WHO Drug Information

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Biomedicines Update

International nomenclature and gene therapy products

The International Nonproprietary Names (INN) Programme is a core activity embedded in the normative functions of the World Health Organization (WHO) and has served the global public health and medicines community for over fifty years. The biotechnology market is expanding throughout many regions of the world with many new and innovative medicinal products reaching the clinical trials stage of development. Among these are gene therapy products.

The WHO Monitoring Group on Gene transfer Medicinal Products was established to monitor developments and draw up appropriate guidance for assuring the quality of gene transfer medicinal products, including nucleic acids, viral and non-viral vectors, and genetically modified cells. Ensuring the quality and safety of these distinctive products also involves the application of a standard nomenclature procedure.

In January 2005, an informal consultation was convened by WHO to consider use of INNs for gene therapy products and to agree the outline of a possible nomenclature system. The meeting involved participation of experts in nomenclature as well as those in biologicals, biotechnology and gene therapy. It was not the intention, at this stage, to develop a complete and detailed INN system for gene therapy medicinal products but to establish a basis for further discussion and activities, with an emphasis on wider consultation. Comments on the present article and recommendations from the meeting are therefore invited and should be addressed to the World Health Organization:

baloccor©who.int, Programme on International Nonproprietary Names (INN),
Quality Assurance and Safety: Medicines (QSM), and
shinj©who.int, Quality Assurance and Safety: Biologicals (QSB)

Current INN policy on biological products

The WHO INN Programme was established to assign nonproprietary names to medicinal substances so that each substance would be recognized globally by a unique name. Such names are needed because chemical descriptions are usually very complex for even relatively small molecules. Unlike trademark names, INNs do not give proprietary rights and can be used freely since they are in the public domain. The INNs provide standardized terminology for the international exchange of scientific information and form an essential part of the regulatory process in many countries where a nonproprietary name is a requirement for licensing.

INNs have been assigned to biological medicines since the early days of the INN Programme and include biotechnology-derived products such as monoclonal antibodies and recombinant DNA-derived plasma derivatives and hormones. INNs have not been assigned to natural human blood products nor to vaccines. Instead, the WHO Expert Committee on Biological Standardization formally assigns scientific names to these biologicals when developing the appropriate WHO recommendations and these become international names.

With novel scientific and biotechnical developments taking place at an increasingly fast pace, biotechnology is expanding and many new biological products are currently being introduced for the prevention, diagnosis or treatment of human disease, with many more anticipated in the future. Indeed, biotechnology-derived medicine is one of the fastest growing sectors of the pharmaceutical market.

The complexity of the biologicals area is well recognized and in January 2002 WHO convened a meeting to review policies used by the INN

Expert Group when naming biological products. The objective of the meeting was to seek specialist advice on nomenclature issues, in particular from the Expert Committee on Biological Standardization (1). The meeting led the way to further cooperation and collaboration between INN and biologicals experts and recognized a future need for assigning INNs to gene therapy products.

Monitoring Group on Gene Transfer Medicinal Products

In parallel to these activities, a WHO Monitoring Group on Gene Transfer Medicinal Products has been established and two meetings have taken place (2–3) to review the situation concerning gene therapy. After reviewing the current situation on product development, the Group recommended global harmonization of regulations for gene transfer medicinal products as a priority activity, and identified a need for WHO guidelines and a nomenclature system.

In this latter regard, the Group discussed a nomenclature system for nonproprietary names for gene therapy products proposed by the United States Approved Names (USAN) Council. It was agreed that the system had interesting potential, but more work would be needed to achieve a flexible, all encompassing and appropriate INN nomenclature system suitable for use with gene transfer medicinal products. In particular, the INN system would need to be sufficiently robust to capture latest developments in biotechnology while covering a varying range of products. It would also need to be adaptable to definitions of gene therapy products used within different jurisdictions.

The need for nomenclature of gene therapy products

On 27 January 2005, an informal consultation was held at WHO to discuss the elements of an INN policy on nomenclature for gene therapy products. Participants reviewed the current range of gene transfer products which could be included in a future INN nomenclature policy. This policy would need to be sufficiently flexible to encompass all desired product types. Three broad types of gene transfer products were identified:

• gene therapy:

• DNA/nucleic acid vaccines (4); and

• live viral vector based vaccines (5).

Although commercial entities and clinical grade materials are available for nucleic acid vaccines, most of the work is at the proof of concept stage in humans. For gene therapy products, however, many clinical trials have been undertaken — the majority being in the cancer field — including for treatment of monogenic diseases, multiple sclerosis and rheumatoid arthritis, or in bone regeneration and angiogenesis. A range of different vectors (adenovirus, adeno-associated virus, herpes virus, pox virus, retroviruses, naked DNA) and genes for antigens, tumour suppressor cytokines, and hormones have all been studied, sometimes using systems involving the transfer of ex-vivo genetically manipulated cells. The field is thus highly complex, with a wide range of potential products, including the same genes in different vectors and different genes in the same vectors.

Even then, it would be expected that each gene and vector combination would have its own specific characteristics. Only a small percentage of clinical trials (2–3%) are presently at the Phase III stage of development but there is no doubt that clinical success is possible in some areas. Recent reports (6 , 7) describe the successful correction by gene therapy of immunodeficiency in children with the X-linked form of severe combined immunodeficiency disease (SCID-X1), which is characterized by a block in the differentiation of T and natural-killer cells as a consequence of defective expression or function of gamma c-cytokine receptor-subunit, or both. Thus, the field of gene transfer has become a clinical reality and serious consideration has to be given to the development of an INN nomenclature policy for these products.

Regulatory policy and nomenclature: country reports

In **Japan**, no consideration has yet been given to developing a policy on systematic names for gene transfer products. The emphasis has been on providing detailed guidance on quality and safety issues, including those issues related to the route of administration. If ex-vivo methods (systems involving the transfer of ex-vivo genetically manipulated cells) are to be used, consideration will be given to the target cells, to donor selection criteria, ex-vivo cell culture, and acceptance criteria and methods of administration of transduced cells. When in vivo administration of the vector/gene is used, target cells are again an issue, as are administration methods and the possible transfer of genes to non-target cells.

Japan had less experience of clinical trials of gene transfer products than the USA and Europe but nevertheless a number of trials have been approved. The range of vectors and disease targets is similar to other countries. Safety is of paramount concern and there is a need to explain clearly the potential risk of severe serious adverse events in the informed consent form. Consideration is also being given to viral shedding and monitoring, and an important goal will be development of vectors with better targeted delivery.

In the **USA**, a nomenclature system which would satisfy statutory requirements is under development. Gene therapy products are regulated as biologics by the Food and Drug Administration (FDA) and no medicinal product can be licensed unless a proper systematic nonproprietary name is in place and displayed on the label. The FDA cannot therefore grant a license to market a biological product that does not meet labelling requirements. A nonproprietary name is thus essential for gene therapy products. The need for an INN is considered to be linked to product safety. If there is a problem in the field, possibly in another country, then it is vital that both vector and gene construct be rapidly identified through a common name.

The FDA considers that the nomenclature system for gene therapy products needs to identify the product as a vector carrying a gene to be transferred, but that the indication should not be part of the name, nor should the name incorporate the finer details of the construct. The simpler the name the better, while avoiding the danger of over simplification. The Center for Biologics Evaluation and Research (CBER) has been discussing potential nomenclature systems with USAN since 2001.

A nomenclature scheme should include four elements in order to distinguish a gene therapy product and convey safety information to the user. These would be:

- Indication of the mechanism of action (pharmacologic class).
- Complete identification of the gene being transfected.
- Vector type.
- Indication of the vector's ability to replicate *in vivo.*

Specific nomenclature elements would include:

A prefix: a distinct compatible syllable or element to provide a unique identification of the molecular entity.

An infix: to identify the gene product's mechanism of action. In many cases existing INN infixes for biological products could be incorporated, such as *"ermin"* for growth factor or *"lim "* for immunomodulator.

A stem: "gen"(e) to serve as a suffix for all gene therapy products.

A qualifier could be added to indicate vector type (plasmid, adenovirus , retrovirus, etc.) and the term "replicating" to indicate a capacity to replicate *in vivo.*

However, problems are foreseen with such a system if multiple genes are incorporated at the same time into one vector. Such products are already at the developmental stage. It is unclear also how vector-modified cells will be named and more thought is needed on this aspect. The FDA sees several benefits of developing a systematic nomenclature system for gene therapy products : it will satisfy regulatory requirements for labelling, standardize the assignment of nonproprietary names, and expand the pool of possible names for related, but unique, molecular entities.

Like the USA, **European Union** legislation foresees reference to the INN, where one exists, in medical product literature. There are advantages to a global harmonized common name, and the INN process is a well respected and recognized system which can serve this purpose. The definition of a gene therapy product in the EU is quite broad and flexible. It considers gene transfer to involve an expression system contained in a delivery system known as a vector, which can be of viral as well as non-viral origin. The vector can also be included in a human or animal cell.

Difficulties to be overcome in developing an INN nomenclature system for gene therapy products would include the best way to impart information on the gene of interest and especially on similar or related genes, the problem of multigenes, the use of different types of vectors and the issue of small but possibly important differences within one type of vector. However, a systematic name should be easy to use and to understand.

Building on the experience gained for other complex biological substances such as fusion protein conjugates, which have a two component system, a two word system could be possible. A two word system would give more flexibility and allow similar genes and vectors to be more easily recognized. The proposal for an INN policy for gene therapy products based on two words involves Word 1 as the name for the gene component and Word 2 as the name for the vector component.

The specific nomenclature elements for each word would include a prefix, infix and suffix in a way similar to that already proposed by the FDA and USAN.

Word 1 (gene component)

Prefix: contributes to the distinctive name: e.g., al- bel- val-

Infix: identifies the gene using, when available, existing infixes for biological products as proposed by the FDA or use similar infix as for the protein for which the gene codes.

Suffix: gen or gene

Word 2 (vector component)

Prefix: contributes to the distinctive name

Infix: lenti (lentivirus), retro (other retroviruses), adeno (adenovirus), herpa (herpes virus), or naked DNA, etc . The infix "mul" could be used in the case of multigenes.

Suffix: to indicate viral vector "vec".

More details of the structure/composition could be given in INN publications in an analogous fashion to other biological products such as recombinant proteins.

A distinction can be made between gene therapy medicinal products where the primary mode of action is the delivery and expression of a gene, and somatic cell therapy medicinal products where the primary mode of action is the delivery of cells with different physiological or other characteristics. It is recognized that gene therapy medicinal products can be administered to a patient's cells ex-vivo; in this scenario, ex-vivo could be considered as the route of administration (i.e the cells are not included in the INN scheme).

Discussion of this issue concluded that the suffix "vec" would be more appropriate. "Vac" could easily be misinterpreted as indicating "vaccine", but "vec" would clearly be seen as indicating "vector " . It was agreed that the suffix for word 2 be "vec". It was also agreed that the suffix for the first word (gene component) should be "gene" not "gen".

Participants reviewed four gene therapy INN requests to evaluate how products and proposals would fit into a two-word INN system. Two of these applications were from USA and one each from Germany and Japan. The exercise proved useful and highlighted the need for some thought to be given to infixes for plasmid vectors.

Conclusions

Several important recommendations emanated from the consultation.

1. It was recommended that a systematic nomenclature system for gene therapy products be developed by WHO within the INN framework.

2. It was recommended that the INN for gene therapy products should be based on a two word system. The first word should describe the expression gene, and the second word the vector component.

3. It was agreed that in the case of gene therapy medicinal products administered by transfecting a patient's cells *ex vivo*, the cells themselves should be seen simply as the route of administration and should not be included in the INN system.

4. It was agreed that, for the present, gene transfer products covered within the INN policy should not include DNA/nucleic acid vaccines nor live viral vector vaccines to be used for prophylaxis. However, discussion should take place on this point including whether therapeutic or cancer vaccines should be included in the INN system.

It was agreed that further work and broader consultation was needed to refine the proposed INN policy on nomenclature of gene therapy products.

References

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4. World Health Organization. Guidelines for assuring quality of DNA vaccines. Annex 3. *WHO Technical Report Series*, No. 878 (1998).

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Safety and Efficacy Issues

Tiagabine: seizures in patients without a history of epilepsy

United States of America — The manufacturer of tiagabine hydrochlorine (Gabitril®) has informed prescribers of important new safety information regarding the risk of new onset seizures and status epilepticus in patients without a history of epilepsy. Since the launch of tiagabine in 1997 through 2004, there have been 59 postmarketing reports of such seizures. Clinicians are advised to carefully review the newly added information. Safety and effectiveness of tiagabine have not been established for any indication other than as adjunctive therapy for partial seizures in adults and children 12 years and older.

Seizures in patients without epilepsy
Post-marketing reports have shown that tiagabine use has been associated with new onset seizures and status epilepticus in patients without epilepsy. Dose may be an important predisposing factor in the development of seizures which have been reported in patients taking daily doses as low as 4 mg/day. In most cases, patients were using concomitant medications (antidepressants, antipsychotics, stimulants, narcotics) that are thought to lower the seizure threshold. Some seizures occurred near the time of a dose increase, even after periods of prior stable dosing.

Dosing recommendations in current labelling for treatment of epilepsy are based on use in patients with partial seizures 12 years of age and older, most of whom were taking enzyme-inducing antiepileptic drugs (AEDs; e.g., carbamazepine, phenytoin, primidone and phenobarbital) which lower plasma levels of tiagabine by inducing its metabolism. Use of tiagabine without enzyme-inducing antiepileptic drugs results in blood levels about twice those attained in the studies on which current dosing recommendations are based.

In nonepileptic patients who develop seizures, tiagabine should be discontinued and patients should be evaluated for an underlying seizure disorder. Seizures and status epilepticus are known to occur with tiagabine overdosage.

Tiagabine is approved for use only as adjunctive therapy in adults and children 12 years and older in the treatment of partial seizures. Because tiagabine has not been systematically evaluated in adequate and well-controlled clinical trials for any other indication, its safety and effectiveness have not been established for any other use. The manufacturer does not recommend the use of tiagabine outside of its approved indication.

Reference: Communication from Cephalon, Inc., 14 February 2005 available on http://www.fda.gov/MedWatch/getforms.htm.

Effect of medroxyprogesterone on bone mineral density

Singapore — New data suggest that women who use medroxyprogesterone acetate for long-term contraception may lose significant bone mineral density (BMD). Medroxyprogesterone acetate (Depo-Provera®) is a progestogen-only injection. It was registered in Singapore in 1989 and is indicated for use in contraception, treatment of endometriosis, menopausal vasomotor symptoms, palliative treatment for recurrent endometrial or renal carcinoma and treatment of hormonal-dependent, recurrent breast cancer in postmenopausal women. Several international regulatory authorities including the US Food & Drug Administration, UK Committee on Safety of Medicines and Health Canada have issued advisories on the new prescribing information of Depo-Provera® on BMD changes.

Several new studies have revealed that prolonged use of medroxyprogesterone acetate may result in significant loss of bone density, and the loss is greater the longer the drug is administered. This BMD loss may not be completely reversible after discontinuation of the drug. In a controlled clinical study, adult women using Depo-Provera® Injection (150 mg IM) for up to 5 years for contraception showed spine, femoral neck and hip BMD mean decrease of 5–6% compared to no significant change in BMD in the control group. The decline in BMD was more pronounced during the first 2 years of use, with smaller declines in subsequent years.

The local package insert of Depo-Provera® will be updated to include the following warnings:

- Since loss of BMD may occur in premenopausal women who use medroxyprogesterone acetate injection long-term, a risk-benefit assessment should be considered.
- Medroxyprogesterone acetate injection should be used as a long-term (e.g. longer than 2 years) birth control methods or endometrial treatment only if other treatments are inadequate.
- Other birth control methods or endometrial treatments should be considered in the risk/benefit analysis for the use of MPA injection in women with osteoporotic risk factors.

Reference: Health Science Authority (HSA). Product Safety Alert 17 March 2005 at http://www.hsa.gov.sg/cda/safetyalerts

Tumour necrosis factor inhibitors: safety update

Singapore —Three tumour necrosis factor (TNF) blocking agents are registered in Singapore and are licensed for the treatment of rheumatoid arthritis: infliximab (Remicade®), etanercept (Enbrel®) and adalimumab (Humira®). These monoclonal antibodies bind to human TNF which is a pro-inflammatory and immunoregulatory cytokine that, when overexpressed, mediates chronic inflammation in diseases such as rheumatoid arthritis.

Several uncommon but serious adverse events have come to light through post-marketing surveillance. The Heath Science Agency would like to highlight some important safety information concerning this class of drugs.

Malignancies — lymphoma
There are more cases of lymphoma amongst patients receiving TNF blocking agents compared with control patients in clinical trials. The standardized incidence ratios of lymphoma are higher in treated patients than expected in the general population.

It should be noted that adverse reaction rates observed in clinical trials of a particular drug cannot be compared directly to the rates in other clinical trials of other TNF blocking agents because the trial designs and patient population studies differ among the three TNF blocking agents and between the various studies. No head-to-head trials for these drugs have been studied. Patients with rheumatoid arthritis, and in particular those with highly active disease, may have a higher risk for the development of lymphoma. Other malignancies beside lymphoma have been observed in patients on TNF blocking therapies. The potential role of TNF blocking agents in the development of malignancies is not known.

Haematological events
Rare cases of pancytopenia including aplastic anaemia, some of which led to fatal outcomes, have been reported in patients receiving TNF blocking agents. Caution should be exercised in patients when using these drugs, particularly in those with a history of blood dyscrasias. Doctors should advise patients to seek immediate medical attention if they develop signs and symptoms suggestive of blood dyscrasias or infection (e.g. persistent fever, sore throat) while on any of these products. Discontinuation of therapy should be considered in patients with confirmed significant haematological abnormalities.

Hepatotoxicity and infliximab
Severe hepatic reactions, including acute liver failure, jaundice, hepatitis and cholestasis have been reported in association with infliximab. However, a causal relationship between infliximab and these events has not been established. These severe reactions were reported to occur from 2 weeks to more than 1 year after initiation of infliximab; elevations in hepatic aminotransferase levels were not noted prior to discovery of the liver injury in many of these cases. Some of these cases were fatal or necessitated liver transplantation. Infliximab should be discontinued if a patient presents with jaundice and/or marked liver enzyme elevations and a thorough investigation of the abnormality should be undertaken. In clinical trials, mild or moderate elevations of ALT and AST have been observed in patients receiving infliximab without progression to severe hepatic injury. The Heath Science Agency is working with the affected companies to make the necessary changes to the local package inserts.

References

1. Update on the TNF blocking agents. Briefing Document for FDA Arthritis Advisory Committee, 4 March 2003.

2. HSA Product Safety Alert 31 March 2005 at http://www.hsa.gov.sg/cda/safetyalerts

Pimecrolimus and tacrolimus linked to cancer increase

United States of America — The Food and Drug Administration (FDA) has advised health care professionals to prescribe pimecrolimus (Elidel®) and tacrolimus (Protopic®) only as directed and only after other eczema treatments have failed to work because of a potential cancer risk associated with their use. In addition, FDA is adding a black box warning to the health professional label for the two products and developing a medication guide for patients.

This action follows recommendations made by the FDA's Pediatric Advisory Committee during its 15 February 2005 meeting, at which findings of cancer in three different animal species were reviewed. The data showed that the risk of cancer increased in line with the amount of drug increase. The data also included a small number of reports of cancers in children and adults treated with pimecrolimus and tacrolimus.

The manufacturers of the products have agreed to conduct research to determine whether there is an actual risk of cancer in humans, and, if so, its extent. Both products are applied to the skin to control eczema by suppressing the immune system. FDA's Public Health Advisory specifically advises physicians to weigh the risks and benefits of these drugs in adults and children and consider the following:

"Pimecrolimus and tacrolimus are approved for short-term and intermittent treatment of atopic dermatitis (eczema) in patients unresponsive to, or intolerant of other treatments. They are not approved for use in children younger than 2 years old. The long-term effect of pimecrolimus and tacrolimus on the developing immune system in infants and children is not known. In clinical trials, infants and children younger than 2 years of age treated with pimecrolimus had a higher rate of upper respiratory infections than those treated with placebo cream."

Pimecrolimus and tacrolimus should be used only for short periods of time, not continuously. The long term safety of these products is unknown. Children and adults with a weakened or compromised immune system should not use pimecrolimus or tacrolimus.

Reference: *FDA Talk Paper* T05-06, 10 March 2005. http://www.fda.gov/medwatch

Erythropoietin: caution in cancer patients

Singapore —There are two erythropoietins (EPO) currently registered in Singapore: epoetin alfa (Eprex®) and epoetin beta (Recormon®). Both products are indicated for:

- treatment of anaemia in patients associated with renal failure;
- to increase yield of autologous blood collection; and
- for use in prevention and treatment of anaemia in cancer patients.

Recent emerging safety concerns of the possibility that some clinical uses of EPOs in patients with cancer may be associated with unanticipated risks, including an increased risk of thrombotic vascular events and/or an adverse effect on tumour progression and duration of survival have prompted the health Sciences Authority and its Pharmacovigilance Advisory Committee (PVAC) to review the use of EPOs in cancer patients. Several international regulatory authorities have also discussed the risk-benefit profile of EPOs in cancer patients due to this emerging safety concern triggered by publication of the following studies.

The ENHANCE study (1), was a double-blind, placebo controlled trial to evaluate whether correction of anaemia in subjects receiving radiation therapy for the treatment of head and neck carcinoma improves tumour control. Patients were randomized to receive either epoetin beta or placebo. Vascular disorders (hypertension, haemorrhage, venous thrombosis/pulmonary embolism, cardiovascular accidents) developed in 5% of the placebo group and in 11% of the epoetin beta arm. It was concluded that epoetin beta treatment was associated with an adverse effect on mortality and tumour progression.

The Breast Cancer Erythropoietin Trial (BEST) was a randomized controlled trial of epoetin alfa versus placebo in patients with metastatic breast cancer receiving chemotherapy which was terminated prematurely (2). The trial was designed to test whether epoetin alfa would improve survival and quality of life. Results showed frequencies of deaths as higher in epoetin alfa-treated subjects (32%) compared to placebo (24%). Thrombotic vascular events (TVEs) could

have been a significant contributing factor to the differences in survival rates between the two treatment groups. Treatment with EPOs has been associated with some increase in the risk for TVEs, and it is assumed that such events may become more frequent when subjects are treated beyond the correction of anaemia (1, 2).

The American Society of Clinical Oncology, the American Society of Hematology (3), and the European Organization for Research and Treatment of Cancer (EORTC) (4) have separately developed evidence-based clinical practice guidelines for the use of EPOs in patients with cancer. Both sets of guidelines recommended that cancer patients receiving chemotherapy and/or radiotherapy, treatment of EPOs if initiated should be at a Hb level of < 11 g/dL.

Based on the risk-benefit assessment of EPOs in cancer patients, the Pharmacovigilance Advisory Committee has recommended the following:

- that the licensed indication of EPOs for prevention of anaemia in cancer patients is no longer appropriate.
- that the target Hb concentration in cancer patients if treated with EPOs should be up to 12 g/dL.

References

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2. *Lancet Oncology*, **4**: 459–460 (2003).

3. *Journal of Clinical Oncology*, **20**: 4083 (2002).

4. *European Journal of Cancer*, **40**: 2201 (2004).

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6. Epoetin alfa and blood clot formation in cancer patients. *WHO Drug Information*, **18**(4): 285 (2004).

Oxcarbazepine: multi-organ hypersensitivity

Canada — The manufacturer of oxcarbazepine (Trileptal®) has communicated new safety information concerning the risk of serious dermatological reactions, including Stevens Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN), as well as multi-organ hypersensitivity reactions in both children and adults, associated with the use of oxcarbazepine. Oxcarbazepine is indicated for treatment of partial seizures in adults and children ages 6–16 with epilepsy.

1. The reporting rate of SJS and TEN with use of oxcarbazepine currently exceeds the background incidence rate estimates by a factor of 3–10 fold. Some patients have required hospitalization with very rare reports of fatal outcome. Most cases occurred within the first month. Estimates of the background incidence rate for these serious skin reactions in the general population range between 0.5 to 6 cases per million person years.

If a patient develops any skin reaction while taking oxcarbazepine, consideration should be given to discontinuing use and prescribing another anti-epileptic. A diagnosis of SJS or TEN requires immediate discontinuation of oxcarbazepine.

2. A limited number of cases of multi-organ hypersensitivity reactions have been reported in both children and adults in association with the use of oxcarbazepine. Many of these cases resulted in hospitalization and some were considered life threatening. Signs and symptoms of this disorder were diverse; however, patients typically, although not exclusively, presented with fever and rash associated with various organ system abnormalities, including liver, kidney and haematological. Other organ symptoms and signs may occur.

If this reaction is suspected, oxcarbazepine should be discontinued immediately and an alternative treatment started.

3. Approximately 25–30% of patients who have had hypersensitivity reactions to carbamazapine will experience hypersensitivity reactions with oxcarbazepine. Hypersensitivity reactions may also occur in patients without a history of hypersensitivity to carbamazapine.

Reference: Health Canada advisory dated 27 April 2005 at http://www.hc-sc.gc.ca

Drotrecogin alfa: single organ dysfunction

United States of America — The manufacturer of drotrecogin alfa (activated) (Xigris®) has communicated new safety information on drotrecogin alfa, a biological therapeutic product indicated for the treatment of adult patients with severe sepsis who are at high risk of death. The

warning is based upon exploratory analyses of the ADDRESS clinical trial database and subsequent reanalysis of the PROWESS (Phase III registration) clinical trial database.

Among the small number of patients enrolled in PROWESS with single organ dysfunction and recent surgery (surgery within 30 days prior to study treatment) all-cause mortality was numerically higher in the drotrecogin alfa group compared to the placebo group.

In a preliminary analysis of the subset of patients with single organ dysfunction and recent surgery from a separate, randomized, placebo-controlled study (ADDRESS) of septic patients at lower risk of death, all-cause mortality was also higher in the drotrecogin alfa group. Patients with single organ dysfunction and recent surgery may not be at high risk of death and therefore may not be among the indicated population. Drotrecogin alfa should be used in these patients only after careful consideration of the risks and benefits.

This observation underscores the importance of accurate severe sepsis diagnosis and assessment of risk of death when considering patients for drotrecogin alfa treatment.

Reference: Communication from Ely Lilly on http://www.fda.gov/medwatch.

Drotrecogin alfa: not indicated for paediatric sepsis

Canada — The manufacturer of drotrecogin alfa (Xigris®), recombinant human activated protein C, rhAPC, has informed healthcare professionals of important safety information. Drotrecogin alfa is indicated for the treatment of adult patients with severe sepsis (sepsis associated with acute organ dysfunction) who have a high risk of death (e.g. as determined by APACHE II score or multiple organ dysfunctions).

The manufacturer has recently stopped enrolment in study EVBP, a randomized, double-blind,

Table. Efficacy and safety of drotrecogin alfa in paediatric severe sepsis (EVBP): Interim analysis

	Xigris® N=201 n (%)	**Placebo N=198 n(%)**
CTCOFRS (Composite Time to Complete Organ Failure Resolution), mean score standard deviation	9.7 + 5.0	9.8 + 5.1
28-day all-cause mortality Deaths attributable to hemorrhage by investigator*	34 (16.9) 1 (0.5)	36 (18.2) 5 (2.5)
Intracranial haemorrhage Days 0–6 (infusion period) Days 0–28 (entire study period)	 4 (2.0) 8 (4.0)	 1 (0.5) 5 (2.5)
Serious Adverse Events Days 0–6 (infusion period) Days 0–28 (entire study period)	 21 (10.4) 35 (17.4)	 23 (11.6) 40 (20.2)
Serious Bleeding Events Days 0–6 (infusion period) Days 0–28 (entire study period)	 8 (4.0) 13 (6.5)	 7 (3.5) 14 (7.1)
At least one intracranial haemorrhage event OR died during 28-day study period.	39 (19.4)	38 (19.2)
Major Amputations	4 (2.0)	6 (3.0)

** Intracranial haemorrhage was the cause of death for the Xigris® fatality and two of the placebo fatalities.*

placebo-controlled trial of drotrecogin alfa (activated) in paediatric patients with severe sepsis. Interim analysis showed that drotrecogin alfa was highly unlikely to show an improvement over placebo in the primary outcome of complete organ failure resolution over 14 days. There was a numerical increase in the rate of intracranial haemorrhage in the drotrecogin alfa versus the placebo group, primarily seen in patients aged 60 days or less. Drotrecogin alfa is not indicated for use in pediatric severe sepsis.

The Data Monitoring Committee also noted a numerical increase in the rate of intracranial haemorrhage in the drotrecogin alfa versus the placebo group. Mortality, the rate of serious adverse events, overall serious bleeding events, and major amputations appeared to be similar in the drotrecogin alfa and placebo groups.

The main findings of the interim analysis are summarized in the table on page 112. Data collection in study EVBP is ongoing. All patients enrolled will be followed for the complete 28–day study period. Full results of the complete dataset will be available in the latter half of 2005 and publicly presented as soon as possible.

Reference: Communication from Lilly. Association of Xigris® with Intracranial Hemorrhage in Pediatric Patients and Discontinuation of Study F1K-MC-EVBP (Investigation of the Efficacy and Safety of Drotrecogin Alfa (Activated) in Pediatric Severe Sepsis) based on failure to reach desired clinical endpoints and an unfavourable benefit/risk profile. http://www.lilly.ca and Health Canada website http://www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/index_advisories_professionals_e.html.6 May 2005.

Interferon beta–1a and hepatic injury

United States of America — Interferon beta-1a (Avonex®) was introduced to the United States market in 1996. In post-marketing experience severe hepatic injury, including hepatic failure, has been reported rarely. In some cases, these events have occurred in the presence of other drugs that have been associated with hepatic injury. The potential for hepatic injury should be considered when interferon beta-1a is used in combination with other products associated with hepatic injury, or when new agents are added to the regimen of patients already on

In March 2005, the prescribing information and medication guide were updated to include this important new safety information including precautions for patients and pregnancy.

Reference: Communication from Biogen dated 1 March 2005, posted on http://www.fda.gov/medwatch

Avascular necrosis with interferon alfa–2b in chronic myelogenous leukaemia

Australia — Out of a total of 426 reports involving interferon alfa-2b (Intron A®), the Adverse Drug Reaction Advisory Committee (ADRAC) has received six reports of avascular necrosis, aseptic necrosis or osteonecrosis in association with the treatment of chronic myelogenous leukaemia (CML). The site was the femoral or humoral head as identified by a bone scan or MRI. Daily doses varied from 3 to 10 million units, and the time to onset was 3–8 weeks.

Three cases of avascular necrosis of the femoral head in CML patients treated with interferon alfa have been described (1). All had thrombocytosis and loss of response (not described in the ADRAC reports). Avascular necrosis has occurred without interferon treatment in CML, but it has been exacerbated by interferon alfa treatment (1).

Since there appear to be no literature reports of avascular necrosis for interferon alfa in other indications, it was concluded that the avascular necrosis may be the result of an interaction between CML and interferon alpha therapy. Interferon alfa can inhibit angiogenesis, which may cause avascular necrosis, and the stress of weight bearing may make the femoral head particularly vulnerable (2). The possibility of avascular necrosis should be considered if bone or joint pain develops in patients with CML given interferon alfa.

Extracted from Australian Adverse Drug Reactions Bulletin, Volume 24, Number 2, April 2005.

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Hylan G–F 20: joint inflammation and pain

Canada — Hylan G-F 20 (Synvisc®) is an elastoviscous fluid containing hylan polymers, which are derivatives of hyaluronan (sodium hyaluronate). It is indicated for the treatment of pain caused by osteoarthritis of the knee in patients who have failed to respond adequately to conservative nonpharmacologic therapy and simple analgesics. Treatment involves intra-articular injection once a week for 3 weeks. The most commonly reported adverse incidents have been pain, swelling and effusion in the injected knee (1).

From March 1996 to January 2005, Health Canada received 31 reports of suspected incidents associated with Synvisc®; 23 were received in 2003–2004. In nine cases, the synovial fluid was not removed before each injection, and in five the course of injection was continued after the occurrence of adverse symptoms. Six of the 23 recent reports described patients who had pain, walking disability and knee swelling with or without effusion after the third injection of the first course. Two of these 23 patients were admitted to hospital.

The occurrence of post-injection effusion may be associated with the number of injections (1). There have been reports in the literature of pseudosepsis (2). In affected patients, pseudosepsis typically occurs after more than one injection. Sepsis or pseudogout should be ruled out. Mononuclear cells are present in the synovial fluid (2). Although the cause of pseudosepsis is not fully understood, there is increasing evidence to suggest an immunologic mechanism (2).

Health care professionals should be aware of these possible adverse incidents and encouraged to follow the labelled procedure, including aspiration of synovial fluid before each injection (1). Patients should be alerted of the occurrence of such events, and those who have severe inflammation of the joint after an injection should be fully evaluated (3).

Extracted from: Canadian Adverse Reaction Newsletter, Volume 15, Issue 2, April 2005.

References

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Galantamine and vascular events

United States of America — The prescribing information for galantamine hydrobromide (Reminyl®) has been updated to reflect the results of two investigational studies in individuals with mild cognitive impairment. Galantamine is approved only for the treatment of mild to moderate Alzheimer disease. No indication is being sought for the treatment of individuals with mild cognitive impairment.

In two randomized, placebo-controlled trials of two years duration in subjects with mild cognitive impairment (MCI), a total of 13 subjects on galantamine and one subject on placebo died. The deaths were due to various causes which could be expected in an elderly population; about half of the galantamine deaths appeared to result from various vascular causes (myocardial infarction, stroke, and sudden death).

Although the difference in mortality between galantamine and placebo-treated groups in these two studies was significant, the results are highly discrepant with other studies of galantamine. Specifically, in these two MCI studies, the mortality rate in the placebo-treated subjects was markedly lower than the rate in placebo-treated patients in trials of galantamine in Alzheimer disease or other dementias.

Although the mortality rate in the galantamine treated MCI subjects was also lower than that observed in galantamine treated patients in Alzheimer disease and other dementia trials, the relative difference was much less. When the Alzheimer disease and other dementia studies were pooled, the mortality rate in the placebo group numerically exceeded that in the galantamine group. Furthermore, in the MCI studies, no subjects in the placebo group died after 6 months, a highly unexpected finding in this population. Individuals with mild cognitive impairment demonstrate isolated memory impairment greater than expected for their age and education, but do not meet current diagnostic criteria for Alzheimer disease.

Reference: Communication from Ortho-McNeil Neurologics on 31 March 2005. http://www.fda.gov/medwatch

Rosuvastatin: revised start doses

United states of America — A revised package insert has been published by the manufacturer of rosuvastatin (Crestor®). Changes to the label reflect results from a Phase IV pharmacokinetic study in Asian-Americans and highlight important information to reduce the risk for myopathy and rhabdomyolysis, especially at the highest approved dose of 40 mg.

Rosuvastatin is a statin approved in August 2003 for use in lowering serum cholesterol. All statins rarely cause serious muscle damage. Physicians are warned to prescribe rosuvastatin with caution, particularly at higher doses, as the risk of myopathy increases with higher drug levels.

In a pharmacokinetic study involving a diverse population of Asians residing in the United States, rosuvastatin drug levels were found to be elevated approximately 2-fold compared with a Caucasian control group. As a result of these findings, the label now states that the 5 mg dose of rosuvastatin should be considered as the start dose for Asian patients and any increase in dose should take into consideration the increased drug exposure in this patient population.

It also emphasizes that the 40 mg dose is not an appropriate start dose and should be reserved only for those patients who have not achieved their cholesterol goals with the 20 mg dose.

Reference: *FDA Public Advisory*, 2 March 2005 http://www.fda.gov/cder/foi/label/2005/21366slr005lbl.pdf

New kidney function test a better predictor of risk

United States of America — Cystatin-C®, a new blood test for kidney function, is a better predictor of death and cardiovascular risk among the elderly than the standard measure of kidney function, according to a National Heart, Lung, and Blood Institute (NHLBI)-funded study published in the *New England Journal of Medicine*. This more sensitive test distinguishes those at low, medium and high cardiovascular risk, which may enable earlier detection.

Investigators for NHLBI's Cardiovascular Health Study compared the two measures of kidney function, cystatin-C® and the standard test creatinine, as predictors of death from all causes, death from cardiovascular causes, and incidence of heart attack and stroke among 4637 elderly participants in the study.

The 20% of participants with the highest levels of cystatin-C had twice the risk of death from all causes as well as death from cardiovascular disease, and a 50% higher risk of heart attack and stroke compared with those who had the lowest levels. In contrast, testing the same participants with creatinine detected a smaller high-risk group — about 10 percent of the participants — and all others appeared to be at average risk. With cystatin-C investigators found that 60% had abnormal kidney function putting them at medium or high risk for cardiovascular complications.

Cystatin-C is FDA-approved for diagnostic use, but the test is not yet widely available or commonly used in clinical settings. This and other studies have shown that cystatin-C may detect moderate kidney disease at earlier stages, before creatinine levels would rise, enabling identification of a much larger group of people at risk for death and cardiovascular complications.

Additional research is needed to determine the exact clinical role for this test, but it may be most useful in high-risk patients with normal creatinine. Evaluating the mechanisms that underlie this strong association between the kidney and cardiovascular disease would be critical for targeting prevention efforts.

References

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2. *NIH News*, 18 May 2005. National Institutes of Health website http://www.nih.gov.

Statins and peripheral neuropathy

Australia — The Adverse Drug Reactions Advisory Committee (ADRAC) has received 281 reports of peripheral neuropathy or symptoms consistent with this diagnosis attributed to statins (see Table below), and first highlighted this association in 1993 (1). Thirteen of the 281 cases

were confirmed by nerve conduction studies. Both sensory and mixed sensorimotor peripheral neuropathies were reported. The time to onset ranged from one dose to 4.5 years.

Many patients requiring statin therapy have conditions which predispose them to peripheral neuropathy, particularly diabetes mellitus and chronic renal failure (2). Thus the observation of an association is not necessarily indicative of causation. However, recovery on withdrawal of the statin was noted in approximately half of the ADRAC cases, including cases where the patient also had diabetes, and some reports describe positive rechallenge. In two cases, symptoms developed after an increase in dose.

Statin-associated peripheral neuropathy may persist for months or years after withdrawal of the statin (2, 3`). In two ADRAC cases of persistent peripheral neuropathy, motor and sensory conduction tests showed minimal recovery 4 and 12 months, respectively, after discontinuation of simvastatin, despite clinical improvement (3). A further 21 cases had not recovered at the time of reporting, between one and eight months after discontinuation of the statin. In two other reports, the problem was persisting after 3 and 5 years, respectively.

The incidence of statin-induced peripheral neuropathy appears to be low. A study, which excluded patients with predisposing disease, attributed 4.5 cases per 10 000 person-years to statin use (4). Consideration should be given to drug withdrawal if patients taking a statin develop sensory or motor disturbances.

Extracted from Australian Adverse Drug Reactions Bulletin, Volume 24, Number 2, April 2005.

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Angioedema : still a problem with ACE inhibitors

Australia — Of over 7000 reports of angioedema received by the Adverse Drug Reactions Advisory Committee (ADRAC) since 1970, ACE inhibitors account for 12.6%. Angioedema may present with acute onset of soft-tissue swelling of part or all of the face (periorbital, peri-oral, lips), tongue, pharynx and neck. Oedema of the gastrointestinal tract resulting in attacks of abdominal pain, vomiting and diarrhoea has also been rarely reported with ACE inhibitors (1). Angioedema can be life-threatening, and may require prompt parenteral administration of adrenaline if the airway is compromised. The cause may not always be obvious as the first occurrence may be

Table: ADRAC cases of peripheral neuropathy with the statins

Drug	Total cases	Sole suspected drug (%)	Recovered (%)
Simvastatin (Zocor®, Lipex®)	136	64 (47%)	59 (43%)
Atorvastatin (Lipitor®)	108	70 (65%)	60 (56%)
Pravastatin (Pravachol®)	26	14 (54%)	17 (65%)
Fluvastatin (Lescol®, Vastin®)	11	6 (54%)	9 (82%)
Total	**281**	**155 (54%)**	**145 (52%)**

after months or even years of ACE inhibitor therapy. Angioedema may also occur episodically with long symptom-free intervals.

ADRAC first advised of the risk of angioedema with ACE inhibitors in 1993 (2) and noted its occurrence with angiotensin II antagonists in 1999 (3). ADRAC now has 119 reports with angiotensin II antagonists. With ACE inhibitors the reaction is thought to be associated with potentiation of bradykinin, causing increased vascular permeability and vasodilation (4). The mechanism with the angiotensin II antagonists is unclear but it has also been postulated to be by bradykinin activation (4, 5) Individuals with a history of angioedema with ACE inhibitors may occasionally develop it with an angiotensin II antagonist as well (4, 5).

Extracted from Australian Adverse Drug Reactions Bulletin, Volume 24, Number 2, April 2005.

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More advice on SSRI use

United Kingdom — The Medicines and Healthcare Products Regulatory Agency (MHRA) has issued a reminder on selective serotonin reuptake inhibitor use (SSRIs). This reminder is prompted by a number of studies on SSRIs published in the *British Medical Journal*.

During the course of 2004, an Expert Working Group convened by the MHRA reviewed evidence on SSRIs. It published its advice, together with the evidence on which that advice was based, in December 2004. The group considered a huge range of evidence, both published and unpublished. The Expert Group published a number of conclusions and recommendations, including the following:

- The balance of risks and benefits remains positive in those groups of patients for whom treatment with SSRIs is indicated. Whilst the evidence suggests that a modest increase in suicidal thoughts and self-harm for SSRIs compared with placebo cannot be ruled out, this needs to be offset against the benefits of treatment with SSRIs, and the risks associated with not treating the condition.

- Careful and frequent monitoring by healthcare professionals and, where appropriate, other carers in the early stages of treatment is necessary. Evidence reviewed by the expert group shows that the risk of self-harm in depressed patients is greatest around the time of presentation to medical services. The advice, based on years of clinical experience, has therefore always been that the risk of self harm may increase in the early stages of treatment for depressive illness.

- The balance of risks and benefits for the treatment of depression in children under the age of 18 is unfavourable in paroxetine, venlafaxine, sertraline, citalopram, escitalopram and mirtazapine. It is not possible to assess the balance of risks and benefits for fluvoxamine due to the absence of paediatric clinical trial data. The balance of risks and benefits is judged to be favourable for fluoxetine. Given that people mature at different rates, the group also advised close monitoring of young adults.

The report of the Expert Working Group on SSRIs can be found at http://www.mhra.gov.uk/news/2004/SSRIfinal.pdf. The advice given to healthcare professionals at the time the report was published can be found at http://www.mhra.gov.uk/news/2004 SSRI_Letter_061204.pdf

Reference: *MHRA highlights its recent advice on SSRIs*, 18 February 2005. http://www.mhra.gov.uk/

Million Women Study: latest HRT data

United Kingdom — The Committee on the Safety of Medicines (CSM) has commented on data from the UK Million Women Study. This adds

important information to growing knowledge of the effects of different types of hormone replacement therapy (HRT) and underlines the need for caution in long term use. However, this new data on endometrial cancer is unlikely to change the overall balance of risks and benefits for the short term use of HRT. Different types of HRT show differing effects on the risk of cancer of the breast and endometrium and both of these need to be considered when deciding the most suitable form of therapy for an individual woman.

Each decision to start or continue HRT should be made with a fully informed patient individually and should take into account any changes in risk factors and personal preferences as follows:

- For the treatment of menopausal symptoms the benefits of short-term HRT are considered to outweigh the risks in the majority of women.
- In all cases, it is good practice to use the lowest effective dose for the shortest possible time and to review the need to continue treatment at least annually.
- For postmenopausal women over 50 years who are at an increased risk of bone fracture, HRT should be used to prevent osteoporosis only in those who are intolerant of, or contraindicated for, other osteoporosis therapies.

The safety of HRT is under continuous review and the product information for all HRT products contains warnings about the risks of breast and endometrial cancer.

Reference: *Press Release*, 29 April 2005 at press.office @mhra.gsi.gov.uk

Tuberculin purified protein derivative (Mantoux) and serious allergic reactions

Canada — Acute allergic reactions including anaphylaxis, angioedema, urticaria and/or dyspnoea have been very rarely reported following intradermal skin testing with tuberculin purified protein derivative (Tubersol®).

These reactions may occur in persons without a prior history of a tuberculin skin test. Epinephrine hydrochloride solution (1:1000) and other appropriate agents should routinely be available for immediate use in case an anaphylactic or other acute hypersensitivity reaction occurs.

Health care providers should monitor the patient for immediate reactions for a period of at least 15 minutes after inoculation for the initial management of anaphylaxis (1).

The Canadian case reports contain such hypersensitivity events as anaphylactic reaction, angioedema, oedema, urticaria, throat swelling/tightness, lip swelling, and hives, including in patients with no prior exposure to tuberculin. Health care professionals are directed to information in the product direction leaflet regarding the need for persons administering tuberculin skin tests to be prepared to treat an immediate systemic allergic reaction should one occur, and to monitor the patient for immediate reactions for a period of at least 15 minutes after inoculation.

References

1 Canadian Immunization Guide 2002. P. 14. http://www.phac-aspc.gc.ca/publicat/cig-gci/pdf/part1-cdn_immuniz_guide-2002-6.pdf

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Ezetimibe: hepatic, muscle, and pancreatic reactions

Canada — Health Canada and the manufacturer of ezetimibe (Ezetrol®), have provided new safety data on this cholesterol absorption inhibitor, used alone or in combination with a statin, because of the entero-hepatic recirculation of one of its metabolites (1). The Product Monograph for Ezetrol® (ezetimibe) has been updated to include information from international post-marketing reports of rare, and in some cases serious, adverse events. The Patient Information section is being updated to inform patients of the signs and symptoms of hepatic, muscle, and pancreatic adverse events, for which early consultation with a physician is recommended. Additional reports of myalgia, many accompanied by elevated creatine phosphokinase (CK) values, have been reviewed by Health Canada.

The following adverse events have occurred in patients taking ezetimibe alone or in combination with a statin: myalgia; rhabdomyolysis; hepatitis; acute pancreatitis; thrombocytopenia; and suspected interaction with warfarin.

- Patients with a history of statin intolerance (myalgia with or without elevated CK levels)

should be closely monitored for adverse muscle events during treatment with Ezetrol® (ezetimibe).

- Patients who experience persistent muscle pain should be instructed to contact their physicians for evaluation of the possibility of rhabdomyolysis. In most reported cases, rhabdomyolysis resolved when the drugs were discontinued.
- Liver function monitoring is recommended. The use of ezetimibe in combination with a statin is contraindicated in patients with active liver disease or unexplained persistent elevations of liver transaminases.
- Physicians should consider the diagnosis of pancreatitis in patients who develop sudden acute abdominal pain during therapy.
- Additional international normalized ratio (INR) measurements are recommended in patients treated with warfarin.

Reference: Communication from Merck Frosst/Schering Pharmaceuticals dated 1 February 2005 posted by Health Canada at http://www.hc-sc.gc.ca

Mefloquine: revised patient information

Canada — Health Canada has advised of the availability of revised patient information for prophylactic use of the antimalarial, mefloquine (Apo-Mefloquine®).

The warnings and contraindications sections have been modified to inform of:

- rare events that may occur with the use of Apo-Mefloquine, including anxiety, paranoia, depression, hallucinations, and psychotic behaviour; as well as suicidal ideation and suicide, for which no causal relationship with the use of Apo-Mefloquine has been confirmed; and
- the contraindication of Apo-Mefloquine for malaria prophylaxis in patients with active depression or a history of psychiatric disturbance (including depression, generalized anxiety disorder, psychosis, schizophrenia, or other major psychiatric disorder) or a history of convulsions.

Reference: Health Canada, 25 January 2005 at http://www.hc-sc.gc.ca

Atomoxatine and liver injury

United States of America — The US Food and Drug Administration (FDA) is advising health care professionals of a new warning for atomoxatine (Strattera®), a drug approved for attention deficit hyperactivity disorder (ADHD) in adults and children. The labelling is being updated with a bolded warning about the potential for severe liver injury following two reports in patients (a teenager and an adult) who had been treated with atomoxatine for several months, both of whom recovered. The labelling warns that severe liver injury may progress to liver failure resulting in death or the need for a liver transplant in a small percentage of patients. It also notes that the number of actual cases of severe liver injury is unknown because of under-reporting of postmarketing adverse events.

Atomoxatine, a selective norepinephrine reuptake inhibitor, has been on the market since 2002 and has been used in more than 2 million patients. In clinical trials of 6000 patients, no signal for liver problems (hepatotoxicity) had emerged.

Reference: *FDA Talk Paper*, T04–60 2004 at http://www.fda.gov/medwatch/

Gefitinib: failure to show survival in lung cancer

United States of America — The Food and Drug Administration (FDA) has reported that a large clinical trial comparing gefitinib (Iressa®) with placebo in patients with non-small cell lung cancer who had failed other courses of cancer therapy showed no survival benefit. Patients currently taking gefitinib should consult their physicians as soon as possible; patients should not change their therapy without first consulting their physicians.

Alternative therapies are available. FDA has approved docetaxel (Taxotere®) and erlotinib (Tarceva®), both of which have been shown in studies to improve survival in patients with non-small cell lung cancer whose cancer has progressed while on previous therapies. Pemetrexed (Alimta®) has received an accelerated approval based on the surrogate endpoint for this use but has not yet demonstrated any survival benefit.

FDA approved gefitinib in 2003 under the Agency's accelerated approval program for the treat-

ment of patients with non-small cell lung cancer who had failed two or more courses of chemotherapy. Gefitinib was approved because the data from clinical trials showed that it caused significant shrinkage in tumours in about 10% of patients, and this was thought likely to increase patients' overall survival time.

After the approval of gefitinib, the manufacturer conducted a study in approximately 1700 patients to determine whether the drug would in fact prolong survival in comparison to patients taking placebo. The results announced indicate that the drug did not prolong survival. FDA will determine whether gefitinib should be withdrawn from the market or if other regulatory actions are appropriate after it has evaluated the recent study results.

Reference: *FDA statement*. (revised version). 17 December 2004. http://www.fda.gov/medwatch/

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.

Regulatory Action and News

Progress on defining borderline pharmaceutical products

European Union — Article 2.2 of Directive 2001/83/EC on the Community code relating to medicinal products for human use aims to address the issue of the borderline products. The new legislation is applicable from 30 October 2005 through implementation in national legislation by Member States (1). The aim of the new provision is to clarify, from a legal point of view, the situation of certain borderline products for which there is uncertainty regarding which regulatory system should be applied. The intention of the new legislation is not to extend the definition of medicinal products currently covered by other legislative frameworks.

The Directive sets out clear rules for the classification of products:

(a) if a product falls clearly under the definition of other product categories, pharmaceutical legislation does not apply.

(b) If a product falls clearly under the definition of a medicinal product, pharmaceutical legislation will apply.

(c) If after due consideration of all relevant criteria and taking into account all characteristics of the product doubt remains whether a product falls within the definition of a medicinal product or of a product covered by other Community legislation, the pharmaceutical legislation will apply.

A workshop was recently organized between the Commission, Member States and industrial sectors for input on how to apply the new provision. It provided a unique opportunity to work on the clarifications needed concerning application of the legal framework and a definition/delimitation of medicinal products, food/food supplements, cosmetics and medical devices. Discussion focused on a variety of issues, including:

- Implementing a consistent legal approach across the European Union; and
- The need to offer a detailed explanation of the term 'modifying physiological functions'.

Member States insisted on the need to develop a 'Commission-driven cooperation mechanism' to overcome the sectorial approaches often prevailing at Member State and Community level.

A Commission report regarding the use of substances other than vitamins and minerals in food supplements is to be prepared by the Commission for 2007.

References

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2. European Union. Official Journal L – 311 of 28/11/2004. http://www.eu.int

Temozolomide approved for glioblastoma multiforme

United States of America — The Food and Drug Administration (FDA) has granted approval of a new indication for temozolomide (Temodar®). The drug, used concurrently with radiotherapy and as maintenance therapy after radiotherapy, can extend the lives of adult patients newly diagnosed with glioblastoma multiforme (GBM), the most common form of malignant brain cancer.

GBM is usually fatal. The annual incidence of GBM is four to five cases per 100 000 persons with 8000 to 10 000 new cases diagnosed per year in North America.

The new approval of temozolomide for GBM was based on efficacy and safety data from a large randomized controlled study conducted by the European Organization for Research and Treatment of Cancer (EORTC) in patients with newly diagnosed GBM. Patients were randomized to treatment with radiation alone or to treatment with radiotherapy plus temozolomide. In the multicentre trial of 573 patients, median survival was improved by two and a half months in the temozolomide group, a significant benefit. The median survival was 14.6 months with radio-

therapy plus temozolomide and 12.1 months with radiotherapy alone.

Temozolomide was previously granted accelerated approval in 1999 for the treatment of adult patients with another form of brain tumour (anaplastic astrocytoma) in relapse after chemotherapy with nitrosurea and procarbazine.

Side effects for temozolomide reported include nausea, vomiting, headaches, fatigue, and anorexia. Preventive treatment for pneumocystis carinii pneumonia is required when temozolomide is administered with radiotherapy.

Reference: *FDA Talk Paper*, T05-07. 16 March 2005

Pramlintide approved for diabetes

United States of America — The Food and Drug Administration (FDA) has approved an injectable medicine, pramlintide acetate (Symlin®), to control blood sugar for adults with type 1 and type 2 diabetes. Pramlintide, a synthetic analogue of the naturally occurring human hormone amylin, is to be used in addition to insulin therapy in patients who cannot achieve adequate control of their blood sugar on intensive insulin therapy alone.

Pramlintide will be the only therapy for the treatment of type 1 diabetes other than insulin. Patients with type 2 diabetes already have several other types of oral therapies available.

The safety and efficacy of pramlintide has been studied in approximately 5000 patients. Overall, pramlintide therapy was associated, in patients with both types of diabetes, with improvement in the control of blood glucose and weight loss. So-called “tight” control of blood sugar is desirable in all patients with diabetes in order to reduce risks for long-term adverse consequences of the disease, including blindness, kidney disease, and vascular disease.

Pramlintide is to be used only in combination with insulin to help lower blood sugar during the 3 hours after meals. pramlintide will have a Medication Guide (FDA-approved patient labelling) and a Risk Minimization Action Plan (RiskMAP) due to three areas of concern. First, the principle risk associated with pramlintide therapy is hypoglycaemia, and this risk is greatest in patients with type 1 diabetes and in patients with gastroparesis (motility problems of the stomach – a long-term complication of diabetes). Second, the potential for medication errors, specifically mixing of pramlintide with insulin in the same syringe, which can alter the activity of the insulin, is addressed in the Medication Guide and in physician labelling. Finally, the potential for off-label use in patients where the benefit/risk profile has not been characterized or demonstrated is also a concern and will be monitored by the sponsor.

Pramlintide should not be used if patients cannot tell when their blood sugar is low, have gastroparesis (slow stomach emptying), or are allergic to pramlintide acetate, metacresol, D-mannitol, acetic acid, or sodium acetate. Side effects associated with pramlintide include but are not limited to nausea, vomiting, abdominal pain, headache, fatigue and dizziness.

Pramlintide has not been evaluated in the pediatric population.

Reference: *FDA Talk Paper*, T05-08. 17 March 2005

Entecavir approved for chronic hepatitis B

United States of America — The Food and Drug Administration (FDA) has announced the approval of entecavir (Baraclude®) tablets and oral solution for the treatment of chronic hepatitis B in adults.

Chronic hepatitis B is a serious disease that can cause lifelong infection, cirrhosis, liver cancer, liver failure, and death. According to the Centers for Disease Control and Prevention, approximately 1.25 million Americans are chronically infected with the HBV virus.

Entecavir slows the progression of chronic hepatitis B by interfering with viral reproduction. Approval was based on the results of three comparison studies with lamivudine. In all three clinical studies, patients treated with entecavir showed significant improvement in the liver inflammation caused by HBV and an improvement in the degree of liver fibrosis.

The major adverse events associated with the use of entecavir include severe, acute exacerbation of hepatitis B after discontinuation of entecavir, headache, abdominal pain, diarrhoea, fatigue, and dizziness. The labelling for entecavir states that patients who discontinue should be monitored at repeated intervals over a period of time for liver function.

Reference: *FDA Talk Paper*, T05-11. 30 March 2005

DNA-based test approved to detect cystic fibrosis

United States of America — The Food and Drug Administration (FDA) has approved the first DNA-based blood test to help detect cystic fibrosis. The Tag-It Cystic Fibrosis Kit® directly analyses human DNA to find genetic variations indicative of the disease. The test will be used to help diagnose cystic fibrosis in children and to identify adults who are "carriers" of the gene variations.

Cystic fibrosis is a serious genetic disorder affecting the lungs and other organs that often leads to an early death. It is the number one cause of chronic lung disease in children and young adults, as well as the most common fatal hereditary disorder affecting Caucasians in the United States. The disease affects about one in 2500–3300 Caucasian babies. Half of the people with cystic fibrosis die by the age of 30.

The Tag-It test® identifies a group of variations in a gene called the "cystic fibrosis transmembrane conductance regulator" or CFTR gene that causes cystic fibrosis. FDA approved Tag-It based on a manufacturer study of hundreds of DNA samples showing that the test identifies the CFTR gene variations with a high degree of certainty. The manufacturer also provided FDA with a broad range of supporting peer-reviewed literature.

Since Tag-It detects a limited number of the more than 1300 genetic variations identified in the CFTR gene, the test should not be used alone to diagnose cystic fibrosis. Physicians should interpret test results in the context of the patient's clinical condition, ethnicity, and family history. Also, patients may need genetic counselling to help them understand their test results.

Reference: *FDA News*, P05-23. 9 May 2005 . http://www.fda.gov

Natalizumab: marketing withdrawal pending evaluation

United States of America — The Food and Drug Administration (FDA) has issued a public health advisory to inform patients and health care providers about the suspended marketing of natalizumab (Tysabri®) while two serious adverse events are evaluated. Natalizumab received accelerated approval from FDA in November 2004 as an innovative treatment for relapsing forms of multiple sclerosis (MS).

FDA received a report from the manufacturer of one confirmed fatal case and one possible case of progressive multifocal leukoencephalopathy (PML). PML is a rare, serious progressive neurologic disease usually occurring in immunosuppressed patients. There is no known effective treatment for PML. The relationship between natalizumab and PML is not known at this time, but because of the serious and often fatal nature of PML, FDA concurred with the company that the drug be voluntarily withdrawn from marketing and that the use of natalizumab in clinical trials be suspended until more is known.

During the review of natalizumab for marketing approval, FDA conducted an intensive analysis of possible adverse events that might be related to effects of the drug on the immune system. No cases of PML were seen in the clinical trials.

Reference: *FDA News*, P05-07. 28 February 2005. http://www.fda.gov/cder/drug/advisory/natalizumab.htm.

Rosiglitazone (Nyracta®): voluntary withdrawal

European Union — On 11 July 2000 the European Commission granted a marketing authorization for the whole European Union to the manufacturers of rosiglitazone (Rosiglitazone is indicated as oral monotherapy in type 2 diabetes mellitus patients, particularly overweight patients, inadequately controlled by diet and exercise for whom metformin is inappropriate because of contraindications or intolerance.

Rosiglitazone is also indicated for oral combination treatment in type 2 diabetes mellitus patients with insufficient glycaemic control despite maximal tolerated dose of oral monotherapy with either metformin or a sulphonylurea:

- in combination with metformin particularly in overweight patients.
- in combination with a sulphonylurea only in patients who show intolerance to metformin or for whom metformin is contraindicated.

Nyracta® was not marketed anywhere in the European Union. On 1 November 2004 the Marketing Authorization Holder notified the European Commission of its decision to voluntarily withdraw the Marketing Authorization for Nyracta® as there were no plans to market this product in the future. It should be noted that there

is still one Community Marketing Authorization valid throughout the European Union for rosiglitazone i.e. Avandia® (2)

References

1. European Medicines Agency Public Statement EMEA/41043/2005 dated 4 April 2005 on http://www.emea.eu.int

2. Thiazolidinediones experience. *WHO Drug Information*, **17**(2): 92 (2003).

Risk management legislation

European Union — As a result of a collaboration between the Heads of the National Medicines Agencies across the EU and the European Medicines Agency (EMEA), two key documents on the European Risk Management Strategy have been published. They set out what has been delivered to date and priorities for the collaborative European Union (EU) system of monitoring the safety of medicines in the future. Their publication comes at a time when the profile of safety issues, in relation to medicines across the EU has never been higher.

The impact of this collaborative work is set out in the 'Progress report of the ad hoc working group on the implementation of the European Risk Management Strategy' which describes measures designed to strengthen the safety monitoring of medicines in the EU. By enabling authorities to better identify, assess and manage risks as they emerge, more effective, coordinated actions and communications across the EU regulatory system can be delivered. It is widely recognized that no effective medicine is without risk. But strong regulation, based on robust scientific decision-making should clearly assess the balance of benefits against the known risks.

The pharmaceutical industry, healthcare professionals and patients all have their part to play. Medicines regulation cannot protect the public from every risk and the Strategy aims at putting in place a coherent approach to the detection, assessment, minimization and communication of risks in Europe. The next steps of the Strategy are set out in an 'Action plan to further progress the European Risk Management Strategy'. This builds on progress made and the need to respond to public concerns over the safety of medicines.

From November 2005 onwards, new EU pharmaceutical legislation will give authorities additional tools for monitoring the safety of medicines, as well as greater scope for urgent regulatory action once the benefit/risk balance of a medicinal product becomes unfavourable. The legislation will also result in increased transparency on safety issues and facilitate communication, with the provision of timely and targeted information to healthcare professionals and the public.

Complementary initiatives to put in place an intensive drug-monitoring system will focus on risk detection, risk assessment, risk minimization and risk communication.

The action plan also highlights the need to make best use of scientific resources and expertise available at EU level, and on enhancing quality assurance. This should lead to a further strengthening of the EU regulatory system overall, resulting in the establishment of a 'network of excellence' for medicines regulation.

Reference: *Progress report of the ad hoc working group on the implementation of the European Risk Management Strategy.* Doc. Ref. EMEA/136253/2005 available from: http://www.emea.eu.int.

New pharmacogenomics guidance

United States of America — As part of an initiative to speed development of new medical products through the science of pharmacogenomics, the Food and Drug Administration (FDA) has issued a guidance document, *Pharmacogenomic Data Submissions*.

Pharmacogenomics allows health care providers to identify sources of an individual's profile of drug response and predict the best possible treatment option for this individual. Until now, this technology has enabled the development of targeted therapies for metastatic breast cancer, chronic myeloid leukaemia and metastatic colorectal cancer.

Instead of the standard hit-or-miss approach to treating patients, where it can take multiple attempts to find the right drug and the right dose, doctors will be able to analyse a patient's genetic profile and prescribe the best available drug therapy and dose from the start. Both the guidance and a new Web page are part of a broad effort under way to foster pharmacogenomics during drug development.

The guidance clarifies how pharmacogenomic data will be evaluated and describes what data

will be needed during the marketing application review process, the format for submissions, and the data that will be used during regulatory decision making. The guidance also explains a new mechanism for industry to voluntarily submit research data to further the scientific exchange of information as we move into more advanced areas of pharmacogenomic research. The voluntary data, which will be reviewed by an internal, agency-wide group and will not be used for regulatory decision making, will help FDA and industry gain valuable experience as this new field continues to evolve.

FDA's new pharmacogenomics Web page is available at http://www.fda.gov/cder/genomics/default.htm. The Web site ("Genomics at FDA") will include detailed information on submitting genomic data, including a decision tree to simplify data submissions, relevant regulatory information, and FDA contact information. The agency has already received several pharmacogenomic data submissions through both the regulatory and voluntary processes, and has recently approved the first laboratory test, the Amplichip Cytochrome P450 Genotyping Test®, which will enable physicians to use genetic information to select the right doses of certain medications for cardiac, psychiatric diseases and cancer.

Reference: *FDA News*, P05-12. 22 March 2005 http://www.fda.gov/cder/genomics/

Current Topics

WHO clinical trial registration initiative

Access to information about ongoing, completed or published clinical research is essential for appropriate decision-making. Researchers, research funders, policy-makers, medical practitioners, patients and the general public need such information to improve research practices, policy, and clinical decision-making.

For several decades, many health researchers have proposed public registration of clinical trial data. Although many registers for ongoing clinical trials now exist, they are designed for a variety of purposes and there has been no comprehensive global registration process until now. Incomplete registration and register fragmentation make it impossible to identify with certainty – even within a narrow field or for a single intervention – all existing controlled trials.

Recently, a consensus for public registration and reporting of clinical trials has been growing following safety concerns involving at least three drugs where availability of relevant clinical trial data could have favourably affected prescribing behaviour outcomes. As a result, several pharmaceutical companies have announced plans or have actually begun their own trial registers. This announcement has further been supported by the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) (see page 129).

The International Committee of Medical Journal Editors (ICMJE) has called for public registration of clinical trials (1) and the ICMJE has stated that as of 1 July 2005, only registered trials will be eligible for journal publication. (See page 128).

The World Health Organization has now established an international clinical trial registration platform (2). This platform will link registers into a comprehensive network, harmonize register and trial registration standards, provide global trial identification and search capability, promote compliance, and help strengthen research monitoring capacity where needed. WHO will undertake this effort with the advice and input from clinical research stakeholders.

A public and readily-searchable register of clinical trials overseen by an objective international body drawing on input from relevant stakeholders will underpin good research practice, assist in making treatment decisions, and increase public trust in clinical research. In April 2005, a consultation was convened by WHO to initiate a framework for development of the international clinical trial registration platform. Progress was made during the meeting on determining essential elements of the strategy and discussion took place on the development of a guide for trial registration. Following are some of the basic provisions of the initiative.

Registration

- Any research project that prospectively assigns human participants or groups to one or more health-related interventions to evaluate the effects on health outcomes should be registered.

- Trials aimed to assess all health and health care interventions, not only medicines and medical devices, should be registered. The intent of this definition is to include trials that could inform health and health care practice.

- Exploratory studies that are not designed to influence health practice and that serve only to set direction for future testing need not be registered.

- When trial sponsors are unsure whether to register or not, registration is recommended.

- Trials should be registered as early as possible, ideally before recruitment of the first participant.

- The informed consent form should include the trial identification number.

Trial characteristics

The minimum data set recommended is set out in the table overleaf. Data set should be reported in English.

Table: minimum registration data set

Item	Comment
1. Unique trial number	The unique trial number will be established be the primary registering entity (the registry).
2. Trial registration date	The date of registration will be established by the primary registering entity.
3. Secondary IDs	May be assigned by sponsors or other interested parties (there may be none).
4. Funding source(s)	Name of the organization(s) that provided funding for the study.
5. Primary sponsor	The main entity responsible for performing the research.
6. Secondary sponsor(s)	The secondary entities, if any, responsible for performing the research.
7. Responsible contact person	Public contact person for the trial, for patients interested in participating.
8. Research contact person	Person to contact for scientific inquiries about the trial.
9. Title of the study	Brief title chosen by the research group (can be omitted if the researchers wish).
10. Official scientific title of the study	This title must include the name of the intervention, the condition being studied, and the outcome
11. Research ethics review	Has the study at the time of registration received appropriate ethics committee approval (yes/no)? (It is assumed that all registered trials will be approved by an ethics board before commencing.)
12. Condition	The medical condition being studied (e.g., asthma, myocar dial infarction, depression).
13. Intervention(s)	A description of the study and comparison/control intervention(s) (For a drug or other product registered for public sale anywhere in the world, this is the generic name; for an unregistered drug the generic name or company serial number is acceptable). The duration of the intervention(s) must be specified.
14. Key inclusion and exclusion criteria	Key patient characteristics that determine eligibility for participation in the study.
15. Study type	Database should provide drop-down lists for selection. This would include choices for randomized vs. non-randomized, type of masking (e.g., double-blind, single-blind), type of controls (e.g., placebo, active), and group assignment, (e.g., parallel, crossover, factorial).
16. Anticipated trial start date	Estimated enrollment date of the first participant.
17. Target sample size	The total number of subjects the investigators plan to enroll before closing the trial to new participants.
18. Recruitment status	Is this information available (yes/no) (If yes, link to information).
19. Primary outcome	The primary outcome that the study was designed to evaluate Description should include the time at which the outcome is measured (e.g., blood pressure at 12 months)
20. Key secondary outcomes	The secondary outcomes specified in the protocol. Description should include time of measurement.

All items listed are required for scientific and ethical reasons. Therefore, all fields in the minimum data set should normally be entered into the register at the time of trial registration. However, one or more of data items 10, 13, 17, 19, 20 may be regarded as sensitive for competitive reasons by the sponsor who may wish to delay release of the information. In this event, all data items should be made publicly available by agreed dates. WHO will convene a group to develop a mechanism to advise on requests to delay release of one or more of data items until a requested date.

Results disclosure standards

The results database will be useful for multiple constituencies (reviewers, patients, and policy-makers). The database is assumed to be an extension of the trial register and the data are meant to complement, but not replace, peer-review and publication. Thus, results disclosure should not be a barrier to peer-review journal publication.

While there is no single agreed definition of study completion, the results should be disclosed within one year of completion as a general rule. Results

of trials of commercially developed drugs (newly registered drugs) should be disclosed within one year of first product launch. In deciding the extent of disclosure, the ICH E3 synopsis is proposed as a guide (with the addition of the trial register number).

The sponsor is responsible for ensuring that results are disclosed. For unsponsored trials, the principal investigator takes responsibility and for marketed products, the license holder is responsible for updates.

References

1. International Committee of Medical Journal Editors (ICMJE) on http://www.bmj.com

2. International Clinical Trials Registry Platform on http://www.who.int/ictrp/background/en/

3. WHO facilitates international collaboration in setting standards for clinical trialk registration. www.thelancet.com online 24 May 2005.

International registration of trial information: Ottawa statement

Registration of trials is essential to ensure all results are publicly available and that ethical obligations to participants are met. Recent evidence of selective reporting of results has eroded public and academic confidence in publications of clinical trials, leading to renewed calls for trial registration. The rationale for registering trials is well known (Box 1). Most importantly, the contribution to social good that justifies research on human participants is not realized when resulting knowledge remains invisible.

The Canadian Institutes of Health Research hosted an open meeting on 4 October 2004 in Ottawa, Canada, to foster international consensus on trial registration. The resulting Ottawa statement issued by the International Committee of Medical Journal Editors (ICMJE) aims to establish internationally recognized principles for registration (1, 2) as a follow-on to the Trials Registration Policy issued in 2004 (3).

Summary of principles

The mandatory registration of all trials has three components:

- Obtaining an internationally unique identification number (unique ID)
- Registering the original protocol along with subsequent amendments
- Registering the trial results.

Key principles

Registering all types of trials: Protocol information and results from all trials related to health or healthcare—regardless of topic, design, outcomes, or market status of interventions examined—should be registered and publicly available.

Timing of public release of protocol information: The public should have cost-free access to the Unique ID, minimum protocol items, and

Box 1: Rationale for registration of clinical trials

Ethical
- Respect the investigator-participant covenant to contribute to biomedical knowledge by making trial methods and results public
- Provide global open access to information
- Reduce unnecessary duplication of invested research resources through awareness of existing trials
- Assure accountability with regard to global standards for ethical research
- Enable monitoring of adherence to ethical principles and process

Scientific
- Increase the reliability and availability of evidence on which healthcare decisions are based
- Improve trial participation
- Increase opportunities for collaboration
- Ensure transparency of trial design and methods
- Provide open review of protocols to improve trial quality and refine methods
- Provide means for identification and prevention of biased under-reporting or over-reporting of research
- Accelerate knowledge creation

consent forms prior to participant enrolment. Registered amendments should be made publicly available as they occur.

Registering unpublished results: At a minimum, results for outcomes and analyses specified in the protocol (as approved by the institutional review boards/independent ethics committees) as well as data on harms, should be registered regardless of whether or not they are published.

References

1. Krleza-Jeric, K, Chan, A., Dickersin, K. et al. Principles for international registration of protocol information and results from human trials of health related interventions: Ottawa statement (part 1). *British Medical Journal*, **330**: 956–958 (2005). http://www.bmj.com

2. De Angelis, C., Drazen, J.M., Frizelle, F.A. et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. *Annals of Internal Medicine*, **141**: 477–478. (2004) and http://www.icmje.org

3. Selective reporting and clinical trial registration & Trials registration policy. *WHO Drug Information*, Volume 18, Number 4, page 278-280 (2004).

Disclosure of information on clinical trials

The research-based pharmaceutical industry has announced principles of disclosure of clinical trial information through clinical trial registries and databases. The International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) has jointly developed these principles together with three other industry associations: the European Federation of Pharmaceutical Industries and Associations (EFPIA), the Japanese Pharmaceutical Manufactures Association (JPMA) and the Pharmaceutical Research and Manufacturers of America (PhRMA).

The Joint Position on the Disclosure of Clinical Trial Information via Clinical Trial Registries and Databases demonstrates the innovative pharmaceutical industry's commitment to increasing the transparency of clinical trials sponsored by their member companies. The industry recognizes that there are important public health benefits, including increased confidence, associated with making clinical trial information more widely available to healthcare practitioners, patients and others. Beginning mid-2005, the industry will make the results public of trials that have taken place — both positive or negative — together with information on those that are just being initiated. This includes all trials except exploratory trials, where results will be published only if they have significant medical importance.

Trial results will be published in a standard, non-promotional summary that will include a description of trial design and methodology, results of primary and secondary outcome measures described in the protocol, and safety results. If the results are published in a peer-reviewed medical journal, the database will include a link to the relevant article. The results will be published within one year after the medicine is approved or, for post-approval trials, within one year of completion.

Reference: IFPMA website at http://www.ifpma.org/News/

Forecasting antiretroviral and diagnostic needs

Over the last four years, access to antiretrovirals (ARVs) and diagnostics for people living with HIV/AIDS (PLWHA) has become easier owing to the availability of more affordable generic products of assured quality supported by public pressure to overcome access barriers. In addition, substantial funding became available through the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM).

The World Health Organization (WHO) and its partners have committed to a goal of 3 million people on ARV treatment by 2005 (the 3 by 5 Initiative). This requires massive scale-up in country-level operations. Setting up services providing diagnosis, care and treatment to HIV patients is complex. Continuous supply of ARVs will be crucial to ensure that no treatment interruptions occur.

Three factors are essential to assure success of the 3 by 5 Initiative:

- government commitment to providing ARVs within public health services;
- availability of guidelines for simplified ARV treatment;
- availability of prequalified generic and fixed-dose combinations (FDCs) of ARVs and prequalified diagnostic test kits.

A WHO-UNICEF technical consultation was held in Geneva, Switzerland, 28-29 June 2004. Thirty-

two participants attended from 14 organizations to review best-practices and identify common problems in quantifying antiretrovirals and diagnostics for treatment of HIV patients. The overall purpose of the meeting was to support efforts towards better forecasting, and to promote the use of software packages to estimate needs within tight budgets.

The Consultation involved formal presentations and six working groups were established to provide recommendations on:

- central versus peripheral quantification;
- quantification of paediatric ARV needs;
- quantification of HIV diagnostics and laboratory equipment;
- specifications of software tools;
- national quantification policy; and
- implementation and capacity-building.

Three software systems currently under development for forecasting and estimating needs were demonstrated. From different country- and industry-perspectives, a number of points surfaced. Of particular importance is the complexity and scale of HIV infection; its status in different countries and varying capacity within the different levels of health systems. Additional issues are raised by the characteristics of supply management for a variety of products with differing indications, administration and shelf-life. A critical requirement being that a patient's treatment should not be discontinued.

Price, availability and donor-community views were also addressed. Accuracy of data and market-intelligence were seen to be key challenges for health services and the pharmaceutical industry. Important gap-analysis pointed to the unsuitability of adult formulations for children and recognition that HIV in children cannot be easily diagnosed, beyond reliance on the local mother-to-child transmission rate. The overwhelming policy decision facing health authorities is to determine who should receive treatment.

As an outcome of the meeting, a Forecasting Technical Consultation Group was established to continue working and sharing information through a restricted access website. A five-point action plan was agreed, including field-testing of newly developed software packages for forecasting and estimating needs in two countries by June 2005. Also, on request, WHO will validate existing quantification software packages in the second half of 2005. One important theme of the Consultation was the continued need for networking to share best-practices among all involved, and to develop capacity building and training for health practitioners. A key outcome is expected to be the sustainable availability and uninterrupted supply of antiretrovirals and diagnostics to patients, based upon improved accuracy of forecasting.

Reference: *Forecasting of antiretrovirals and diagnostics*. WHO-UNICEF Technical Consultation 28-29 June 2005, Geneva. Available on http://whq1ibdoc.who.int/publications or who.int/medicines/library/doseng

ATC/DDD classification (temporary)

The following anatomical therapeutic chemical (ATC) classifications and defined daily doses (DDDs) were agreed by the WHO International Working Group for Drug Statistics Methodology in April 2005. Comments or objections to the decisions should be forwarded to the WHO Collaborating Centre for Drug Statistics Methodology at whocc@nmd.no before 1 September 2005. If no objections are received before this date, the new ATC codes and DDDs will be considered final and 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. 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):		
	HMG CoA reductase inhibitors in combinations with other lipid modifying agents	C10BA
	HMG CoA reductase inhibitors, other combinations	C10BX
	Lipid modifying agents, combinations	C10B
New ATC 5th level codes:		
	agomelatine	N06AX22
	benfotiamine	A11DA03
	bivalirudin	B01AE06
	cilansetron	A03AE03
	clofarabine	L01BB06
	combinations	R07AA30
	dexmedetomidine	N05CM18
	efaproxiral	L01XD06
	enalapril and calcium channel blockers	C09BB02
	entecavir	J05AF10
	fumagillin	P01AX10
	galsulfase	A16AB08
	loteprednol	S01BA14
	olopatadine	R01AC08
	palifermin	V03AF08
	paricalcitol	A11CC07
	pegaptanib	S01XA17
	posaconazole	J02AC04
	ranolazine	C01EB18
	rotigotine	N04BC09
	rufinamide	N03AF03
	simvastatin and ezetimibe	C10BA02
	tipranavir	J05AE09
	zofenopril and diuretics	C09BA15

ATC code changes:

INN/common name	Previous ATC	New ATC
anagrelide	B01AC14	L01XX35
atorvastatin and amlodipine	C10AA55	C10BX03[1)]
lovastatin and nicotinic acid	C10AA52	C10BA01[1)]
pravastatin and acetylsalicylic acid	C10AA53	C10BX02[1)]
simvastatin and acetylsalicylic acid	C10AA51	C10BX01[1)]
loteprednol	S01BA14	

ATC name changes

Previous	New	ATC code
Agents used in photodynamic therapy	Sensitizers used in photodynamic/ radiation therapy	L01XD
Cholesterol and triglyceride reducers	Lipid modifying agents, *plain*	C10A
Other cholesterol and triglyceride reducers	Other lipid modifying agents	C10AX
Serum lipid reducing agents	Lipid modifying agents	C10

New DDDs:

INN/common name	DDD	Unit	Adm.R	ATC code
acemetacin	0.12	g	O	M01AB11
acetyldihydrocodeine	30.0	mg	O	R05DA12
ambroxol	0.12	g	O	R05CB06
artemether	120.0	mg	P	P01BE02
bivalirudin	0.25	g	P	B01AE06
ciclesonide	0.16	mg	Inhal. aerosol	R03BA08
cinacalcet	90.0	mg	O	H05BX01
citalopram	20.0	mg	P	N06AB04
darifenacin	7.5	mg	O	G04BD10
dexketoprofen	75.0	mg	P	M01AE17
duloxetine	60.0	mg	O	N06AX21
efalizumab	10.0	mg	P	L04AA21
eplerenone	50.0	mg	O	C03DA04
ibandronic acid	2.5	mg	O	M05BA06
lanthanum carbonate	2.25	g[2)]	O	V03AE03
sevelamer	6.4	g	O	V03AE02
strontium ranelate	2.0	g	O	M05BX03
treprostinil	4.3	mg	P	B01AC21
zolmitriptan	2.5	mg	N	N02CC03

2) expresses as lanthanum

Change of DDDs

(Note that the changes will not be implemented before January 2006).

INN/common name	Previous DDD	New DDD	ATC Code
amprenavir	2.4 g O	1.2 g O	J05AE05
sirolinus	6 mg O	3 mg O	L04AA10

ATC/DDD classification (final)

The following anatomical therapeutic chemical (ATC) classifications and defined daily doses (DDDs) were agreed by the WHO International Working Group for Drug Statistics Methodology in October 2004. They came into force on 1 March 2005 and 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. 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):		
	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
	fatifloxacin	S01AX21
	histamine dihydrochloride	L03AX14
	ibritumomab tiuxetan [^{90}Y]	V10XX02
	iodoform	D09AA13
	ivabradine	C01EB17
	measles, combinations with mumps, rubella and 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 name changes

Previous	New	ATC code
histamine	histamine phosphate	V04CG03
anti-parathyroid hormones	Aanti-parathyroid 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
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
nicotine	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*

Recent Publications and Sources of Information

Sources and prices of malaria medicines and products

WHO has released a report entitled *Sources and Prices of Selected Products for the Prevention, Diagnosis and Treatment of Malaria* which provides market information on products reviewed for the prevention, diagnosis and treatment of malaria from 80 manufacturers in 20 countries. It gives purchasers of malaria-related products a range of choices related to suppliers and affordability. The medicines included were selected on the basis of WHO treatment recommendations. The list is not exhaustive but covers the most commonly used antimalarials, with paediatric forms included wherever possible.

This report follows a similar format as *Sources and Prices of Selected Medicines and Diagnostics for People Living with HIV/AIDS* and was commenced in 2004 by Roll Back Malaria Partnership Secretariat (RBM), WHO, UNICEF, Population Services International (PSI), and Management Sciences for Health (MSH). The report includes sections on antimalarial medicines, mosquito nets, diagnostic tests, insecticides, insecticide spraying equipment, and resistance test kits.

There is also a section on the registration status of products. This information will be useful for countries that are in the process of granting marketing authorization to malaria-related products. Detailed information is provided on the artemisinin-based combination therapy, and on how to place an order for Coartem(R) through WHO and UNICEF.

Sources and Prices of Selected Products for the Prevention, Diagnosis and Treatment of Malaria is available on the following web sites:

RBM Partnership: http://rbm.who.int/mmss
UNICEF: http://www.unicef.org
WHO: http://www.who.int/medicines
PSI: http://www.psi.org
MSH: www.msh.org

Available in haard copy from WHO at edmdoccentre@who.int

Launch of a searchable online database of adverse reactions

Health Canada has announced the launch of a searchable online database that will, for the first time, allow immediate, direct access to the latest reported adverse reactions to health products as recorded in Health Canada's Canadian Adverse Drug Reaction Information System (CADRIS).

Health Canada receives reports of suspected adverse reactions from consumers, health care professionals and product manufacturers. This information is then recorded in the CADRIS, the information source for the new database. Before the launch of Health Canada's new online database, adverse reaction reports from CADRIS were available only by request, with a minimum wait time of two weeks.

The database can be searched by the name of the product or active ingredient, the date a report was received, patient age and gender, and the outcome of the adverse reaction. The online database does not include confidential information such as patient identity.

Health Canada's Adverse Reaction Monitoring Program receives reports through its national office and seven regional reporting centres across the country. The program collects and assesses adverse reactions for prescription and non-prescription drugs, natural health products, biological products (including vaccines), and radiopharmaceuticals.

A report of a particular reaction does not necessarily mean that the reaction was caused by the suspected health product, and individuals should check other sources of safety information concerning health products. They should also consult a health care professional before making treatment decisions.

The database can be consulted at http://www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/cadrmp-pcseim/index_e.html.

Recently published European Union guidelines

Common Technical Document (CTD) format: adopted guidelines

CHMP/EWP/252/03
Guideline on clinical investigation of medicinal products intended for the treatment of neuropathic pain. Effective: 1 June 2005

CPMP/BWP/5180/03
Guideline on assessing the risk for virus transmission - new chapter 6 of the note for guidance on plasma - derived medicinal products (CPMP/BWP/269/95). Effective: 20 May 2005

CPMP/EWP/788/01
Note for Guidance on Clinical Investigation of Medicinal Products for the Treatment of Migraine. Effective: 20 May 2005

CPMP/EWP/2863/99
Points to Consider on Adjustment for Baseline Covariates. Effective: 20 May 2005

CPMP/EWP/3020/03
Note for guidance on clinical investigation of medicinal products in the treatment of lipid disorders. Effective: 20 May 2005

CPMP/BWP/CPMP/5136/03
Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to VCJD risk. Effective: 12 May 2005

EMEA/CPMP/3097/02
Guideline on Comparability of Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-Clinical and Clinical Issues. Effective: 12 May 2005

CPMP/EWP/612/00
Note for Guidance on Clinical Investigation of Medicinal Products for Treatment of Nociceptive Pain. Effective: 12 May 2005

EMEA/CPMP/BWP/3207/00, rev 1
Guideline on Comparability of Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Quality Issues. Effective: 2 May 2005

CPMP/SWP/2599/02, rev 1
Position Paper on Non-Clinical Safety Studies to Support Clinical Trials with a Single Microdose. Effective: 2 May 2005

EMEA/CPMP/BWP/3794/03
Guideline on the Scientific Data Requirements for a Plasma Master File (PMF). Effective: 18 February 2005

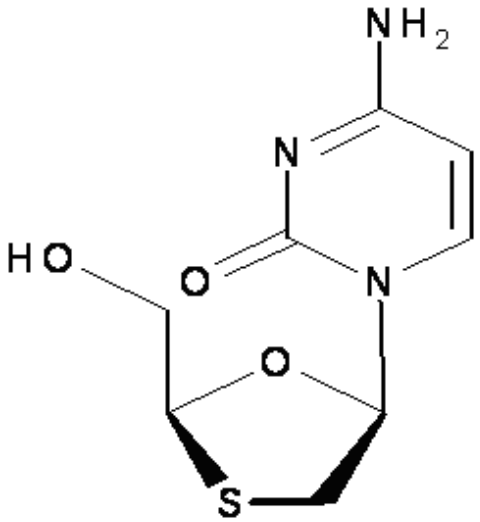
Available from: http:www.emea.eu.int/whatsnewp.htm

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. A draft for lamivudine is provided below for comment.

Lamivudinum
Lamivudine (first draft)



$C_8H_{11}N_3O_3S$

Relative molecular mass. 229.3

Chemical name. (-) 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one; CAS Reg. NO. 134678-17-4.

Description. A white or almost white powder.

Solubility. Soluble in water; sparingly soluble in methanol R; insoluble in acetone R.

Category. Antiretroviral (nucleoside reverse transcriptase inhibitor).

** Refers to The International Pharmacopoeia*

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

Manufacturer. The production method is validated to demonstrate that the substance, if tested, would comply with a limit of not more than 0.3% for 2S, 5R lamivudine enantiomer using a suitable chiral chromatographic method.

[Note from WHO Secretariat: This statement could be included, if reference to enantiomeric purity is considered advisable based on the relative toxicity of the 2S, 5R enantiomer. Such a statement would avoid the need to include a chiral chromatographic test within the analytical requirements of the monograph. The status of statements under the heading Manufacture will be defined in the General Notices of The International Pharmacopoeia.]

REQUIREMENTS

Lamivudine contains not less than 97.0% and not more than 103.0% of $C_8H_{11}N_3O_3S$, 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) 5 mg of the test substance per ml and (B) 5 mg of lamivudine 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) 5 mg of the test substance per ml and (B) 5 mg of lamivudine 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 the final solution prepared for the Assay, when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 577 to 637.

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 lamivudine RS or with the *reference spectrum* of lamivudine.

Specific optical rotation. Use a 10 mg/ml solution in methanol R and calculate with reference to the dried substance; $[\alpha]_D^{25°C}$ = -136° to -144°.

** Refers to The International Pharmacopoeia*

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 mg/g.

Sulfated ash. Not more than 2.0 mg/g.

Loss on Drying. Dry for 3 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 octadecylsilyl silica gel for chromatography R (Stationary phase A) (5μm) (Waters Hypersil BDS is suitable). As the mobile phase, use a mixture of 5 volumes of methanol R and 95 volumes of 1.9 g/l solution of ammonium acetate R, buffer adjusted to pH 3.8 with glacial acetic acid R.

Prepare the following solutions. For solution (1) prepare 0.5 mg/ml solution of test substance in the mobile phase. For solution (2) dilute 1.0 ml of solution (1) to 100 ml with mobile phase and then dilute 1.0 ml of this solution to 10 ml. For solution (3) dissolve 25 mg of salicylic acid R in 100 ml of mobile phase. Then dilute 1.0 ml of this solution to 500 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 277 nm.

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

Inject alternately 10 μl each of solutions (1), (2) and (3). Record the chromatograms for about 3 times the retention time of lamivudine in solution (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1), (2) and (3) and calculate the content of the related substances as a percentage.

In the chromatogram obtained with solution (1), the area of the peak (at a relative retention time of about 0.4) is not greater than 3 times the area of the peak in the chromatogram obtained with solution (2) (0.3%). The area of the peak (at a relative retention time of about 0.9) is not greater than 2 times the area of the peak in the chromatogram obtained with solution (2) (0.2%). The area of the peak corresponding to salicylic acid is not greater than that of the corresponding peak in the chromatogram obtained with solution (3) (0.1%). The area of any other peak apart from the principal peak is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.1%). The total area of all the peaks apart from the principal peak obtained in the chromatogram with solution (1) is not greater than 6 times the area of the peak obtained with solution (2) (0.6%). Disregard any peak with an area less than 0.5 times the area of the principal peak obtained with solution (2) (0.05%).

Assay: Weigh accurately about 25 mg of the test substance into a 200-ml volumetric flask. Add about 180 ml of water and dissolve by using an ultrasonic bath if necessary. Cool to room temperature and dilute to volume with water and mix.

Dilute 4 ml of this solution to 50 ml with 0.1M H_2SO_4 and mix. For the blank, use a solution prepared by mixing 4 ml of water with 50 ml of 0.1M H_2SO_4.

Measure the absorbance of a 1-cm layer of the final solution at a maximum about 280 nm against a solvent cell containing the blank. Calculate the content of $C_8H_{11}N_3O_3S$ using the absorptivity value of 60.7 ($A^{1\%}_{1cm}$ = 607).

** Refers to The International Pharmacopoeia*

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.

[Note from WHO Secretariat: Chemical structures will be included in the next version.]

A. *cis*-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid and enantiomer

B. 4-amino-1-[*trans*-2-(hydroxymethyl)-1,3-oxathiolan-5yl]pyrimidin-2(1*H*)-one

C. salicylic acid

D. 4-amino-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5yl]pyrimidin-2(1*H*)-one

E. 4-aminopyrimidin-2(1*H*)-one

F. pyrimidine-2,4(1*H*,3*H*)-dione

G. 4-amino-1-[(2*R*,3*S*,5*S*)-2-(hydroxymethyl)-3-oxo-1,3λ⁴-oxathiolan-5-yl]pyrimidin-2(1*H*)-one

H. 4-amino-1-[(2*R*,3*R*,5*S*)-2-(hydroxymethyl)-3-oxo-1,3λ⁴-oxathiolan-5-yl]pyrimidin-2(1*H*)-one

I. 4-amino-1-[(2*S*,4*S*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl]pyrimidin-2(1*H*)-one

J. 1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2,4(1*H*,3*H*)-dione

Reagent

Salicylic acid R. 2-hydroxybenzoic acid; $C_7H_6O_3$.

A commercially available reagent of suitable grade.

Storage. Keep protected from light.

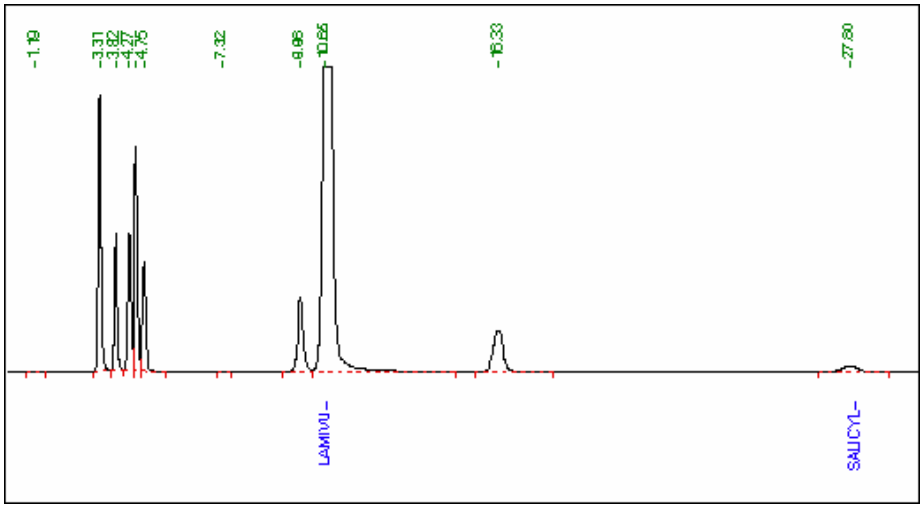


Figure 1: **HPLC chromatogram of lamivudine and its related impurities**

** Refers to The International Pharmacopoeia*

Monographs for antiretrovirals

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Nelfinavir mesilas pulvis oralis (first draft)
Nelfinavir mesilate oral powder

Category. Antiretroviral (protease inhibitor).

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

Labelling. The designation on the container of nelfinavir mesilate oral powder should state that the active ingredient is in the mesilate form, and the quantity should be indicated in terms of the equivalent amount of nelfinavir. Expiry date.

Additional information. Strength in the current WHO Model List of Essential Medicines: 50 mg of nelfinavir (as mesilate) per g.

Requirements

Complies with the monograph for “Oral Powders”.

Nelfinavir mesilate oral powder contains not less than 90.0 % and not more than 110.0 % of the amount of $C_{32}H_{45}N_3O_4S$ stated on the label.

Identity tests

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 the following 2 solutions in methanol R: (A) shake a quantity of oral powder equivalent to about 21 mg of nelfinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg nelfinavir 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 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 the following 2 solutions in methanol R: (A) shake a

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quantity of oral powder equivalent to about 21 mg of nelfinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg of nelfinavir 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. Spray the plate 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. To a quantity of the oral powder equivalent to about 20 mg of nelfinavir add 50 ml of methanol R, shake, and filter. Dilute 5 ml of the filtrate to 50 ml with the same solvent. The absorption spectrum of the resulting solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 253 nm.

Uniformity of mass

Weigh individually 20 doses taken at random from one or more containers with the measuring device provided and determine the individual and average masses. Not more than 2 of the individual masses deviate from the average mass by more than 10 % and none deviates by more than 20 %.

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: 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 (%)	Mobile phase B (%)	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

For solution (1) mix and transfer a quantity equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 ml volumetric flask. Add about 20 ml of methanol R, sonicate for about 15 minutes, allow to

** Refers to The International Pharmacopoeia*

cool to room temperature, and make up the volume using mobile phase A. Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of the filtered solution. For solution (2) dilute a suitable volume of solution A to obtain a concentration equivalent to 10.0 μg of nelfinavir per ml of mobile phase A. For solution (3) use 100 μg of methanesulfonic acid per ml of mobile phase A.

For the system suitability test: prepare solution (4) using 2 ml of solution (1) and 5 ml of sulphuric 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 225 nm.

Maintain the column at 35 °C.

Inject 20 μl of solution (4). The test is not valid unless the resolution between the principal peak (retention time = about 27 minutes) and the peak with a retention time relative to the principal peak of about 0.2 is not less than 15. The test is also not valid unless the resolution between the last two peaks out of three peaks, which are growing during decomposition, 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 in both mobile phases A and B, or adjust the gradient programme.

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). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than two times the area of the principal peak obtained with solution (2) (1.0 %). Not more than two peaks are greater than the area of the principal peak obtained with solution (2) (0.5 %). Not more than one other peak is greater than 0.4 times the area of the principal peak obtained with solution (2) (0.2 %). The sum of the areas of all peaks, other than the principal peak, is not greater than four times the area of the principal peak obtained with solution (2) (2.0 %). Disregard any peak with retention time less than 5 min and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %). Disregard any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3).

Assay

Either method A or method B may be applied.

A. 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).

As the mobile phase, use a solution prepared as follows: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of purified water. Prepare the phosphate buffer by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of distilled water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

For solution (1) mix and transfer a quantity of contents of oral powder equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 ml volumetric flask. Add about 20 ml of methanol, sonicate for about 15 minutes, allow to cool to room temperature, and make up the volume using the mobile phase.

** Refers to The International Pharmacopoeia*

Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of filtered solution. For solution (2) use 2 mg of nelfinavir mesilate RS per ml prepared in the same manner.

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

Maintain the column temperature at 35 ˚C.

Inject 20 μl of solution (2) in six replicate injections into the chromatographic system. The relative standard deviation for the peak area of nelfinavir is not more than 2.0 %.

Inject alternatively 20 μl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of nelfinavir.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of $C_{32}H_{45}N_3O_4S$.

B. Mix and transfer a quantity of the contents of oral powder equivalent to about 20 mg of nelfinavir, accurately weighed, to a 50 ml volumetric flask. Add about 25 ml of methanol R, sonicate for about 5 minutes, allow to cool to room temperature, and make up the volume using the same solvent. Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 50.0 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 253 nm against a solvent cell containing methanol R.

Calculate the content of $C_{32}H_{45}N_3O_4S$ using an absorptivity value of 15.7 ($A^{1\%}_{1\,cm}$ = 157).

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

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

Sodium dihydrogen phosphate, anhydrous

Molecular formula: NaH_2PO_4
Description: White powder, hygroscopic.
Storage: in an airtight container.

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).

** Refers to The International Pharmacopoeia*

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Nelfinavir mesilas compressi
Nelfinavir mesilate tablets (first draft)

Category. Antiretroviral (protease inhibitor).

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

Labelling. The designation on the container of nelfinavir mesilate tables should state that the active ingredient is in the mesilate form, and the quantity should be indicated in terms of the equivalent amount of nelfinavir. Expiry date.

Additional information. Strength in the current WHO Model List of Essential Medicines: 250 mg of nelfinavir (as mesilate).

Requirements

Complies with the monograph for “Tablets” (Vol. 4, P. 26*).

Nelfinavir mesilate tablet contains not less than 90.0 % and not more than 110.0 % of $C_{32}H_{45}N_3O_4S$ stated on the label.

Identity tests

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 the following 2 solutions in methanol R: (A) shake the quantity of powdered tablets equivalent to about 21 mg of nelfinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg nelfinavir 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 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 the following 2 solutions in methanol R: (A) shake the quantity of powdered tablets equivalent to about 21 mg of nelfinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg nelfinavir 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. Spray the plate with basic potassium permanganate (5 g/l) TS. Examine the chromatogram in daylight.

** Refers to The International Pharmacopoeia*

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

B. To a quantity of the tablets equivalent to about 20 mg of nelfinavir add 50 ml of methanol R, shake, and filter. Dilute 5 ml of the filtrate to 50 ml with the same solvent. The absorption spectrum of the resulting solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 253 nm.

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: 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 dihydrogenphosphate 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 (%)	Mobile phase B (%)	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

For solution (1) weigh and powder 20 tablets, and transfer a quantity equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 ml volumetric flask. Add about 20 ml of methanol R, sonicate for about 15 minutes, allow to cool to room temperature, and make up the volume using mobile phase A. Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of the filtered solution. For solution (2) dilute a suitable volume of solution A to obtain a concentration equivalent to 10.0 μg of nelfinavir per ml of mobile phase A. For solution (3) use 100 μg of methanesulfonic acid per ml of mobile phase A.

For the system suitability test: prepare solution (4) using 2 ml of solution (1) and 5 ml of sulphuric 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 spectro- photometer set at a wavelength of 225 nm.

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Maintain the column at 35 ˚C.

Inject 20 µl of solution (4). The test is not valid unless the resolution between the principal peak (retention time = about 27 minutes) and the peak with a retention time relative to the principal peak of about 0.2 is not less than 15. The test is also not valid unless the resolution between the last two peaks out of three peaks, which are growing during decomposition, 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 in both mobile phases A and B, or adjust the gradient programme.

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). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than two times the area of the principal peak obtained with solution (2) (1.0 %). Not more than two peaks are greater than the area of the principal peak obtained with solution (2) (0.5 %). Not more than one other peak is greater than 0.4 times the area of the principal peak obtained with solution (2) (0.2 %). The sum of the areas of all peaks, other than the principal peak, is not greater than four times the area of the principal peak obtained with solution (2) (2.0 %). Disregard any peak with retention time less than 5 min and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %). Disregard any peak due to methanesulfonic acid, corresponding to the principal peak in the chromatogram obtained with solution (3).

Assay

Either method A or method B may be applied.

A. 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).

As the mobile phase, use a solution prepared as follows: 27 volumes of acetonitrile R, 20 volumes of methanol R, 28 volumes of phosphate buffer pH 3.4 and 25 volumes of purified water. Prepare the phosphate buffer 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.

For solution (1) weigh and powder 20 tablets, and transfer a quantity equivalent to about 0.10 g of nelfinavir, accurately weighed, into a 50 ml volumetric flask. Add about 20 ml of methanol R, sonicate for about 15 minutes, allow to cool to room temperature, and make up the volume using the mobile phase. Filter a portion of this solution through a 0.45 µm filter, discarding the first few ml of filtered solution. For solution (2) use 2 mg of nelfinavir mesilate RS per ml prepared in the same manner.

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

Maintain the column temperature at 35 ˚C.

Inject 20 µl of solution (2) in six replicate injections into the chromatographic system. The relative standard deviation for the peak area of nelfinavir is not more than 2.0 %.

Inject alternatively 20 µl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of nelfinavir.

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Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of $C_{32}H_{45}N_3O_4S$.

B. Weigh and powder 20 tablets. Transfer a quantity of the contents of tablets equivalent to about 20 mg of nelfinavir, accurately weighed, to a 50 ml volumetric flask. Add about 25 ml of methanol R, sonicate for about 5 minutes, allow to cool to room temperature, and make up the volume using the same solvent. Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 50.0 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 253 nm against a solvent cell containing methanol R.

Calculate the content of $C_{32}H_{45}N_3O_4S$ using an absorptivity value of 15.7 ($A^{1\%}_{1\,cm}$ = 157).

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

Sodium dihydrogen phosphate, anhydrous
Molecular formula: NaH_2PO_4
Description: White powder, hygroscopic.
Storage: in an airtight container.

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).

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. A draft for saquinavir mesilate capsules is provided below for comment.

Saquinavirum mesilas capsulae
Saquinavir mesilate capsules (first draft)

Category. Antiretroviral (protease inhibitor).

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

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Labelling. The designation on the container of saquinavir mesilate capsules should state that the active ingredient is in the mesilate form, and the quantity should be indicated in terms of the equivalent amount of saquinavir. Expiry date.

Additional information. Strength in the current WHO Model List of Essential Medicines: 200 mg of saquinavir.

REQUIREMENTS

Complies with the monograph for "Capsules" (Vol. 4, p.32*)

Saquinavir mesilate capsules contain not less than 90.0 % and not more than 110.0 % of the amount of $C_{38}H_{50}N_6O_5$ stated on the label.

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, 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) shake a quantity of the contents of the capsules equivalent to 22 mg of saquinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg 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, 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) shake a quantity of the contents of capsules equivalent to 22 mg of saquinavir with 5 ml, filter, and use the clear filtrate; and (B) 5 mg 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. To a quantity of the contents of capsules equivalent to about 20 mg saquinavir mesilate add 100 ml of methanol R, shake, and filter. Dilute 5 ml of the filtrate to 100 ml with the same solvent. The absorption spectrum of resulting solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 239 nm.

C. To a quantity of the contents of capsules equivalent to 50 mg of saquinavir mesilate add 10 ml of methanol R, shake to dissolve, and filter. Evaporate the filtrate to dryness under vacuum. Carry out the examination with the residue as described under ''Spectrophotometry in the infrared region'' (Vol. 1, p. 40*). The infrared absorption spectrum is concordant with the spectrum obtained in a similar way from saquinavir mesilate RS or with the *reference spectrum* of saquinavir mesilate.

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[Note from WHO Secretariat: Feedback on the applicability of this method would be much appreciated.]

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 and 2 parts of methanol, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile, 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) mix the content of 20 capsules and transfer a quantity equivalent to about 0.025 g of saquinavir, accurately weighed, into a 50 ml glass-stoppered flask. Add about 40 ml mobile phase A, sonicate for about 5 minutes, allow to cool to room temperature, and make up the volume using the same solvent. Filter a portion of this solution through a 0.45 μm filter, discarding the first few ml of filtered solution. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 2.5 μg of per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 5 ml of sufuric acid (475 g/l), heat in a water bath at 100 ˚C for 30 minutes.

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

Maintain the column temperature at 30 ˚C.

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 are increasing during decomposition, is not less than 2. The ratio of the retention times of these two peaks relative to

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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 that obtained with solution (2) (0.5 %). Not more than one peak is greater than half the area of the principal peak obtained with solution (2) (0.25 %). 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 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1 %).

Assay

Either method A or method B may be applied.

A. 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).

As the mobile phase, use a solution prepared as follows: 50 volumes of a mixture of 5 parts of acetonitrile and 2 parts of methanol, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water. Prepare the phosphate buffer by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of distilled water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

Prepare the following solutions using the mobile phase as diluent. For solution (1) mix the content of 20 capsules and transfer a quantity equivalent to about 0.025 g of saquinavir, accurately weighed, into a 50 ml glass-stoppered flask. Add about 40 ml mobile phase, sonicate for about 5 minutes, allow to cool to room temperature, and make up the volume using the same solvent. Filter a portion of this solution through a 0.45 µm filter, discarding the first few ml of filtered solution. For solution (2) use 0.5 mg of saquinavir RS per ml mobile phase.

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

Maintain the column temperature at 30 °C.

Inject 20 µl of solution (2) in six replicate injections into the chromatographic system. The relative standard deviation for the peak area of saquinavir is not more than 2.0 %.

Inject alternatively 20 µl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of saquinavir.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of $C_{38}H_{50}N_6O_5$.

B. Mix the contents of 20 capsules and transfer a quantity equivalent to about 0.020 g of saquinavir, accurately weighed, to a 100 ml glass stopperd flask. Add about 50 ml of methanol R, sonicate for 5 minutes, allow to cool to room temperature, and make up the volume using the same solvent. Filter a portion of this solution through a 0.45 µm filter, discarding the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 100.0 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 239 nm. Calculate the content of $C_{38}H_{50}N_6O_5$ using an absorptivity value of 71.5 ($A^{1\%}_{1\,cm}$ = 715).

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Reagents

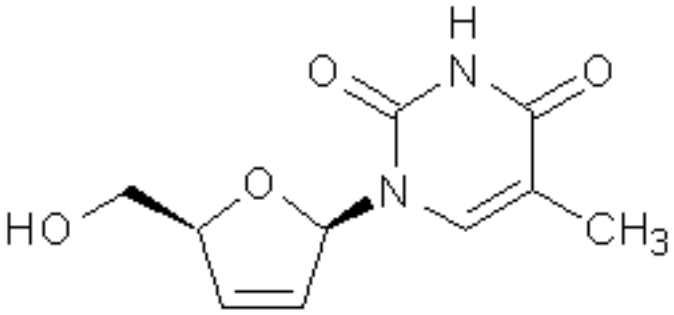
Silica gel for chromatography, octadecylsilyl, base deactivated
A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Potassium permanganate, basic (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. A draft for stavudine is provided below for comment.

Stavudinum
Stavudine (first draft)



$C_{10}H_{12}N_2O_4$

Relative molecular mass. 224.2

Chemical name. 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; 1-(2,3-dideoxy-ß-D-*glycero*-pent-2-enofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (D4T); CAS Reg. No.3056-17-5.

Description. A white to almost white powder.

Solubility. Soluble in water and ethanol (~750 g/l) TS (ethanol (95 per cent) R).

Category. Antiretroviral (nucleoside reverse transcriptase inhibitor).

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

Additional information. Stavudine shows polymorphism.

** Refers to The International Pharmacopoeia*

Requirements

Stavudine contains not less than 97.0% and not more than 103.0% of $C_{10}H_{12}N_2O_4$, 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 2 ml of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of stavudine 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 2 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of stavudine 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 the final solution prepared for the Assay, when observed between 210 nm and 300 nm, exhibits one maximum at about 266 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 412 and 458.

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 stavudine RS or with the *reference spectrum* of stavudine.

If the spectra are not concordant, use stavudine RS. Dissolve the sample in a small amount of ethanol (~750 g/l) TS (ethanol (95 per cent) R, evaporate to dryness and carry out the IR spectrum with the residue as mentioned above. Treat stavudine RS in the same way. The infrared absorption spectrum is concordant with the spectrum obtained from stavudine RS.

Specific optical rotation. Use a 7 mg/ml solution and calculate with reference to the dried substance; $[\alpha]_D^{25°C}$ = -39° to -45°.

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 20 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

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Loss on drying. Dry for 3 hours at 105 °C; it loses not more than 5 mg/g.

Related substances

(Note: Prepare the solutions immediately before use and maintain at 2–8 °C until use)

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 octadecylsilyl silica gel for chromatography R (Stationary phase A) (5mm) (Supelcosil LC-18-DB is suitable). As mobile phase A, use a mixture of 35 volumes of acetonitrile R and 965 volumes of a 0.77 g/l solution of ammonium acetate R. As mobile phase B, use a mixture of 250 volumes of acetonitrile R and 750 volumes of a 0.77 g/l solution of ammonium acetate R.

Use the following gradient elution system:

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Comments
0–10	100	0	Isocratic
10–20	100 to 0	0 to 100	Linear
20–30	0	100	Isocratic
30–35	0 to 100	100 to 0	Linear
35–40	100	0	Isocratic

Prepare the following solutions. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute 1.0 ml of this solution to 200 ml. For solution (3) Dilute 10 ml of solution (2) to 50 ml.

Operate with a flow rate of 2 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Maintain the column temperature between 20–25 °C.

Inject alternately 10µl each of solutions (1), (2), (3) and (4).

In the chromatogram obtained with solution (1), the following peaks are eluted at the following retention times ratio with reference to stavudine: impurity A = about 0.26; impurity B = about 0.49; impurity C = about 0.52; impurity D = about 0.69; impurity E = about 1.1 and impurity F = about 1.3.

[Note from WHO Secretariat: Details on solution (4) will be added as soon as the availability of the test mix has been confirmed.]

The test is not valid unless in the chromatogram obtained with solution (4) the resolution between the peaks corresponding to impurities B and C is greater than 1.5 and between impurity E and stavudine is greater than 1.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1), (2) and (3) and calculate the content of related substances as a percentage. For the calculation of limit contents, multiply the peak area of impurity A by a correction factor of 0.69.

In the chromatogram obtained with solution (1), the area of the peak corresponding to impurity A is not greater than the principal peak in the chromatogram obtained with solution (2) (0.5%). For any other impurity, the peak area is not greater than the principal peak in the chromatogram obtained with solution (3) (0.1%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any

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peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (3) (0.05%).

Assay: Weigh accurately about 25 mg of the test substance into a 50 ml volumetric flask. Add about 40 ml of water and shake to dissolve. Dilute to volume with water and mix. Dilute 3 ml of this solution to 100 ml with 0.1M H_2SO_4 and mix. For the blank use 0.1M H_2SO_4.

Measure the absorbance of a 1-cm layer of the final solution at a maximum about 266 nm against a solvent cell containing the blank. Calculate the content of $C_{10}H_{12}N_2O_4$ using the absorptivity value of 43.5 ($A^{1\%}_{1cm}$ = 435).

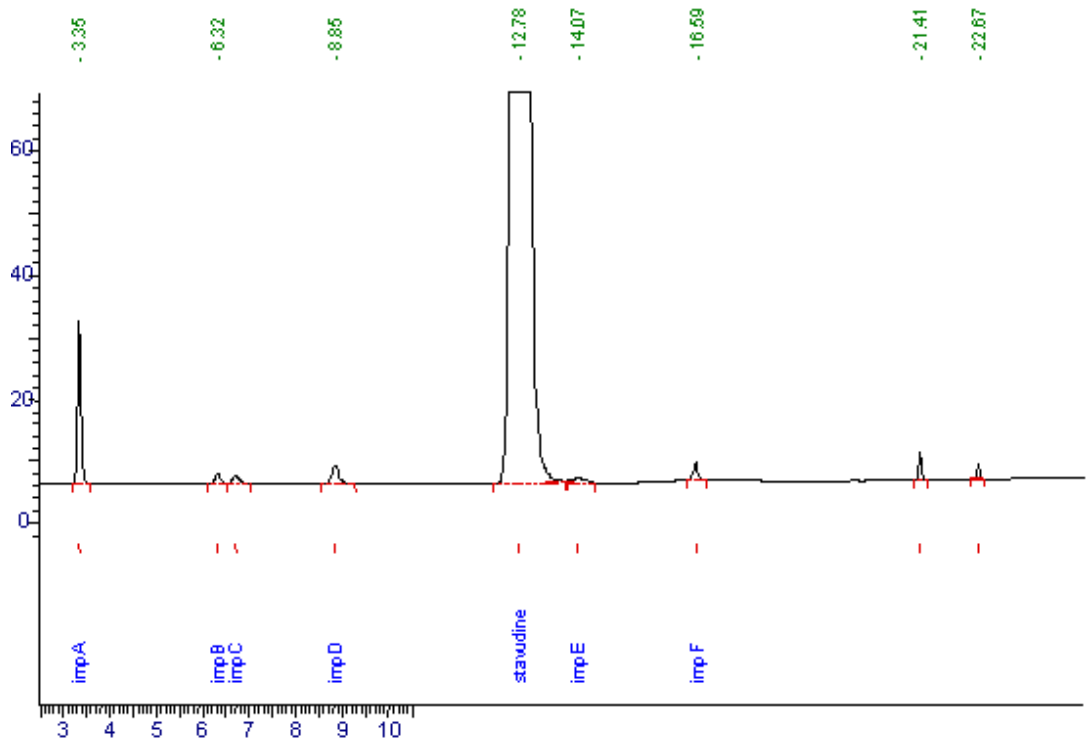
Impurities

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.

[Note from WHO Secretariat: Chemical structures will be included in the next version.]

A. Thymine
B. Thymidine epimer
C. Thymidine
D. Stavudine lactone
E. Stavudine anomer alpha
F. 3',5'-anhydrothymidine

A typical chromatogram obtained for stavudine (Refer to the monograph text for chromatographic conditions in "Related substances")



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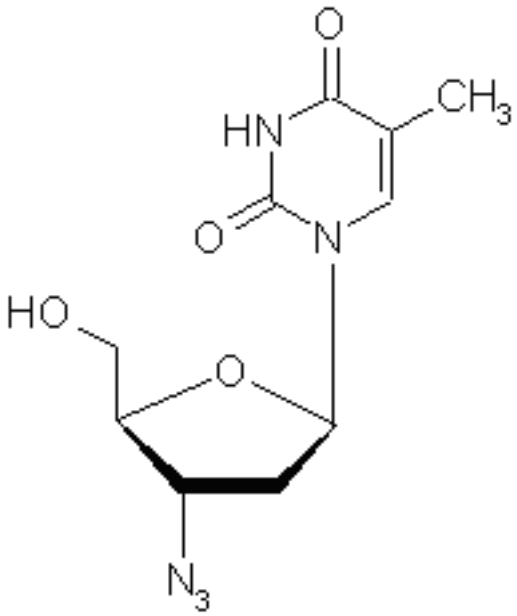
Monographs for antiretrovirals

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. A draft for zidovudine is provided below for comment.

Zidovudinum
Zidovudine (first draft)



$C_{10}H_{13}N_5O_4$

Relative molecular mass. **267.2**

Chemical name. 1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methyl-pyrimidine-2,4(1*H*,3*H*)-dione; 1-(3-azido-2,3-dideoxy-b-D-*erythro*-pentofuranosyl)-5-methyl-pyrimidine-2,4(1*H*,3*H*)-dione; 3'-azido-3'-deoxythimidine (AZT); CAS Reg. NO.30516-87-1.

Description. A white or almost white powder.

Solubility. Soluble in ethanol (~750 g/l) TS (ethanol (95 per cent) R), sparingly soluble in water.

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

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[Note from Secretariat: USP revised the 'storage conditions' by adding the following sentence: "Store at 25 °C, excursions permitted between 15 °C and 30 °C." Definition for "room temperature" in the International Pharmacopoeia. "When nothing is mentioned, the storage of the substance is at room temperature."]

Additional information. Zidovudine shows polymorphism.

Requirements

Zidovudine contains not less than 97.0% and not more than 103.0% of $C_{10}H_{13}N_5O_4$, 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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid 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 zidovudine 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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid 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 zidovudine 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 15 μg/ml solution in methanol R, when observed between 210 nm and 300 nm, exhibits one maximum at about 266 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 360 to 398.

[Note from Secretariat: The specific absorbance range has been defined within +/-5% limits as agreed by the EC. Test B may be referred to the UV assay and not described here. Please comment.]

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 zidovudine RS or with the *reference spectrum* of zidovudine.

If the spectra are not concordant, use zidovudine RS. Dissolve the sample in a small amount of ethanol (~750 g/l) TS (ethanol (95 per cent) R), evaporate to dryness and carry out the IR spectrum

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with the residue as mentioned above. Treat zidovudine RS in the same way. The infrared absorption spectrum is concordant with the spectrum obtained from zidovudine RS.

Melting range. 124–126 °C.

Specific optical rotation. Use a 10 mg/ml solution in ethanol (~750 g/l) TS (ethanol (95 per cent) R) and calculate with reference to the dried substance; $[\alpha]_D^{25°C}$ = +60° to +63°.

Heavy metals. Use 1.0 g 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.

[Note from Secretariat: additional information needed:

- The method used, for instance in the European Pharmacopoeia and Indian Pharmacopoeia, is more complex (combustion method). Please comment.

- Which solvent has to be used (e.g. ethanol 95% R) if procedure 2 is retained?]

Sulfated ash. Not more than 2 mg/g.

Loss on drying. Dry for 3 hours at 105 °C; it loses not more than 5 mg/g.

[Note from Secretariat: The limit for 'loss on drying' is more stringent in this monograph than, for example, in the European Pharmacopoeia, USP and Indian Pharmacopoeia (0.5% instead of 1.0%). Please comment.]

Related substances

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p.8*), using silica gel R4 as the coating substance and a mixture of 90 volumes of dichloromethane R and 10 volumes of methanol R as the mobile phase. Apply separately to the plate 10 μl of each of the 2 solutions in methanol R containing (A) a mixture containing 0.1 mg per ml each of zidovudine RS and triphenylmethanol R and (B) 20 mg per ml of the test substance. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air. Examine the chromatogram in ultraviolet light (254 nm).

[Note from Secretariat: This method is described in the USP. However, chloroform has been changed to dichloromethane in the mobile phase (International Pharmacopoeia policy).]

Any spot obtained with solution (B), other than the principal spot, is not more intense and not larger than the principal spot obtained with solution A (0.5%). Furthermore, the sum of intensities of the secondary spots obtained with solution B does not exceed 3.0%.

[Note from Secretariat: It is suggested that the last sentence above referring to the sum of spot intensities be deleted (difficult interpretation).]

Spray the plate with a mixture of 0.5 g of carbazole R in 95 volumes of ethanol (~750 g/l) TS (ethanol (95 per cent) R) and 5 volumes of sulfuric acid R, heat for 10 minutes at 120 °C.

Any spot corresponding to triphenylmethanol R (R_f value about 2.3 relative to the R_f value of zidovudine) is not more intense than the corresponding spot in solution A (0.5%). Any other spot obtained with solution B, other than the principal spot, is not more intense and not larger than the principal spot corresponding to zidovudine obtained with solution A (0.5%). Furthermore, the sum of intensities of the secondary spots obtained with solution (B) does not exceed 3.0%.

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[Note from Secretariat: It is suggested that the last sentence above referring to the sum of spot intensities be deleted (difficult interpretation).]

B. 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 octadecylsilyl silica gel for chromatography R (Stationary phase A) (5μm) (Waters Hypersil BDS is suitable). As the mobile phase, use a mixture of 20 volumes of methanol R and 80 volumes of water.

Prepare the following solutions. For solution (1) prepare 1 mg/ml solution of the test substance in the mobile phase. For solution (2) dilute 1.0 ml of solution (1) to 5 ml with the mobile phase. For solution (3) dissolve 2 mg of zidovudine impurity C (thymine R) in 10 ml of methanol R. Then dilute 2 ml to 20 ml with the mobile phase. For solution (4), dissolve 2 mg of impurity B RS (1-(3-chloro-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) in 10 ml of mobile phase. Mix 5 ml of this solution with 5 ml of solution (2) into a 10-ml volumetric flask. For solution (5) dilute 2 ml of solution (4) to 20 ml with the mobile phase. For solution (6) dilute 0.5 ml of solution (1) to 100 ml with the mobile phase.

Operate with a flow rate of 1.2 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 265 nm.

Inject alternately 10 μl each of solutions (1), (3), (5) and (6). Record the chromatogram for 4 times the retention time of zidovudine in solution (1).

The retention times ratio with reference to zidovudine are about 0.26 for zidovudine impurity C (thymine R), 1 and 1.18 for zidovudine related impurity B (1-(3-chloro-2,3-dideoxy-ß-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione). The test is not valid unless the resolution factor between the peaks corresponding to zidovudine and zidovudine impurity B obtained with solution (5) is greater than 2.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1), (3), (5) and (6).

In the chromatogram obtained with solution (1) the area of the peak corresponding to zidovudine impurity C (thymine R) is not greater than the area of the principal peak obtained with solution (3) (2.0%). The area of the peak corresponding to zidovudine impurity B RS is not greater than the area of the corresponding peak in the chromatogram obtained with solution (5) (1.0%). The area of any other peak, other than the principal peak, is not greater than the area of the peak obtained with solution (6) (0.5%). The sum of the areas of all peaks, other than the principal peak, obtained with solution (1) is not greater than 6 times the area of the principal peak obtained with solution (6) (3.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak obtained with solution (6) (0.05%).

Assay. Weigh accurately about 40 mg of sample into a 200 ml volumetric flask. Add about 160 ml of a mixture consisting of 20 volumes of methanol R and 80 volumes of water and dissolve by using an ultrasonic bath. Dilute to volume with the same solvent and mix. Dilute 5 ml of this solution to 50 ml with 0.1M H_2SO_4 and mix. For the blank, use 5 ml of a mixture consisting of 20 volumes of methanol R and 80 volumes of water diluted to 50 ml with 0.1M H_2SO_4.

Measure the absorbance of a 1-cm layer of the final solution at a maximum about 266 nm against a solvent cell containing the blank. Calculate the content of $C_{10}H_{13}N_5O_4$ using the absorptivity value of 38.0 ($A^{1\%}_{1cm}$ = 380).

[Note from Secretariat: The UV wavelength has been changed from 267 to 266 nm to be consistent with identity test B. The specific absorbance has been experimentally determined by 2 different laboratories. It would be good to have additional experimental feedback to confirm this value. Otherwise the use of a reference substance is an alternative. Please comment.]

** Refers to The International Pharmacopoeia*

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.

Note from Secretariat: Chemical structures to come.

A. 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione

B. (1-(3-chloro-2,3-dideoxy-b-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione)

C. 5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine)

D. triphenylmethanol

Reagents

Carbazole R.
Dibenzopyrolle
$C_{12}H_9N$.
A commercially available reagent of suitable grade.
Melting point: about 245 °C.

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).

Thymine R.
5-methylpyrimidine-2,4(1*H*,3*H*)-dione; $C_5H_6N_2O_2$.
A commercially available reagent of suitable grade.
Description. Short needles or plates.
Solubility. Slightly soluble in cold water, soluble in hot water. It dissolves in dilute solution of alkali hydroxide.

Triphenylmethanol R.
Triphenylcarbinol; $C_{19}H_{16}O$.
A commercially available reagent of suitable grade.
Description. Colourless crystals.
Solubility. Practically insoluble in water, freely soluble in ethanol (~750 g/l) TS (ethanol (95 per cent) R).