a minority of cases, the events occurred on the first day of dosing or shortly after an increase in dose or a switch from another drug in the structural class. Deaths were reported for patients both naïve or chronically exposed to amphetamine-related central nervous system stimulants. The decision was not based on reported deaths that were associated with overdose, misuse or abuse.

Of the 20 reported deaths, there were cases that occurred in patients without a documented history of structural or other cardiac abnormalities/ disease. In a few cases, other drugs, including antidepressants, clonidine and/or anti-psychotics, were concomitant medications. Exercise was an associated event in some of the reports of death. None of the reported deaths occurred in Canada.

Health Canada has requested manufacturers of other stimulants approved for the treatment of ADHD to provide a thorough review of their worldwide safety data. Information updates will be provided as they become available.

Reference: Communication dated 9 February 2005 from Health Canada at http://www.hc-sc.gc.ca

Tolcapone: return to market

United Kingdom —Tolcapone (Tasmar®) is a catechol-*O*-methyltransferase (COMT) inhibitor medicine developed for use in Parkinson disease.

In February 1999, the Committee on Safety of Medicines reported the withdrawal of tolcapone following serious hepatic reactions and neuroleptic malignant syndrome (NMS) (1). At its April 2004 meeting, the European Committee for Proprietary Medicinal Products recommended lifting the suspension of the marketing authorization for tolcapone. This followed review of the available clinical evidence including evidence of increased efficacy for tolcapone over entacapone in the control of motor fluctuations in patients with advanced Parkinson disease.

Tolcapone will be made available with the restricted indication in patients "who failed to respond to or are intolerant of other COMT inhibitors".

The following conditions will be applied to use of tolcapone :

- More frequent liver function monitoring and closer attention to the monitoring of possible signs and symptoms of underlying liver disease.
- Contraindication in patients with severe dyskinesia or with a previous history of NMS.
- Restriction on prescribing to physicians experienced in the management of advanced Parkinson's disease.

The safety of tolcapone will be closely monitored.

1. Committee on Safety of Medicines, *Current Problems in Pharmacovigilance*, **25**: 2 (1999).

Patient reporting and public access to safety data

United Kingdom — Patients and researchers will be able to access data on the safety of different medicines as a move to further improve the drug side effect reporting system — the Yellow Card Scheme — used to monitor the safety of medicines in the United Kingdom.

The Medicines and Healthcare products Regulatory Agency (MHRA) will publish anonymous data on suspected adverse drug reactions on their website. Researchers will also be able to access more detailed data and measures will be put in place to prevent potential abuse of the information. Every request will be reviewed by an independent committee to make sure it is ethically and scientifically sound and protects patient confidentiality.

A first pilot phase of a project for patient reporting of unexpected effects of drugs to the regulator were also launched. Forms to report unexpected drug reactions will be available in 4000 physician surgeries across the UK and patients will also be able to make reports online.

The Yellow Card System is recognized as one of the best spontaneous reporting schemes for adverse drug reactions in the world. Expansion of the scheme nationally is planned for later in the year.

The new measures are key recommendations made by experts who reviewed the yellow card scheme last year and a public consultation.

Reference: Medicines and Healthcare products Regulatory Agency. 2005/0015. 17 January 2005. http://www.yellowcard. gov.uk

Australia and Canada agree mutual recognition

The Governments of Australia and Canada have signed a Mutual Recognition Agreement (MRA) that enables both countries to accept each other's good manufacturing practice (GMP) audits and inspection of the makers of prescription and over the counter medicines. GMP regulation is the cornerstone of ensuring that medicines on the market have been manufactured to the highest standards of safety and efficacy. The agreement will enable access to the latest products assessed to best regulatory standards in as short a timeframe as possible. The MRA will allow the manufacturer's batch certifications to be recognized by the other party without re-analysis at the point of import.

The Therapeutic Goods Administration (TGA) of Australia has signed a number of multi-sectoral MRAs over the past few years, including with the European Community, the European Free Trade Association and Singapore. The TGA also has a cooperative arrangement with the US Food and Drug Administration on GMP inspections, recalls, adverse product trends, health hazard evaluations and alert system information.

The MRA reflects the significant growth in pharmaceutical exports. The total trade between Australia and Canada in regulated medicines for human use was more than $67 million in 2004. Australian imports from Canada amounted to more than $17 million in 2004 while in the same year Australian exports to Canada were some $50 million. By reducing regulatory costs and duplication, the MRA will significantly speed up the process of getting life saving drugs onto the market of both countries.

Reference: TGA Press statement. CP013/05. 16 March 2005. http://www.tga.gov.au

Didanosine–tenofovir interaction: safety concerns

France — The Agency for Health Products Safety (AFSSAPS), in collaboration with the European Medicines Agency (EMEA), has carried out a review of available data with regard to reporting of increased reactions and lack of efficacy following concomitant use of two antiretrovirals, didanosine (Videx®) and tenofovir (Viread®).

EMEA has issued the following recommendations:

- Concomitant administration of didanosine and tenofovir is not recommended, in particular among those patients with high viral load and low CD4 cell count.
- rare, sometimes fatal, cases of pancreatitis and lactic acidosis have been reported with co-administration of tenofovir and didanosine.
- If co-administration is considered unavoidable, patients should be closely monitored for efficacy and signs of intolerance or adverse reactions.

References:

1. AFSSAPS Press release. 3 March 2005. http://www.agmed.sante.gouv.fr/htm/10/filltrpsc/indlp3.htm

2. European Medicines Agency. EMEA/62331/2005. 3 March 2005.

Prequalification of Medicines

Improving medicines quality through prequalification

WHO, UNICEF and other organizations are involved in the procurement of pharmaceutical products for use in public health programmes, such as those to combat HIV/AIDS, malaria and tuberculosis. Because low-cost pharmaceutical products of assured quality offer the greatest potential for maximizing the impact of efforts to combat such diseases, the quality of pharmaceutical products has become a major concern at both international and country level. Efforts to accelerate access to pharmaceutical products used in the treatment of these major impact diseases through price negotiation and generic competition have highlighted the fundamental importance of developing a robust quality assurance system for pharmaceutical products and diagnostics. Without a quality assurance system, public health agencies risk sourcing substandard, counterfeit and contaminated pharmaceutical products leading to product complaints and recalls, waste of money and health risk to patients.

WHO Prequalification Project

The prequalification project, set up in 2001, is a service provided by the World Health Organization to facilitate access to medicines for HIV/AIDS, malaria and tuberculosis that meet international standards of quality, safety and efficacy. From the outset, the project was established by WHO, UNAIDS, UNICEF, UNFPA, with the support of the World Bank, as a concrete contribution to the United Nations priority goal of addressing widespread diseases in countries with limited access to quality medicines.

Prequalification by WHO involves assessing data provided by the manufacturer and inspecting sites involved in manufacturing, clinical or bioequivalence testing for compliance with WHO requirements. The coordination of the project, including dossier assessments and site inspections are impartial. Neither the external assessors and inspectors nor the WHO prequalification team are engaged in product procurement, supply, distribution, or policy-making. The manufacturer is responsible for the development of the products and must ensure that a product dossier is submitted and that any sites involved in manufacturing and clinical testing comply with good manufacturing pactice (GMP), good clinical practice (GCP) and good laboratory practice (GLP). In the event that compliance is not confirmed, the manufacturer will be expected to take corrective action and generate new data as required. If the corrective actions are confirmed as satisfactory by WHO, the product – as manufactured at a particular site — will be included in the WHO list of prequalified products (1).

Achievements and challenges

In May 2004, the World Health Assembly requested measures to strengthen the Prequalification Project and to ensure that the prequalification review process and results of inspections and assessment reports of listed products are made publicly available (2). The Prequalification Project has optimized its resources to avoid any backlog in its pipeline. It now takes around 3–4 months from submission of a dossier to prequalification of the product, provided the dossier is complete at the time of submission and the sites comply with WHO requirements. By regulatory standards this is a very short turnaround.

The time taken to produce and submit data however depends on a number of factors, many of which are unrelated to the prequalification process, such as the manufacturer's own resources and prioritizations, time schedules of any partners involved, the time needed to write anyt reports, etc. WHO is not in control of these factors and cannot make decisions in this regard on behalf of the manufacturer. In other words, any time delays are intimately linked to the manufacturer's willingness and ability to take prompt and appropriate action in order to become compliant or to complement deficient dossiers with a minimum of delay.

The prequalification team has been strengthened and additional resources made available. This will enable WHO to convene additional expert assessment meetings in 2005 and thus handle more products and possibly reduce timelines even further. However, quality cannot be as-

sessed, tested or inspected in the final product. It must be built in. This process is fully in the hands of manufacturers. In order to prepare manufacturers from developing countries to apply for prequalification successfully, appropriate training is needed. WHO has unique experience obtained in assessing a great number of generic products from different countries. During 2005, two workshops for local manufacturers and national regulators have been held focusing on GMP and the quality and bioequivalence requirements for the product dossier. The workshop in Malaysia focused on drugs to treat tuberculosis and the other in China on antiretrovirals. Other workshops will follow to satisfy needs. These efforts will develop understanding of what is required to improve the quality, safety and efficacy of products and ensure that only good quality products are supplied. Manufacturers will also gain insight into which areas need improvement and how to comply with international regulatory requirements.

The importance of prequalification has been underscored by different parties (3, 4) and is supported by the international regulatory community as reflected in recent recommendations from the Tenth and Eleventh International Conferences of Drug Regulatory Authorities (5). Indeed, without the contribution and collaboration of over 40 national regulatory authorities that provide expertise in the form of assessors and inspectors, WHO would not be able to maintain such a high level of technical capability.

Immediate outcomes of WHO prequalification include the list of prequalified products for treating priority diseases (see prequalification website at: http://mednet3.who.int/prequal/), harmonization of quality requirements for international procurement and strengthening of collaboration between WHO, other UN agencies, related organizations and drug regulatory authorities. Collaboration and coordination is improved through joint inspections, assessment of product dossiers, training of inspectors and assessors, and making information on prequalification inspections available on the prequalification website (6). Meanwhile, ongoing monitoring, review and updating of quality-related systems and programmes, and more effective networking and information exchange on drug regulatory and drug quality issues are constantly under development.

How to participate in prequalification

Products identified as being of public health significance are listed on the prequalification website under an “invitation for expression of interest”. Products are selected from the respective WHO treatment guidelines and are largely consistent with the WHO Model List of Essential Medicines. Any manufacturer or supplier of such a product is eligible for assessment in the prequalification project and can apply by submitting a product dossier together with the site master file and a sample of the product (7). Products submitted for prequalification should meet WHO requirements as set out below.

Data and information: generic products

For multisource (generic) products, dossiers should meet the specifications as summarized in the guideline for the preparation of a product dossier. Multisource (generic) products must satisfy the same quality standards as those of the originator product. In addition, assurance must be provided that they are clinically interchangeable with the originator products as shown by bioequivalence or relevant clinical data. Information is required as follows (7):

- Details of the product.
- Registration in other countries.
- Active pharmaceutical ingredient(s) – API
  - Properties of the API(s)
  - Sites of manufacture
  - Route of synthesis
  - Specification
    API described in a pharmacopoeia
    API not described in a pharmacopoeia
  - Stability testing
- Finished product.
  - Formulation
  - Sites of manufacture
  - Manufacturing procedure
  - Specifications for excipients (in addition to API)
  - Specifications for finished product
  - Container/closure system(s) and other packaging
  - Stability testing
  - Container labelling
  - Product information
  - Patient information and package inserts
  - Justification for any differences to the product if different to the product authorized in the country of origin.
- Interchangeability.
  - Bio-equivalence study (or other relevant clinical studies).
- Summary of pharmacology, toxicology and efficacy of the product if applicable.

Data and information: innovator products

For innovator products licensed in the USA, European Union or Japan (ICH countries) or country with similarly requirements:

- A WHO-type Certificate of a Pharmaceutical Product issued by the regulatory authority, together with the summary of product characteristics (SPC).
- Assessment report(s) issued by the regulatory authority.
- WHO-type batch certificate from the manufacturer.
- Stability testing data, if the packaging of the product is different from that approved by the drug regulatory authority.
- Arguments and/or data to support the applicability of the certificate(s) in the event that the formulation, strength, or specifications are different from the product for which the WHO-type Certificate of a Pharmaceutical Product was issued.

Assessment of dossiers

Following submission of documentation, a team of WHO appointed assessors drawn from national regulatory authorities will assess the product data. Teams are drawn from both developed and developing authorities, offering a unique opportunity to benefit from each other's expertise and experience. Manufacturers are informed of the outcome of the evaluation and given the opportunity to submit additional information if required. Samples are evaluated and may be sent for independent testing at quality control laboratories.

Inspection and monitoring

Products cannot be prequalified before the manufacturing site has been inspected by a team appointed by WHO and generally includes an inspector from one of the Pharmaceutical Inspection Cooperation Scheme (PIC/S) countries, a WHO representative (with experience and training as a GMP inspector), and an inspector(s) from the drug regulatory authority of the country in which the manufacturing site is located.

If inspection has already been carried out by a stringent regulatory authority, the reports should be submitted to WHO. If these are satisfactory, an inspection may not be necessary. In cases where a manufacturing site was inspected by a stringent regulatory authority, compliance is acceptable on the basis of documentary evidence, which should also include a WHO-type Certificate of a Pharmaceutical Product as issued by the national drug regulatory authority. However, in the event that the product is licensed and manufactured for export purposes only, an inspection by WHO may be required.

Inspections normally take a minimum of three consecutive days and involve verification of all aspects of GMP including premises, equipment, materials, documentation, validation, personnel, production, quality control, HVAC (heating, ventilation, and air conditioning), water systems, utilities, and production and control.

WHO prequalification requirements:

- Product dossier assessment (including quality and bioequivalence parts).
- Inspection of manufacturing site of finished pharmaceutical product for compliance with WHO GMP requirements.
- Assessment of drug master file and inspection of API manufacturing site for compliance with WHO GMP requirements.
- Inspection of the CRO used by the applicant for clinical (bioequivalence) studies for compliance with WHO GCP and GLP requirements.
- Completion of WHOPAR and WHOPIR.

WHO Public Assessment Reports

In May 2004, the World Health Assembly requested WHO to "ensure that the prequalification review process and the results of inspection and assessment reports of the listed products, aside from proprietary and confidential information, are made publicly available" (2). WHO Public Assessment Reports (WHOPAR) are summaries of the assessment of the product data and information as submitted by the manufacturer.

Documents supporting a WHOPAR are requested as part of the initial submission for prequalification. These include package leaflets, summary of product characteristics, labelling and in certain

cases a separate summary of the product's quality, safety and efficacy data. A draft guideline with detailed information to manufacturers on how to submit information for the WHOPAR is available on the prequalification website (6).

WHO Public Inspection Report

A WHO Public Inspection Report (WHOPIR) is a summary of the inspection report covering either a manufacturing site for an active pharmaceutical ingredient (API), a manufacturing site for a finished product (FP), or an organization such as a contract research organization where a bioequivalence or other clinical study has been performed

The WHOPIR is a summary of the findings of inspection, and excludes confidential and proprietary information. It is important to note that the observations contained in the WHOPIRs reflect general findings, and that generally any "non-compliance" will have been corrected. Verification of corrective action is conducted by WHO through a documentation review or a follow-up inspection. Public inspection reports are published on the WHO Prequalification web page. Requests for copies of the complete inspection reports should be made to individual manufacturers.

Re-qualification process

Manufacturers are required to apply for re-qualification of their products three years after prequalification. Re-qualification involves re-assessment of the product data and information as provided in the original product dossier, and re-inspection of the relevant site(s) for compliance with WHO requirements. The following is needed for re-qualification:

Generic (multisource) products

• submission of fully updated information to WHO

List of documents which should be available at the CRO

No	Document
1	Organizational chart
2	Job descriptions
3	Updated and signed CVs of responsible persons
4	Training records and course certificates of responsible persons
5	List of persons who have access to archives
6	List of persons who have access to computer database
7	Specification of data protection methods (virus testing, backup copies)
8	List of clinical laboratory normal ranges
9	Accreditation certificate of clinical laboratory
10	Sample contract forms for investigators
11	Application form for ethics committee approval
12	Adverse event registration form
13	Serious adverse event registration form
14	SOP for monitoring, data verification check-lists for monitor
15	SOP for designing CRF, standard CRF modules
16	Record for biological sample collection times
17	SOP for labelling of samples
18	Record for storage of biological samples (temperature log)
19	List of persons who have access to investigational drugs
20	Drug accountability forms (receipt, storage, randomization, dispensing, administration to subjects, destruction or return to sponsor of remaining medication)
21	SOP for data entry and data validation procedure
22	Documentation (validation) of the pharmacokinetic computer program
23	Documentation (validation) of the statistical computer program
24	Trial report template
25	SOP's for calibration and quality control of the apparatus. (Depends on apparatus0
26	SOP's for analytical method validation
27	Procedures for raw data handling, archiving and labelling
28	SOP for laboratory QA audit
29	List of signatures of responsible persons (including any delegations).
30	Raw data
31	Case Report Forms
32	Informed consent forms
33	Proof of independent ethical review of the study undertaken.

including mention of any changes that could impact the safety, efficacy or quality of the product, such as changes relating to sourcing of the active pharmaceutical ingredient (API), formulation, manufacturing method, manufacturing equipment or manufacturing site or new clinical data or changes in the safety profile of the product.

- request for a WHO inspection of the manufacturing sites and the CRO or other site used for the bioequivalence study.
- a sample of the product and its commercial presentations.

Innovator products

- submission of a fully updated dossier for each product with a covering letter summarizing any changes since the previously submitted data and information on the products. For example, information on changes to API sourcing, formulation, manufacturing method, equipment or site or new clinical data/changes to safety profile. When there are no changes, a letter confirming this will suffice.
- in some cases, latest inspection reports by the competent drug regulatory authority may be required.

Guidelines for organizations conducting bioequivalence studies

Products submitted for prequalification are often multisource (generic) products. In such cases, therapeutic equivalence is generally demonstrated by performing a bioequivalence study carried out by an independent organization, company, or academic institution, a research organization, or laboratory.

Recently, certain contract research organizations (CROs) were found to be deficient with discrepancies in bioequivalence data and non-compliance with WHO good clinical practices (GCP) or good laboratory practices (GLP) requirements. As a consequence, it is now a prerequisite for prequalification of medicinal products that the CRO used by the sponsor for bioequivalence or other clinical studies is also inspected.

Draft guidelines for organizations involved in the conduct of *in vivo* bioequivalence studies are now in the final stages of consultation. The guidelines cover general recommendations for conducting bioequivalence studies; analysis of clinical trial samples; facilities and equipment; and documents and records. The guideline also provides information on organization and management; clinical phases, bio-analysis; pharmacokinetic and statistical analysis; and study report. The guideline does not replace existing WHO GCP or GLP guidelines, but may be useful as an additional guidance for organizations to help them conduct bioequivalence studies properly.

Monitoring programme

Random samples are taken for quality control analysis from batches of products supplied by UN procurement agencies as part of an ongoing monitoring programme. Comparative dissolution testing is also conducted as appropriate. In addition, inspections are performed of manufacturing sites supplying batches of products. During these inspections, compliance with the specifications as approved by prequalification is verified. Batch manufacturing records are reviewed and assessed, and compared against master specifications, dossier information, validation protocols, reports, records and data. This is part of an ongoing quality assurance programme to ensure that batches contain the API listed in the product dossier, as well as the same product formula, manufacturing method, process and equipment. This procedure verifies that the products supplied are the same as those described in the product dossier and tested in the bioequivalence study, ensuring batch-to-batch consistency.

References

1. Participation in prequalification. http://mednet3.who.int/prequal/

2. World Health Assembly. Resolutions and Decisions. WHA57.14. Scaling up treatment and care within a coordinated and comprehensive response to HIV/AIDS. http://www.who.int/governance/en/

3. The important world of drug prequalification (Editorial). *Lancet*, **364**, (2004).

4. M. Weinberg. Generic HIV Drugs — Enlightened Policy for Global Health (Perspective). *New England Journal of Medicine*, **352**, (2005).

5. 10th and 1th International Conference of Drug Regulatory Authorities. http://www.who.int/medicines/icdra.shtml

6. WHOPAR and WHOPIR. http://mednet3.who.int/prequal/

7. World Health Organization. *Marketing Authorization of Pharmaceutical Products with Special Reference to Multisource (Generic) Products: a Manual for a Drug Regulatory Authority*, WHO/DMP/RGS/98.5 at: http://www.who.int/medicines

ATC/DDD Classification (Final)

The following final anatomical therapeutic chemical (ATC) classifications and defined daily doses (DDDs) were agreed at a meeting of the WHO International Working Group for Drug Statistics Methodology which took place in March 2004. They came into force on 1 September 2004 and will be included in the January 2005 issue of the ATC index. The inclusion of a substance in the lists does not imply any recommendation of use in medicine or pharmacy. The WHO Collaborating Centre for Drug Statistics Methodology can be contacted through e-mail: whocc@nmd.no.

ATC level	INN/Common name	ATC code
New ATC level codes (other than 5th level):		
	First-generation cefalosporins	J01DB
	Second-generation cefalosporins	J01DC
	Third-generation cefalosporins	J01DD
	Fourth-generation cefalosporins	J01DE
New ATC 5th level codes:		
	anecortave	S01XA16
	atorvastatin, combinations	C10AA55
	bevacizumab	L01XC07
	cefoperazone, combinations	J01DD62
	cromoglicic acid	D11AX17
	darifenacin	G04BD10
	eplerenone	C03DA04
	hydroxybutyric acid	N07XX04
	insulin detemir	A10AE05
	mecobalamin	B03BA05
	melatonin	N05CM17
	olmesartan medoxomil & diuretics	C09DA08
	pemetrexed	L01BA04
	pravastatin, combinations	C10AA53
	rasagiline	N04BD02
	sulfamerazine and trimethoprim	J01EE07
	typhoid - hepatitis A	J07CA10
	ziconotide	N02BG08

ATC code changes:

INN/common name	Previous ATC	New ATC
cefacetrile	J01DA34	J01DB10
cefaclor	J01DA08	J01DC04
cefadroxil	J01DA09	J01DB05
cefalexin	J01DA01	J01DB01
cefaloridine	J01DA02	J01DB02
cefalotin	J01DA03	J01DB03
cefamandole	J01DA07	J01DC03
cefapirin	J01DA30	J01DB08

ATC code changes (continued):

INN/common name	Previous ATC	New ATC
cefatrizine	J01DA21	J01DB07
cefazedone	J01DA15	J01DB06
cefazolin	J01DA04	J01DB04
cefdinir	J01DA42	J01DD15
cefepime	J01DA24	J01DE01
cefetamet	J01DA26	J01DD10
cefixime	J01DA23	J01DD08
cefmenoxime	J01DA16	J01DD05
cefmetazole	J01DA40	J01DC09
cefodizime	J01DA25	J01DD09
cefonicide	J01DA17	J01DC06
cefoperazone	J01DA32	J01DD12
cefotaxime	J01DA10	J01DD01
cefotetan	J01DA14	J01DC05
cefotiam	J01DA19	J01DC07
cefoxitin	J01DA05	J01DC01
cefpiramide	J01DA27	J01DD11
cefpirome	J01DA37	J01DE02
cefpodoxime	J01DA33	J01DD13
cefprozil	J01DA41	J01DC10
cefradine	J01DA31	J01DB09
cefroxadine	J01DA35	J01DB11
cefsulodin	J01DA12	J01DD03
ceftazidime	J01DA11	J01DD02
ceftezole	J01DA36	J01DB12
ceftibuten	J01DA39	J01DD14
ceftizoxime	J01DA22	J01DD07
ceftriaxone	J01DA13	J01DD04
ceftriaxone, combinations	J01DA63	J01DD54
cefuroxime	J01DA06	J01DC02
latamoxef	J01DA18	J01DD06
loracarbef	J01DA38	J01DC08

ATC name changes

	Level name	ATC code
Previous:	Morbilli vaccines	
New:	Measles vaccines	J07BD
Previous:	Morbilli, combinations with parotitis & rubella, live attenuated	
New:	Measles, combinations with mumps & rubella, live attenuated	J07BD52
Previous:	Morbilli, combinations with parotitis, live attenuated	
New:	Measles, combinations with mumps, live attenuated	J07BD51
Previous:	Morbilli, combinations with rubella, live attenuated	
New:	Measles, combinations with rubella, live attenuated	J07BD53
Previous:	Morbilli, live attenuated	
New:	Measles, live attenuated	J07BD01
Previous:	Parotitis vaccines	
New:	Mumps vaccines	J07BE
Previous:	Parotitis, live attenuated	
New:	Mumps, live attenuated	J07BE01
Previous:	Rubella, combinations with parotitis, live attenuated	
New:	Rubella, combinations with mumps, live attenuated	J07BJ51

New DDDs:

INN/common name	DDD	Unit	Adm.R	ATC code
aprepitant	95	mg	O	A04AD12
aripiprazole	15	mg	O	N05AX12
atomoxetine	80	mg	O	N06BA09
abenzathine benzylpenicillin	3.6	g	P	J01CE08
benzathine phenoxy metylpenicillin	2	g	O	J01CE10
brodimoprim	0.2	g	O	J01EA02
buprenorphine	1.2	mg	TD	N02AE02
cefmenoxime	2	g	P	J01DD05
ceftezole	6	g	P	J01DB12
cloperastine	60	mg	O	R05DB21
clotiapine	80	mg	O,P	N05AX09
flumequine	1.2	g	O	J01MB07
flurithromycin	0.75	g	O	J01FA14
levosulpiride	0.4	g	O	N05AL07
methotrexate	2.5	mg	O	L04AX03
nesiritide	1.5	mg	P	C01DX19
rufloxacin	0.2	g	O	J01MA10
sodium folinate	60	mg	P	V03AF06
solifenacin	5	mg	O	G04DB08
sulfamazone	1.5	g	O,R	J01ED09

Change of DDDs:

	INN/common name	DDD	Unit	Adm.R	ATC code
Previous:	alosetron	2	mg	O	
New:		1	mg	O	A03AE01
Previous:	amoxicillin & enzyme inhibitor	1	g	P	
New:		3	g	P	J01CR02
Previous:	esomeprazole	20	mg	O	
New:		30	mg	O	A02BC05
Previous:	fentanyl	0.6	mg	TD	
New:		1.2	mg	TD	N02AB03
Previous:	levetiracetam	2	g	O	
New:		1.5	g	O	N03AX14

ATC/DDD Classification (temporary)

The following anatomical therapeutic chemical (ATC) classifications and defined daily doses (DDDs) were agreed at a meeting of the WHO International Working Group for Drug Statistics Methodology which took place on 28 October 2004. Comments or objections to the decisions from the meeting should be forwarded to the WHO Collaborating Centre for Drug Statistics Methodology, e-mail: whocc@nmd.no. They will be included in the January 2006 issue of the ATC index. The inclusion of a substance in the lists does not imply any recommendation of use in medicine or pharmacy.

ATC level	INN/Common name	ATC code
New ATC level codes (other than 5th level):		
	Other anti-parathyroid agents	H05BX
New ATC 5th level codes:		
	abetimus	L04AA22
	acetyl dihydrocodeine	R05DA12
	alglucosidase alfa	A16AB07
	anidulafungin	J02AX06
	atazanavir	J05AE08
	brivudine	J05AB15
	cefditoren	J01DD16
	ceforanide	J01DC11
	cinacalcet	H05BX01
	dimethoxanate	R05DB28
	duloxetine	N06AX21
	erlotinib	L01XX34
	fenetylline	N06BA10
	gadoxetic acid	V08CA10
	gatifloxacin	S01AX21
	histamine dihydrochloride	L03AX14
	ibritumomab tiuxetan [^{90}Y]	V10XX02
	iodoform	D09AA13
	ivabradine	C01EB17
	measles, combinations with mumps, rubella & varicella, live attenuated	J07BD54
	natalizumab	L04AA23
	pregabalin	N03AX16
	prulifloxacin	J01MA17
	risedronic acid and calcium	M05BB02
	roflumilast	R03DX07
	spiramycin, combinations with other antibacterials	J01RA04
	sulfamerazine	D06BA06
	sulfanilamide	D06BA05
	treprostinil	B01AC21
ATC code changes:		
(implementation January 2006)		
Previous:	piribedil	C04AX13
New:	piribedil	N04BC08

ATC name changes

	ATC level	ATC code
Previous:	Histamine	
New:	Histamine phosphate	V04CG03
Previous:	Ointment dressings with antiinfectives	
New:	Medicated dressings with antiinfectives	D09AA
Previous:	Antiparathyroid hormones	
New:	Antiparathyroid agents	H05B

New DDDs:

INN/common name	DDD	Unit	Adm.R	ATC code
atazanavir	0.3	g	O	J05AE08
azithromycin	0.5	g	P	J01FA10
brivudine	0.125	g	O	J05AB15
ceforanide	4	g	P	J01DC11
emtricitabine	0.2	g	O	J05AF09
esomeprazole	30	mg	P	A02BC05
fosamprenavir	1.4	g	O	J05AE07
ibandronic acid	2.5	mg	O	M05BA06
iloprost	0.15	mg	inhal	B01AC11
levodopa, decarboxylase inhibitor and COMT-inhibitor	0.45	g*	O	N04BA03
melagatran	6	mg	P	B01AE04
moxifloxacin	0.4	g	P	J01MA14
nncotine	30	mg	SL	N07BA01
omalizumab	16	mg	P	R03DX05
oxybutynin	3.9	mg	TD	G04BD04
pregabalin	0.3	g	O	N03AX16
trospium	40	mg	O	G04BD09
ximelagatran	48	mg	O	B01AE05
zonisamide	0.2	g	O	N03AX15

* as levodopa

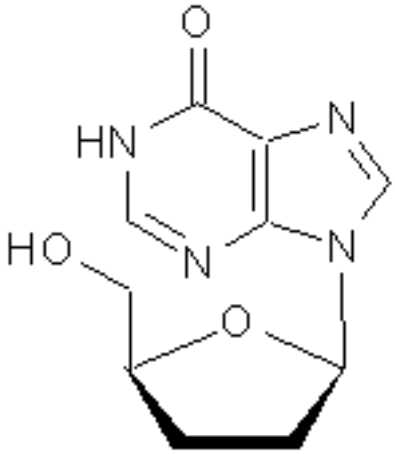
The International Pharmacopoeia

Monographs for antiretrovirals

Within the framework of the Procurement, Quality and Sourcing Project for HIV, Tuberculosis and Malaria (http://www.who.int/prequal), *The International Pharmacopoeia* is collaborating with manufacturers, independent analytical drug quality control laboratories, national and regional pharmacopoeial bodies, research, governments, and regulatory bodies to provide specifications and monographs for the following antiretroviral agents: abacavir, didanosine, efavirenz, indinavir, lamivudine, nelfinavir, nevirapine, ritonavir, saquinavir, stavudine, zidovudine. The final text for didanosine is provided below.

Didanosinum (final)

Didanosine



$C_{10}H_{12}N_4O_3$

Relative Molecular Mass. 236.2

Chemical name. 9-[(2*R*,5*S*)-5-(hydroxymethyl)tetrahydrofuran-2-yl]-1,9-dihydro-6*H*-purin-6-one; 9-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one; 2',3'-dideoxyinosine (DDI); CAS Reg. No. 69655-05-6.

Description. A white to almost white powder.

Solubility. Sparingly soluble in water; slightly soluble in methanol R and ethanol (95 per cent) R

Category. Antiretroviral (nucleoside reverse transcriptase inhibitor).

Storage. Didanosine should be kept in a tightly closed container.

** Refers to The International Pharmacopoeia*

Requirements

Didanosine contains not less than 98.5% and not more than 101.0% of $C_{10}H_{12}N_4O_3$, calculated with reference to the dried substance.

Identity test

Either tests A and B, or test C may be applied.

A. Carry out test A.1. or , where UV detection is not available , test A.2.

A.1. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83*), using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of didanosine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of didanosine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120 °C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 10 μg/ml solution in methanol R, when observed between 210 nm and 300 nm, exhibits one maximum at about 250 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 435 to 485.

C. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS or with the reference spectrum of didanosine.

If the spectra are not concordant, use didanosine RS. Dissolve the sample in a small amount of methanol R, evaporate to dryness and carry out the IR spectrum with the residue as mentioned above. Treat didanosine RS in the same way. The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{20°C}$ –24° to –28°.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118*); determine the heavy metals content according to Method A (Vol. 1, p. 119*); not more than 20 μg/g.

** Refers to The International Pharmacopoeia*

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 4 hours at 105 ˚C; it loses not more than 5.0 mg/g.

Related substances

Note: Prepare fresh solutions and perform the tests without delay

Carry out the test as described under “High-performance liquid chromatography” (Vol. 5, p. 257*), using a stainless steel column (25cm x 4.6mm), packed with octadecylsilyl base-deactivated silica gel for chromatography R (5μm) (hypersil BDS is suitable).

Maintain the column temperature at 20 – 25 ˚C.

The mobile phases for gradient elution consist of a mixture of aqueous phase (Mobile phase A) and methanol (Mobile phase B), using the following conditions :

Mobile phase A: A 0.05 M solution of ammonium acetate R adjusted to pH 8.0 using ammonia (~260 g/l) TS.

Mobile phase B: Methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	92	8
18	92	8
25	70	30
45	70	30
50	92	8
60	92	8

Prepare the following solutions in a mixture of 92 volumes of mobile phase A and 8 volumes of mobile phase B (dissolution solvent).

For solution (1) dissolve 5.0 mg of hypoxanthine R in the dissolution solvent and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 20.0 ml with the same solvent. For solution (2) dissolve 5 mg of didanosine for system suitability RS (containing impurities A to F) in the dissolution solvent and dilute to 10 ml with the same solvent. For solution (3) dissolve 25 mg of the test substance in the dissolution solvent and dilute to 50.0 ml with the same solvent. For solution (4) dilute 5.0 ml of solution (3) to 50.0 ml with the dissolution solvent. Then dilute 5.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Use the chromatogram supplied with didanosine for system suitability RS and the chromatogram obtained with solution (2) to identify the peaks due to impurities A to F.

Inject 20 μl of solution (2). The test is not valid unless the resolution factor between the peaks due to impurity (C) (2'-deoxyinosine) and impurity D (3'-deoxyinosine) is greater than 2.5, if necessary reduce the amount of methanol in the mobile phase and adjust the proportion of aqueous phase pH 8.0 accordingly.

** Refers to The International Pharmacopoeia*

Inject separately 20 μl of solution (4) in replicate injections in the chromatographic system. The relative standard deviation for peak areas of didanosine in replicate injections of solution (4) is not more than 5.0%.

Inject separately 20 μl each of solutions (1) and (3) and 20 μl of dissolution solvent in the chromatographic system. Examine the mobile phase chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (3).

In the chromatogram obtained with solution (3), the following peaks are eluted at the following retention times ratio with reference to didanosine (retention time = about 13-15 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.44; impurity D = about 0.48; impurity E = about 0.5; impurity F = about 0.8; impurity I = about 1.4; impurity G = about 1.6; impurity H = about 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1), (3) and (4), and calculate the content of related substances as a percentage.

In the chromatogram obtained with solution (3) the area of any peak corresponding to impurity A (hypoxanthine) is not greater than the area of the principal peak obtained with solution (1) (0.5%). The area of any individual peak corresponding to impurities B, C, D, E, F or G is not greater than 0.2 times the area of the principal peak obtained with solution (4) (0.2%). The area of any other impurity peak is not greater than 0.1 times the area of the principal peak obtained with solution (4) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than the area of the principal peak obtained with solution (4) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (4) (0.05%).

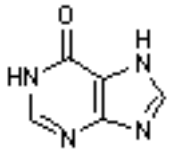
Assay

Dissolve about 0.200 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”; Method A (Vol. 1, p.131*) determining the end point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 23.62 mg of $C_{10}H_{12}N_4O_3$.

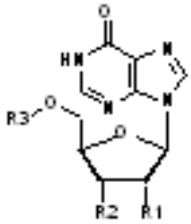
Impurities

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.



A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine)

** Refers to The International Pharmacopoeia*

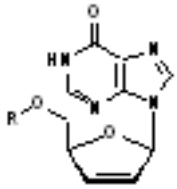


B. R1 = R2 = OH, R3 = H
9-ß-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine)

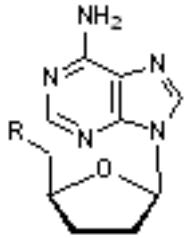
C. R1 = R3 = H, R2 = OH
9-(2-deoxy- ß-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2'-deoxyinosine)

D. R1 = OH, R2 = R3 = H
9-(3-deoxy-ß-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (3'-deoxyinosine)

E. R1 + R2 = O, R3 = H
9-(2,3-anhydro-ß-D-ribofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2',3'-anhydroinosine)



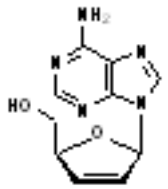
F. R = H
9-(2,3-dideoxy-ß-D-*glycero*-pent-2-enofuranosyl]-1,9-dihydro-6*H*-purin-6-one; (2',3'-didehydro-2',3'-dideoxyinosine)



G. R = OH
9-(2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2',3'-dideoxyadenosine)

H. R = H
9-(2,3,5-trideoxy-ß-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2',3',5'-trideoxyadenosine)

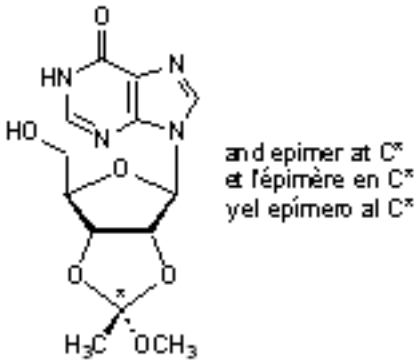
** Refers to The International Pharmacopoeia*



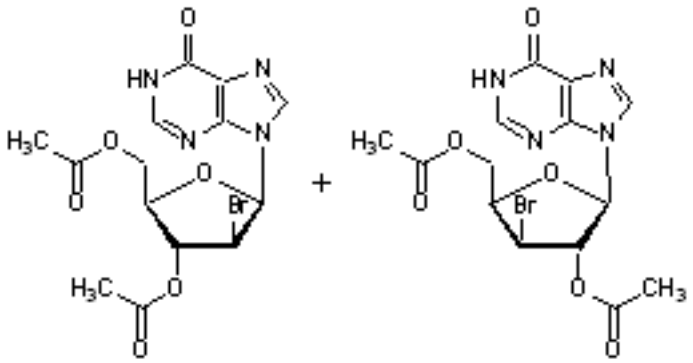
I. 9-(2,3-dideoxy-ß-D-*glycero*-pent-2-enofuranosyl)-9*H*-purin-6-amine (2',3'-dideoxy-2',3'-didehydroadenosine)

J. structure as shown for impurities B to E where R1= R2 = H, R3 = $CO\text{-}CH_3$
9-(5-*O*-acetyl-2,3-dideoxy-ß-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (didanosine acetate)

K. structure as shown for impurity F where R = $CO\text{-}CH_3$
9-(5-*O*-acetyl-2,3-dideoxy-ß-D-*glycero*-pent-2-enofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2',3'-didehydrodidanosine acetate)



L.9-[2,3-*O*-[(1*RS*)-1-methoxyethylene]-ß-D-ribofuranosyl]-1,9-dihydro-6*H*-purin-6-one (2',3'-*O*-(1-methoxyethylidene)inosine; (“dioxalane”)



** Refers to The International Pharmacopoeia*

M. mixture of 9-(3,5-di-*O*-acetyl-2-bromo-2-deoxy-ß-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one and 9-(2,5-di-*O*-acetyl-3-bromo-3-deoxy-ß-D-xylofuranosyl)-1,9-dihydro-6*H*-purin-6-one ("bromoesters")

Reagents

Hypoxanthine R. 1,7-dihydro-6*H*-purin-6-one; $C_5H_4N_4O$.

A commercially available reagent of suitable grade.

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water, sparingly soluble in boiling water, soluble in dilute acids and in dilute alkali hydroxide solutions.

Melting point. Decomposes without melting at about 150 °C.

Thin-Layer Chromatography. Examine as prescribed in the monograph on Mercaptopurine (Vol. 4, p.77-79*); the chromatogram shows only one principal spot.

Silica gel for chromatography, octadecylsilyl, base-deactivated

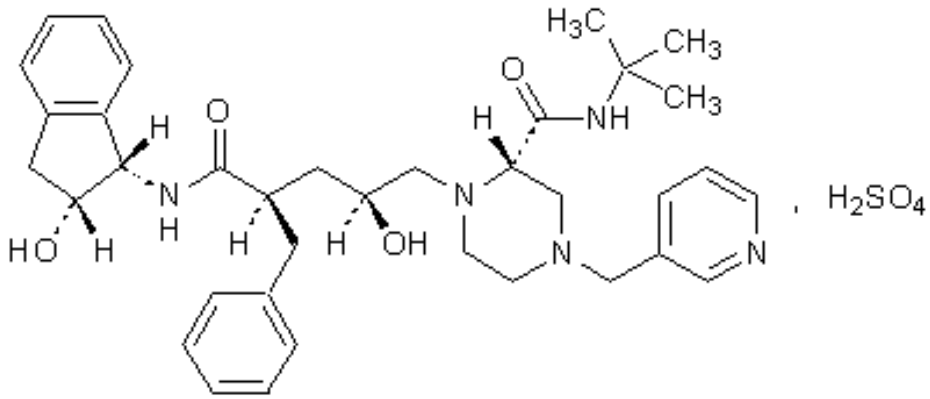
A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups to minimize the interaction with basic components.

Monographs for antiretrovirals

Within the framework of the Procurement, Quality and Sourcing Project for HIV, Tuberculosis and Malaria (http://www.who.int/prequal), *The International Pharmacopoeia* is collaborating with manufacturers, independent analytical drug quality control laboratories, national and regional pharmacopoeial bodies, research, governments, and regulatory bodies to provide specifications and monographs for the following antiretroviral agents: abacavir, didanosine, efavirenz, indinavir, lamivudine, nelfinavir, nevirapine, ritonavir, saquinavir, stavudine, zidovudine. The final text for Indinavir sulfate is provided below.

Indinaviri sulfas (final)

Indinavir sulfate



$C_{36}H_{47}N_5O_4,H_2O_4S$

Relative molecular mass. 711.9

** Refers to The International Pharmacopoeia*

Chemical name. (2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide sulfate; CAS Reg. No. 157810-81-6.

Description. A white or almost white powder.

Solubility. Freely soluble in water, soluble in methanol.

Category. Antiretroviral (protease inhibitor).

Storage. Indinavir sulfate should be kept in a tightly closed container, protected from light.

Additional information. Indinavir sulfate occurs as the monoethanolate which is hygroscopic. It converts to the hydrate upon loss of ethanol and exposure to moist air.

REQUIREMENTS

Indinavir sulfate contains not less than 98.5% and not more than 101.0% of $C_{36}H_{47}N_5O_4,H_2O_4S$ calculated on anhydrous, ethanol free basis.

Identity tests

Either tests A, B and D, or tests C and D may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of indinavir sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of indinavir sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 0.100 mg/ml solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 260 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 56 and 65.

C. Carry out the examination as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from indinavir sulfate RS or with the reference spectrum of indinavir sulfate.

** Refers to The International Pharmacopoeia*

D. A 20 mg/ml solution yields reaction A described under “General identification tests” as characteristic of sulfates (Vol. 1, p. 115*).

Specific optical rotation. Use a 10.0 mg/ml solution and calculate with reference to the anhydrous and ethanol free substance; $[a]_D^{20^\circ C}$ = +27° to +31°.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 1 (Vol. 1, p. 118*); determine the heavy metals content according to Method A (Vol. 1, p. 119*); not more than 10 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under ‘‘Determination of water by the Karl Fischer method’’, Method A (Vol. 1, p. 135*), using 0.5 g of the substance; the water content is not more than 15 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 2.8-3.2.

Ethanol content. Determine by “Gas chromatography with static head-space injection”. Use a fused-silica capillary or wide bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with macrogol 20 000 R (film thickness: 0.25 μm).

As detector use a flame ionization detector.

Use nitrogen for chromatography R or helium for chromatography R as the carrier gas at an appropriate pressure and a split ratio 1:5 with a linear velocity of about 35 cm/sec.

The following head-space injection conditions may be used:

Equilibration temperature (°C)	80
Equilibration time (min)	60
Transfer line temperature (°C)	85
Pressurization time (s)	30
Injection volume (ml)	1

Maintain the temperature of the column at 30 °C for 7 min, then raise the temperature at a rate of 35 °C per min to 180°C and maintain for 10 min, maintaining the temperature of the injection port at 140 °C and that of the flame ionization detector at 250 °C.

Test solution. Dissolve 0.200 g of the test substance in purified water and dilute to 20.0 ml with the same solvent. Introduce 5.0 ml of this solution and 1.0 ml of purified water into a headspace vial. Prepare two more vials.

Reference solutions. Add 0.200 g of ethanol R to purified water and dilute to 200.0 ml with the same solvent. Transfer respectively 2.0 ml, 3.0 ml and 4.0 ml in separate headspace injection vials and bring the volume to 6.0 ml with purified water.

Blank solution. Introduce 6.0 ml of purified water into a headspace vial.

Analyse the blank solution and then alternatively three times the test solution and the three reference solutions.

The test is not valid unless the relative standard deviation on the areas of the peaks obtained from the test solutions is not more than 5%.

Calculate the ethanol content by using the results obtained with the test solution and with the reference solutions; the ethanol content is not less than 50 mg/g and not more than 80 mg/g.

** Refers to The International Pharmacopoeia*

Related substances. Carry out the test as described under "High–performance liquid chromatography" (Vol. 5, p. 257*), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5μm).

Use the following conditions for gradient elution:

Mobile phase A: 30 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 65 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 25 volumes of purified water.

Prepare the phosphate buffer pH 7.5 by dissolving 1.4 g of anhydrous disodium hydrogen phosphate in 50 ml of purified water, adjust the pH to 7.5 by adding phosphoric acid (105 g/l) and dilute it to 100 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–5	93	7	Isocratic
5–25	93 to 20	7 to 80	Linear gradient
25–30	20	80	Isocratic
30–35	20 to 93	80 to 7	Return to the initial conditions
35–45	93	7	Isocratic re-equilibration

Prepare the following solutions. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 2 μg of indinavir sulfate per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 2 ml of sulfuric acid (190 g/l), heat carefully in a water bath at 80°C for 60 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 40 °C, using, for example, a water-bath.

Inject 20 μl of solution (3). The test is not valid unless the resolution factor between the two major peaks, with a retention time between 15 and 20 min, is not less than 3.5. If necessary adjust the amount of acetonitrile in mobile phase A, or adjust the gradient programme.

Inject alternatively 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than the area of the principal peak obtained with solution (2) (0.1 %). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.300 g, accurately weighed, in 50 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 35.59 mg of $C_{36}H_{47}N_5O_4,H_2O_4S$; calculate with reference to the anhydrous and ethanol free substance.

** Refers to The International Pharmacopoeia*

Impurities Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.

Reagents

Silica gel for chromatography, octadecylsilyl, base deactivated
A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups to mini mize the interaction with basic compounds.

Macrogol 20 000 R. Polyethyleneglycol 20 000
Description. White or almost white solid with a waxy or paraffin-like appearance.

Solubility: Very soluble in water and dichloromethane R. Practically insoluble in alcohol and in fatty oils and mineral oils.

Nitrogen for chromatography.
Contains not less than 99.95% v/v of N_2.

Helium for chromatography.
Contains not less than 99.995% v/v of He.

Monographs for antiretrovirals

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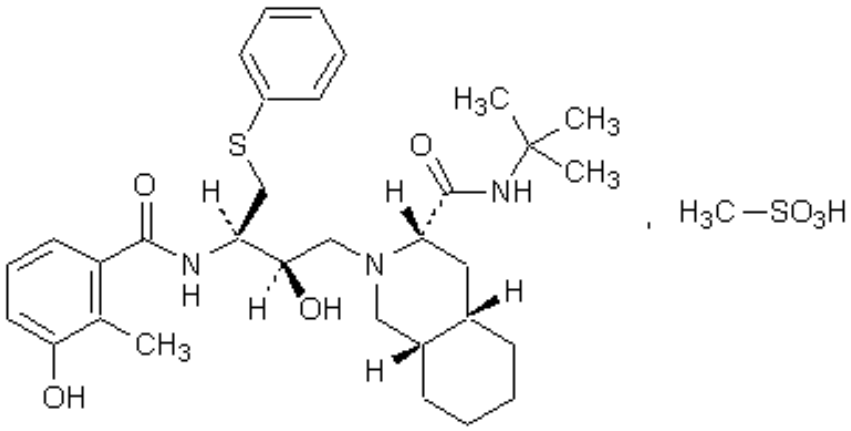
Nelfinaviri mesilas (final)

Nelfinavir mesilate



** Refers to The International Pharmacopoeia*

$C_{32}H_{45}N_3O_4S,CH_4O_3S$

Relative molecular mass. 663.9

Chemical name. (3*S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)-2-[(2*R*,3*R*)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylsulfanyl)butyl]decahydroisoquinoline-3-carboxamide methanesulfonate; CAS reg. No. 159989-65-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Nelfinavir mesilate should be kept in a tightly closed container, protected from light.

Additional information. Nelfinavir mesilate is hygroscopic.

Requirements

Nelfinavir mesilate contains not less than **98.5%** and not more than **101.0%** of $C_{32}H_{45}N_3O_4S,CH_4O_3S$, calculated with reference to the dried substance.

Identity tests

Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol containing(A) 1 mg of the test substance per ml and (B) 1 mg of nelfinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with basic potassium permanganate (5 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 40 μg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits a maximum at about 253 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 124 to136.

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C. Carry out the examination as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from nelfinavir mesilate RS or with the *reference spectrum* of nelfinavir mesilate.

Specific optical rotation. Use a 10.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[a]_D^{20\,^\circ C}$ = -105° to -125 °.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under ''Limit test for heavy metals'', Procedure 2, (Vol. 1, p.118*); determine the heavy metals content according to Method A (Vol. 1, p.119*); not more than 20 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 100 °C; it loses not more than 30 mg/g.

Related substances. Carry out the test as described under "High–performance liquid chromatography" (Vol. 5, p. 257*), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5μm) (hypersil BDS C18 is suitable). Use the following conditions for gradient elution:

Mobile phase A: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of purified water.

Mobile phase B: 41 volumes of acetonitrile R, 31 volumes of methanol R and 28 volumes of phosphate buffer pH 3.4.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute it to 1000 ml with purified water.

Time(min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–27	100	0	Isocratic
27–60	100 to 0	0 to 100	Linear gradient
60–75	0	100	Isocratic
75–80	0 to 100	100 to 0	Return to the initial conditions
80–90	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 10.0 μg of Nelfinavir mesilate per ml. For solution (3) use 100 μg of methanesulfonic acid R per ml.

For the system suitability test: prepare solution (4) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 225 nm.

Maintain the column at 35 °C using, for example, a water-bath.

Inject 20 μl of solution (4). The test is not valid unless the resolution factor between the principal peak (retention time = about 27 minutes) and the peak with a retention time relative to the principal peak of

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about 0.2 is not less than 15. The test is also not valid unless the resolution factor between the last two peaks out of three peaks, which increase during the decomposition process, is not less than 4.0. The ratio of the retention times of these two peaks relative to the principal peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile R in both mobile phases A and B, or adjust the gradient program.

Inject 20 μl of solution (3).

Inject alternatively 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of related substances as a percentage. In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than that obtained with solution (2) (0.5 %). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (1.0 %). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%) and any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3).

Assay. Dissolve about 0.50 g, accurately weighed, in 50 ml of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Perform a blank determination and make the necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 66.39 mg of $C_{32}H_{45}N_3O_4S.CH_4O_3S$.

Impurities Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.

Reagents

Silica gel for chromatography, octadecylsilyl, base-deactivated

A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Methanesulfonic acid R

Molecular formula. CH_4O_3S
Description. Colourless and corrosive liquid, strong irritant.
Solubility. Miscible with water.
Density (d). ~1.48.
Melting point. About 20 °C.

Potassium permanganate, basic (5 g/l) TS

A solution of potassium permanganate R containing about 5 g of $KMnO_4$ per litre of sodium hydroxide (1 mol/l).

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Monographs for antiretrovirals

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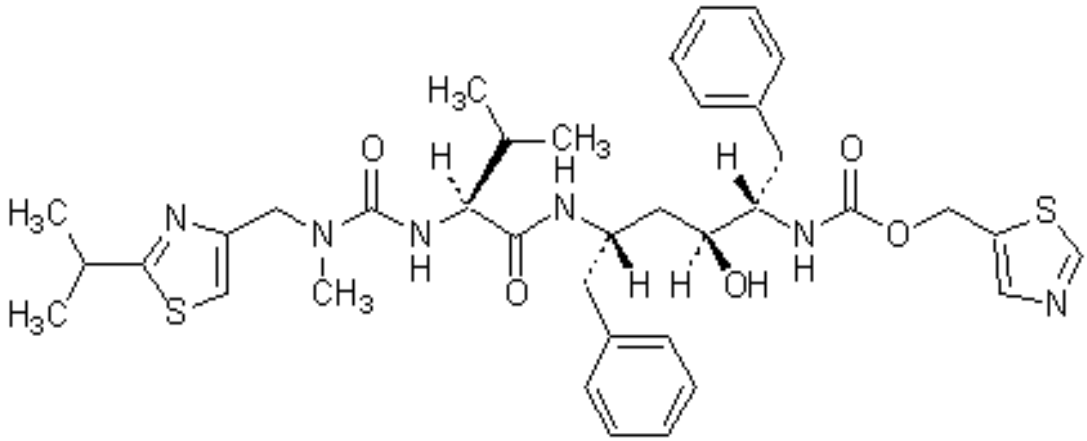
Ritonavirum (final)
Ritonavir



$C_{37}H_{48}N_6O_5S_2$

Relative molecular mass. 721.0

Chemical name. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate; CAS Reg. NO.155213-67-5.

Description. A white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methanol R, sparingly soluble in acetone R and very slightly soluble in acetonitrile R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Ritonavir should be kept in a well-closed container, protected from light.

Additional information. Ritonavir may exhibit polymorphism.

Requirements

Ritonavir contains not less than 98.5 % and not more than 101.0 % of $C_{37}H_{48}N_6O_5S_2$, calculated with reference to the dried substance.

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Identity tests

Either tests A and B or test C may be applied.

A. Note: TLC to be added before publication in 4th Edition*; meanwhile use test C.

B. The absorption spectrum of a 40 μg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 240 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 116 to128.

C. Carry out the examination as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from ritonavir RS or with the *reference spectrum* of ritonavir.

If the spectra obtained in the solid-state show differences, dissolve the test substance and the reference substance separately in a minimal amount of methanol R, crystallise by adding just enough water drop by drop, filter and dry for about one hour and record the spectra again.

Specific optical rotation. Use a 20.0 mg/ml solution in methanol R; $[\alpha]_D^{20\,^\circ C} = +7^\circ$ to $+10.5^\circ$.

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under ''Limit test for heavy metals'', Procedure 2, (Vol. 1, p. 118*); determine the heavy metals content according to method A (Vol. 1, p. 119*); not more than 20 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 2 hours at 105 ˚C; it loses not more than 5 mg/g.

Related substances. Carry out the test as described under "High–performance liquid chromatography" (Vol. 5, p. 257*), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 μm).

Use the following conditions for gradient elution:

Mobile phase A: 35 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 37 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 2 volumes of purified water.
Prepare the sodium phosphate buffer pH 4.0 by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 1.88 g of sodium hexanesulfonate R in 800 ml of purified water, adjust the pH to 4.0 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–20	70	30	Isocratic
20–30	70 to 0	30 to 100	Linear gradient
30–40	0	100	Isocratic
40–45	0 to 70	100 to 30	Linear gradient
45–50	70	30	Isocratic re-equilibration

** Refers to The International Pharmacopoeia*

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 μg of ritonavir per ml.

For the system suitability test: prepare solution (3) using 5 ml of solution (1) and 1 ml of sulfuric acid (475 g/l), heat in a boiling water bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 240 nm.

Maintain the column temperature at 35° C using, for example, a water-bath.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the principal peak (retention time = about 22 minutes) and the peak with a retention time relative to the principal peak of about 0.8 is not less than 3.5. The test is also not valid unless the resolution between the principal peak and the peak with a retention time relative to the principal peak of about 1.5 is not less than 9.0. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than three times the area of the principal peak obtained with solution (2) (0.3%). In the chromatograms obtained with solution (1), the areas of not more than two peaks, other than the principal peak, are greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the areas of not more than four such peaks are greater than the area of the principal peak obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than ten times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.25 g, accurately weighed, in 30 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under ''Non aqueous titration'' Method A (Vol. 1, p. 131*). Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.05 mg of $C_{37}H_{48}N_6O_5S_2$.

Impurities. Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.

Reagents

Silica gel for chromatography, octadecylsilyl, base deactivated

A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to mini mise the interaction with basic compounds.

Sodium dihydrogen phosphate dihydrate

[Sodium biphosphate]; sodium phosphate, monobasic; NaH_2PO_4, $2H_2O$

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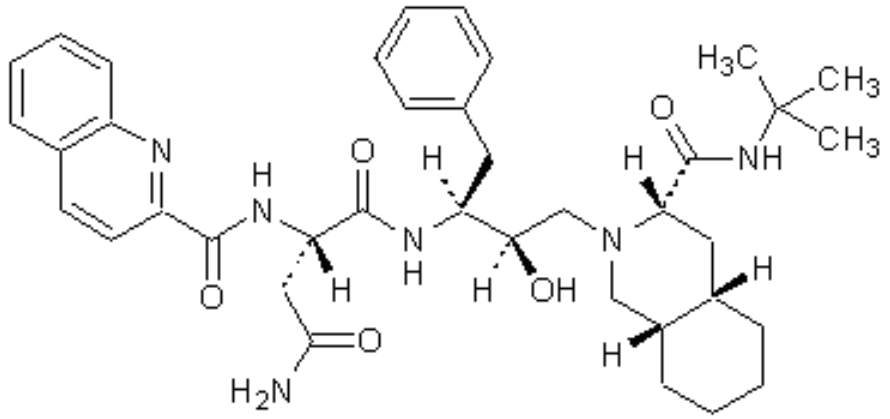
Saquinaviri (final)

Saquinavir



$C_{38}H_{50}N_6O_5$

Relative molecular mass. 670.8

Chemical name. (2*S*)-*N*[2]-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1 dimethylethyl)carbamoyl] octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide; CAS Reg. NO.127779-20-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol.

Category. Antiretroviral (Protease Inhibitor).

Storage. Saquinavir should be kept at 2–8 ˚C in a tightly-closed container, protected from light.

Additional information. Saquinavir is slightly hygroscopic.

Requirements

Saquinavir contains not less than 98.5 % and not more than 101.0 % of $C_{38}H_{50}N_6O_5$, calculated with reference to the dried substance.

** Refers to The International Pharmacopoeia*

Identity tests

Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol R containing (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 μl of each of 2 solutions in methanol R containing (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in dilute basic potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 20 μg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 238 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 670 to 730.

C. Carry out the examination as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from saquinavir RS or with the reference spectrum of saquinavir.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R; $[\alpha]_D^{20\,^\circ C}$ = - 50 ° to - 56 °

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under '' Limit test for heavy metals'', Procedure 2, (Vol. 1, p. 118*); determine the heavy metals content according to Method A (Vol. 1, p. 119*); not more than 10 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 20 mg/g.

Related substances. Carry out the test as described under "High–performance liquid chromatography" (Vol. 5, p. 257*), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 μm).

Use the following conditions for gradient elution:

Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

** Refers to The International Pharmacopoeia*

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comments
0–5	100	0	Isocratic
25–45	100 to 45	0 to 55	Linear gradient
45–55	45	55	Isocratic
55–60	45 to 100	55 to 0	Linear gradient
60–70	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 μg of saquinavir per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 30 °C using, for example, a water-bath.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time = about 21 minutes) and the peak of similar size with a retention time of about 0.45 relative to the saquinavir peak is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 4.0. The ratio of the retention times of these two peaks relative to the saquinavir peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient program.

Inject alternatively 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the area of not more than one such peak is greater than the area of the principal peak obtained with solution (2) (0.1 %). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.300 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under ''Non aqueous titration'' method A (Vol.1, p. 131). Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.54 mg of $C_{38}H_{50}N_6O_5$; calculate with reference to the dried substance.

Impurities

Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.

** Refers to The International Pharmacopoeia*

Reagents

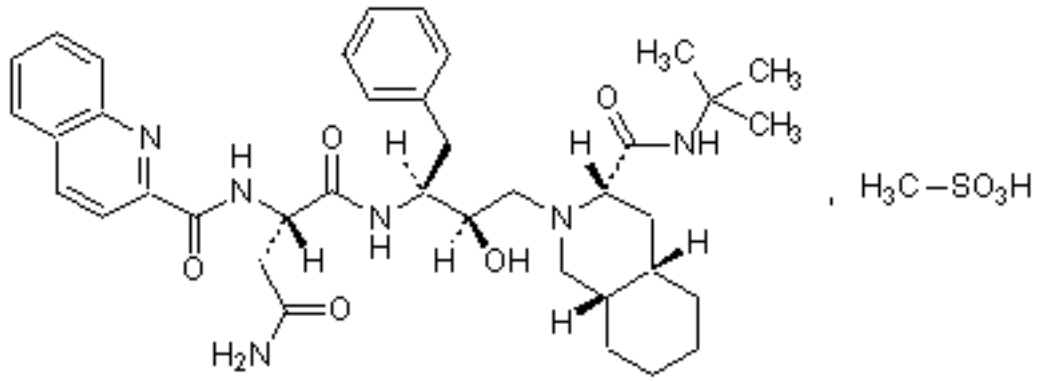
Silica gel for chromatography, octadecylsilyl, base deactivated
A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Potassium permanganate, basic, dilute (1 g/l) TS
A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre of sodium hydroxide (1 mol/l).

Monographs for antiretrovirals

Within the framework of the Procurement, Quality and Sourcing Project for HIV, Tuberculosis and Malaria (http://www.who.int/prequal), *The International Pharmacopoeia* is collaborating with manufacturers, independent analytical drug quality control laboratories, national and regional pharmacopoeial bodies, research, governments, and regulatory bodies to provide specifications and monographs for the following antiretroviral agents: abacavir, didanosine, efavirenz, indinavir, lamivudine, nelfinavir, nevirapine, ritonavir, saquinavir, stavudine, zidovudine. The final text for saquinavir mesilate is provided below.

Saquinaviri mesilas (final)
Saquinavir mesilate



$C_{38}H_{50}N_6O_5.CH_4O_3S$

Relative molecular mass. 767.0

Chemical name. (2*S*)-*N*[*]-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide methanesulfonate; CAS Reg. No. 149845-06-7.

Description. A white or almost white powder.

Solubility. Very slightly soluble in water and sparingly soluble in methanol R.

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Category. Antiretroviral (Protease Inhibitor).

Storage. Saquinavir mesilate should be kept in a tightly-closed container, protected from light.

Additional information. Saquinavir mesilate is slightly hygroscopic.

Requirements

Saquinavir mesilate contains not less than **98.5 %** and not more than **101.0 %** of $C_{38}H_{50}N_6O_5.CH_4O_3S$ calculated with reference to the dried substance.

Identity tests

Either tests A and B or test C may be applied.

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 μl of each of the following 2 solutions in methanol (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ''Thin-layer chromatography'' (Vol. 1, p. 83*), using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 μl of each of the following 2 solutions in methanol (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir mesilate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in dilute basic potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 10 μg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 239 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 580 to 640.

C. Carry out the examination as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained from saquinavir mesilate RS or with the *reference spectrum* of saquinavir mesilate.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = - 33 ° to - 39 °.

Heavy metals. Use 0.5 g in 30 ml of methanol R for the preparation of the test solution as described under ''Limit test for heavy metals'', Procedure 2, (Vol. 1, p. 118*); determine the heavy metals content according to Method A (Vol. 1, p. 119*); not more than 20 μg/g.

** Refers to The International Pharmacopoeia*

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 10 mg/g.

Related substances. Carry out the test as described under "High–performance liquid chromatography" (Vol. 5, p. 257*), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 μm).

Use the following conditions for gradient elution:

Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts of methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Comments
0–25	100	0	Isocratic
25–45	100 to 45	0 to 55	Linear gradient
45–55	45	55	Isocratic
55–60	45 to 100	55 to 0	Linear gradient
60–70	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 μg of saquinavir per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 30 °C using, for example, a water-bath.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time = about 21 minutes) and the peak of similar size with a retention time of about 0.45 relative to the saquinavir peak is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 4.0. The ratio of the retention times of these two peaks relative to the saquinavir peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme. Inject alternatively 20 μl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than the area of the principal peak obtained with solution (2) (0.1 %). The sum of the areas

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of all such peaks is not greater than five times the area of the principal peak obtained with solution (2) (0.5 %). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05 %).

Assay. Dissolve about 0.500 g, accurately weighed, in 70 ml of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS determining the end point potentiometrically. Perform a blank determination and make the necessary correction. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 76.70 mg of $C_{38}H_{50}N_6O_5.CH_4O_3S$; calculate with reference to the dried substance.

Impurities Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.

Reagents

Silica gel for chromatography, octadecylsilyl, base deactivated

A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Potassium permanganate, basic, dilute (1 g/l) TS

A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre of sodium hydroxide (1 mol/l).

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