WHO Drug Information

Contents

WHO Prequalification Programmes

WHO Prequalification of Medicines Programme: survey of service quality provided to manufacturers

Established in 2001, the Prequalification of Medicines Programme (PQP) is a service provided by the World Health Organization (WHO) to facilitate access to quality medicines for treating priority diseases. In order to be prequalified, medicines must meet WHO-specified standards for quality, safety and efficacy. PQP is supported by various United Nations agencies (e.g., UNAIDS, UNICEF, UNFPA) and the World Bank.

WHO prequalification of medicines is a multi-step process whereby a manufacturer submits extensive information that is then evaluated by a WHO assessment team with respect to product quality, safety and efficacy, site(s) of manufacture and any clinical studies that may have been carried out during development. Products that successfully pass PQP evaluation are listed on the WHO List of Prequalified Medicinal Products (see: http://www.who.int/prequal). This list provides UN agencies with a single source of reference for quality-assured priority medicines and is also used by a variety of entities, both country-specific and international, that purchase medicines in bulk quantities.

The Prequalification of Medicines Programme has conducted a comprehensive survey among pharmaceutical manufacturers to assess its level of service. PQP assessment and inspection activities reflect those carried out by national regulatory authorities before granting marketing authorization (registration) for a medicine. The assessment review was therefore designed to maximize the diagnostic capability of the survey: to measure both the level of service provided by PQP and the levels of service expected from regulatory authorities by manufacturers. The survey included items relevant to the assessment of product dossiers and on-site inspections of manufacturing facilities in a way that separated service design (the service process) from service delivery (or the "people" aspects of service). The results of this survey provide direction for improvements to the current Programme and have implications for future strategic development.

The survey

In administering the Prequalification of Medicines Programme (PQP), WHO interacts with a number of stakeholder groups and organizations whose objectives are dependent upon, or related to, WHO prequalification of medicines: manufacturers, government agencies (including national and regional medicines regulatory authorities) and donor organizations. In 2009, one such donor organization recommended that WHO undertake a process optimization review with respect to PQP – an exercise that would include feedback from manufacturers with experience of WHO prequalification of medicines.

To provide guidance in developing a survey of manufacturers, implementing the survey and analysing the results, PQP retained the services of a market research consulting company, Interclarity

Research & Consulting, Inc. Interclarity Research created a survey development process with both internal and external components that resulted in a survey whose contents were relevant to manufacturers and, when assessed by those manufacturers, actionable by PQP. The survey was undertaken with the clear understanding that PQP must operate in accordance with respective WHO and other relevant international technical standards. It was also understood that the results of the survey should neither serve to "lower" PQP's technical standards nor to "relax" the requirements for the sustained quality, safety and efficacy of any medicine that WHO had prequalified.

Survey objectives

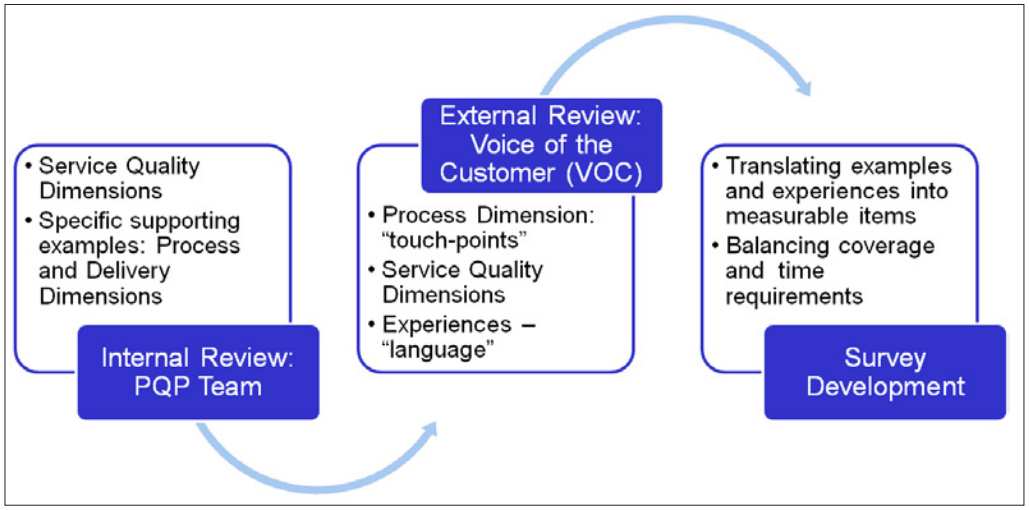
PQP identified three main objectives in designing and conducting a survey of pharmaceutical manufacturers:

- **Implement a comprehensive approach to assessing the level of service quality delivered to pharmaceutical manufacturers by PQP** by (i) incorporating aspects of both service design (process) and service delivery (people) and (ii) including services provided during both the assessment of product dossiers and on-site inspections of manufacturing facilities.
- **Maximize the diagnostic ability of the survey** by incorporating questions that use manufacturer experience with major regulatory agencies (US FDA, EMA, etc.) to measure expectations of service quality and prioritize areas for improvement.
- **Provide a unified framework for measurement.** While the intent of the initial survey of pharmaceutical manufacturers was to include only those companies that have had at least one product prequalified, PQP was also interested in developing a survey format that could be extended to companies involved for the first time (at some stage in the approval process or awaiting final approval) and companies that are considering participation.

Survey development process

Without the benefit of previous self-assessment efforts, PQP had little to work with in terms of survey content in January

Figure 1. Survey development



2010. The area of service delivery is rich in both academic research and practical application [1]; the area of service design, however, is specific to the organization, application, and recipients of the service. Figure 1 on page 294 illustrates the process used by PQP to identify and codify important services related to the prequalification of medicines.

A survey development process was designed to obtain input from within the organization and from regulatory and quality assurance professionals in pharmaceutical companies. The overall aim of this process was to capture and include the knowledge and experience of PQP so that, when combined with the "voice of the customer", survey contents would be both relevant and actionable.

Interclarity Research developed topic guides and facilitated discussions, interviews and working sessions for both internal and external phases of the process. Internal working sessions, both in conference call and in-person formats, were conducted with key PQP members and groups (e.g. programme management, PQP assessors and PQP inspectors). Following the internal review, in-depth telephone interviews were conducted with industry professionals familiar with PQP. In both phases of the survey development process, the emphasis was on identifying, from a participant's point of view, important stages in the WHO prequalification process — aspects that work well and those that fall short of participant expectation. To gain greater clarity around important issues associated with service delivery, efforts were made in the "voice of the customer" to identify detailed descriptions of service delivery for both favourable and unfavourable events, as experienced by PQP manufacturer participants [2, 3].

A list of candidate survey items related to the PQP service process was developed from the findings of internal and external qualitative research. Further analysis of the qualitative findings suggested that service delivery aspects of PQP would be well-represented by items included in the SERVQUAL scale of service quality [4]. SERVQUAL is a widely-applied scale for measuring the quality of service delivery. Developed in the 1980s, it has been refined and applied over the past 25 years.

Survey construction and deployment
The survey was divided into four main sections:

1. Experience with PQP.

2. PQP Service Design ("process").

3. PQP Service Delivery ("people").

4. Other Aspects of PQP.

The first section includes an introduction to the survey and questions that provide indicators and measures of previous experience with PQP. Section 2 (Service Design) is "custom" to PQP – developed as a result of a process review with PQP staff and the series of interviews with pharmaceutical manufacturers. This section includes two multi-item questions on PQP aspects common to both assessment and inspection environments, and two separate multi-item questions each for regulatory affairs and quality assurance professionals respectively. In Section 3 (Service Delivery), measuring the people component with SERVQUAL requires five separate multi-item questions each for the assessment of product dossiers and on-site inspections respectively. The items in Section 4 are also custom to PQP and include measures related to the advocacy, training and compliance aspects of PQP.

Incorporating both common and application-specific items in a single survey made deploying the questionnaire on the Internet advantageous. The appropriate programming logic was developed to

display the questions common to all respondents and the application-specific questions (i.e. assessment of product dossiers and on-site inspections) only to those respondents with the relevant background and PQP experience. In addition, since a relatively large number of respondents in both the Indian and Chinese markets were anticipated, native language versions of the questionnaire for these two markets were made available, along with the English language version. An option was included in the survey introduction that allowed respondents to select a language option.

Measurement

WHO service performance was measured using a 7-point rating scale, where a "1" indicates "low performance" and a "7" indicates "high performance". The numbers 2 – 6 are varying degrees in between. To increase the diagnostic value of the survey results, two supplemental questions [4] were included to describe manufacturer expectations around each service item:

1. *Minimum Service Level.* The minimum level of service performance that manufacturers are willing to accept from ANY major regulatory agency.

2. *Desired Service Level.* The level of service that a regulatory agency should deliver to manufacturers.

Data Collection

A list of potential survey participants was compiled by PQP Staff using internal records. The list included regulatory affairs professionals (assessment of product dossiers) and quality assurance professionals (on-site inspections) who had participated in WHO prequalification of medicines during the period 2006 – 2010. Individuals on the contact list represented manufacturers of both branded and generic pharmaceutical medicines who have had a medicinal product or products prequalified by WHO. After cleaning and validation, the initial list of 75 contacts was revised to a final list of 62 prospective survey respondents. All contacts on the final list received an e-mail invitation from Interclarity Research on behalf of WHO. Each e-mail included an embedded link to the survey questionnaire on a secure web site. A total of 41 completed surveys was collected: 18 responses from regulatory affairs professionals and 23 responses from quality assurance professionals.

Key Findings

- Both PQP assessors and inspectors are meeting or exceeding manufacturer expectations for service delivery.

- The structure of PQP generally delivers levels of service at, or above, those expected by manufacturers. However, the service process is falling short of manufacturer expectations with respect to:

  - Review/reply time for product dossiers.
  - Opportunities for in-person communication during the assessment process.
  - Question/problem resolution during assessment.
  - Consistency of membership in the team of assessors throughout the process.
  - Local/national representation in on-site inspection teams,

- Most manufacturers view PQP GMP requirements as more stringent than those of the US Food and Drug Administration or European Medicines Agency.

- Reducing the time required to prequalify a product requires a joint effort between the manufacturer and the PQP.

Conclusions

Overall, the findings from this survey indicate that pharmaceutical manufacturers consider PQP to be a well-designed, well-executed programme. PQP assessors and inspectors are meeting or exceeding manufacturer expectations for service delivery in the process. However, pharmaceutical manufacturer applicants place a premium on feedback, communications and problem resolution during the prequalification process – with particular emphasis on the assessment of product dossiers – and these are potential improvement areas in the service design of PQP.

Based on the survey results, WHO is now working on improvements to the Programme and these will be implemented in the coming year.

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WHO initiates pilot prequalification of active pharmaceutical ingredients

In October 2010, the WHO Prequalification of Medicines Programme (PQP) started to pilot prequalification of selected active pharmaceutical ingredients (APIs) for products for treating HIV and related diseases, malaria and tuberculosis. Its first “Invitation to Manufacturers of Active Pharmaceutical Ingredients to Submit an Expression of Interest (EOI) for Evaluation” is available at http://who.int/prequal/

Globalization of pharmaceutical production has led to diversification of API sources and made verification of API quality more difficult. WHO’s decision to prequalify APIs responds to the increasing concern expressed by medicines regulators regarding API quality, including the manner in which APIs are manufactured.

PQP already assesses API master files (APIMFs) as part of its evaluation of finished pharmaceutical products (FPPs). This can include inspection of the manufacturing site to assess compliance with WHO good manufacturing practices (GMP), if risk assessment indicates that an on-site inspection is necessary. An API submitted for evaluation will undergo both dossier assessment and inspection of the manufacturing site.

Each prequalified API — including details of the supplier and manufacturing site(s) — will be added to the WHO List of Prequalified Active Pharmaceutical Ingredients. The List will be of great interest to FPP manufacturers seeking to ensure the good quality of APIs used in FPP production, and to national medicines regulatory authorities who wish to verify the standard of APIs that have been used to manufacture nationally registered medicines.

It is expected that the time taken to reach prequalification will be made shorter for FPPs that are manufactured using WHO-prequalified APIs, than for FPPs that are manufactured using APIs that have not previously been evaluated by WHO PQP.

An APIMF that has already been accepted by WHO in relation to the prequalification of an FPP may be included in the WHO List of Prequalified APIs without reassessment or re-inspection. This is contingent upon the APIMF meeting certain administrative criteria and the relevant manufacturing site having passed inspection by WHO or a stringent regulatory authority.

Selection of APIs for inclusion in the first Invitation was based on APIs for which APIMFs have already been submitted in connection with evaluation of an FPP. PQP anticipates that future Invitations will be expanded to incorporate additional APIs. New Invitations will be posted on the PQP web site and manufacturers are therefore encouraged to regularly access http://www.who.int/prequal.

New on-line database for WHO prequalified vaccines

A new database for vaccines prequalified by WHO by type of vaccine, manufacturer and country of manufacture is now available on WHO's web site (1). This will enable immunization programme managers, procurement agencies, regulatory authorities, and other partners to consult summary pages for each prequalified vaccine and seek information such as date of prequalification, vaccine presentation, route of administration, shelf life, packaging, and cold chain requirements.

The ultimate goal of WHO's prequalification programme for vaccines is to ensure that all countries have access to vaccines that meet international standards of quality, safety and efficacy and are appropriate for the target population. The rigourous prequalification process includes

- review of comprehensive documentation on production methods, vaccine composition, quality control and clincial experience;
- independent testing for consistency by WHO-accredited laboratories; and
- site audits of the manufacturer to ensure that vaccine and production methods conform to international standards.

Complaints about vaccine quality and reports of adverse events following immunization are investigated by WHO. Furthermore, United Nations procurement agencies only purchase vaccines that have been prequalified by WHO.
The system is widely credited with contributing to the growing number and proportion of quality vaccines being supplied by companies in developing countries, such as Brazil, Cuba, India, Indonesia and Senegal.

The prequalification pages on WHO's web site contain sections on overall goals, principles, process and priority-setting for WHO vaccine prequalification, together with guidance documents for vaccine manufacturers.

References

1. Database on prequalified vaccines at http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/index.html

2. Further information on the WHO Prequalification of Vaccines Programme at http://www.who.int/immunization_standards/vaccine_quality/vq_index/en/index.html

Safety and Efficacy Issues

H1N1 influenza vaccine: narcolepsy

European Union — The European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) has reviewed all available data on the suspected link between narcolepsy and Pandemrix® an H1N1 influenza vaccine. The Committee concluded that the available evidence was insufficient to determine whether there is any link between Pandemrix® and reports of narcolepsy, and that further studies were necessary to fully understand this issue.

The Committee agreed that at present the benefit-risk balance for Pandemrix® continues to be positive and that while the review is still ongoing there was no need for Europe-wide restrictions on use. Narcolepsy is a rare sleep disorder that causes a person to fall asleep suddenly and unexpectedly. Its precise cause is unknown, but it is generally considered to be triggered by a combination of genetic and environmental factors.

As per 17 September 2010, there were 81 reports from healthcare professionals suggestive of narcolepsy, all collected through spontaneous reporting systems. Of these, 34 reports come from Sweden, 30 from Finland, 10 from France, six from Norway and one from Portugal. In addition, there are a further 13 consumer reports from Sweden and two from Norway. The age range of patients is between four and 52 years.

Reference: *EMA Press Release*, EMA/CHMP/588294/2010, 23 September 2010 at http://www.ema. europa.eu

Statins: interstitial lung disease

Canada — Interstitial lung disease (ILD) is a heterogeneous group of disorders that can be acute or chronic and may lead to pulmonary fibrosis and pulmonary insufficiency (1, 2). Signs and symptoms include difficulty breathing, nonproductive cough and diffuse crackles heard on auscultation. ILD has been reported in association with several drugs, such as amiodarone, azathioprine, carbamazepine, cyclophosphamide, methotrexate and nitrofurantoin (1, 2).

During the past 15 years, 29 cases of ILD suspected of being associated with HMG-CoA reductase inhibitors (statins) have been published (3–14). Of these, 16 describe a positive dechallenge with or without immunosuppressive treatment (3,4, 6, 8–11,14) and three cases described a positive rechallenge (4, 9). In some of these reports, ILD was part of systemic clinical features consistent with potential drug-induced diseases such as lupus (7), polymyositis (4, 12), dermatomyositis (5) and Churg-Strauss syndrome (14).

A systematic review of the suspected association between ILD and statins has recently been published (15). Although the mechanism of potential statin-induced ILD is unknown, some authors suggested it could be mediated by the inhibition of phospholipases; an effect of the statins on mitochondrial metabolism; or immune mediated (15).

As of 31 March 2010, Health Canada received 8 adverse reaction reports of ILD, or pathologies associated with ILD,

suspected of being associated with atorvastatin, pravastatin, rosuvastatin and simvastatin. Two of the cases received by Health Canada were published (14).

Extracted from the Canadian Adverse Reaction Newsletter, Volume 20, Number 4, October 2010

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Tocilizumab: risk of fatal anaphylaxis

Canada — The manufacturer of tocilizumab (Actemra®) has informed healthcare professionals of important safety information.

Tocilizumab is a recombinant humanized anti-human interleukin 6 (IL-6) receptor monoclonal antibody of the immunoglobulin (Ig) IgG1 subclass with a H2L2 polypeptide structure. It is authorized for intravenous use to reduce the signs and symptoms of moderately to severely active rheumatoid arthritis in adult patients who have inadequate response to one or more disease modifying antirheumatic drugs (DMARDs) and/or tumour necrosis factor (TNF) antagonists.

A case of fatal anaphylaxis has been reported in a patient with rheumatoid arthritis treated with tocilizumab. No Canadian cases of anaphylactic reaction have been reported.

As hypersensitivity reactions can occur with the administration of tocilizumab, patients need to be closely monitored throughout the infusion for signs and symptoms of hypersensitivity.

If a hypersensitivity reaction is suspected, infusion is to be stopped immediately and appropriate treatment should be administered.

Reference: Communication from Hoffmann-La Roche Limited, dated 13 September 2010 at http://www.hc-sc.gc.ca/

Pioglitazone: potential bladder cancer

United States of America — The Food and Drug Administration (FDA) is reviewing data from an ongoing, ten-year epidemiological study designed to evaluate whether pioglitazone (Actos®), is associated with an increased risk of bladder cancer. Findings from studies in animals and humans suggest this is a potential safety risk that needs further study. Pioglitazone is used in adults with type 2 diabetes mellitus.

Bladder cancer is estimated to occur in 20 per 100 000 persons per year in the United States and is thought to be higher in diabetics.

FDA has not concluded that pioglitazone increases the risk of bladder cancer. The Agency is reviewing information related to the safety concern and will update the public when additional information is available.

Reference: *FDA Drug Safety Communication,* 17 September 2010 at http://www/fda.gov

Angiotensin receptor blockers and cancer: safety review

United States of America — The Food and Drug Administration (FDA) is conducting a review of the class of medications known as angiotensin receptor blockers (ARBs) after a recently published study suggested they may be associated with a small increased risk of cancer.

ARBs are used in patients with high blood pressure and other conditions. Brand names include Atacand®, Avapro®, Benicar®, Cozaar®, Diovan®, Micardis®, and Teveten®. ARBs are also sold in combination with other medications.

The Agency plans to review the available data on these medications, and evaluate additional ways to better assess a possible link between use of ARBs and cancer. FDA will update the public when this review is complete.

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GnRH agonists, diabetes and cardiovascular disease

United States of America — The Food and Drug Administration (FDA) has notified the manufacturers of gonadotropin-releasing hormone (GnRH) agonists of the need to add new safety information to the *Warnings and Precautions* section of drug labels. This new information warns about increased risk of diabetes and certain cardiovascular diseases (heart attack, sudden cardiac death, stroke) in men receiving these medica-

tions for the treatment of prostate cancer. This action is based on the Agency's review of several published studies (1–7), described in the Agency's ongoing safety review of GnRH agonists and possible increased risk of diabetes and certain cardiovascular diseases, issued in May 2010.

GnRH agonists are approved for palliative treatment of advanced prostate cancer. The benefits of GnRH agonist use for earlier stages of non-metastatic prostate cancer have not been established.

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8. *FDA Drug Safety Communication,* 20 October 2010 at http://www/fda.gov

Gadolinium-based contrast agents: kidney dysfunction

United States of America — The Food and Drug Administration (FDA) is requiring changes to the drug labelling for gadolinium-based contrast agents (GBCAs) to minimize the risk of nephrogenic systemic fibrosis (NSF), a rare but serious condition associated with the use of GBCAs in certain patients with kidney dysfunction.

These label changes are intended to help ensure these drugs are used appropriately, and that patients at risk for NSF who receive GBCAs are actively monitored for the development of NSF. Symptoms of NSF include scaling, hardening and tightening of the skin, red or dark patches on the skin, and stiffness. NSF can also cause fibrosis of internal organs which may lead to death. There is no effective treatment for NSF.

Reference: *FDA Drug Safety Communication,* 9 September 2010 at http://www/fda.gov

Lamotrigine: aseptic meningitis

United States of America — The Food and Drug Administration (FDA) has informed the public that lamotrigine (Lamictal®), a medication commonly used for seizures in children two years and older and bipolar disorder in adults, can cause aseptic meningitis. FDA is revising the drug label and the patient medication guide to include information about this risk.

The decision to revise the Lamictal® label is based on FDA's identification of 40 cases of aseptic meningitis in patients from December 1994 to November 2009.

Reference: *FDA Drug Safety Communication,* 12 August 2010 at http://www/fda.gov

Tinzaparin sodium: renal Impairment in elderly

Canada — The manufacturer of tinzaparin sodium (Innohep®) has informed healthcare professionals of important safety information related to results from a clinical study that was stopped prematurely (IRIS – Innohep® in Renal Insufficiency Study) due to the observance of increased mortality. This study involved the use of therapeutic doses of tinzaparin sodium for the treatment of acute venous thromboembolism (VTE) in elderly patients with renal impairment.

Tinzaparin sodium is a low molecular weight heparin. It is authorized for the prevention of postoperative VTE in patients undergoing orthopaedic surgery and in patients undergoing general surgery who are at high risk of developing postoperative VTE; the treatment of deep vein thrombosis (DVT) and/or pulmonary embolism (PE); and the prevention of clotting in indwelling intravenous lines for haemodialysis and extracorporeal circulation in patients without high bleeding risk. Based on the observations in IRIS:

- The study was halted by the Data Safety Monitoring Committee due to an interim finding of an increase in all-cause mortality in patients who received tinzaparin sodium compared to unfractionated heparin (UFH).
- Tinzaparin sodium is not recommended in elderly patients over 70 years of age with renal impairment.
- Tinzaparin sodium should be used with caution in patients with moderate to severe renal impairment and in all cases of impaired renal function patients should be closely monitored.

The IRIS study was an international, multicentre, prospective, open, centrally randomized, parallel group study comparing treatment doses of tinzaparin sodium and UFH for the initial treatment of DVT and/or PE in elderly patients with renal impairment.

Reference: Communication from LEO Pharma Inc., dated 14 October 2010 at http://www.hc-sc.gc.ca/

Tamoxifen: drug interactions involving CYP2D6 genetic variants

United Kingdom — CYP2D6 genetic polymorphisms and concomitant use of potent CYP2D6 inhibitors may be associated with variability in clinical response in patients treated with tamoxifen for breast cancer. Therefore, concomitant use of medicines known to be potent CYP2D6 inhibitors should be avoided whenever possible in patients treated with tamoxifen. Current data for the effect of genetic polymorphisms are insufficient to support recommending genotyping of patients.

Tamoxifen is a selective oestrogen-receptor modulator indicated for palliative and adjuvant treatment of oestrogen-receptor-positive breast cancer in premenopausal and postmenopausal women. Tamoxifen is a prodrug, and the formation of the active metabolite, endoxifen, is mediated by the CYP2D6 enzyme. Several articles have recently been published regarding the potential effect of CYP2D6 genetic variants on clinical response to tamoxifen treatment in patients with breast cancer.

In patients with inherited non-functional alleles of the CYP2D6 gene ("poor metabolisers") or in patients concomi-

tantly treated with CYP2D6 enzyme inhibitors, concentrations of the tamoxifen metabolites that most strongly bind to the oestrogen receptor may be reduced.

Reference: *Drug Safety Update,* Volume 4, Issue 4 November 2010 at http://www.mhra.gov.uk/Safetyinformation/DrugSafetyUpdate/index.htm

Zoledronic acid solution: renal dysfunction

Canada — The manufacturer of zoledronic acid 5 mg/100 mL solution for intravenous infusion (Aclasta®) has informed healthcare professionals of important safety information. As of 30 April 2010, 265 spontaneous reports of renal impairment have been received following administration of Aclasta®, corresponding to a reporting rate of approximately 20 cases per 100 000 patient-years of exposure.

The following precautions should be taken to minimize the risk of renal adverse reactions.

- Zoledronic acid should not be used in patients with severe renal impairment.
- Zoledronic acid should be used with caution when concomitantly used with other drugs that could impact renal function.
- Creatinine clearance should be calculated before each treatment followed by periodic monitoring of serum creatinine in patients with risk factors. Transient increase in serum creatinine may be greater in patients with underlying impaired renal function.
- Patients should be appropriately hydrated, especially elderly patients and those receiving diuretic therapy.
- A single dose of Aclasta® should not exceed 5 mg and the duration of infusion should be no less than 15 minutes.

Reference: Communication from Novartis Pharmaceuticals Canada Inc. dated 12 October 2010 at http://www.hc-sc.gc.ca/

Regulatory Action and News

Influenza vaccines: 2011 southern hemisphere

World Health Organization — It is expected that pandemic A(H1N1), A(H3N2) and B viruses will co-circulate and the following viruses are recommended for influenza vaccines for the 2011 southern hemisphere influenza season:

- an A/California/7/2009 (H1N1)-like virus.
- an A/Perth/16/2009 (H3N2)-like virus. (A/Wisconsin/15/2009 and A/Victoria/210/2009 are A/Perth/16/2009-like viruses.)
- a B/Brisbane/60/2008-like virus.

As in previous years, national or regional control authorities approve the composition and formulation of vaccines used in each country. National public health authorities are responsible for making recommendations regarding the use of the vaccine.

Reference: Recommended viruses for influenza vaccines for use in the 2011 southern hemisphere influenza season. *Weekly Epidemiological Record*, **85**:401–402 (2010) at http://www.who.int/wer

Rosiglitazone–containing antidiabetes medicines: suspension of marketing authorization

European Union — The European Medicines Agency has recommended suspension of the marketing authorizations for the rosiglitazone-containing antidiabetes medicines Avandia®, Avandamet® and Avaglim®.

Patients who are currently taking these medicines should make an appointment with their doctor to discuss suitable alternative treatments. Patients are advised not to stop their treatment without speaking to their doctor.

Doctors should stop prescribing rosiglitazone-containing medicines. Patients taking rosiglitazone-containing medicines should be reviewed in a timely manner to amend their treatment.

The current review of rosiglitazone by the Agency's Committee for Medicinal Products for Human Use (CHMP) was initiated on 9 July 2010 following the availability of new studies questioning the cardiovascular safety of the medicine.

Since its first authorization, rosiglitazone has been recognized to be associated with fluid retention and increased risk of heart failure and its cardiovascular safety has always been kept under close review. Consequently, the use of rosiglitazone was restricted to second-line treatment and contraindicated in patients with heart failure or a history of heart failure when it was first granted a marketing authorization as Avandia® in 2000.

Data from clinical trials, observational studies and meta analyses of existing studies that have become available over the last three years have suggested a possibly increased risk of ischaemic heart disease associated with the use of rosiglitazone. Further restrictions on the use of these medicines in patients with ischaemic heart disease were introduced. The availability of recent studies has added to the knowledge about rosiglitazone and overall, the accumulated data

support an increased cardiovascular risk of rosiglitazone.

In view of the restrictions already in place on the use of rosiglitazone, the Committee could not identify additional measures that would reduce the cardiovascular risk. The Committee therefore concluded that the benefits of rosiglitazone no longer outweigh its risks and recommended the suspension of the marketing authorization of the medicines.

Reference: *EMA Press Release*, EMA/585784/2010, 23 September 2010, at http://www. ema.europa.eu

Modified-release oral opioids: suspension of marketing authorization

European Union — The European Medicines Agency has finalized a review of modified-release oral opioids of the WHO level III scale for the management of pain. The Agency's Committee for Medicinal Products for Human Use (CHMP) concluded that the benefits of most of these medicines continue to outweigh their risks, but that the existing warnings on the interaction of these medicines with alcohol should be made consistent across the class. However, for modified-release oral opioids that contain a polymethacrylate-triethylcitrate controlled-release system the Committee recommended suspension of marketing authorization until they have been reformulated to be more stable in alcohol.

Modified-release oral opioids of the WHO level III scale for the management of pain were reviewed due to concerns that they may be unstable in alcohol and that the active substance may be released too quickly when patients take them together with alcohol. This could put patients at risk of serious side effects such as respiratory depression.

Reference: *EMA Press Release,* EMA/463702/2010, dated 23 July 2010 at http://www.ema. europa.eu

Human normal immunoglobulin: suspension of marketing authorization

European Union — The European Medicines Agency has recommended the suspension of the marketing authorizations for human normal immunoglobulin (Octagam®).

Octagam® is an intravenous solution used to strengthen the body's immune system, including people with primary immunodeficiency syndrome, or children born with acquired immune deficiency syndrome (AIDS). It is also used in people with certain immune disorders such as idiopathic thrombocytopenic purpura (ITP) and in patients who have had a bone marrow transplant.

The CHMP reviewed Octagam® because Germany and Sweden had suspended the marketing authorization of these medicines following an unexpected increase in reports of thromboembolic reactions, including stroke, myocardial infarction and pulmonary embolism in patients. This increase is thought to be related to problems with the medicine's manufacturing process.

The suspension will remain in place until the marketing authorization holder has rectified the problem.

Reference: *EMA Press Release,* EMA/CHMP/591722/2010, 24 September 2010 at http://www.ema. europa.eu

Propoxyphene: recommendation against use

United States of America — The Food and Drug Administration (FDA) is recommending against continued prescribing

and use of the pain reliever propoxyphene because new data show that the drug can cause serious toxicity to the heart, even when used at therapeutic doses. FDA has requested that companies voluntarily withdraw propoxyphene from the United States market.

Propoxyphene is an opioid pain reliever used to treat mild to moderate pain. It is sold under various names as a single-ingredient product (e.g., Darvon®) and as part of a combination product with acetaminophen (e.g., Darvocet®).

The recommendation is based on all available data including data from a new study that evaluated the effects that increasing doses of propoxyphene have on the heart. FDA has concluded that the safety risks of propoxyphene outweigh its benefits for pain relief at recommended doses.

Reference: *FDA Drug Safety Communication,* 19 November 2010 at http://www/fda.gov

Sitaxentan: worldwide withdrawal

Australia — The Therapeutic Goods Administration (TGA) has advised that the supply of the prescription medicine sitaxentan (Thelin®) will be suspended. The company that supplies the medicine has announced that it will withdraw Thelin® from the market globally.

Sitaxentan is a prescription-only medicine used to treat pulmonary hypertension. Patients currently taking sitaxentan should contact their physician as soon as possible to organize the supply of a different medicine but should not cease their use of sitaxentan until they have been assessed and switched to another medication.

This action has been taken in response to a review of safety data in clinical trials that showed patients were at risk of acute liver failure that in some cases was not reversible. The TGA has received 10 adverse event reports of abnormal liver function in Australian patients receiving sitaxentan.

Reference: *TGA Safety Alert,* 10 December 2010 at http://www.tga.gov.au/alerts/medicines/thelin-withdrawal.htm

Sibutramine: suspension of sales

Singapore — The Health Sciences Authority (HSA) has taken a regulatory decision to suspend the sales of sibutramine products following a benefit-risk assessment which took into consideration the findings from the Sibutramine Cardiovascular Outcomes (SCOUT) study, use of the product in the local context and developments in other international jurisdictions.

Sibutramine is licensed in Singapore since 2001 for use as an adjunctive therapy to diet and exercise for obese patients with a body mass index (BMI) $\geq$30kg/m^2, or for overweight patients with a BMI $\geq$27kg/m^2 with other obesity-related risk factors such as Type 2 diabetes mellitus or dyslipidaemia.

The SCOUT study was a randomized, double-blind, placebo-controlled, multicentre study conducted in approximately 10 000 patients aged ≥55 who were obese or overweight and had a history of cardiovascular (CV) disease and/or type 2 diabetes with at least one other CV risk factor treated over a six year period. The study results showed that there was a 16% increase in the risk of a primary outcome event of nonfatal myocardial infarction (MI), nonfatal stroke, resuscitated cardiac arrest and CV death in the sibutramine group as compared with the placebo group. This was driven primarily by increase in rates of nonfatal MI and nonfatal stroke seen in the sibutramine group. A review of the serious adverse

events also showed that there were significantly more reports of myocardial ischaemia and ischaemic stroke in subjects taking sibutramine as compared to placebo.

The weight loss achieved in the SCOUT study was modest. At the end of 12 months, the mean weight loss achieved with sibutramine was up to 2.4 kg more than placebo. After 12 months of treatment, no additional mean weight loss was achieved and it was not clear if the effect on weight loss could be maintained when sibutramine was stopped.

Based on these findings and the overall assessment, HSA has concluded that the benefits of sibutramine do not outweigh their risks and has recommended that the sales of sibutramine be suspended. This suspension will remain in place until the company can provide sufficient data to identify a group of patients for whom sibutramine's benefits clearly outweigh its risks.

In view of the cessation of marketing of sibutramine in Singapore with immediate effect, healthcare professionals are advised to stop prescribing sibutramine, to review the therapy of existing patients who have been prescribed sibutramine and to consider suitable alternatives where appropriate.

Reference: *HSA Safety Alert,* 11 October 2010 at http://www.hsa.gov.sg

Sibutramine-containing medicines: withdrawal

Mexico — The Federal Committee for Protection against Health Risks (COFEPRIS) has requested manufacturers of products containing sibutramine to begin withdrawal of these products from the market. Sibutramine is used for the treatment of obesity and overweight. Evidence now exists of a link to cardiovascular events.

The Committee has reminded the public of the risk incurred with self-medication of products claiming weight reduction.

Reference: Communication to Public and Healthcare Professionals, Comision Federal para la Proteccion contra Riesgos Sanitarios, 8 October 2010 at http://www.cofepris.gob.mx

Testosterone transdermal patch: withdrawal of extension of indication application

European Union — The European Medicines Agency (EMA) has been formally notified by the manufacturer of its decision to withdraw its application for an extension of indication for the centrally authorized testosterone transdermal patch (Intrinsa®).

On 10 August 2009, the manufacturer submitted an application to extend the marketing authorization for Intrinsa® to include the treatment of hypoactive sexual desire disorder in menopausal women. Intrinsa® is currently authorized for the treatment of hypoactive sexual desire disorder in women who have had their uterus and both ovaries removed. It is used in patients already taking an oestrogen.

Reference: *EMA Press Release,* EMA/601877/2010, 29 September 2010 at http://www.ema. europa.eu

Aliskiren/valsartan: withdrawal of marketing authorization application

European Union — The European Medicines Agency has been formally notified by the manufacturer of its decision to withdraw the application for a centralized marketing authorization for aliskiren/valsartan (Rasival®), 150/160 mg and 300/320 mg film-coated tablets.

This medicine was intended to be used for the treatment of essential hypertension as a substitution therapy in adults whose blood pressure is adequately controlled with aliskiren and valsartan, given as single components concurrently, at the same dose level as in the combination.

The company stated that its decision to withdraw the application was based on their inability to address the CHMP's requests and provide additional data within the timeframe allowed in the centralized procedure.

Reference: *EMA Press Release,* EMA/583880/2010, 17 September 2010 at http://www.ema. europa.eu

Mometasone furoate/formoterol fumarate: withdrawal of marketing authorization application

European Union — The European Medicines Agency has been formally notified by the manufacturer of its decision to withdraw the application for a centralized marketing authorization for mometasone furoate/formoterol fumarate (Zenhale®) 50/5, 100/5 or 200/5 mg, pressurised inhalation.

This medicine was intended to be used for long-term, twice-daily maintenance treatment of asthma, including reduction of asthma exacerbations, in adults and children aged 12 years or older.

The decision to withdraw the application was based on the manufacturer's inability to address CHMP requests to provide additional data within the timeframe allowed in the centralized procedure.

Reference: *EMA Press Release,* EMA/700091/2010, 9 November 2010 at http://www.ema. europa.eu

EMA and US FDA extend confidentiality arrangements indefinitely

European Union/United States of America — The European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) have extended their confidentiality arrangements related to medicinal products for human and veterinary use, following the positive experience gained since the initial arrangements were signed in September 2003. This cooperation will now continue indefinitely without the need for further renewal.

The confidentiality arrangements allow both Agencies to exchange confidential information as part of their regulatory and scientific processes. Their aim is to promote public and animal health and to protect European and US patients. The types of information covered by the arrangements relate to scientific advice, orphan drug designation, paediatric development, good manufacturing practice (GMP) and good clinical practice (GCP) inspection planning and reports, marketing authorization procedures and subsequent changes to the marketing authorizations together with post-marketing surveillance.

Reference: *EMA Press Release,* EMA/579151/2010, 15 September 2010 at http://www.ema. europa.eu

Recent Publications, Information and Events

US Government to share patents with Medicines Patent Pool

United States of America — A Presidential Policy Directive on Global Development has been signed that focuses on sustainable development outcomes and places a premium on broad-based economic growth, democratic governance, game-changing innovations, and sustainable systems for meeting basic human needs. The new Policy aims to leverage innovation to solve long-standing development challenges, encourage new models for innovation and to increase developing country utilization of science and technology.

The initial contribution by the National Institutes of Health (NIH) and co-patent owner, the University of Illinois at Chicago, takes an important step toward making affordable and appropriate HIV medicines available to patients around the world. It builds on the President's previous commitment to support humanitarian licensing policies to ensure that medications developed with US taxpayer dollars are available off-patent in developing countries. The patents have previously been licensed for the HIV drug darunavir. The license to the Medicines Patent Pool stipulates that the technology will be available for the benefit of all low- and middle-income countries, as defined by the World Bank, and is royalty-free.

The Medicines Patent Pool is supported by UNITAID, an innovative global health financing mechanism that was co-founded by Brazil, Chile, France, Norway and the United Kingdom at the United Nations General Assembly in 2006. It is a voluntary mechanism through which pharmaceutical patent holders can choose to license their patents to the Pool.

Reference: *Fact Sheet,* 30 September 2010 at http://www.WhiteHouse.gov

Clinical trials and global medicines development

European Union — In September 2010, The European Medicines Agency (EMA) held an international workshop with a broad cross section of stakeholders from around the world to discuss a way forward for a global framework of clinical trials that has at its heart the protection of the rights, safety and wellbeing of patients participating in clinical trials anywhere in the world.

The workshop was part of the consultation process on the Agency's 'Reflection Paper on Ethical and Good Clinical Practice (GCP) Aspects of Clinical Trials of Medicinal Products for Human Use Conducted in Third Countries and submitted in Marketing Authorization Applications to the EMA'.

Some 170 participants from 50 countries provided feedback on the Reflection Paper and discussed international cooperation. They represented patient organisations, health-related nongovernmental organizations, clinical trial sponsors, pharmaceutical industry, ethics committees, regulatory authorities from all continents and intergovernmental organizations.

In marketing authorization applications submitted to the Agency between 2005

and 2009, only 38.8% of patients enrolled in pivotal clinical trials, received their treatments at clinical trial sites within the EU and EEA. These trials involved more than 44 000 clinical trial sites in 89 countries. The data generated was used to support 347 marketing authorization applications as well as some applications for a variation or a line extension of the existing marketing authorization.

References

1. *Draft Reflection Paper on Ethical and Good Clinical Practice (GCP) Aspects of Clinical Trials of Medicinal Products for Human Use Conducted in Third Countries and submitted in Marketing Authorization Applications to the European Medicines Agency*, at http:// www.ema.europa.eu/docs/en_GB/document_ library/Regulatory_and_procedural_guideline/ 2010/06/WC500091530.pdf

2. *EMA Press Release*, EMA/559074/2010, 6 September 2010 at http:// www.ema. europa.eu

Evaluation of future nanomedicines

European Union — The European Medicines Agency (EMA) has hosted the first international scientific workshop on nanomedicines in September 2010. Some 200 European and international participants from 27 countries including Australia, Canada, India, Japan and the United States discussed benefits and challenges arising from the application of nanotechnologies to medicines. Participants included representatives from patient organizations, health care professional organizations, academia, regulatory authorities and the pharmaceutical industry.

Nanotechnologies have a wide and still only partially exploited potential in the development of medicines. They provide scope for engineered nano-systems that could lead to a spectrum of useful functions such as refined drug delivery, advanced combined diagnostics/therapeutic functions, matrices and support structures for regenerative medicines. Some eighteen marketing authorization applications for nanomedicines have been reviewed by the EMA so far.

Emerging therapies give rise to questions on the appropriateness of current regulatory frameworks, the relevance and adequacy of existing requirements and guidelines, and on the availability of adequate expertise to regulators. Scientific challenges arise from the limitations of current testing methods and the reliability of novel ones, because of the 'nanosize' and the unique behaviour of such nano-systems in biological structures.

Reference: European public assessment reports for nanomedicines and presentations of the keynote speakers at the workshop (EMA/567306/2010) at: http://www.ema. europa.eu

Reporting on opioid inaccessibility

Strong opioids such as morphine are rarely accessible in low- and middle-income countries, even for patients with the most severe pain. The three cases recently reported in the *Journal of Pain and Palliative Care Pharmacotherapy* from three diverse countries provide examples of the terrible and unnecessary suffering that occurs everyday when this essential, inexpensive, and safe medication is not adequately accessible by patients in pain. The reasons for this lack of accessibility are explored, and ways to resolve the problem are proposed.

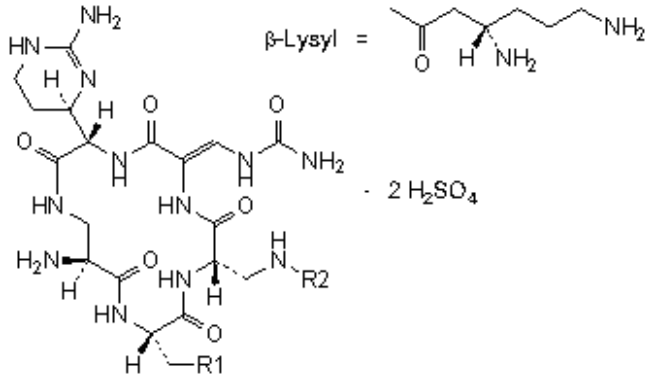
Reference: Krakauer EL, Wenk R, Buitrago R, Jenkins P, and Scholten W . Opioid inaccessibility and its human consequences: Reports From the Field. *Journal of Pain and Palliative Care Pharmacotherapy,* 2010;**24**(3):239–243.

Consultation Documents

The International Pharmacopoeia

Capreomycini sulfas
Capreomycin sulfate

Draft proposal for *The International Pharmacopoeia* (August 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.



Component	R_1	R_2
Capreomycin IA	OH	-Lysyl
Capreomycin IB	H	-Lysyl
Capreomycin IIA	OH	H
Capreomycin IIB	H	H

Capreomycin IA: $C_{25}H_{48}N_{14}O_{16}S_2$; Capreomycin IB: $C_{25}H_{48}N_{14}O_{15}S_2$; Capreomycin IIA: $C_{19}H_{35}N_{12}O_{15}S_2$; Capreomycin IIB: $C_{19}H_{35}N_{12}O_{14}S_2$

Relative molecular mass. Capreomycin IA: 864.9; Capreomycin IB: 848.9, Capreomycin IIA: 735.7; Capreomycin IIB: 719.7

Chemical name. Capreomycin IA: (3*S*)-3,6-diamino-N-[[(2*S*,5*S*,8Z,11*S*,15*S*)-15-amino-11-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-8-[(carbamoylamino)

methylidene]-2-(hydroxymethyl)-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide; sulfuric acid.

Capreomycin IB: (3*S*)-3,6-diamino-N-[[(2*S*,5*S*,8*Z*,11*S*,15*S*)-15-amino-11-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-8-[(carbamoylamino)methylidene]-2-methyl-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentazacyclohexadec-5-yl]methyl]hexanamide; sulfuric acid.

Capreomycin IIA: [(*Z*)-[(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-12-(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-6-ylidene]methyl]urea; sulfuric acid.

Capreomycin IIB: [(*Z*)-[(3*S*,9*S*,12*S*,15*S*)-15-amino-9-(aminomethyl)-3-[(4*R*)-2-amino-3,4,5,6-tetrahydropyrimidin-4-yl]-12-methyl-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentazacyclohexadec-6-ylidene]methyl]urea; sulfuric acid.

CAS Reg. No. 1405-37-4 (capreomycin sulfate).

Description. A white or almost white powder.

Solubility. Very soluble in water, practically insoluble in ethanol (~750 g/l) TS and in ether.

Category. Antituberculosis drug.

Storage. Capreomycin sulfate should be kept in a tightly closed container or, if sterile, in a hermetically closed container.

Labelling. The label states, where applicable:

(1) that the substance is free from bacterial endotoxins,
(2) that the substance is sterile.

Requirements

Definition. Capreomycin sulfate is the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*. It contains not less than 93.0% and not more than 102.0% of capreomycin sulfate, calculated with reference to the dried substance and taking into account the sum of capreomycins sulfate IA, IB, IIA and IIB. The content of capreomycins sulfate IA and IB is not less than 90%.

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

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from capreomycin sulfate RS or with the *reference spectrum* of capreomycin sulfate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 30 volumes of phenol R, 10 volumes

of water R and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 4 μl of each of the following two solutions in water R. For solution (A), use 10 mg of the test substance per ml and for solution (B), use 10 mg of capreomycin sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air. Spray with triketohydrindene/methanol TS and heat the plate for 3 minutes at 120 °C. Examine the chromatogram in daylight. The spots obtained with solution A correspond in position, appearance, and intensity with those obtained with solution B.

C. The absorption spectrum of a 20 μg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits one maximum at about 268 nm; the specific absorbance ($A^{1\%}_{1\ cm}$) is about 300.

D. The absorption spectrum of a 20 μg/ml in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a major maximum at about 287 nm; the specific absorbance ($A^{1\%}_{1\ cm}$) is about 200.

E. A 20 mg/ml solution yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

pH value (1.3). pH of a 30 mg/ml solution in carbon-dioxide-free water R, 4.5-7.5.

Loss on drying. Dry for 4 hours at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 100 mg/g.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 30 μg/g.

Sulfated ash (2.3). Not more than 10.0 mg/g.

Bacterial endotoxins. If intended for use in the manufacture of a parenteral dosage form, carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.35 IU of endotoxin per mg of capreomycin.

Sterility. If intended for use in the manufacture of either a parenteral or other sterile dosage form without a further appropriate sterilization procedure, complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given under Assay, Method A.

Prepare the following solutions using water R as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 10 μg of capreomycin sulfate per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 μl of solution (1). The test is not valid unless the resolution between the two major peaks corresponding to capreomycin IA and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The test is also not valid unless the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB, with a relative retention of 0.53 and 0.63, respectively, is at least 3.5.

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

In the chromatogram obtained with solution (1), the area of any peak, other than the four major peaks corresponding to capreomycins IA, IB, IIA and IIB, is not greater than 4 times the sum of the areas of the four major peaks obtained with solution (2) (2.0%). The area of not more than one such peak is greater than twice the sum of the areas of the four major peaks obtained with solution (2) (1.0%). The sum of the areas of all peaks, other than the four major peaks, is not greater than 14 times the sum of the areas of the four major peaks obtained with solution (2) (7.0%). Disregard any peak with an area less than 0.1 times the sum of the areas of the four major peaks in the chromatogram obtained with solution (2) (0.05%).

Assay

Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Hypersil BDS column has been found suitable).

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

Mobile phase A: 5 volumes of acetonitrile R and 95 volumes of phosphate buffer pH 2.3.
Mobile phase B: 15 volumes of acetonitrile R and 85 volumes of phosphate buffer pH 2.3.

Prepare the phosphate buffer pH 2.3 by dissolving 54.4 g of potassium dihydrogen phosphate R in 1500 ml of water R, adjust the pH to 2.3 by adding phosphoric acid (~105 g/l) TS, add 9.4 g of sodium hexanesulfonate R and dilute to 2000 ml with water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–25	55 to 52	45 to 48	Linear gradient
25–40	52	48	Isocratic
40–60	30	70	Isocratic
60–70	55	45	Isocratic re-equilibration

Prepare the following solutions using water R as diluent. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) use 2.0 mg of capreomycin sulfate RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 μl of solution (1). The assay is not valid unless the resolution between the two major peaks corresponding to capreomycin IA and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The assay is also not valid unless the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB, with a relative retention of 0.53 and 0.63, respectively, is at least 3.5.

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of capreomycin sulfate (sum of the four peaks corresponding to capreomycins IA, IB, IIA and IIB) from the declared content of capreomycin sulfate in capreomycin sulfate RS.

B. Dissolve about 40 mg, accurately weighed, in hydrochloric acid (0.1 mol/l) VS to produce 20 ml. Dilute 1 ml of this solution to 100 ml with the same solvent. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 268 nm, and calculate the content of capreomycin sulfate, using the absorptivity value of 30.0 ($A^{1\%}_{1\ cm}$ = 300).

Capreomycini add injectionem
Capreomycin for injection

Draft proposal for *The International Pharmacopoeia* (August 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Description. A white or almost white powder.

Category. Antituberculosis drug.

Storage. Capreomycin for injection should be stored in a well-closed container.

Labelling. The designation on the container of capreomycin for injection should state that the active ingredient is in the sulfate form, and the quantity should be indicated in terms of the equivalent amount of capreomycin.

Additional information. Strength in the current WHO Model List of Essential Medicines: 1 g. Strength in the current WHO Model List of Essential Medicines for children: 1 g.

The injection is reconstituted by dilution of Capreomycin powder for injections in Water for injections.

Requirements

The powder for injection and the reconstituted injection comply with the monograph for "Parenteral preparations".

Definition. Capreomycin for injection is a sterile powder containing Capreomycin sulfate. It contains not less than 90.0% and not more than 115.0% of the amount of capreomycin stated on the label, taking into account the sum of capreomycins IA, IB, IIA and IIB.

Identity tests

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

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from capreomycin sulfate RS or with the *reference spectrum* of capreomycin sulfate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 30 volumes of phenol R, 10 volumes of water R and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 4 μl of each of the following two solutions in water R. For solution (A), dissolve a quantity of the powder to obtain a solution containing 10 mg of the test substance per ml. For solution (B), use 10 mg of capreomycin sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air. Spray with triketohydrindene/methanol TS and heat the plate for 3 minutes at 120 °C. Examine the chromatogram in daylight.

The spots obtained with solution A correspond in position, appearance, and intensity with those obtained with solution B.

C. Dissolve a quantity of the powder in hydrochloric acid (0.1 mol/l) VS to obtain a solution containing the equivalent of 20 μg of capreomycin per ml. The absorption spectrum of this solution, when observed between 230 nm and 350 nm, exhibits one maximum at about 268 nm.

D. Dissolve a quantity of the powder in sodium hydroxide (0.1 mol/l) VS to obtain a solution containing the equivalent of 20 μg of capreomycin per ml. The absorption spectrum of this solution, when observed between 230 nm and 350 nm, exhibits a major maximum at about 287 nm.

E. A solution of the powder containing the equivalent of 20 mg of capreomycin per ml yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Clarity of solution. A freshly prepared solution of the powder containing the equivalent of 1 g of capreomycin in 10 ml of carbon-dioxide-free water R is clear.

pH value (1.3). pH of a solution of the powder containing the equivalent of 0.3 g of capreomycin in 10 ml of carbon-dioxide-free water R, 4.5–7.5.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.35 IU of endotoxin per mg of capreomycin.

Related substances. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given under Assay.

Prepare the following solutions using water R as diluent. For solution (1) dissolve a quantity of the powder to obtain a solution containing the equivalent of 2.0 mg of capreomycin per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 10 μg of capreomycin per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 μl of solution (1). The test is not valid unless the resolution between the two major peaks corresponding to capreomycin IA and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The test is also not valid unless the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB, with a relative retention of 0.53 and 0.63, respectively, is at least 3.5.

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

In the chromatogram obtained with solution (1), the area of any peak, other than the four major peaks corresponding to capreomycins IA, IB, IIA and IIB, is not greater than 4 times the sum of the areas of the four major peaks obtained with solution (2) (2.0%). The area of not more than one such peak is greater than twice the sum of the areas of the four major peaks obtained with solution (2) (1.0%). The sum of the areas of all peaks, other than the four major peaks, is not greater than 14 times the sum of the areas of the four major peaks obtained with solution (2) (7.0%). Disregard any peak with an area less than 0.1 times the sum of the areas of the four major peaks in the chromatogram obtained with solution (2) (0.05%).

Assay

Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Hypersil BDS column has been found suitable).

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

Mobile phase A:	5 volumes of acetonitrile R and 95 volumes of phosphate buffer pH 2.3.
Mobile phase B:	15 volumes of acetonitrile R and 85 volumes of phosphate buffer pH 2.3.

Prepare the phosphate buffer pH 2.3 by dissolving 54.4 g of potassium dihydrogen phosphate R in 1500 ml of water R, adjust the pH to 2.3 by adding phosphoric acid (~105 g/l) TS, add 9.4 g of sodium hexanesulfonate R and dilute to 2000 ml with water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–25	55 to 52	45 to 48	Linear gradient
25–40	52	48	Isocratic
40–60	30	70	Isocratic
60–70	55	45	Isocratic re-equilibration

Prepare the following solutions using water R as diluent. For solution (1) dissolve a quantity of the powder to obtain a solution containing the equivalent of 2.0 mg of capreomycin per ml. For solution (2) use an amount of capreomycin sulfate RS equivalent to 2.0 mg of capreomycin per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 268 nm.

Inject 20 l of solution (1). The assay is not valid unless the resolution between the two major peaks corresponding to capreomycin IA and capreomycin IB, with a relative retention of 0.89 and 1, respectively, is at least 2.0. The assay is also not valid unless the resolution between the peaks corresponding to capreomycin IIA and capreomycin IIB, with a relative retention of 0.53 and 0.63, respectively, is at least 3.5.

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of capreomycin (sum of the four peaks corresponding to capreomycins IA, IB, IIA and IIB) from the declared content of capreomycin in capreomycin sulfate RS.

[Note from Secretariat: it is proposed that the ICRS will have its content expressed on the label in terms of capreomycin base and capreomycin sulfate]

Efavirenz tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Antiretroviral (Non-nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Strengths in the current WHO Model List of Essential Medicines: 600 mg. Strengths in the current WHO Model List of Essential Medicines for Children: 600 mg.

Requirements

Comply with the monograph for “Tablets”.

Definition. Efavirenz tablets contain Efavirenz. They contain not less than 90.0% and not more than 110.0% of the amount of Efavirenz ($C_{14}H_9ClF_3NO_2$) stated on the label.

Identity tests

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

A. To a quantity of the powdered tablets containing 25 mg of Efavirenz, add 10 ml of methanol R, shake to dissolve and filter. Evaporate the filtrate to dryness. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from efavirenz RS or with the *reference spectrum* of efavirenz.

If the spectra thus obtained are not concordant, repeat the test using the test residue and the residue obtained by dissolving efavirenz RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from efavirenz RS.

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

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, 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 the following two solutions in methanol R. For solution (A) shake a quantity of the powdered tablets containing 5 mg of Efavirenz with 5 ml, filter and use the clear filtrate. For solution (B) use 1 mg of efavirenz 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.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described under test A.1 but using silica gel R5 as the coating substance. Spray the plate with 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.

C. See the test described under Assay method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

D. The absorption spectrum of the final solution prepared for Assay method B, when observed between 210 nm and 300 nm, exhibits one maximum at about 247 nm.

Related substances. Prepare fresh solutions and perform the test without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay Method A.

Prepare the following solutions in the dissolution solvent, a mixture of equal volumes of acetonitrile R and water R.

For solution (1) transfer a quantity of the powdered tablets containing about 25 mg of Efavirenz into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 25.0 ml with the same solvent. Filter a portion of this solution through a 0.45-μm filter, discarding the first few ml of the filtrate. For solution (2) dilute 1.0 ml of solution (1) to 50.0 ml with the dissolution solvent and dilute 5.0 ml of the resulting solution to 100.0 ml with the same solvent. For solution (3) dissolve about 5 mg of efavirenz RS in 5 ml of a solution prepared as follows: dissolve 1 mg of efavirenz impurity B RS in the dissolution solvent and dilute to 10 ml with the same solvent. Dilute 1 ml of the resulting solution to 25 ml with the dissolution solvent.

Inject separately 35 μl each of solutions (1), (2) and (3) and of the dissolution solvent in the chromatographic system. Examine the blank chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (1).

In the chromatogram obtained with solution (3), the peak due to impurity B is eluted at a relative retention of about 0.9 with reference to Efavirenz (retention time about 20 minutes). The test is not valid unless the resolution factor between the peaks due to impurity B and Efavirenz is at least 3.

In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity B is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%), the area of any other peak, apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%) and the area of not more than three such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than eight times the area of the principal peak in the chromatogram obtained with solution (2) (0.8%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

[Note from Secretariat: retention times and resolution factor to be confirmed.]

Assay

Either method A or method B may be applied.

A. Carry out the assay as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm x 4.6 mm), packed with cyanopropyldimethylsilane monolayer (3.5 μm). (Zorbax® SB-CN has been found suitable.)

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

Mobile phase A: 90 volumes of a 0.05% solution of trifluoroacetic acid R and 10 volumes of methanol R.
Mobile phase B: 10 volumes of a 0.05% solution of trifluoroacetic acid R and 90 volumes of methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–16	60 to 50	40 to 50	Linear gradient
16–23	50 to 35	50 to 65	Linear gradient
23–28	35 to 30	65 to 70	Linear gradient
28–29	30 to 20	70 to 80	Linear gradient
29–31	20	80	Isocratic
31–32	20 to 60	80 to 40	Return to initial composition
32–40	60	40	Re-equilibration

Prepare the following solutions in the dissolution solvent, a mixture of equal volumes of acetonitrile R and water R

For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 25 mg of Efavirenz, accurately weighed, into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 25.0 ml with the same solvent. Filter a portion of this solution through a 0.45-μm filter, discarding the first few ml of the filtrate. Dilute 1.0 ml of the resulting solution to 100.0 ml with the dissolution solvent. For solution (2) dissolve 25 mg of efavirenz RS in the dissolution solvent and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of the resulting solution to 100.0 ml with the dissolution solvent. For solution (3) dissolve about 5 mg of efavirenz RS in 5 ml of a solution prepared as follows: dissolve 1 mg of efavirenz impurity B RS in the dissolution solvent and dilute to 10 ml with the same solvent. Dilute 1 ml of the resulting solution to 25 ml with the dissolution solvent.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 250 nm.

Maintain the column temperature at 40 °C.

Inject separately 35 μl each of solutions (1), (2) and (3). In the chromatogram obtained with solution (3), the peak due to impurity B is eluted at a relative retention of about 0.9 with reference to efavirenz (retention time about 20 minutes). The assay is not valid unless the resolution factor between the peaks due to impurity B and efavirenz is at least 3.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of efavirenz ($C_{14}H_9ClF_3NO_2$) in the tablets.

[Note from Secretariat: retention times and resolution factor to be confirmed.]

B. Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 25 mg of Efavirenz, 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 to 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 1.0 ml of this solution to 50.0 ml with methanol R. Measure the absorbance (1.6) of a 1-cm layer of this solution at the maximum at about 247 nm. Calculate the content of efavirenz ($C_{14}H_9ClF_3NO_2$) in the tablets using an absorptivity value of 55.0 ($A^{1\%}_{1\ cm}$ = 550).

Impurities. The impurities limited by the requirements of this monograph include those listed in the monograph for Efavirenz.

Efavirenz, emtricitabine and tenofovir tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Antiretroviral (Non-nucleoside/Nucleoside/Nucleotide Reverse Transcriptase Inhibitors).

Storage. Efavirenz, emtricitabine and tenofovir tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model List of Essential Medicines: 600 mg Efavirenz, 200 mg Emtricitabine and 300 mg Tenofovir disoproxil fumarate.

Requirements

Comply with the monograph for “Tablets”.

Definition. Efavirenz, emtricitabine and tenofovir tablets contain Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of efavirenz ($C_{14}H_9ClF_3NO_2$), emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) stated on the label.

Manufacture. The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the tablets. They ensure that, if tested, the tablets would comply with a water content limit of not more than 60 mg/g when determined as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the powdered tablets.

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 1.14.1 Thin-layer chromatography, 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 l of each of the following four solutions in methanol R. For solution (A) disperse a quantity of the powdered tablets to obtain a concentration of 5 mg of Emtricitabine per ml, filter and use the filtrate. For solution (B) use 5 mg of emtricitabine RS per ml. For solution (C) use 7.5 mg of tenofovir disoproxil fumarate RS per ml. For solution (D) use 15 mg of efavirenz RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).
The three principal spots obtained with solution A correspond in position, appearance and intensity with those obtained with solutions B, C and D.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Stain the plate with iodine vapour and examine the chromatogram in daylight.

The three principal spots obtained with solution A correspond in position, appearance and intensity with those obtained with solutions B, C and D.

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

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 l of each of the following two solutions in ethanol R. For solution (A) disperse a quantity of the powdered tablets to obtain a concentration of 10 mg of Tenofovir disoproxil fumarate per ml, filter and use the filtrate. For solution (B) use 2 mg of fumaric acid R per ml. Develop the plate in an unsaturated tank over a path of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test B.1 but using silica gel R5 as the coating substance. Spray lightly with a 16 g/l solution of potassium permanganate R and examine the chromatogram in daylight.

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

C. See the test described under Assay. The retention times of the principal peaks due to efavirenz, emtricitabine, tenofovir disoproxil and fumarate in the chromatogram obtained with the test solution are similar to those in the chromatogram obtained with the reference solution.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 1000 ml of a 2% solution of sodium dodecyl sulfate R, and rotating the paddle at 100 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool to room temperature and dilute if necessary [solution (1)]. Prepare solution (2) using 0.60 mg of efavirenz RS, 0.20 mg of emtricitabine RS and 0.30 mg of tenofovir disoproxil fumarate RS per ml of dissolution medium. Determine the content of efavirenz ($C_{14}H_9ClF_3NO_2$), emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) as described under Assay using solution (1) and solution (2).

For each of the six tablets tested, calculate the total amount of efavirenz ($C_{14}H_9ClF_3NO_2$), emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) in the medium from the results obtained. The amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and no tablet contains less than 60%.

Tenofovir monoester. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay.

After preparation, keep the solutions at about 6 °C, or use an injector with cooling. Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 100 mg of Tenofovir disoproxil fumarate, accurately weighed in 100 ml of the diluent and filter. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 5 μg of Tenofovir disoproxil fumarate per ml. For solution (3) heat carefully 1 mg of tenofovir disoproxil fumarate RS per ml of water R in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Inject 20 μl of solution (3). The peak due to tenofovir monoester elutes at a relative retention of about 0.9 with reference to tenofovir disoproxil (retention time about 18 minutes). The test is not valid unless the resolution between these peaks is at least 5. Inject separately 20 μl each of solutions (1) and (2).

In the chromatogram obtained with solution (1), the area of any peak due to tenofovir monoester, is not greater than 7 times the area of the principal peak obtained with solution (2) (3.5%).

Assay

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 m).

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

Mobile phase A: 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 95 volumes of water R.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 25 volumes of water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–9	93	7	Isocratic
9–15	93 to 0	7 to 100	Linear gradient
15–19	0	100	Isocratic
19–19.1	0 to 93	100 to 7	Return to initial composition
19.1–30	93	7	Re-equilibration

After preparation, keep the solutions at about 6 °C, or use an injector with cooling. Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Tenofovir disoproxil fumarate, accurately weighed in 100 ml of the diluent and filter. For solution (2) use 0.2 mg of efavirenz RS, 0.1 mg of tenofovir disoproxil fumarate RS and 66.7 μg of emtricitabine RS per ml of diluent. For solution (c) use 0.02 mg of fumaric acid R per ml of water R.

If necessary adapt the concentration of solution (2) according to the ratio of Efavirenz, Emtricitabine and Tenofovir disoproxil fumarate in the tablets.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Inject separately 20 μl each of solutions (1), (2) and (3).

The test is not valid unless in the chromatograms obtained with solutions (1) and (2), four principal peaks, that elute at the following retention times, are shown: fumarate (about 2.5 minutes), emtricitabine (about 9 minutes), tenofovir disoproxil (about 18 minutes) and efavirenz (about 22 minutes).

Calculate the content of efavirenz ($C_{14}H_9ClF_3NO_2$), emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) in the tablets.

Emtricitabinum capsulae
Emtricitabine capsules

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Emtricitabine capsules should be kept in a tightly closed container.

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

Requirements

Comply with the monograph for “Capsules”.

Definition. Emtricitabine capsules contain Emtricitabine. They contain not less than 90.0% and not more than 110.0% of the amount of emtricitabine ($C_8H_{10}FN_3O_3S$) stated on the label.

Identity tests

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

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

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, 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 the following two solutions in methanol R. For solution (A) disperse a quantity of the contents of the capsules to obtain a concentration of 5 mg of Emtricitabine per ml, filter and use the filtrate. For solution (B) use 5 mg of emtricitabine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of 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 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Stain the plate with iodine vapour and examine in daylight.

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

B. Disperse the quantity of contents of the capsules containing about 50 mg of Emtricitabine with 40 ml of methanol R, dilute to 50 ml and filter. Dilute 1 ml of the filtrate to 50 ml with methanol R. The absorption spectrum (1.6) of the resulting solution, when observed between 220 and 350 nm, exhibits two maxima at about 242 nm and 284 nm.

C. See the test described under Assay, Method A. The retention time of the principal peak in the chromatogram obtained with the test solution is similar to that in the chromatogram obtained with the reference solution.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (~4 g/l) TS and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1-cm layer of the resulting solution, suitably diluted if necessary, at the maximum at about 280 nm. Determine the content of emtricitabine ($C_8H_{10}FN_3O_3S$) in the medium from the absorbance obtained from a solution of known concentration of emtricitabine RS.

For each of the six capsules tested, calculate the total amount of emtricitabine ($C_8H_{10}FN_3O_3S$) in the medium from the results obtained and from the declared content of $C_8H_{10}FN_3O_3S$ in emtricitabine RS. The amount in solution for each capsule is not less than 80% of the amount declared on the label. If the amount of emtricitabine obtained for one of the six capsules is less than 80%, repeat the test using a further six capsules; the average amount of emtricitabine for all 12 capsules tested is not less than 75% and no capsule contains less than 60%.

Related substances
Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay, Method A.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and mix the contents of 20 capsules and disperse a quantity containing about 50 mg of Emtricitabine in 80 ml of the diluent, dilute to 100 ml with the diluent, filter and use the filtrate. For solution (2) dilute a suitable volume of solution (1) with the diluent to obtain a concentration of 0.50 μg of Emtricitabine per ml.

For the system suitability test: prepare solution (3) using 5 ml of solution (1) and 2 ml of phosphoric acid (~105 g/l) TS, heat carefully in a boiling water-bath for 15 minutes. Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to emtricitabine (retention time about 9 minutes) and the peak with a relative retention of about 1.3 is not less than 6.

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

In the chromatogram obtained with solution (1) the area of any peak eluting before the principal peak is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%); the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%); the area of any peak eluting after the principal peak is not greater than 7 times the area of the principal peak in the chromatogram obtained with solution (2) (0.7%); the area of not more than two such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (1%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay

Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). (Hypersil BDS C18 has been found suitable.)
.
The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 95 volumes of water R.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 25 volumes of water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–9	93	7	Isocratic
9–15	93 to 0	7 to 100	Linear gradient
15–19	0	100	Isocratic
19–19.1	0 to 93	100 to 7	Return to initial composition
19.1–30	93	7	Re-equilibration

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1): weigh and mix the contents of 20 capsules and disperse a quantity containing about 50 mg of Emtricitabine in 80 ml of the diluent, dilute to 100 ml with the diluent, filter and use the filtrate.

For solution (2) use 0.5 mg of emtricitabine RS per ml of the diluent.

For the system suitability test: prepare solution (3) using 5 ml of solution (1) and 2 ml of phosphoric acid (~105 g/l) TS, heat carefully in a boiling water-bath for 15 minutes. Inject 20 l of solution (3). The test is not valid unless the resolution between the peak due to emtricitabine (retention time about 9 minutes) and the peak with a relative retention of about 1.3 is not less than 6.

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of emtricitabine ($C_8H_{10}FN_3O_3S$).

B. Weigh and mix the contents of 20 capsules and disperse a quantity containing about 50 mg of Emtricitabine in 40 ml of methanol R, dilute to 50 ml with methanol R and filter. Dilute 1 ml of the filtrate to 50 ml with methanol R.

Measure the absorbance (1.6) of the resulting solution in a 1-cm layer at the maximum at about 284 nm.

Calculate the content of emtricitabine ($C_8H_{10}FN_3O_3S$), using the absorptivity value of 33.2 ($A_{1cm}^{1\%} = 332$).

* * *

New reagent to be added to The International Pharmacopoeia:

Hydrochloric acid (~4 g/l) TS.

Dilute 10 ml of hydrochloric acid (~420 g/l) TS with sufficient water to produce 1000 ml (approximately 0.1 mol/l).

Emtricitabini et tenofoviri compressi
Emtricitabine and tenofovir tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Antiretroviral (Nucleoside/Nucleotide Reverse Transcriptase Inhibitor).

Storage. Emtricitabine and tenofovir tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 200 mg Emtricitabine and 300 mg Tenofovir disoproxil fumarate.

Requirements

Comply with the monograph for "Tablets".

Definition. Emtricitabine and tenofovir tablets contain Emtricitabine and Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amounts of emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) stated on the label.

Manufacture. The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the tablets. They ensure that, if tested, the tablets would comply with a water content limit of not more than 60 mg/g when determined as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the powdered tablets.

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 1.14.1 Thin-layer chromatography, 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 the following three solutions in methanol R. For solution (A) disperse a quantity of powdered tablets to obtain a concentration of 5 mg of Emtricitabine per ml, filter and use the filtrate. For solution (B) use 5 mg of emtricitabine RS. For solution (C) use 7.5 mg of tenofovir disoproxil fumarate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the two principal spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B and the other one corresponds with that obtained with solution C.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Stain the plate with iodine vapour and examine the chromatogram in daylight.

One of the two principal spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B and the other one corresponds with that obtained with solution C.

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

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 μl of each of the following two solutions in ethanol R. For solution (A) disperse a quantity of powdered tablets to obtain a concentration of 10 mg of Tenofovir disoproxil fumarate per ml, filter and use the filtrate. For solution (B) use 2 mg of fumaric acid R per ml. Develop the plate in an unsaturated tank over a path of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test B.1 but using silica gel R5 as the coating substance. Spray lightly with a 16 g/l solution of potassium permanganate R and examine the chromatogram in daylight.

One of the spots obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

C. See the test described under Assay. The retention times of the principal peaks in the chromatogram obtained with the test solution are similar to those due to emtricitabine, tenofovir disoproxil and to fumarate in the chromatogram obtained with the reference solution.

Dissolution
Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (~0.4 g/l) TS, and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium and filter. Allow the filtered sample to cool to room temperature and dilute if necessary [solution (1)]. Prepare solution (2) using 0.22 mg of emtricitabine RS and 0.33 mg of tenofovir disoproxil fumarate RS per ml of dissolution medium. Determine the content of emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) as described under Assay using solution (1) and solution (2).

For each of the six tablets tested, calculate the total amount of emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) in the medium from the results obtained. For both substances, the amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and no tablet contains less than 60%.

Tenofovir monoester. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay.

After preparation, keep the solutions at about 6 °C, or use an injector with cooling.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 100 mg of Tenofovir disoproxil fumarate, accurately weighed, in 100 ml of the diluent and filter. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 5 μg of Tenofovir disoproxil fumarate per ml. For solution (3) heat carefully 1 mg of tenofovir disoproxil fumarate RS per ml of water R in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Inject 20 μl of solution (3). The peak due to tenofovir monoester elutes at a relative retention of about 0.9 with reference to tenofovir disoproxil (retention time about 18 minutes).

Inject separately 20 μl each of solutions (1) and (2). The test is not valid unless in the chromatogram obtained with solution (1), three principal peaks are shown.

In the chromatogram obtained with solution (1), the area of any peak due to tenofovir monoester, is not greater than seven times the area of the principal peak obtained with solution (2) (3.5%).

Assay
Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Hypersil BDS column.)

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

Mobile phase A: 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 95 volumes of water R.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of potassium dihydrogen phosphate (27.2 g/l) TS and 25 volumes of water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–9	93	7	Isocratic
9–15	93 to 0	7 to 100	Linear gradient
15–19	0	100	Isocratic
19–19.1	0 to 93	100 to 7	Return to initial composition
19.1–30	93	7	Re-equilibration

After preparation, keep the solutions at about 6 °C, or use an injector with cooling.

Prepare the following solutions using a mixture of 20 volumes of acetonitrile R and 80 volumes of water R as a diluent. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Tenofovir disoproxil fumarate, accurately weighed in 100 ml of the diluent and filter. For solution (2) use 0.1 mg of tenofovir disoproxil fumarate RS and 66.7 μg of emtricitabine RS per ml of diluent. If necessary, adapt the concentration of solution (2) according to the ratio of Emtricitabine and Tenofovir disoproxil fumarate in the tablets. For solution (3) use 0.02 mg of fumaric acid R per ml of water R.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm.

Maintain the column temperature at 35 °C.

Inject separately 20 μl each of solutions (1), (2) and (3).

The test is not valid unless in the chromatograms obtained with solutions (1) and (2), three principal peaks are shown and the resolution factor between those peaks is at least 5.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of emtricitabine ($C_8H_{10}FN_3O_3S$) and tenofovir disoproxil fumarate ($C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$) in the tablets.

[Note from Secretariat: a test for related substances is not available, due to the overlapping of the impurities from both APIs and the non-availability of reference substances for the impurities that would be required to allow the identification of the peaks.]

New reagent to be added to The International Pharmacopoeia:

Hydrochloric acid (~0.4 g/l) TS.

Levamisole tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Anthelminthic.

Storage. Levamisole tablets should be kept in a tightly closed container.

Labelling. The designation on the container of Levamisole tablets should state that the active ingredient is in the hydrochloride form and the quantity should be indicated in terms of equivalent amount of levamisole.

Additional information. Strengths in the current WHO Model List of Essential Medicines: 50 mg, 150 mg. Strengths in the current WHO Model List of Essential Medicines for Children: 50 mg, 150 mg

Requirements

Comply with the monograph for “Tablets”.

Definition. Levamisole tablets contain Levamisole hydrochloride. They contain not less than 90.0% and not more than 110.0% of the amount of levamisole ($C_{11}H_{12}N_2S$) stated on the label.

Identity tests

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

A. Shake a quantity of the powdered tablets containing the equivalent of about 450 mg of levamisole with 30 ml of water R, filter. Wash the filter with 20 ml of water R and add the washings to the filtrate. To the combined filtrate add ammonia (~100 g/l) TS to make it alkaline and extract with two quantities, each of 25 ml and 15 ml, of dichloromethane R. Combine the dichloromethane extracts and evaporate to dryness. Add 0.5 ml of hydrochloride acid (~420 g/l) TS, heat on a water-bath to dryness. Dissolve the residue in 10 ml of hydrochloric acid (0.1 mol/l) VS. The optical rotation of the resulting solution is not less than -5°

B. See the test described below under Related substances using ultraviolet light (254 nm) to examine the chromatogram. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution D.

C. See the test described below under Assay, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

D. Shake a quantity of the powdered tablets containing the equivalent of about 100 mg of levamisole with 40 ml of water R and filter. The filtrate yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

Related substances

Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as coating substance and a mixture of 60 volumes of toluene R, 40 volumes of acetone R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following four solutions in methanol R. For solution (A) shake a quantity of the powdered tablets containing the equivalent of about 85 mg of levamisole with 5 ml, filter, and use the filtrate. For solution (B) dilute 1 ml of solution A to 10 ml. For solution (C) dilute 1 ml of solution B to 20 ml. For solution (D) use 2.0 mg of levamisole hydrochloride RS per ml. After removing the plate from the chromatographic chamber, dry it at 105 °C for 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

Expose the plate to iodine vapour in a tightly closed chamber for 15 minutes and examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

Assay

Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 μm). (Phenomenex Gemini C18 has been found suitable.)

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

Mobile phase A: acetonitrile R

Mobile phase B: a 0.75% solution of monobasic ammonium phosphate R in water R adjusted to pH 7 with diisopropylamine R

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–5	20 to 80	80 to 20	Linear gradient
5–7	80	20	Isocratic
7–8	80 to 20	20 to 80	Return to initial composition
8–12	20	80	Re-equilibration

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing the equivalent of about 170 mg of levamisole, accurately weighed, with 100 ml of water R, and filter. Dilute a suitable volume of the filtrate with methanol R to obtain a concentration equivalent to 0.17 mg of levamisole per ml. For solution (2) use 0.2 mg of levamisole hydrochloride RS per ml in methanol R. For solution (3) dissolve the equivalent of 17 mg of levamisole in 5 ml of a 0.1 mol/l solution of sodium hydroxide R in a test tube, and heat in a water bath at 100 °C for 5 hours. Allow to cool and dilute 1 ml of the resulting solution to 25 ml with methanol R.

Operate with a flow rate of 2 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 215 nm.

Inject 10 µl of solution (3). In the chromatogram obtained with solution (3), the peak due to the major degradation product elutes at the following relative retention with reference to levamisole (retention time about 3 minutes): about 1.3. The test is not valid unless the resolution between the peak due to levamisole and the peak due to the major degradation product with a relative retention of about 1.3 is at least 6.0.

Inject separately 10 µl, each of solutions (1) and (2).

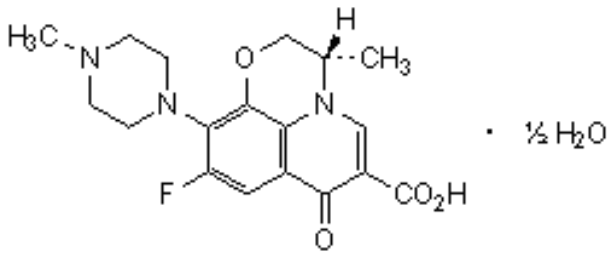
Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of levamisole ($C_{11}H_{12}N_2S$) in the tablets.

B. Weigh and powder 20 tablets. To a quantity of the powdered tablets containing the equivalent of about 170 mg of levamisole, accurately weighed, add 30 ml of water R and shake for 10 minutes. Filter, wash the filter with 20 ml of water R and add the washings to the filtrate. To the combined filtrate add ammonia (~100 g/l) TS to make it alkaline and extract with three quantities, each of 25 ml, 15 ml and 15 ml, of dichloromethane R. Filter each quantity through cotton wool covered with a layer of anhydrous sodium sulfate R and wash the filter with 15 ml of dichloromethane R. Combine the dichloromethane extracts and evaporate to dryness. Dissolve the residue in 50 ml of anhydrous glacial acetic acid R. Titrate with perchloric acid (0.1 mol/l) VS, as described under 2.6 Non-aqueous titration, Method A, using crystal violet/acetic acid TS solution as indicator.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 20.43 mg of $C_{11}H_{12}N_2S$.

Levofloxacin

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.



$C_{18}H_{20}FN_3O_4,{}^1/_2\,H_2O$

Relative Molecular Mass. 370.4

Chemical name. (*S*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid hemihydrate; CAS Reg. No. 138199-71-0.

Description. Yellowish white to bright yellow, crystals or crystalline powder.

Solubility. Slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in dichloromethane, slightly soluble in methanol.

Category. Antibacterial.

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

REQUIREMENTS

Definition. Levofloxacin contains not less than 99.0% and not more than 101.0% of levofloxacin ($C_{18}H_{20}FN_3O_4$) calculated with reference to the anhydrous substance.

Manufacture. The production method is validated to ensure that the substance is the (*S*-enantiomer).

Identity test

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

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levofloxacin RS or with the *reference spectrum* of levofloxacin.

B. Carry out the test as described under 1.14.1. Thin layer chromatography, using silica gel R6 as the coating substance and a mixture of 10 volumes of dichloromethane R, 5 volumes of methanol R and 1 volume of ammonia solution 1% as the mobile phase. Apply separately to the plate 5 µl of each of the following two solutions in a mixture of 1 volume of methanol R and 4 volumes of dichloromethane R. For solution (A) use 5 mg of Levofloxacin per ml. For solution (B) use 5 mg of levofloxacin 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.

C. Transfer 25 mg of Levofloxacin to a 50-ml volumetric flask. Add about 20 ml of hydrochloric acid (~4 g/l) TS, sonicate for about 5 minutes, allow to cool to room temperature and make up to 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 1.0 ml of this solution to 100.0 ml using water R. The absorption spectrum (1.6) of the resulting solution, when observed between 210 and 350 nm, exhibits two maxima at about

294 nm and at about 327 nm. The specific absorbance ($A^{1\%}_{1\ cm}$) at 294 nm is between 876 and 948.

D. Determine the specific optical rotation (1.4) using a 30 mg/ml solution dissolved in a mixture of 10 volumes of methanol R and 40 volumes of dichloromethane R and calculate with reference to the anhydrous substance; $[\alpha]^{20\ °C}$ = -12° to -11°.

[Note from Secretariat: suitability of carrying out the Optical rotation test in methanol under investigation and corresponding limits to be confirmed.]

Heavy metals

[Note from Secretariat: suitable test for heavy metals under investigation.]

Sulfated ash (2.3). Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by Karl Fischer Method, Method A. Use 1.0 g of the test substance. The water content is not less than 21 mg/g and not more than 27 mg/g.

Impurity A
Carry out the test as described under 1.14.1. Thin layer chromatography, using silica gel R6 as the coating substance and a mixture of 10 volumes of glacial acetic acid R, 10 volumes of water R and 20 volumes of ethyl acetate R. Apply separately to the plate 10 l of each of the two following solutions in the dissolution solvent prepared by mixing 10 volumes of methanol R and 40 volumes of dichloromethane R. For solution (A) use 50 mg of Levofloxacin per ml. For solution (B) use 0.1 mg of levofloxacin impurity A RS per ml. After removing the plate from the chromatographic chamber, allow to dry in air. Examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A corresponding impurity A is not more intense than the principal spot obtained with solution B.

Other related substances
Prepare fresh solutions, protected from light and perform the tests without delay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm), packed with particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Symmetry 150 x 46 mm (5 μm) is suitable.)

Maintain the column temperature at 45 °C.

Prepare the mobile phase as follows: dissolve 4.0 g of ammonium acetate R and 7.0 g of sodium perchlorate R in water R and dilute to 1300 ml; adjust to pH 2.2 with phosphoric acid R and add 240 ml of acetonitrile R.

Prepare the following solutions in the dissolution solvent prepared in mixing 10 volumes of acetonitrile R and 60 volumes of water R.

For solution (1) dissolve 10 mg of Levofloxacin in the dissolution solvent and dilute to 50.0 ml with the same solvent. For solution (2) dilute 1.0 ml of solution (1) to 50.0 ml

with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with the same solvent. For solution (3) dissolve 10 mg of levofloxacin impurity E RS in the dissolution solvent and dilute to 100.0 ml with the same solvent. Mix 10 ml with 5 ml of solution (1) and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 294 nm.

Inject 20 μl of solution (3). The test is not valid unless the resolution factor between the peaks due to impurity E and Levofloxacin is at least 2.

Inject separately 20 μl each of solutions (1), (2) and of the dissolution solvent in the chromatographic system.

In the chromatogram obtained with solution (1), the following impurity peaks, if present, are eluted at the following relative retention with reference to Levofloxacin (retention time about 17 minutes): impurity B about 0.36; impurity C about 0.57; impurity D about 0.75; impurity E about 0.91; impurity F about 1.50.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 2.6, is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 4.2, is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any peak corresponding to impurity C, E or F is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any other impurity peak is not greater than 0.5 times the area of the principal peak obtained with solution (2) (0.1%);

- the sum of the areas (corrected, where necessary) of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak obtained with solution (2) (0.05%).

[Note from Secretariat: following information to be confirmed:
- correction factors for impurities B and D,
- limit for individual unspecified impurities,
- limit for total of impurities]

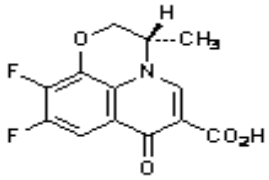
Assay

Dissolve about 0.300 g, accurately weighed, in 100 ml of glacial acetic acid and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6. Non aqueous titrations,

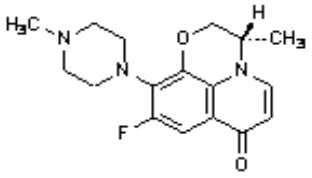
Method A determining the end point potentiometrically. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

Impurities

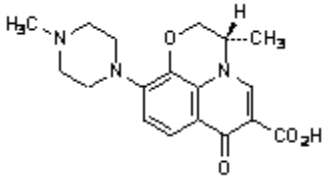
The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information:



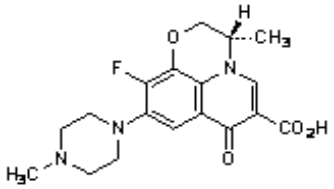
A. (3*S*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



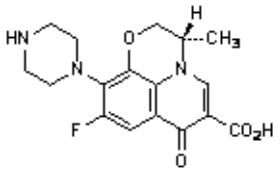
B. (3*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-7-one,



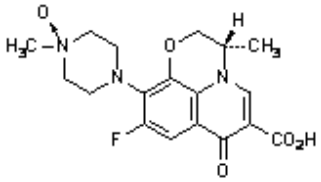
C. (3*S*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



D. (3*S*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



E. (3*S*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



F. 4-[(3*S*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-10-yl]-1-methylpiperazine 1-oxide.

New reagent to be added to The International Pharmacopoeia:

Hydrochloric acid (~4 g/l) TS.

Dilute 10 ml of hydrochloric acid (~420 g/l) TS with sufficient water to produce 1000 ml (approximately 0.1 mol/l).

Levofloxacin tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Antibacterial.

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

Labelling. The designation of the container of Levofloxacin tablets should state that the active ingredient is the hemihydrate form and the quantity should be indicated in terms of the equivalent amount of Levofloxacin.

Additional information. Strengths in the current WHO Model List of Essential Medicines: 200 mg, 400 mg. Strengths in the current WHO Model List of Essential Medicines for Children: 200 mg, 400 mg.

Requirements

Comply with the monograph for “Tablets”.

Definition. Levofloxacin tablets contain Levofloxacin. They contain not less than 90.0% and not more than 110.0% of the amount of Levofloxacin ($C_{18}H_{20}FN_3O_4$) stated on the label.

Identity test

Either test A alone or any two of tests B, C and D may be applied

A. To a quantity of the powdered tablets containing 100 mg of Levofloxacin, add 10 ml of acetonitrile R, shake, filter and evaporate to dryness. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from levofloxacin RS or with the *reference spectrum* of levofloxacin.

B. Carry out the test as described under 1.14.1. Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 10 volumes of dichloromethane R, 5 volumes of methanol R and 1 volume of ammonia solution 1% as the mobile phase. Apply separately to the plate 5 μl of each of the two following solutions in a mixture of 1 volume of methanol R and 4 volumes of dichloromethane R. For solution (A) shake a quantity of the powdered tablets containing 25 mg of Levofloxacin with 5 ml, filter and use the clear filtrate. For solution (B) use 5 mg of levofloxacin 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.

C. See the test described under Assay method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

D. The absorption spectrum of the final solution prepared for Assay method B, when observed between 210 and 350 nm, exhibits two maxima at about 294 nm and at about 327 nm.

[Note from Secretariat: a specific optical rotation test to differentiate levofloxacin from ofloxacin is under investigation, with the possibility to include such test under a Manufacture section.]

Dissolution test

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (~ 4 g/l) TS, and rotating the paddle at 100 revolutions per minute. At 30 minutes withdraw a sample of about 5 ml of the medium through an in-line filter. Measure the absorbance (1.6) of a 1-cm layer of the filtered sample at the maximum at about 294 nm. At the same time,

measure the absorbance at the maximum at about 294 nm of a suitable solution of levofloxacin RS in hydrochloric acid (~4 g/l) TS using hydrochloric acid (~4 g/l) TS as a blank.

For each of the six tablets, calculate the total amount of Levofloxacin ($C_{18}H_{20}FN_3O_4$), in the medium. The amount in solution for each tablet is not less than 75% of the amount declared on the label. If the amount obtained for one of the six tablets is less than 75%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 70% and the amount obtained for no tablet is less than 55%.

[Note from Secretariat: dissolution conditions and limits to be confirmed.]

Related substances
Prepare fresh solutions and perform the tests without delay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay, method A.

For solution (1) transfer a quantity of the powdered tablets containing about 10 mg of Levofloxacin into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 50.0 ml with the same solvent. Filter a portion of this solution through a 0.45-μm filter, discarding the first few ml of the filtrate. For solution (2) dilute 1.0 ml of solution (1) to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with the same solvent. For solution (3) dissolve 10 mg of levofloxacin impurity E RS in the dissolution solvent and dilute to 100.0 ml with the same solvent. Mix 10 ml with 5 ml of solution (1) and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the same solvent.

Inject 20 μl of solution (3). The test is not valid unless the resolution factor between the peaks due to impurity E and Levofloxacin is greater than 2.

Inject separately 20 μl each of solutions (1) and (2) and of the dissolution solvent in the chromatographic system. Examine the dissolution solvent chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (1).

In the chromatogram obtained with solution (1), the following impurity peaks, if present, are eluted at the following relative retention with reference to Levofloxacin (retention time about 17 minutes): impurity B about 0.36; impurity C about 0.57; impurity D about 0.75; impurity E about 0.91; impurity F about 1.50.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 2.6, is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 4.2, is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any peak corresponding to impurity C, E or F is not greater than the area of the principal peak obtained with solution (2) (0.2%);

- the area of any other impurity peak is not greater than 0.5 times the area of the principal peak obtained with solution (2) (0.1%);

- the sum of the areas (corrected, where necessary) of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak obtained with solution (2) (0.5%). Disregard any peak with an area less than 0.25 times the area of the principal peak obtained with solution (2) (0.05%).

[Note from Secretariat: as for the API monograph, following information to be confirmed:

- correction factors for impurities B and D,
- limit for individual unspecified impurities
- limit for total of impurities.]

Assay

Either method A or method B may be applied.

A. Prepare fresh solutions and perform the tests without delay. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm), packed with particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Symmetry 150 x 46 mm (5 μm) is suitable).

Maintain the column temperature at 45 °C.

Prepare the mobile phase as follows: dissolve 4.0 g of ammonium acetateR and 7.0 g of sodium perchlorate R in water R and dilute to 1300 ml; adjust to pH 2.2 with phosphoric acid R and add 240 ml of acetonitrile R.

Prepare the following solutions in the dissolution solvent prepared by mixing 10 volumes of acetonitrile R and 60 volumes of water R.

For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 20 mg of Levofloxacin, accurately weighed, into about 20 ml of the dissolution solvent, sonicate for 5 minutes, allow to cool to room temperature and dilute to 50.0 ml with 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 ml of this solution to 25 ml with the dissolution solvent.

For solution (2) dissolve 2.0 mg of ofloxacin RS in the dissolution solvent and dilute to 25.0 ml with the same solvent.

For solution (3) dissolve 10 mg of levofloxacin impurity E RS in the dissolution solvent and dilute to 100.0 ml with the same solvent. Mix 10 ml with 5 ml of solution (1) and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 294 nm.

Inject 20 μl of solution (3). The test is not valid unless the resolution factor between the peaks due to impurity E and Levofloxacin is at least 2.

Inject separately 20 μl each of solutions (1) and (2) and of the dissolution solvent in the chromatographic system.

In the chromatogram obtained with solution (1), the following peaks are eluted at the following relative retention with reference to Levofloxacin (retention time about 17 minutes): impurity B about 0.36; impurity C about 0.57; impurity D about 0.75; impurity E about 0.91; impurity F about 1.50.

Measure the areas of the peak responses in the chromatograms obtained with solutions (1) and (2). Calculate the content of Levofloxacin ($C_{18}H_{20}FN_3O_4$).

B. Weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 25 mg of Levofloxacin, accurately weighed, to a 50-ml volumetric flask. Add about 20 ml of hydrochloric acid (~4 g/l) TS, sonicate for about 5 minutes, allow to cool to room temperature and make up to 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 1.0 ml of this solution to 100.0 ml using water R. Measure the absorbance (1.6) of a 1-cm layer of this solution at the maximum at about 294 nm. Calculate the content of Levofloxacin in the tablets using an absorptivity value of 91.2 ($A^{1\%}_{1\ cm}$ = 912).

Impurities. The impurities limited by the requirements of this monograph include impurities B to F listed in the monograph for Levofloxacin.

* * *

New reagent to be added to The International Pharmacopoeia:

Hydrochloric acid (~4 g/l) TS.

Dilute 10 ml of hydrochloric acid (~420 g/l) TS with sufficient water to produce 1000 ml (approximately 0.1 mol/l).

Levonorgestrel tablets

Draft proposal for *The International Pharmacopoeia* (September 2010). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax +41227914730 or e-mail to mendyc@who.int. A subscriber mailing list is now available to speed up consultation. For more information please contact bonnyw@who.int.

Category. Contraceptive.

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

Additional information. Strength in the current WHO Model List of Essential Medicines: 30 μg, 750 μg, 1.5 mg.

Requirements

Comply with the monograph for “Tablets”.

Definition. Levonorgestrel tablets contain Levonorgestrel. They contain not less than 90.0% and not more than 110.0% of the amount of levonorgestrel ($C_{21}H_{28}O_2$) stated on the label.

Identity tests

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

A. To a quantity of the powdered tablets containing 37.5 mg of Levonorgestrel, add 5 quantities of dichloromethane R, each of 40 ml. After each addition, stir thoroughly and filter through a sintered-glass filter (G4). Wash the residue and the filter with dichloromethane R, combine the filtrates, evaporate to dryness on a water-bath with the aid of a stream of air and allow to cool. Dissolve the residue in 5 ml of dichloromethane R and measure the optical rotation. The optical rotation of the resulting solution is not less than -0.18°.

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

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 7 volumes of cyclohexane R and 3 volumes of acetone R as the mobile phase. Apply separately to the plate 10 l of each of the following two solutions in acetonitrile R. For solution (A) shake a quantity of the powdered tablets containing 1.5 mg of Levonorgestrel with 5 ml, filter, and use the clear filtrate. For solution (B) use 0.30 mg of levonorgestrel 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 at 254 nm. The principal spot obtained with solution A corresponds in position, appearance and intensity to that obtained with solution B.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described under test B.1 but using silica gel R5 as the coating substance. Spray with a mixture of equal volumes of sulfuric acid TS and ethanol (~750 g/l) TS. Heat the plate for a few minutes at 105 °C. Examine the chromatogram in daylight.

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

C. See the method described below under the test for Dextronorgestrel. The retention time of the principal peak in the chromatogram obtained with solution (2) is similar to that in the chromatogram obtained with the solution (3).

Dissolution test
Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 500 ml of 0.1% solution of sodium dodecyl sulfate R

in hydrochloride solution (0.1 mol/l) VS, and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of 10 ml of the medium through an in-line filter and use the filtrate. Prepare standard solution as follows: add a suitable volume of ethanol (~750 g/l) TS to dissolve a suitable amount of levonorgestrel RS, then add a suitable volume of the dissolution medium to obtain a concentration of 6 μg per ml.

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the chromatographic conditions as described under Assay.

For each of the six tablets, calculate the total amount of levonorgestrel ($C_{21}H_{28}O_2$), in the medium. The amount of levonorgestrel in solution for each tablet is not less than 80% of the amount declared on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and no tablet contains less than 60%.

[Note from Secretariat: possible alternative dissolution method for the 30 μg tablets, which will not use sodium dodecyl sulfate is under investigation.]

Dextronorgestrel
Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Hypersil ODS is suitable.) As the mobile phase, use a solution prepared as follows: dissolve 5.0 g of gamma-cyclodextrin R in 500 ml of water R and dilute to 1000.0 ml with methanol R.

Prepare the following solutions in a dissolution solvent prepared by mixing 80 volumes of methanol R and 20 volumes of water R. For solution (1) transfer a quantity of powdered tablets containing about 3.0 mg of Levonorgestrel to a 25-ml volumetric flask. Add about 15 ml of the dissolution solvent Cheat in a water-bath at 60 °C for 10 minutes, shaking occasionally. Allow to equilibrate to room temperature, dilute to volume with the dissolution solvent and mix. Filter through a 0.45-μm filter. For solution (2), dilute a suitable volume of solution (1) to obtain a concentration of 6 μg of Levonorgestrel per ml. For solution (3) use 6 μg of levonorgestrel RS per ml. For solution (4), use 12 μg of norgestrel RS per ml. For solution (5), use 0.12 μg of Levonorgestrel RS per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 242 nm.

Inject 20 μl of solution (3). In the chromatogram obtained with solution (3), the test is not valid unless the resolution factor between the peaks due to levonorgestrel and dextronorgestrel is at least 1.5.

Inject separately 20 μl, each of solutions (1), (2), (3), (4) and (5).

In the chromatogram obtained with solution (1) the area of the peak due to dextronorgestrel , is not greater than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

Related substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Spherisorb ODS 2 is suitable). As the mobile phase, use a solution prepared as follows: mix 15 volumes of methanol R, 35 volumes of acetonitrile R and 50 volumes of water R.

Prepare the following solutions in a dissolution solvent prepared by mixing equal volumes of methanol R and water R. For solution (1), transfer a quantity of powdered tablets containing about 0.18 mg of Levonorgestrel, accurately weighed, in 5 ml. Sonicate for 30 minutes, stir vigorously for 15 minutes, centrifuge and use the supernatant liquid. For solution (2), dilute a suitable volume of solution (1) to obtain a concentration of 0.36 g of Levonorgestrel per ml. For solution (3) use 4 μg of ethinylestradiol RS and 4 μg of levonorgestrel RS per ml.

Operate with a flow rate of 1.2 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 30 °C.

Inject 100 μl of solution (3). Record the chromatogram for twice the retention time of levonorgestrel (retention time about 26 minutes). The test is not valid unless, in the chromatogram obtained with solution (3), the resolution factor between the peaks due to ethinylestradiol and levonorgestrel is at least 12.

Inject separately 100 μl of each of solutions (1) and (2). Record the chromatogram for twice the retention time of levonorgestrel.

In the chromatogram obtained with solution (1) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%).

Assay

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Uniformity of content

The tablets comply with the test for 5.1 Uniformity of content for single-dose preparations, using the following method of analysis.

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Spherisorb ODS 2 is suitable.)

As the mobile phase, use a solution prepared by mixing equal volumes of acetonitrile R and water R.

Prepare the following solutions. For solution (1), transfer one powdered tablet to a stoppered test-tubes, add 5.0 ml of the mobile phase, sonicate for 45 minutes, shake for 15 minutes, and centrifuge. Dilute a suitable volume to produce a solution containing 6 μg of Levonorgestrel per ml. For solution (2), accurately weigh 12 mg of levonorgestrel RS, dissolve in sufficient mobile phase to produce 100.0 ml, and mix. Dilute 5.0 ml of this solution to 100.0 ml with the same solvent.

Operate with a flow rate of 1.3 ml per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Inject 25 μl, each solution (1) and (2). The retention time of levonorgestrel is about 7.9 minutes. The test is not valid unless the column efficiency, determined for the peak due to levonorgestrel using solution (2) is at least 5000. The symmetry factor of the peak due to levonorgestrel is not more than 1.6.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of levonorgestrel ($C_{21}H_{28}O_2$) in each tablet.