# WHO Drug Information

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## International Harmonization

Integration and application of equivalent regulatory assessments from other countries

## Recent Publications, Information and Events

- Marketing authorization of pharmaceutical products
- Guidelines for medicine donations
- Quality control methods for herbal materials
- European Union and Andean Community trade agreements
- Pharmaceutical reform: improving performance and equity

## Consultation Documents

**The International Pharmacopoeia**

- Bulk density and tapped density of powders
- Tablet friability
- Test for bacterial endotoxins
- Test for sterility
- Chewable albendazole tablets
- Artenimol
- Medroxyprogesterone injection
- Ritonavir tablets

## International Nonproprietary Names

Proposed List No. 106
WHO Drug Information
&
digital library
are available online at:
http://www.who.int/druginformation
Seven years of EU pharmaceutical regulation in Malta*

In an era of globalization, governments are expected to provide safety and welfare for citizens while ensuring a level playing field and boosting competitiveness for businesses. Public authorities regulate in the public interest to protect health, ensure patient access to safe medicines, stimulate innovation, encourage a competitive market, and preserve the environment (1).

Malta is an island in the south of Europe with a population of 417,608 (2010). The country became a Member State of the European Union (EU) on 1 May 2004, when a total of 10 European countries joined the EU.

During the process of Malta’s preparation for accession to the EU, the country transposed European pharmaceutical legislation into national legislation, leading to publication of the Medicines Act and subsidiary legislation. This established the Licensing Authority — a function which is vested in the Superintendent of Public Health — and the Medicines Authority (2).

The mission of the Medicines Authority is the protection of public health in Malta through the regulation of medicinal products and pharmaceutical activities. The Authority is committed to being an effective and supportive regulator with loyalty towards its core values: protection of public health as the purpose of regulation; public trust through an impartial, consistent, disciplined and transparent approach; excellence through competent personnel with integrity, which is based on customer focused, robust, resilient and secure processes and infrastructure.

Pharmaceutical regulatory framework

The main objectives of medicines regulation can be defined as:

- Ensuring that only medicines of good quality and favourable benefit/risk profile are authorized and available.
- Optimizing timely, equitable and affordable access to essential medicines.
- Enhancing the safe and rational use of medicines through independent information which helps prescribers and patients make informed decisions on the choice and use of medicines.
- Supporting innovation and competitiveness through effective, efficient, proportionate and consistent regulation and provision of excellent scientific and regulatory advice.

Pharmaceutical regulation supports the various components of a national pharmaceutical policy, mainly research and development, authorization, production, distribution and use of medicinal products. The tangible public health outcomes of these processes result in

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*Article by Patricia Vella Bonanno, CEO, Medicines Authority, and Gavril Flores, Operations and Regulatory Affairs Manager, Medicines Authority, Malta
### Figure 1. Model of the pharmaceutical framework

<table>
<thead>
<tr>
<th>Resources</th>
<th>Activities</th>
<th>Outputs</th>
<th>Stakeholders</th>
<th>Outcomes of the process</th>
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<tr>
<td>Structural &amp; Human</td>
<td>Legislation</td>
<td></td>
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<tr>
<td>Marketing authorization</td>
<td>R&amp;D, registration, safety</td>
<td>Patients</td>
<td></td>
<td>√</td>
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<tr>
<td>holders</td>
<td></td>
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<td></td>
<td>√</td>
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<tr>
<td>Regulatory authorities</td>
<td>Registration, safety, enforcement</td>
<td>Public health</td>
<td>Consumers</td>
<td>√</td>
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<tr>
<td>Pricing authorities</td>
<td>Setting of price &amp; profit</td>
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<tr>
<td>Reimbursement authorities</td>
<td>Establishment of reimbursement list</td>
<td>Free movement of goods</td>
<td>Healthcare professionals</td>
<td>√</td>
</tr>
<tr>
<td>Manufacturers &amp; importers</td>
<td>Manufacturing/distribution</td>
<td>Open competition</td>
<td>Pharmacies</td>
<td>√</td>
</tr>
<tr>
<td>Wholesale dealers</td>
<td>Distribution</td>
<td></td>
<td>Wholesale dealers</td>
<td>√</td>
</tr>
<tr>
<td>Pharmacies</td>
<td>Distribution</td>
<td>A true single market</td>
<td>Manufacturers &amp; importers</td>
<td>√</td>
</tr>
<tr>
<td>Healthcare professionals</td>
<td>Prescribing/ dispensing/</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>administration</td>
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<tr>
<td>Patient/consumer</td>
<td>Administration</td>
<td></td>
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<td>√</td>
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access, availability and affordability of medicinal products which are of good quality, and safe and efficacious when used rationally (Figure 1).

Medicinal products are not always available on the market. Affordable prices are an important prerequisite to ensuring access to essential medicines in the public and private sectors. Affordability is influenced through promotion of competition, through policies that encourage the use of generic medicinal products, alternative and therapeutic substitution, good procurement practices and regulation/control and information on prices and empowerment of healthcare professionals and consumers (4).

The rational use of medicines ensures that patients receive medicines appropriate to their clinical needs in doses that meet their individual requirements for an adequate period of time at an affordable cost. Continuous education of healthcare providers, patients and consumers, and independent and unbiased medicines information empower and support decision making to enhance health outcomes. Regulatory and managerial strategies should help to optimize safe and rational use of medicines and to minimize any
interventions which negatively impact on rational medicines use (4).

**Medicines regulation within EU**
The principles of medicines regulation in the EU support public health, the free movement of goods and people, compliance with legal requirements and the concepts of harmfulness and therapeutic efficacy. The essential aim of any rules governing these areas is to additionally safeguard public health through a framework which does not stifle the development of the pharmaceutical industry, innovation, investment or trade in medicinal products within the community (5).

EU legislation is implemented on a national level through Directives which are transposed into national legislation, while Regulations are implemented in full.

Within the EU regulatory framework, a medicinal product is authorized following evaluation to ensure that it has a positive benefit to risk balance: that is, the product should be of good quality, efficacious and safe. In these evaluations, the products are assessed to ensure that the recommended indications and any user safety restrictions are evidence based, that generic products are bio-equivalent to the reference product, that labels, patient information leaflets and packaging are appropriate and patient-friendly and that information for health care professionals (the summary of product characteristics) reflects authorized information. During this process, products are also assessed for aspects related to quality, expiry date and recommendations for storage conditions, etc. Medicinal products are classified either as prescription-only medicines (POM) or over-the-counter (OTC).

**Figure 2. Framework for medicines regulation within EU**\(^{(6)}\)
A medicinal product can only be placed on the market if the marketing authorization holder submits an application for the product to be registered in that Member State. Medicines in certain categories are authorized through a single marketing authorization that is valid in all European Union and European Economic Area countries. Unless a medicinal product is authorized, it will not become accessible to patients.

Due to the lack of economic viability of small markets, marketing authorization holders may choose not to register medicinal products in small markets. Article 126a of Directive 2001/83/EC as amended by Directive 2004/27/EC was introduced specifically to address this issue (5). Article 5 of the same Directive allows for supply of specific medicinal products on a named-patient basis, with the prescriber of the product taking full clinical responsibility for its use. The holder of a marketing authorization for a medicinal product and the distributors of the said medicinal product actually placed on the market in a Member State shall, within the limits of their responsibilities, ensure appropriate and continued supplies of that medicinal product. The provisions, which were aimed to support the availability of medicines on different markets, are implemented to different extents in different Member States (7).

While ensuring quality, safety and efficacy of medicines in the EU, regulatory procedures can pose difficulties on the availability of medicines. In addition there are several other thresholds of an economic nature that limit availability and economic operators need to overcome them before making a medicine available on a national market (7). The EU Pharmaceutical Forum (2008) recommended potential ways to facilitate availability of medicines in small European markets. These included: administrative changes, recommendations for manufacturing, packaging and labelling and recommendations for transport and wholesale dealing (8).

Pharmaceutical regulation in Malta

Optimizing access to good quality, efficacious and safe medicines

Until 2002, the procedure by which medicinal products were allowed on the Maltese market involved the submission of a World Health Organization (WHO) Certificate of a Pharmaceutical Product (CPP). No evaluation of the product was conducted locally. Following EU accession in May 2004, only those products which satisfied the requirements of EU pharmaceutical legislation were allowed to remain on the market and new products were to be in line with EU legislation. A transitional period was granted to this effect.

By the end of the transition period (December 2006), following evaluation of products on the market in line with EU legislation, the number of products authorized was 2410 covering 776 active ingredients (excluding centrally authorized products). This reduction in the number of products which could be placed on the market caused a problem with local access to medicines.

As a concerned Member State, Malta started participating in different EU procedures through its Medicines Authority such as the mutual recognition procedure (MRP) and decentralized procedure (DCP). In 2008, Malta acted for the first time as a Reference Member State in this procedure. In cases where an authorization for a medicinal product does not yet exist in any of the EU Member States, identical dossiers are submitted in all Member States where a marketing authorization is sought by the applicant and the Reference Member State prepares the draft assessment documents which, once approved, lead to an authorization in all the concerned Member States. In 2009, Malta also became involved as a rapporteur for centralized products.
It is a prerogative of marketing authorization holders whether to register a medicinal product on the market in a specific Member State. In order to attract marketing authorization holders to register their products in the country, the Maltese authorities ensure that they impose no additional burden on registration of products in Malta, while product packaging and labelling can be in either Maltese or English. Malta also encourages marketing authorization holders to submit joint packs covering Malta and the United Kingdom and/or Ireland (i.e., packs can then be sold on the respective markets).

Figure 3 shows the number of procedures in which each Member State was included as Reference Member State and as a Concerned Member State over the period January 2010 to June 2011. As is the case for other small Member States, the inclusion of Malta as a Concerned Member State is low. In 2010, Malta was involved in 32% of the average procedures per Member State. In order to increase the participation of Malta in European procedures, an agreement was concluded with the United Kingdom and Irish national authorities whereby Marketing Authorization Holders who submit applications to them are invited to include Malta as a Concerned Member State in authorization procedures. This cooperation has had a positive impact and, in 2011, Malta was included as a Concerned Member State in 51% of the average procedures.

Following the update of Directive 2001/83/EC through Directive 2004/27/EC in October 2005, Malta utilized the provisions of article 126a in order to address the public health need. This has proved to be beneficial to cover gaps in therapeutic availability, particularly for products which may not be economically attractive.

As shown in Figure 4, the number of medicinal products authorized to be placed on the local market increased as compared to the situation in December 2006 and, by the end of June 2011, 3691 products were authorized, covering 1339 active ingredients. In addition, 599
products were authorized by the European Commission through the centralized procedure. These measures have had a significant impact in addressing the problem of access with an increase of 168% in the number of products available and 72% increase in the number of active ingredients.

Taking into consideration national and EU authorizations, most of the chemical/therapeutic/pharmacological subgroups as defined by the WHO Anatomical Therapeutic Chemical (ATC) Classification are covered by at least one product with an authorization. At ATC Classification Level 4 there is an average of 68% coverage. Sub-groups with lowest coverage are those in Level P (antiparasitic products, insecticides and repellents) which covers a number of products which are not generally required in developed countries and Level V (various) which is a miscellaneous group. At Level 3, there is less coverage of combination products, however, the individual components are better covered. Good coverage enables patient access to needed medicines and provides greater choice of treatment.

The use of article 5 of Directive 2001/83/EC (use of products on a named-patient basis as a clinical responsibility of the prescriber) for products which are authorized in other EU Member States is applied as a last resort. This article is applied for medicines which are not registered on the market and is particularly useful to cover gaps in registration of certain medicines restricted for hospital use only.

Participation in clinical trials as well as the possibility for prescribing medicines
on a compassionate basis\(^1\) allows faster access to novel treatments to the benefit of patients within a controlled system. Application of this provision in Malta is low.

**Ensuring medicines of good quality and benefit/risk profile**

Following authorization, the Medicines Authority continues to monitor the quality, safety and efficacy of medicinal products and clinical trials. During 2010, the Authority finalized over two thousand post authorization procedures, including variations, transfers, renewals and notifications. The Authority also monitors and processes adverse drug reactions of medicinal products. All adverse reactions sent by patients and healthcare professionals are transmitted to a centralized European database to enable effective signal detection and evaluation of the associated risks inherent in medicinal products.

The monitoring of authorized medicinal products and ongoing communication with the industry and similar agencies which form part of the network of regulation of medicines in Europe may lead to regulatory actions and restrictions, including suspension and withdrawal of marketing authorizations. Table 1 lists the medicinal products whose marketing authorization has been withdrawn or suspended due to safety reasons.

Products which are not of good quality are recalled from the market. Malta has averaged seven recalls per year due to quality defects. Usually, these recalls are batch specific and are either initiated by the regulator or by the marketing authorization holder.

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Active Ingredient</th>
<th>Date of withdrawal</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Raptiva®</td>
<td>Efalizumab</td>
<td>2009</td>
<td>Withdrawn. Increased risk of progressive multifocal leukoencephalopathy.</td>
</tr>
<tr>
<td>Avandia®</td>
<td>Rosiglitazone</td>
<td>2010</td>
<td>Suspended in Europe. Increased risk of heart attack and death.</td>
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\(^1\) A treatment option for patients suffering from a disease for which no satisfactory authorized alternative therapy exists or who cannot enter a clinical trial, may be use of an unauthorized medicinal product in a compassionate use programme. Compassionate use programmes are intended to facilitate the availability to patients of new treatment options under development. Regulation No. 726/2004/EC.
The Medicines Authority carries out a market surveillance programme of the products on the market and sends an average of twenty medicinal products to an official medicines control laboratory for analysis per year. This is done in line with an annual sampling plan, through ad hoc sampling and to investigate complaints received. These products are tested to ensure that products on the market are of good quality and in accordance with the marketing authorization.

Control of the medicines supply chain is an important aspect of ensuring the integrity of medicinal products on the market. Such efforts also ensure that falsified medicines do not enter the market and reach patients. All operators have to follow strict requirements including good clinical practice, good manufacturing practice, good distribution practice and pharmacy standards. A new directive on falsified medicines has been published to respond to an increasing threat from such medicines. New measures will include an obligatory authenticity feature on the outer packaging of certain medicinal products; strengthened requirements for control and inspections of plants manufacturing active pharmaceutical ingredients; strengthened record-keeping requirements for wholesale distributors; strengthened rules on inspections and an obligation for manufacturers and distributors to report any suspicion of falsified medicines.

**Safe and rational use of medicines**

The Medicines Authority is committed to enhancing the safe and rational use of medicines through information which helps prescribers and patients make informed decisions on the use of medicines.

The main source of public information on medicinal products is the package leaflet and the summary of product characteristics. The Medicines Authority ensures that all package leaflets and summaries of product characteristics for products authorized on the market are reviewed as part of the assessment process so that information contained is in line with the benefit-risk assessment carried out and that these documents are written in clear and understandable terms. The Authority publishes information on all medicinal products authorized to be placed on the market in Malta by means of the Malta Medicines List at http://www.maltamedicineslist.com. This list supports empowerment of patients and their participation in decisions with regard to different treatment options. The list provides a search function for alternative products by ATC code and active ingredient, listing options available for each category.

During 2010, the Authority consolidated its efforts in this area and launched a set of new initiatives entitled *Know Your Medicines*. A survey with 200 consumers and patients was conducted between September 2010 and January 2011 to identify participant knowledge on medicines, awareness on the medicines lifecycle, trust in medicines and awareness on choice of medicines.

The Authority also has a medicines helpline through which consumers and healthcare professionals can ask for information and send feedback, comments and complaints. This helpline aims to supplement, not replace, the advice given by healthcare professionals. The Authority updates healthcare professionals through objective information on the safety of medicinal products through regularly issued circulars to healthcare professionals.

Pharmaceutical legislation also regulates advertising of medicinal products and the promotion of medicinal products by the pharmaceutical industry. Clear guidelines complementing legislation have been issued and a system of self-regulation established. Initially there was concern regarding the regulation of activities of medical representatives with prescribers such as restriction on the use of samples
and participation at sponsored activities but a system has now been agreed.

**Supporting innovation and competitiveness**

The Medicines Authority is committed to support innovation and competitiveness through effective, efficient, proportionate and consistent regulation and provision of scientific and regulatory advice.

Pharmaceutical activities in Malta have adapted and developed themselves in line with the standards as stipulated by EU pharmaceutical legislation. The Medicines Authority also took a number of initiatives to support innovation and competitiveness such as expansion of activities in line with industry and public health requirements and a mutual recognition agreement for inspectorate activities. Such mutual recognition provides easier access to new markets for local manufacturers.

As shown in figure 5, the number of pharmaceutical activities licensed in line with good manufacturing practice (GMP) standards has increased from five in 2004 to a total of 29 in 2011. Manufacturing is mainly of generic oral solid dosage forms. There are two manufacturers which manufacture medical gases and two manufacturers of active pharmaceutical ingredients (APIs) which are GMP licensed. There is one independent laboratory which is GMP authorized providing services for the industry, although a number of other GMP authorized manufacturing sites have testing facilities which may also contract out. Growth has been mainly in the number of repackers and importers – with a number of sites being authorized for both activities. Repackaging/relabelling concerns mainly ‘partial manufacturing’ activities covering labelling of products in accordance with national marketing authorizations and of parallel imports. Importers are mainly

![Figure 5. Number of licensed pharmaceutical activities](image-url)

*As at June 2011*
active in the area of importation of medicinal products from third-party countries into the EU and their release into the European market, an activity which is on the increase.

As shown in Table 2, wholesale dealing and activity at pharmacy level, there have been limited changes in the number of operators. The model for the supply chain in Malta has not changed significantly: marketing authorization holders are represented by local agents. The concept of full-line wholesale dealing has not developed at all. The number of community pharmacies has increased slightly following publication of new legislation in 2007, which brought about a change in demographic criteria for the opening of new pharmacies. In community pharmacies there was a gradual roll out of the Pharmacist of Your Choice Scheme whereby private community pharmacies started distributing medicines which were previously distributed by public pharmacies and this brought about the need for increased regulation over pharmacies to ensure that they continued to maintain their standards in spite of increased stock holding.

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<tr>
<th></th>
<th>2004</th>
<th>2005</th>
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<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011*</th>
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<tr>
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<td>66</td>
<td>69</td>
<td>74</td>
<td>72</td>
<td>71</td>
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<tr>
<td>Community pharmacies</td>
<td>208</td>
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<td>207</td>
<td>208</td>
<td>209</td>
<td>211</td>
<td>213</td>
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</table>

* As at June 2011

Since publication of the Clinical Trials Directive (9) and its transposition into Maltese legislation, clinical trials have been regulated in Malta. At EU level, the regulation of clinical trials came into force in 2004 — at the same time as Malta’s accession into the EU. Up to June 2011, the Medicines Authority evaluated the scientific and regulatory parts of twenty-one applications, mainly phase III trials. Information on clinical trials authorized in Malta is published at http://www.clinicaltrialsregister.eu. It is expected that this area will grow once the new life sciences park, aimed at consolidating and boosting Malta’s research and innovation capacity, is completed in 2013.

Malta actively participates in discussions at EU level in the area of pharmaceutical policy and legislation. Also, the country actively took part in discussions on information on medicinal products, pharmacovigilance, falsified medicines and the impact of pharmaceutical EU legislations on small Member States in areas such as availability of medicinal products. Active participation at this level impacts both public health and also competitiveness of locally operating industry.

A strong regulatory framework safeguards public health and also provides a level playing field for economic operators. The Medicines Authority is also committed to the principles of better regulation to ensure simplification, improved policy making and a reduction in administrative burden.
Conclusion

Regulation of medicinal products has increased trust in products available on the local market. A robust system is in place to ensure that products are of good quality, safe and efficacious, their integrity is maintained up to patient level and the risk of falsified medicines entering into the legal supply chain is reduced.

The Medicines Authority is committed to informing and empowering healthcare professionals and patients in the choice of treatments and use of medicines. The Authority will continue to support innovation and competitiveness through effective, efficient, proportionate and consistent regulation.

The impact of EU accession on availability has been significant, particularly given the size of the local market. Malta has adopted legislative and regulatory measures available within the current legislative framework to support availability, access and affordability of products on the local market. Local stakeholders acknowledge that compliance opens opportunities at European and international levels. Challenges with compliance arose mainly due to lack of knowledge and understanding and the Medicines Authority has endeavoured to inform and support stakeholders both at group as well as individual operator level.

The Medicines Authority will continue strengthening and expanding its role within the EU pharmaceutical regulatory framework. A consistent approach towards pharmaceutical regulation has resulted in enhanced and protected patient health and consumer trust, effective and fair competition and opportunities for improved health and industry development.

References


International Harmonization

Integration and application of equivalent regulatory assessments from other countries*

Regulators attending the Fourteenth International Conference of Drug Regulatory Authorities held in Singapore, 30 November – 3 December 2010, proposed a series of recommendations to harness implementation of the processes discussed during the conference proceedings, including conclusions made following the two-day pre-ICDRA meeting Effective collaboration: the future for medicines regulation. Within this framework, a session was organized on how regulators may constructively utilize resources made available by other countries. The resulting recommendation states:

WHO should encourage regulators operating schemes that openly and transparently utilize data from other countries (e.g., Canada, New Zealand, Singapore and Switzerland) to, inter alia, document their processes and experiences to provide a resource for use by other countries.

Swissmedic, the Swiss Agency for Therapeutic Products, has responded to this recommendation in the following article.

Utilization of data originating from other countries in national marketing authorizations

Requirements for the demonstration of quality, safety and efficacy of medicinal products have been harmonized to a great extent between ICH1 and associated countries and these regulatory authorities apply equivalent standards to ensure quality, safety and efficacy of a medicinal product worldwide. Therefore, the question may be asked whether it would not make sense to consider elements of the assessment made by another authority in one’s own decision-making processes — particularly in times when resources are scarce.

Different categories of assessing an action that has been made by another regulatory agency range from simply taking note; taking into consideration, and partial or complete recognition of the decision.

During the pre-ICDRA meeting, Canada, New Zealand, Singapore and Switzerland made presentations of their specific approaches during a session dedicated to the question on how to deal with assessments made in other countries during the decision-making process.

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*Article by Cordula Landgraf, Head of Networking, Swissmedic, Swiss Agency for Therapeutic, Products, Berne, Switzerland

1 The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a forum bringing together the regulatory authorities and pharmaceutical industry of the European Union, Japan and USA to discuss scientific and technical aspects of medicines registration requirements. ICH technical guidelines are voluntarily implemented by the ICH parties and countries associated with ICH by other voluntary commitments such as Australia, Canada, Singapore and Switzerland.
Situation in Switzerland

In Switzerland, the Federal Act on Therapeutic Products (ATP) which entered into force in 2002 (1) specifies in Article 13 that if a medicinal product or procedure is already authorized in a country having equivalent medicinal product control, the results of tests carried out for this purpose shall be taken into account.

At the time of the pandemic H1N1 influenza situation in 2009, political discussions in Switzerland centered on Article 13 and the question of how to consider regulatory assessments made in other countries to ensure access as early as possible to the respective pandemic vaccines. Swissmedic needed to respond urgently and put forward a strategy on how to implement the provisions of Article 13 of the ATP in an appropriate way.

The strategy is based upon four pillars and laid down in a new Article of the Ordinance on Medicinal Products (2).

Competence for cooperation

To retain its competency and scientific expertise and to be an attractive international partner authority, marketing authorization applications for new active substances and/or new indications (innovative medicinal products) will still be reviewed by Swissmedic. Upon request, the review can be reduced appropriately but only in duly justified cases.

High performance

Marketing authorization applications for known active substances are fully eligible for the provisions of Article 13 of the ATP. In general, the review is reduced to the final assessment report of the reference authority.

Risk-based approach

The agency follows a risk-based approach making use of resources where most needed.

Ex officio reduced evaluation

In justified cases, the Agency may also carry out an ex officio reduced evaluation of its own, namely when it is in the public health interest to expedite the procedure and ensure early market access, e.g., for authorization of a vaccine in a pandemic situation.

Countries (3) regarded as having an equivalent medicinal product control system are listed on the Swissmedic web site and include Australia, Canada, European Union, European Economic Area, Japan, New Zealand, Singapore and USA. The main criteria to qualify for the list are (i) arrangements for the sharing of (confidential) information between Swissmedic and the respective authority of the foreign country and (ii) application of the ICH guidelines.

The provisions of Article 13 of the ATP are laid down in an implementing guideline (4) and apply to first authorizations, including major variations, for human and veterinary medicinal products. Variations to the product information, recognition of batch release and variations requiring notification only are not within the scope of the guideline.

Application of Article 13 is made upon request by the applicant who must specify in the application form that Article 13 should be applied. When validating the submission, Swissmedic checks whether all requirements for application of Article 13 are fulfilled.

To ensure state-of-the-art assessment, the marketing authorization granted by the reference authority or the last updated version of the entire documentation that was authorized must not be older than five years. The complete basis for decision making of the reference authority must be submitted. Documents can be submitted in one of the official Swiss languages (German, French and Italian) or in English. The applicant is required to state in the application letter that the documentation submitted is identical to that upon which the reference authority based its approval. Minor deviations from the documentation submitted in the
country of authorization are possible if sufficiently justified, e.g., different name, packaging size, primary or secondary packaging. Specific requirements for Switzerland have to be met, e.g., labeling and language requirements. They are submitted in a Swiss module 1 of the common technical document (CTD).

If a medicinal product has been approved by more than one country with comparable medicinal product control, the applicant should submit copies of all official decisions from the respective authorities that granted the marketing authorization. However, the applicant can choose a reference authority and submit complete documentation and results of the assessment of this reference authority only. In the event that both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have granted approval, the applicant is required to submit complete documentation and assessment results of both agencies.

In principle, Swissmedic restricts evaluation of a product with a known active substance to the quality, safety and efficacy assessment reports of the reference authority. If concerns arise, Swissmedic will carry out its own assessment of the documentation focusing on the items of concern.

In the event that the product in question is approved by both FDA and EMA, Swissmedic refrains from evaluating the assessment reports. An evaluation of the assessment reports only takes place should there be material differences in the decision making, i.e., approval by one authority and rejection by the other, or if there has been a previous rejection or withdrawal by Swissmedic.

To retain its competency and scientific expertise, Swissmedic conducts a comprehensive scientific assessment of applications for marketing authorizations for medicinal products containing new active substances as well as line extensions. The assessment can be reduced in the following justified cases:

1. Medicinal product with orphan drug status granted by EMA or FDA.
2. Medicinal products fulfilling all of the following requirements:
   - Treatment of a life-threatening or debilitating disease.
   - Treatment possibilities with authorized medicinal products do not exist or are unsatisfactory.
   - Considerable therapeutic advantage from using the product is expected.

*Data up to October 2011*
• The product is already approved by EMA and/or FDA and there are no major divergences regarding their decision making.

In the event that all requirements for the application of Article 13 are met and Swissmedic’s decision can be entirely based upon the reference authority’s evaluation, the target processing time for the evaluation will be reduced by one-third with the exception of the validation phase, i.e., the evaluation time for first authorizations will only be 200 instead of 300 calendar days. Similarly, the fees payable will be reduced by one-third. (Figure 1).

Figure 2 identifies the change in strategy made on 1 July 2010 when the focus for use of Article 13 was placed on known active substances, including generics.

**Conclusion**
The legal basis for Swissmedic to consider other regulatory assessments is clear. Political will and Swissmedic strategy have been aligned.

Consideration of another country’s assessments has much to do with trust and confidence in abilities. This is built over time and strengthened through working in close collaboration with colleagues. Implementation of guidelines on how to consider other regulatory assessments should therefore recognize the importance of mutual cooperation and creating mechanisms within which assessors can work together, get to know each other and fully appreciate each others’ methods of working.

**References**


3. List of all countries with comparable control of medicinal products at http://www.swissmedic.ch/org/00063/00628/00654/index.html?lang=enZA000_00_001e_VZ

Safety and Efficacy Issues

Asenapine maleate: serious allergic reactions

United States of America — The Food and Drug Administration (FDA) has warned the public that serious allergic reactions have been reported with the use of the antipsychotic medication asenapine maleate (Saphris®). Labelling has been revised to include information about this risk and that it should not be used in patients with a known hypersensitivity to the drug.

A search of the FDA’s Adverse Event Reporting System (AERS) database identified 52 cases of Type I hypersensitivity reactions. Signs and symptoms of Type I hypersensitivity reactions may include anaphylaxis, angioedema, low blood pressure, rapid heart rate, swollen tongue, difficulty breathing, wheezing, or rash.


Lenalidomide: positive benefit-risk balance

European Union — The European Medicines Agency (EMA) has confirmed that the benefit-risk balance for lenalidomide (Revlimid®) remains positive within its approved patient population but advises doctors of the risk of new cancers as a result of treatment with the medicine.

Lenalidomide was reviewed following the results of three new studies showing a higher rate of new cancers in patients with newly diagnosed multiple myeloma who were being treated with lenalidomide and received other treatments concomitantly. The studies showed a four-fold increase in the number of new cancers in patients being treated with lenalidomide, including solid tumours and cancers of the blood and the immune system. Although the studies were carried out in patients for whom lenalidomide is not currently indicated, the Agency’s Committee for Medicinal Products for Human Use (CHMP) was concerned that the results could also be relevant for the approved patient population.


Citalopram: dose-related cardiac risk

Canada — Health Canada is reviewing the heart-related safety of the prescription antidepressant citalopram. The review is in light of new study data suggesting that high doses (60 mg/day) can affect the electrical activity of the heart which could potentially lead to serious, possibly fatal abnormal heart rhythms.

Citalopram, an SSRI, is used to treat depression. The current Canadian prescribing information recommends 20 mg/day of citalopram in adults. Some people who have not responded to this dose are prescribed 40 or even 60 mg/day.

Terpenic derivatives: new contraindications

**European Union** — The European Medicines Agency (EMA) has recommended updating the product information for suppositories containing terpenic derivatives with new contraindications following the finalization of a review of their use in children under 30 months by the Agency’s Committee for Medicinal Products for Human Use (CHMP).

The CHMP concluded that there was a risk of these medicines inducing neurological disorders, especially convulsions, in infants and small children and recommended that their use should be contraindicated in children under 30 months and in children with a history of epilepsy or febrile convulsion. It also concluded that there was a risk of these medicines causing local anorectal lesions and recommended their use should be contraindicated in children with a recent history of anorectal lesion.

Suppositories containing terpenic derivatives (including camphor, cineole, niaouli, wild thyme, terpineol, terpine, citral, menthol and essential oils of pine needle, eucalyptus and turpentine) are typically indicated for supportive treatment for mild acute bronchial disorders, particularly productive and non-productive cough.

The review procedure was initiated after the French Safety of Medicines Agency (Afssaps) expressed concerns about the safety of suppositories containing terpenic derivatives, particularly the risk of serious neurological side-effects such as convulsions in young children.

The Committee reviewed all available data including the benefit-risk assessment carried out by France and information requested from the companies that market suppositories containing terpenic derivatives in the European Union. This included study data supporting marketing authorizations and safety data including reports of side effects from post-marketing surveillance and the published literature.


Gonadotropin-releasing hormone: cardiovascular risk

**Canada** — Health Canada is informing health professionals and patients about a possible increased risk of certain heart-related events in men being treated for prostate cancer with a gonadotropin-releasing hormone (GnRH) agonist. Based on information collected from scientific literature, the risk appears to be low.

When determining an appropriate prostate cancer treatment, physicians should weigh the benefits of androgen deprivation therapy against the potential cardiovascular risk of GnRH agonists, along with any additional factors that may put a patient at increased risk for heart-related events. Patients receiving a GnRH agonist should be monitored for signs and symptoms suggestive of development of cardiovascular disease, and managed according to current clinical practice. The labelling for GnRH agonist drugs has been updated to warn of the potential increased risk of heart-related side-effects.

**Reference:** *Information Update 2011-122, 8 September 2011* at http://www.hc-sc.gc.ca

Uromitexan: infant fatal gasping syndrome

**Canada** — The manufacturer of uromitexan (Mesna®) has informed healthcare professionals of cases of fatal gasping syndrome in neonates and infants associated with the use of drug products containing benzyl alcohol as the preservative.

Uromitexan, multi-dose vials, is indicated for the reduction and prevention of hemorrhagic cystitis in adults and children
13 years and older only. However, this product has been reported to be used off-label in younger patients.

The multi-dose vials of uromitexan contain the preservative benzyl alcohol, which can cause serious or fatal reactions. The new multi-dose vials should not be used in paediatric patients 12 years old and younger because of their benzyl alcohol content.


**Dasatinib: pulmonary arterial hypertension**

**Canada** — The manufacturer of dasatinib (Sprycel®) has informed healthcare professionals of important new safety information regarding reports of serious pulmonary arterial hypertension (PAH) in patients treated with dasatinib.

Dasatinib belongs to the class of protein-tyrosine kinase inhibitors. It is marketed for adult patients with newly diagnosed Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) in chronic phase. Dasatinib has also received marketing authorization for the treatment of adult patients with Ph+ chronic, accelerated, or blast phase CML and adult patients with Ph+ acute lymphoblastic leukemia (ALL) who are resistant or intolerant to previous therapies.

A total of 60 serious pulmonary hypertension (PH) cases have been reported worldwide between June 2006 and June 2011, including 12 cases of PAH) confirmed by right heart catheterization, in association with dasatinib treatment. No Canadian cases of PH or PAH have been reported during this time period.

Patients should be evaluated for signs and symptoms of underlying cardiopulmonary disease before initiating dasatinib therapy. Patients who develop symptoms suggestive of PAH such as dyspnea and fatigue after initiation of treatment with dasatinib should be evaluated for more common etiologies and treatment should be withheld during evaluation if symptoms are severe.

Improvements in haemodynamic and clinical parameters have been observed in patients with PAH following cessation of dasatinib therapy and this should be permanently discontinued if the diagnosis of PAH is confirmed.


**Drospirenone: increased risk of blood clots**

**United States of America** — The Food and Drug Administration (FDA) remains concerned about the potential increased risk of blood clots with the use of drospirenone-containing birth control pills.

The FDA has completed its review of the two 2011 studies that evaluated the risk of blood clots for women who use drospirenone-containing contraceptives, previously mentioned in FDA’s Drug Safety Communication issued on 31 May 2011. The FDA is continuing its review of a separate FDA-funded study that evaluated the risk of blood clots in users of several different hormonal contraceptives.

Preliminary results of the FDA-funded study suggest an approximately 1.5-fold increase in the risk of blood clots for women who use drospirenone-containing birth control pills compared to users of other hormonal contraceptives.

Given the conflicting nature of the findings from six published studies evaluating this risk (1–6), as well as preliminary data from the FDA-funded study, the FDA has scheduled a joint meeting of the Reproductive Health Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee to discuss the risks and benefits and specifically the risk
of blood clots of drospirenone-containing birth control pills (7).

References


Ondansetron: QT prolongation

United States of America — The Food and Drug Administration (FDA) is informing the public of an ongoing safety review of the anti-nausea drug ondansetron (Zofran®), ondansetron hydrochloride and their generics. Ondansetron may increase the risk of developing abnormal changes in the electrical activity of the heart, which can result in a potentially fatal abnormal heart rhythm.

Ondansetron and ondansetron hydrochloride are 5-HT3 receptor antagonists used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy and surgery (1).

The manufacturer of Zofran® is being required to conduct a thorough QT study to assess the potential to prolong the QT interval. Results from this study are expected in the summer of 2012. Label changes may result after the additional information has been reviewed. Ondansetron labelling already contains information about the potential for QT prolongation. Additionally, published articles describe QT interval prolongation with ondansetron or droperidol (2–4).

References


TNFα blockers: *Legionella* and *Listeria*

**United States of America** — The Food and Drug Administration (FDA) has informed healthcare professionals that the boxed warning for the entire class of tumour necrosis factor-alpha (TNFα) blockers has been updated to include the risk of infection from two bacterial pathogens, *Legionella* and *Listeria*. In addition, the labelling for all of TNFα blockers has been revised to contain information about the risk of serious infections and associated disease-causing pathogens.

TNFα blockers are used to treat Crohn disease, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and/or juvenile idiopathic arthritis. Since TNFα blockers are immunosuppressants, patients are at increased risk of serious infections. These can involve various organ systems and sites due to bacterial, mycobacterial, fungal, viral and other opportunistic pathogens.

**References**


on the safety of antipsychotic drugs in pregnancy. A search of the US Food and Drug Administration's (FDA) Adverse Event Reporting System database identified 69 suspected cases of neonatal EPS or withdrawal associated with antipsychotic drugs (1).

Symptoms reported include agitation, hypertonia, hypotonia, tremor, somnolence, respiratory distress and feeding disorders, which may occur anytime from birth to one month after birth. The severity of the symptoms may vary, with some neonates recovering within hours or days without specific treatment, while others required intensive care support and prolonged hospitalization.

It was not possible to determine if the events were a result of antipsychotic drug toxicity or withdrawal since blood levels of the implicated drugs were not provided. The majority of the cases were confounded by other factors, including concomitant use of other drugs known to be associated with withdrawal symptoms (such as antidepressants, benzodiazepines, non-benzodiazepine hypnotics and opioids), prematurity, congenital malformations, and obstetrical and perinatal complications (e.g., placental problems, pre-eclampsia). However, there were some suspected cases which suggest that neonatal EPS and withdrawal may occur with antipsychotic drugs alone.

Similarly, in June 2011, Health Canada has also informed healthcare professionals and consumers that the prescribing information for antipsychotic drugs is being updated to reflect safety information on the potential risk of EPS or withdrawal in newborns whose mothers were treated with antipsychotic drugs during the third trimester of pregnancy (2). To date, HSA has not received any local reports of neonatal EPS or withdrawal associated with the use of antipsychotic drugs during the third trimester of pregnancy.
References


3. Health Sciences Authority. Risk of neonatal extrapyramidal signs and/or withdrawal symptoms with antipsychotic drug use during third trimester of pregnancy. HSA Safety Information, 26 Sep 2011 at http://www.hsa.sg

Strontium ranelate: cardiovascular and cutaneous toxicity

European Union — The European Medicines Agency (EMA) has started a review of the osteoporosis medicines containing strontium-ranelate (Protelos® and Osseor®), to determine whether cases of venous thromboembolism and drug rash with eosinophilia and systemic symptoms have an impact on the benefit-risk profile and conditions of use.

Protelos® and Osseor®, from Les Laboratoires Servier, were authorized on 21 September 2004 and are indicated for treatment of postmenopausal osteoporosis to reduce the risk of vertebral and hip fractures.

Venous thromboembolism (VTE) and drug rash with eosinophilia and systemic symptoms (DRESS) are known risks of these medicines. The risk of VTE was identified in clinical trials and the risk of DRESS through spontaneous reporting soon after the granting of the initial marketing authorization, and warnings are included in the product information. The risks are addressed in the risk-management plan and have been kept under close review by the Agency’s Committee for Medicinal Products for Human Use (CHMP).

A study analysing the side effects associated with strontium ranelate spontaneously reported in France from January 2006 to March 2009 noted 199 severe adverse reactions, of which 52% were cardiovascular (most frequently VTE events) and 26% were cutaneous. The authors concluded that DRESS syndrome is unpredictable, but that the VTE risk could be reduced by adding a contraindication for patients with a history of VTE and by stopping treatment if a new VTE risk situation occurs. Based on a recent pharmacovigilance update and pending an EU-wide review, the French Safety of Medicines Agency (Afssaps) recommended restricting the use of strontium ranelate to those patients who are under 80 years of age, at high risk of fractures and who cannot take bisphosphonates.

The CHMP is now reviewing all relevant data on cardiovascular and cutaneous safety concerns.

References


Bevacizumab: ovarian failure

New Zealand — Medsafe has advised prescribers to discuss the possibility of ovarian failure with all female patients prior to treatment with bevacizumab (Avastin®), a recombinant monoclonal antibody that inhibits tumour angiogenesis and tumour growth.

This advice follows the publication of a recent study, NSABP-C08, which found ovarian failure occurs commonly in association with bevacizumab use. Although ovarian failure is a well recognized com-
Orlistat-containing medicines under review

European Union — The European Medicines Agency (EMA) has started a review of orlistat-containing anti-obesity medicines to determine whether very rare cases of hepatic injury have an impact on their benefit-risk profile and conditions of use.

The review includes the centrally authorized prescription-only medicine Xenical®, 120 mg and the centrally authorized over-the-counter-medicine Alli®, 60 mg as well as a number of medicines containing orlistat that have either already or are in the process of being authorized at national level.

Overall, between 1997 and January 2011 there were 21 cases of suspected serious liver toxicity for which a causal link to orlistat cannot be excluded, although these cases do not provide strong evidence of a link to orlistat as alternative explanations for liver injury are present in many of the cases. Furthermore, the number of cases needs to be considered in the context of cumulative usage of these medicines in 38 million patients.

The Committee for Medicinal Products for Human Use (CHMP) is now reviewing all relevant data on the risk of hepatic toxicity of orlistat-containing medicines and will issue an opinion on whether or not the marketing authorizations for these medicines should be revoked, suspended or changed.


Romiplostim: acute myelogenous leukaemia

United Kingdom — Available data from a randomized clinical study of patients with thrombocytopenia associated with myelodysplastic syndrome (MDS) has
shown an increase in the number of cases of disease progression to acute myelogenous leukaemia (AML) and transient increases in blast cell counts in patients treated with romiplostim (Nplate®) compared to placebo.

A positive benefit/risk for Nplate® is established only for the treatment of thrombocytopenia associated with chronic immune (idiopathic) thrombocytopenic purpura (ITP). Nplate® must not be used in other clinical conditions associated with thrombocytopenia.

The diagnosis of ITP in adults and elderly patients should be confirmed by the exclusion of other clinical entities presenting with thrombocytopenia. The diagnosis of MDS must be excluded.

A bone marrow aspirate and biopsy should normally be carried out before initiating Nplate® treatment and over the course of the disease and treatment, particularly in patients over 60 years of age, and those with systemic symptoms or abnormal signs, such as increased peripheral blast cells.


International Pharmaceutical Federation: pharmacovigilance seminar in India

The International Pharmaceutical Federation (FIP) held its 71st International Congress in Hyderabad in September 2011.

Prior to the congress, the FIP Pharmacy Information Section organized a one-day seminar entitled “Pharmacovigilance and Medicines Information to Enhance Patient Safety”. The Congress provides an opportunity for pharmacists from a diverse range of countries to discuss issues of global importance. The seminar had a focus on India and the role of pharmacists in improving patient safety.

The seminar aimed to link the clinical role of pharmacists managing adverse drug reactions with local and international pharmacovigilance responsibilities. It also covered factors which can lead to medication errors, and how to change systems to improve safety. This was an opportunity to emphasize the extended scope of pharmacovigilance which now includes:

- Adverse effects.
- Patient effects of inadequate use (including medication errors, dependence and abuse, and poisoning).
- Safety challenges of mass treatment campaigns such as immunization programmes and other public health programmes.

In order for pharmacists to understand the global dimensions of pharmacovigilance, the scope of the WHO Programme for International Drug Monitoring was explained as well as the manner in which WHO works with national centres and its collaborating centres in Uppsala and Accra. WHO’s responsibility is to develop relevant policies, norms, standards and guidelines for pharmacovigilance while the role of the Uppsala Centre is to collect and analyse reports from all global sources, communicate potential safety issues, and provide support and training for pharmacovigilance programmes. The Accra Centre provides ground and country level support and focuses on public health programmes. Although it has a strong presence in Africa, its activities extend to other countries where support is required.
In recent years WHO pharmacovigilance activities have become more patient centered. The traditional approach has focused on problems relating to medicines but the overall aim is to minimize harm to patients. Current systems can be complemented by a public health perspective which rapidly addresses key safety issues, provides rates of adverse effects, monitoring of adverse effects in special populations (e.g., children) and supporting risk management plans.

The various challenges confronting pharmacovigilance were outlined. The level of spontaneous reporting by health professionals remains low compared to its potential. Interpretation of the event is limited by the level of clinical details provided, and delays in processing and forwarding reports can impair the process of identifying signals.

Cohort event monitoring is being developed as an alternative to spontaneous reporting. This involves prospectively checking for adverse events in a defined population before and after the mass deployment of a new drug or in the event of new drugs introduced which have safety concerns. Cohort monitoring aims to:

- Characterize known reactions and identify signals for unrecognized reactions and interactions.
- Measure risks and identify risk factors.
- Provide utilization data.
- Assess safety in pregnancy and lactation.
- Detect inefficacy.

Current cohort monitoring programmes are following antimalarials and antiretrovirals in a number of countries.

Targeted spontaneous reporting is an approach where a particular treatment is followed in detail as part of routine care. Where facilities are available, electronic longitudinal surveillance can link and follow prescribing with patient outcomes.

This approach can characterize the duration of adverse effects after onset and distinguish transient effects from those which persist during continuing drug exposure.

Initiatives to improve the level of pharmacovigilance in India were outlined. Since the national pharmacovigilance programme was launched in 2004 there has been a major effort to enhance and coordinate activity. Current plans are for a National Coordinating Centre, 40 reporting centres, and four zonal offices (to provide operational and logistic support). Focused adverse reaction monitoring is proposed for medicines or vaccines which have safety concerns of particular relevance to India.

As a result of globalization, there has been a trend for pharmaceutical manufacturers to outsource their pharmacovigilance operations to India. This is attractive partly because of the variation between the requirements for mandatory monitoring in different countries. Indian companies specializing in pharmacovigilance can provide the skills and technology required and are familiar with global pharmacovigilance requirements because of the export focus of the industry.

For the clinical pharmacist, pharmacovigilance is as much about protecting patients as about generating data for global databases. In hospital pharmacy practice, pharmacists need to be concerned that individual patients are protected from future exposure to drugs which have caused a problem (usually an allergy). Therefore, the focus in training pharmacists should be on patient safety. This can be facilitated by creating alerts within the healthcare system as well as providing information which will help to protect the patient from future exposure, e.g. warning cards and medical alerts.

Preventing medication errors is a fundamental responsibility of pharmacists. The human elements which predispose to
errors were outlined together with sys-
tems approaches to improving safety. It
is important for health professionals to be
able to report errors easily and without
fear of punishment. Pharmacists should
be a part of a multidisciplinary team to
implement changes which will help to
reduces future risks.

The power of the media when dealing
with pharmacovigilance issues was
demonstrated and led to animated dis-
cussion. Pharmacist interaction with the
media was also highlighted in a dedicated
symposium later in the Congress.

The seminar effectively highlighted the
interest and concerns of pharmacists
from many different practice environ-
ments. Most importantly, it demonstrated
the links between individual patient care
and patient safety at a global level.

Reference: WHO Pharmaceuticals Newslet-
ter, No. 5, 2011 at http://www.who.int/medi-
cines

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

Recommended composition of influenza vaccines: 2012 southern hemisphere

World Health Organization — It is recommended that the following viruses be used for influenza vaccines in the 2012 influenza season (southern hemisphere):

- an A/California/7/2009 (H1N1)pdm09-like virus;
- an A/Perth/16/2009 (H3N2)-like virus;
- a B/Brisbane/60/2008-like virus.

As in previous years, national or regional 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.

WHO has published recommendations on the prevention of influenza and Influenza surveillance information is updated on the WHO web site at http://www.who.int/influenza/en.


Aprotinin: resumed marketing

Canada — Health Canada has published the outcome of a comprehensive safety review of the heart-surgery drug aprotinin (Trasylol®) where it concludes that the benefits of aprotinin outweigh the risks when used as authorized. As a result of this assessment, the manufacturer can resume marketing.

Health Canada has requested that strong warnings be added to the prescribing information emphasizing that there have been reports of an increased risk of death in some studies associated with aprotinin use outside of its authorized indication and that aprotinin should only be used as authorized after careful consideration of the potential benefits and risks. Warnings have also been added emphasizing that physicians should adhere to the recommended procedures for the management of blood clotting and that aprotinin increases the risk of kidney problems.

Aprotinin marketing was temporarily suspended in November 2007 at Health Canada’s request after a clinical trial, the Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) study, was stopped due to a higher number of deaths in patients receiving aprotinin relative to two drugs also used to reduce blood loss.


First ever PUMA for buccal midazolam

United Kingdom/European Union — The 2007 European Paediatric Regulation aims to improve the availability of medicines for children in suitable dosage forms. As an incentive for the development of existing out-of-patent medicines specifically for use in children, the Regulation introduced a new type of marketing authorization known as a PUMA (Paediatric Use Marketing Authorization) with the same exclusivity rights as a completely new medicine.

Buccal midazolam (Buccolam®) is a treatment for prolonged acute convulsive seizures. It may be considered as an alternative to rectal diazepam for the treatment of prolonged seizures. Several factors should be considered when transferring patients to the authorized product...
when an unlicensed medicine other than Buccolam® has been used previously

Buccal midazolam should only be used by parents or carers where the patient has been diagnosed with epilepsy. For infants aged 3–6 months, treatment should only be in hospital, where monitoring is possible and resuscitation equipment is available. In particular:

- Hypersensitivity to midazolam, benzodiazepines, or to any of the excipients may occur.
- Midazolam should be used with caution in patients with chronic respiratory insufficiency because it may further depress respiration.
- Midazolam may accumulate in patients with chronic renal failure, or impaired hepatic or cardiac function and should therefore be used with caution in these individuals.

The most common adverse reactions in clinical trials of buccal midazolam were sedation, somnolence, depressed levels of consciousness, respiratory depression, and nausea and vomiting.


**Fidaxomicin approved for Clostridium difficile infection**

**European Union** — The European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP) has recommended the authorization of the first antibiotic in a new class to treat infections with *Clostridium difficile*. Fidaxomicin (Dificlir®), a first-in-class macrocyclic antibiotic, promises to improve current treatment of the inflammation of the gut and severe diarrhoea caused by *C. difficile*.

Over the past 20 years, the number of cases has been increasing, particularly among the elderly. The severity of infections has also been on the increase following the emergence of a new, more virulent strain of the bacterium that has spread to at least 17 European countries.


**Dronedarone: restricted use**

**European Union** — The European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP) has recommended restricting the use of dronedarone (Multaq®). The anti-arrhythmic medicine should only be prescribed for maintaining heart rhythm in patients with paroxysmal or persistent atrial fibrillation for the maintenance of sinus rhythm after successful cardioversion. Due to an increased risk of liver, lung and cardiovascular adverse events, dronedarone should only be prescribed after alternative treatment options have been considered. The Committee also recommended a number of other risk minimization measures to reduce the risk of injuries to liver, lung and cardiovascular system.

Dronedarone is an anti-arrhythmic medicine authorized in 2009 for use in adults who have had atrial fibrillation in the past or who currently have non-permanent fibrillation.


**Voclosporin: withdrawal of marketing authorization application**

**European Union** — The European Medicines Agency (EMA) has been notified by the manufacturer of its decision to withdraw the marketing authorization application for voclosporin (Luveniq®), 10 mg soft capsules.
Voclosporin was intended to be used for the treatment of patients with chronic non-infectious uveitis involving the posterior or intermediate segments of the eyes as characterized by a high degree of inflammation and in whom corticosteroids are inappropriate, do not provide adequate control, or cannot be tapered below 10 mg/day. Voclosporin was designated an orphan medicinal product on 14 September 2007.


Lacosamide syrup: change in formulation

European Union — The European Medicines Agency’s Committee for Medicinal Products for Human Use (CHMP) has recommended that Vimpat® 15 mg/ml syrup should no longer be marketed. The CHMP’s recommendation follows the voluntary recall of Vimpat® 15 mg/ml syrup on 15 September 2011. The recall was initiated because of a quality defect in some batches leading to uneven distribution of the active substance, lacosamide, in the syrup. As this defect could not be remediated, the Committee concluded that the benefit does not outweigh the risk that patients might receive either too much or too little of the active substance, and therefore recommended the permanent discontinuation of Vimpat® 15 mg/ml syrup.

Availability of a liquid formulation is essential for some patients. The Agency has therefore worked with the company to continue to make this important alternative formulation available to patients who need it. The application for a marketing authorization for a 10 mg/ml liquid formulation is currently under review by the CHMP.


New database of European experts

European Union — Coinciding with the entry into force of new rules on how to handle conflicts of interests of scientific experts, the European Medicines Agency (EMA) has launched a database of experts which allows a search for the declaration of interest of all experts who have been nominated by competent authorities for medicines regulation across the European Union to be involved in the Agency’s activities.

The launch of the new data base is a major building block of the Agency’s new rules on the handling of conflicts of interests of its scientific experts, which aim at protecting the Agency’s scientific opinion-making processes from the influence of any improper interests.

The experts’ database is accessible on the Agency’s web site at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/about_us/landing/experts.jsp&murl=menus/about_us/about_us.jsp&mid=WC0b01ac058043244a

Recent Publications, Information and Events

Marketing authorization of pharmaceutical products

World Health Organization — In 1999, WHO published a manual entitled Marketing authorization of pharmaceutical products with special reference to multisource (generic) products — a manual for a drug regulatory authority. The manual aims at providing technical advice to countries intending to strengthen their pre-marketing evaluation and marketing authorization system.

After an initial distribution of two thousand copies to regulatory authorities, more than three thousand additional copies have been disseminated. The manual has become known as the «Blue Book».

Feedback has since been received and a new edition has been developed using the many practical suggestions made by regulatory officials which have been taken into account and incorporated.

The new edition now includes a series of documents that will be of practical assistance to regulatory authorities and covering checklists, flow charts, model evaluation reports and model correspondence. Relevant WHO guidelines (such as those concerning stability and bioequivalence testing) are referenced in the text. All of the relevant guidelines are available from the WHO web site at http://www.who.int/medicines.


Guidelines for medicine donations

World Health Organization — The Third edition of Guidelines for medicine donations has been developed in cooperation with major international agencies active in humanitarian relief and development assistance. The guidelines are intended to improve the quality of medicine donations in international development assistance and emergency aid. Good medicine donation practice is crucial to both donors and recipients.


Quality control methods for herbal materials

World Health Organization — Quality control methods for medicinal plant materials was first published in 1998 to establish quality standards and specifications for herbal materials within the overall context of quality assurance and control of herbal medicines.

The purpose of this updated edition remains unchanged from that of the first. It supports the development of national standards based on local market conditions with due regard to existing national legislation and national and regional norms. It describes a series of internationally harmonized tests for assessing the quality of herbal materials, including determination of pesticide residues, arsenic and toxic heavy metals, micro-organisms and aflatoxins.

A collection of recommended test procedures for assessing the identity, purity,
and content of herbal materials, intended to assist national laboratories engaged in pharmaceutical quality control, the manual responds to the growing use of herbal medicines, the special quality problems they pose and the corresponding need for international guidance on reliable methods for quality control.

This new edition will continue to serve as key technical training material for national capacity-building and in setting standards for herbal medicines.


**European Union and Andean Community trade agreements**

The bilateral trade agreement negotiations between the EU and Peru and Colombia have concluded and now implementation is pending. Health Action International (HAI) Europe, a member of the Latin-American and Caribbean Global Alliance for Access to Medicines, has prepared a briefing paper, *The European Union and Andean Community Trade Agreements, Intellectual Property and Public Health*, providing an overview of the agreement history: the initial European demands on intellectual property, their implications for access to medicines, negotiation developments and final outcomes.

This policy paper is available in English and Spanish. As a complement, HAI Europe has also developed a Chronology which traces back the EU-Andean Community trade agreement since June 2007.


**Pharmaceutical reform: improving performance and equity**

A specific training guide for pharmaceutical reform using the Harvard/World Bank Flagship course model has been developed and has now been published by the World Bank in a book which is available as a free download at [http://go.worldbank.org/SRNT5208J0](http://go.worldbank.org/SRNT5208J0). An interactive textbook version is also available at: [http://www.worldbank.org/pdt](http://www.worldbank.org/pdt)

*Pharmaceutical reform: a guide to improving performance and equity* is a practical guide for developing effective policies that will improve the performance of the pharmaceutical sector in low- and middle-income countries. The guide is designed to provide policy makers with the skills and information they need to develop policy initiatives that will produce real results in their own national settings.

The electronic version allows communities of practice and colleagues working in sectors and regions, as well as students and teachers, to share notes and related materials for an enhanced, multimedia learning and knowledge-exchange experience.

**Consultation Documents**

**The International Pharmacopoeia**

**Bulk density and tapped density of powders**


[Note from the Secretariat: This new general method text is proposed for inclusion in the Supplementary information section of The International Pharmacopoeia (Ph.Int.). The text is based on the internationally harmonized test on bulk density and tapped density of powders.]

It is intended to revise the Supplementary Information section of the Ph.Int. (structure and contents). In the proposal currently being reviewed, a new section on Test Methods used during pharmaceutical development and/or manufacture of dosage forms is included. The general method for bulk and tapped density of powders would, therefore, be included in this section and a specific number will be assigned to this method once the proposed format and the methods considered for this section are adopted.]

**Bulk density**

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per millilitre (g/ml) although the international unit is kilogram per cubic metre (1 g/ml = 1000 kg/m³) because the measurements are made using cylinders.

It may also be expressed in grams per cubic centimetre (g/cm³).

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e., how it was handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined by measuring the volume of a known mass of powder sample, that may have been passed through a sieve, into a graduated cylinder (Method A), or by measuring the mass of a known volume of powder that
has been passed through a volumeter into a cup (Method B) or a measuring vessel (Method C). Method A and method C are favoured.

**Method A. Measurement in a graduated cylinder**

*Procedure.* Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated cylinder of 250 ml (readable to 2 ml), gently introduce, without compacting, approximately 100 g of the test sample \((m)\) weighed with 0.1% accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume \((V_0)\) to the nearest graduated unit. Calculate the bulk density in \((g/ml)\) using the formula \(m/V_0\). Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 ml or less than 150 ml, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as test sample such that its untapped apparent volume is 150 ml to 250 ml (apparent volume greater than or equal to 60% of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 ml and 100 ml a 100 ml cylinder readable to 1 ml can be used; the volume of the cylinder is specified in the expression of results.

**Method B. Measurement in a volumeter**

*Apparatus.* The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90. (Figure 1) It consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounted directly

*Figure 1. Volumeter*
below it. The cup may be cylindrical (25.00 ± 0.05 ml volume with an inside diameter of 30.00 ± 2.00 mm) or cubical (16.39 ± 0.20 ml volume with inside dimensions of 25.400 ± 0.076 mm).

Procedure. Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm$^3$ of powder with the cubical cup and 35 cm$^3$ of powder with the cylindrical cup. Carefully, scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the side of the cup and determine the mass ($M$) of the powder to the nearest 0.1%. Calculate the bulk density (g/ml) using the formula $M/V_0$ in which $V_0$ is the volume of the cup and record the average of 3 determinations using 3 different powder samples.

Method C. Measurement in a vessel

Apparatus. The apparatus consists of a 100 ml cylindrical vessel of stainless steel with dimensions as specified in Figure 2.

Procedure. Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method B. Determine the mass ($M_0$) of the powder to the nearest 0.1% by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/ml) using the formula $M_0/100$ and record the average of 3 determinations using 3 different powder samples.

Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample. After observing the initial powder
volume or mass, the measuring cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop, under its own mass, a specified distance by either of 3 methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

**Method A**

*Apparatus.* The apparatus (Figure 3) consists of the following:

- a 250 ml graduated cylinder (readable to 2 ml) with a mass of 220 ± 44 g; and
- a settling apparatus capable of producing, in 1 minute, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

*Procedure.* Proceed as described above for the determination of the bulk volume (V₀). Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V₁₀, V₅₀₀ and V₁₂₅₀ to the nearest graduated unit. If the difference between V₅₀₀ and V₁₂₅₀ is less than or equal to 2 ml, V₁₂₅₀ is the tapped volume. If the difference between V₅₀₀ and V₁₂₅₀ exceeds 2 ml, repeat in increments such as 1250 taps, until the difference between succeeding
measurements is less than or equal to 2 ml. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/ml) using the formula \(m/V_f\) in which \(V_f\) is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 ml graduated cylinder (readable to 1 ml) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

**Method B**

*Procedure.* Proceed as directed under Method A except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per minute.

**Method C**

*Procedure.* Proceed as described in method C for measuring the bulk density using the measuring vessel equipped with the cap shown in Figure 2. The measuring vessel with the cap is lifted 50-60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrape excess powder from the top of the measuring vessel as described in Method C for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the 2 masses obtained after 200 and 400 taps exceeds 2%, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2%. Calculate the tapped density (g/ml) using the formula \(M_f/100\) where \(M_f\) is the mass of powder in the measuring vessel. Record the average of 3 determinations using 3 different powder samples. The test conditions including tapping height are specified in the expression of the results.

**Measures of powder compressibility**

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the Compressibility index or the Hausner ratio.

The Compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio.

Compressibility index:

\[
\frac{100 (V_0 - V_f)}{V_0} = \text{unsettled apparent volume.}
\]

\[
V_0 = \text{final tapped volume.}
\]
Hausner Ratio:

\[ V_0 \]

\[ V_f \]

Depending on the material, the compressibility index can be determined using \( V_{10} \) instead of \( V_0 \). If \( V_{10} \) is used, it is clearly stated in the results.

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**Tablet friability**


[Note from Secretariat: the Expert Committee on Specifications for Pharmaceutical Preparations adopted in October 2009 a revision of the general monograph on Tablets where a reference is made under the Manufacture section to a method on friability testing. Based on the internationally harmonized tablet friability test, a general method text is, therefore, proposed for inclusion in the Supplementary Information section of the Ph.Int.]

This method text provides guidance for the friability determination of compressed, uncoated tablets. The test procedure presented is generally applicable to most compressed tablets. Measurement of tablet friability supplements other physical strength measurements, such as tablet breaking force.

Use a drum with an internal diameter between 283 and 291 mm and a depth between 36 and 40 mm, of transparent synthetic polymer with polished internal surfaces, and subject to minimum static build-up (see Figure 1 for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outer diameter of the central ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or onto each other.

For tablets with a unit weight equal to or less than 650 mg, take a sample of whole tablets \( n \) corresponding as near as possible to 6.5 g. For tablets with a unit weight of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the weight loss is greater than the targeted value, the test...
should be repeated twice and the mean of the three tests determined. A maximum mean weight loss from the three samples of not more than 1.0% is considered acceptable for most products.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with the horizontal and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.

Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, an appropriate humidity-controlled environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum, for the running of multiple samples at one time, are also permitted.

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**Test for bacterial endotoxins**

Draft proposal for inclusion of a General Method in the 4th Edition of the International Pharmacopoeia. Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail mendyc@who.int. All working documents are available for comment at http://www.who.int/medicines/publications/pharmacopoeia/mono_dev/en/index.html

**Note from the Secretariat:** PDG harmonization of general methods was discussed by the WHO Expert Committee on Specifications for Pharmaceutical Preparations at its 42nd meeting in October 2007 where it was decided that “the relevant method texts of The International Pharmacopoeia [Ph.Int.] should be reviewed alongside the
The bacterial endotoxins test (BET) is a test to detect or quantify endotoxins from Gram-negative bacteria using amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are three methods for this test:

Method A. The gel-clot technique, which is based on gel formation.

Method B. The turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate.

Method C. The chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Proceed by any of the three methods for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot limit test unless otherwise indicated in the monograph for the product being tested.

The test is carried out in a manner that avoids endotoxin contamination.

**Apparatus**

Depyrogenate all glassware and other heat stable materials in a hot air oven using a validated process. A commonly used minimum time and temperature is 30 minutes at 250 °C. If employing plastic apparatus such as microplates and pipet tips for automatic pipetters, use apparatus shown to be free of detectable endotoxin and which does not interfere in the test.

**Note:** In this chapter the term “tube” includes any other receptacle such as a microtitre well.

**Reagents and test solutions**

**Amoebocyte lysate**

A lyophilized product obtained from the lysate of amebocytes (white blood cells) from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*).

**Note:** Amoebocyte lysate reacts to some β-glucans in addition to endotoxins. Amebocyte lysate preparations which do not react to glucans are available: they are prepared by removing the G factor reacting to glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate and may be used for the endotoxin testing in the presence of glucans.

**Lysate TS**

Dissolve amebocyte lysate in water BET or in a buffer recommended by the lysate manufacturer, by gentle stirring. Store the reconstituted lysate, refrigerated or frozen, according to the specifications of the manufacturer.
Water BET
Water for injections or water produced by other procedures that shows no reaction with the lysate employed, at the detection limit of the reagent.

Preparation of Standard Endotoxin Stock Solution
A Standard Endotoxin Stock Solution is prepared from an endotoxin reference standard that has been calibrated to the current endotoxin RS. Follow the specifications in the package leaflet and on the label for preparation and storage of the Standard Endotoxin Stock Solution.

Endotoxin is expressed in International Units (IU) of endotoxin.

Note: One International Unit (IU) of endotoxin is equal to one Endotoxin Unit (EU).

Preparation of Standard Endotoxin Solution
After mixing the Standard Endotoxin Stock Solution vigorously, prepare appropriate serial dilutions of Standard Endotoxin Solution, using water BET.

Use dilutions as soon as possible to avoid loss of activity by adsorption.

Preparation of sample solutions
Prepare sample solutions by dissolving or diluting drugs using water BET. Some substances or preparations may be more appropriately dissolved or diluted in other aqueous solutions. If necessary, adjust the pH of the solution to be examined (or dilution thereof) so that the pH of the mixture of the lysate TS and sample solution falls within the pH range specified by the lysate manufacturer, usually 6.0 to 8.0. The pH may be adjusted by the use of acid, base, or suitable buffer as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with water BET in containers free of detectable endotoxin. Buffers must be validated to be free of detectable endotoxin and interfering factors.

Determination of maximum valid dilution
The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample at which the endotoxin limit can be determined.

Determine the MVD from the following equation:

\[
MDV = \frac{\text{Endotoxin Limit} \times \text{Concentration of sample solution}}{\lambda}
\]

Endotoxin Limit
The endotoxin limit for parenteral preparations, defined on the basis of dose, equals K/M, where K is a threshold pyrogenic dose of endotoxin per kg of body weight, and M is equal to the maximum recommended bolus dose of product per kg of body weight. When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.

The endotoxin limit for parenteral preparations is specified in units such as IU/ml, IU/mg, IU/Unit of biological activity, etc., in the individual monograph.
Concentration of sample solution
Mg/ml in the case of endotoxin limit specified by weight (IU/mg).

Units/ml in the case of endotoxin limit specified by unit of biological activity (IU/Unit)
ml/ml when the endotoxin limit is specified by volume (IU/ml).

\( \lambda \): the labeled lysate sensitivity in the gel-clot technique (IU/ml) or the lowest concentration used in the standard curve for the turbidimetric or chromogenic techniques.

Method A: gel clot technique
The gel-clot technique is for detecting or quantifying endotoxins based on clotting of the lysate TS in the presence of endotoxin. The minimum concentration of endotoxin required to cause the lysate to clot under standard conditions is the labelled sensitivity of the lysate TS. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate sensitivity and for interfering factors as described below under Preparatory testing.

Preparatory testing

Test for confirmation of labelled lysate sensitivity
Confirm in four replicates the labeled sensitivity, \( \lambda \), expressed in EU/ml of the lysate prior to use in the test. The test for confirmation of the lysate sensitivity is to be carried out when a new lot of lysate is used or when there is any change in the test conditions which may affect the outcome of the test.

Prepare standard solutions having at least four concentrations equivalent to \( 2\lambda \), \( \lambda \), 0.5\( \lambda \) and 0.25\( \lambda \) by diluting the Standard Endotoxin Stock Solution with water BET.

Mix a volume of the lysate TS with an equal volume of one of the standard solutions (such as 0.1 ml aliquots) in each tube. When single test vials or ampoules, containing lyophilized lysate are employed, add solutions of standards directly to the vial or ampoule. Incubate the reaction mixture for a constant period according to directions of the lysate manufacturer (usually at 37±1°C for 60 ± 2 minutes), avoiding vibration. Test the integrity of the gel for tests carried out in tubes, take each tube in turn directly from the incubator and invert it through approximately 180 degrees in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed.

The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests.

The endpoint is the lowest concentration in the series of decreasing concentrations of standard endotoxin that clots the lysate. Determine the geometric mean endpoint concentration by calculating the mean of the logarithms of the endpoint concentrations of the four dilution series, take the antilogarithm of this value, as indicated in the following formula:

\[
\text{Geometric Mean Endpoint Concentration} = \text{antilog} \left( \frac{\sum e}{f} \right)
\]

\( \sum e \) = the sum of the log endpoint concentrations of the dilution series used

\( f \) = the number of replicate test tubes
Table 1.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Endotoxin concentration/Solution to which endotoxin is added</th>
<th>Diluent</th>
<th>Dilution factor</th>
<th>Endotoxin concentration</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None / Sample solution</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>2λ / Sample solution</td>
<td>Sample solution</td>
<td>1</td>
<td>2λ</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>1λ</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.5λ</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.25λ</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>2λ / Water BET</td>
<td>Water BET</td>
<td>1</td>
<td>2λ</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>1λ</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.5λ</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.25λ</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>None / Water BET</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

Note:
Solution A: a sample solution of the preparation under test that is free of detectable endotoxins
Solution B: test for interference
Solution C: control for labeled lysate sensitivity
Solution D: negative control of water BET.

The geometric mean endpoint concentration is the measured sensitivity of the lysate (IU/ml). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used in tests performed with this lysate.

**Test for interfering factors**

Usually prepare the solutions (A–D) in Table 1, and perform the inhibition/enhancement test on the sample solutions at a dilution less than the MVD not containing any detectable endotoxins, operating as described above under Test for confirmation of labeled lysate sensitivity.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described above under Test for confirmation of labeled lysate sensitivity.

The test for interfering factors must be repeated when any condition changes which is likely to influence the result of the test.

The test is considered valid when all replicates of solutions A and D show no reaction and the result of solution C confirms the labeled sensitivity.

If the sensitivity of the lysate determined in the presence of solution B is not less than 0.5λ and not greater than 2λ, the sample solution does not contain factors which interfere under the experimental conditions used. Otherwise the sample solution to be examined interferes with the test.
If the preparation under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. The use of a more sensitive lysate permits a greater dilution of the preparation to be examined and this may contribute to the elimination of interference.

Interference may be overcome by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which standard endotoxin has been added and which has then been submitted to the chosen treatment.

**Limit test**

**Procedure**
Prepare the solutions A, B, C and D according to Table 2, and perform the test on these solutions following the procedure in Test for confirmation of labeled lysate sensitivity under Preparatory testing.

**Table 2.**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Endotoxin concentration/ Solution to which endotoxin is added</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None / Diluted sample solution</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>2λ / Diluted sample solution</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>2λ / Water BET</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>None / Water BET</td>
<td>2</td>
</tr>
</tbody>
</table>

**Note:** Prepare the solution A and the positive product control solution B using a dilution not greater than the MVD and treatments as for the Test for interfering factors under Preparatory testing. The positive control solutions B and C contain the standard endotoxin preparation at a concentration corresponding to twice the labelled lysate sensitivity. The negative control solution D consists of water BET.

**Interpretation**
The test is considered valid when both replicates of solution B and C are positive and those of solution D are negative.

When a negative result is found for both replicates of solution A, the preparation under test complies with the test.

When a positive result is found for both replicates of solution A, the preparation under test does not comply with the test.

When a positive result is found for one replicate of solution A and a negative result is found for the other, repeat the test. In the repeat test, the preparation under test complies with the test if a negative result is found for both replicates of solution A. The preparation does not comply with the test if a positive result is found for one or both replicates of solution A.
However, if the preparation does not comply with the test at a dilution less than the MVD, the test may be repeated using a greater dilution, not exceeding the MVD.

**Quantitative Test**

**Procedure**

The test quantifies bacterial endotoxins in sample solutions by titration to an endpoint. Prepare the solutions A, B, C and D following Table 3, and test these solutions according to the procedure in Test for confirmation of labeled lysate sensitivity under Preparatory testing.

**Note:**

*Solution A: Sample solution under test at the dilution, not to exceed the MVD, with which the test for interfering factors was completed. Subsequent dilution of the sample solution must not exceed the MVD. Use water BET to make a dilution series of four tubes containing the sample solution under test at concentrations of 1, 1/2, 1/4 and 1/8 relative to the concentration used in the test for interfering factors. Other dilutions up to the MVD may be used as appropriate.*

*Solution B: Solution A containing standard endotoxin at a concentration of 2λ (positive product control)*

*Solution C: A dilution series of four tubes of water BET containing the standard endotoxin at a concentration of 2λ, λ, 0.5λ and 0.25λ, respectively*

*Solution D: Water BET (negative control)*

Table 3.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Endotoxin concentration/Solution to which endotoxin is added</th>
<th>Diluent</th>
<th>Dilution factor</th>
<th>Endotoxin concentration</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None / Sample solution</td>
<td>Water BET</td>
<td>1</td>
<td>-</td>
<td>2</td>
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<tr>
<td>B</td>
<td>2λ / Sample solution</td>
<td></td>
<td>1</td>
<td>2λ</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>2λ / Water BET</td>
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<td>λ</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.5λ</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>0.25λ</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>None / Water BET</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

**Note:**

*Solution A: Sample solution under test at the dilution, not to exceed the MVD, with which the test for interfering factors was completed. Subsequent dilution of the sample solution must not exceed the MVD. Use water BET to make a dilution series of four tubes containing the sample solution under test at concentrations of 1, 1/2, 1/4, and 1/8 relative to the concentration used in the test for interfering factors. Other dilutions up to the MVD may be used as appropriate.*
1/4 and 1/8 relative to the concentration used in the test for interfering factors. Other dilutions up to the MVD may be used as appropriate.

Solution B: Solution A containing standard endotoxin at a concentration of 2\(\lambda\) (positive product control)

Solution C: A dilution series of four tubes of water BET containing the standard endotoxin at a concentration of 2\(\lambda\), \(\lambda\), 0.5\(\lambda\) and 0.25\(\lambda\), respectively

Solution D: Water BET (negative control)

**Calculation and interpretation**

The test is considered valid when the following three conditions are met.

1: Both replicates of the negative control solution D are negative.

2: Both replicates of the positive product control solution B are positive.

3: The geometric mean endpoint concentration of the solution C is in the range of 0.5 \(\lambda\) to 2 \(\lambda\).

To determine the endotoxin concentration of solution A, calculate the endpoint concentration for each replicate by multiplying each endpoint dilution factor by \(\lambda\).

The endotoxin concentration in the sample solution is the endpoint concentration of the replicates. If the test is conducted with a diluted sample solution, calculate the concentration of endotoxin in the original sample solution by multiplying by the dilution factor.

If none of the dilutions of sample solution is positive in a valid assay, report the endotoxin concentration as less than \(\lambda\) (if diluted sample was tested, report as less than the smallest dilution factor of the sample multiplied by 1). If all dilutions are positive, the endotoxin concentration is reported as equal to or greater than the largest dilution factor multiplied by \(\lambda\) (e.g. initial dilution factor multiplied by 8 and by \(\lambda\) in Table 3). The preparation under test meets the requirements of the test if the concentration of endotoxin in both replicates is less than that specified in the individual monograph.

**Photometric quantitative techniques**

**Method B. Turbidimetric technique**

This technique is a photometric assay measuring increases in reactant turbidity. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-turbidimetric assay or a kinetic-turbidimetric assay.

The endpoint-turbidimetric assay is based on the quantitative relationship between the concentration of endotoxins and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation period.

The kinetic-turbidimetric assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance or transmission of the reaction mixture, or the rate of turbidity development.
The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually 37 ± 1°C).

**Method C. Chromogenic technique**
This technique is an assay to measure the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with lysate.

On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-chromogenic assay or a kinetic-chromogenic assay.

The endpoint-chromogenic assay is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period.

The kinetic-chromogenic assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance of the reaction mixture, or the rate of color development.

The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually 37 ± 1°C).

**Preparatory testing**
To assure the precision or validity of the turbidimetric and chromogenic techniques, preparatory tests are conducted to show that the criteria for the standard curve are valid and that the sample solution does not interfere with the test.

Validation for the test method is required when conditions change which are likely to influence the result of the test.

**Assurance of criteria for the standard curve**
The test must be carried out for each lot of the lysate. Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations within the range indicated by the lysate manufacturer to generate the standard curve. Perform the assay using at least three replicates of each standard endotoxin concentration according to the manufacturer’s instructions for the lysate (volume ratios, incubation time, temperature, pH etc.).

If the desired range is greater than two logs in the kinetic methods, additional standards should be included to bracket each log increase in the range of the standard curve.

The absolute value of the correlation coefficient, |r|, must be greater than or equal to 0.980, for the range of endotoxin concentrations set up.

**Test for interfering factors**
Select an endotoxin concentration at or near the middle of the endotoxin standard curve.

Prepare solutions A, B, C and D shown in Table 4. Perform the test on solutions A-D in at least duplicate according to the instructions for the lysate employed, for example, concerning volume of sample solution and lysate TS, volume ratio of sample solution to lysate TS, incubation time, etc.
Table 4.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Endotoxin concentration</th>
<th>Solution to which endotoxin is added</th>
<th>Number of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>Sample solution</td>
<td>Not less than 2</td>
</tr>
<tr>
<td>B</td>
<td>Middle concentration of the standard curve</td>
<td>Sample solution</td>
<td>Not less than 2</td>
</tr>
<tr>
<td>C</td>
<td>At least 3 concentrations (lowest concentration is designated $\lambda$)</td>
<td>Water BET</td>
<td>Each not less than 2</td>
</tr>
<tr>
<td>D</td>
<td>None</td>
<td>Water BET</td>
<td>Not less than 2</td>
</tr>
</tbody>
</table>

Note:

Solution A: The sample solution may be diluted not to exceed the MVD.

Solution B: The preparation under test at the same dilution as solution A, containing added endotoxin at a concentration equal to or near the middle of the standard curve.

Solution C: The standard endotoxin at the concentrations used in the validation of the method described in Assurance of criteria for the standard curve under Preparatory testing (positive controls)

Solution D: Water BET (negative control)

The test is considered valid when the following conditions are met.

1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.

2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

Calculate the mean recovery of the added endotoxin by subtracting the mean endotoxin concentration in the solution, if any (Solution A, Table 4), from that containing the added endotoxin (Solution B, Table 4).

In order to be considered free of factors that interfere with the assay under the conditions of the test, the measured concentration of the endotoxin added to the sample solution must be within 50-200% of the known added endotoxin concentration after subtraction of any endotoxin detected in the solution without added endotoxin.

When the endotoxin recovery is out of the specified range, the sample solution under test is considered to contain interfering factors. Then repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. To establish that the treatment chosen effectively eliminates interference without loss of endotoxins, perform the assay described above using the preparation to be examined to which standard endotoxin has been added and which has then been submitted to the chosen treatment.
Test

Procedure
Follow the procedure described in Test for interfering factors under Preparatory testing.

Calculation
Calculate the endotoxin concentration of each of the replicates of test solution A using the standard curve generated by the positive control solution C. The test is considered valid when the following three requirements are met.

1. The results of the positive control solution C comply with the requirements for validation defined in Assurance of criteria for the standard curve under Preparatory testing.

2. The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in the solution A, is within the range of 50–200%.

3. The result of the negative control solution D does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate employed.

Interpretation
In photometric assays, the preparation under test complies with the test if the mean endotoxin concentration of the replicates of solution A, after correction for dilution and concentration, is less than the endotoxin limit for the product.

*******

New/revised reagents to be added to Ph.Int

Amoebocyte lysate
A lyophilized product obtained from the lysate of amebocytes (white blood cells) from the horseshoe crab (Limulus polyphemus or Tachypleus tridentatus).

Note: Amoebocyte lysate reacts to some β-glucans in addition to endotoxins. Ameobocyte lysate preparations which do not react to glucans are available: they are prepared by removing the G factor reacting to glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate and may be used for the endotoxin testing in the presence of glucans.

Lysate TS
Dissolve amebocytelysate in water BET or in a buffer recommended by the lysate manufacturer, by gentle stirring. Store the reconstituted lysate, refrigerated or frozen, according to the specifications of the manufacturer.

Water BET
Water for injections or water produced by other procedures that shows no reaction with the lysate employed, at the detection limit of the reagent.
Test for sterility


[Note from Secretariat: During its meeting in October 2010 the Expert Committee on Specifications for Pharmaceutical Preparations recommended revision of 3.2 Test for sterility using as basis the internationally harmonized test for sterility. It is, therefore, proposed to replace the current method 3.2.1 Test for sterility of non-injectable preparations and 3.2.2 Sterility testing of antibiotics by the internationally harmonized test for sterility. Testing of surgical materials is not included in the revision. If the proposed revision is accepted consideration should be given to the need for additional advice concerning the testing of antibiotics within the Supplementary Information section of The International Pharmacopoeia (Ph.Int.). Further, it will be necessary to change all references to 3.2.1 and 3.2.2 in Ph.Int. monographs. A proposal to this effect is attached as Appendix 1.

The clause “unless otherwise prescribed, justified and authorized” in the harmonized text is used also in the draft proposal for Ph.Int. The meaning of “justified and authorized” in the context of the Ph.Int needs explanation. It is proposed to include the following wording in the General Notices of the Ph.Int.: The expression “unless otherwise justified and authorized” means that the requirements have to be met or instructions to be followed, unless the relevant national or regional authority authorizes an exemption or modification, where justified in a particular case.]

The test is applied to substances or preparations which, according to the pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined in the conditions of the test.

Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any microorganisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

Culture media and incubation temperatures

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.

The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.
**Fluid thioglycollate medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cystine</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>0.75 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Glucose monohydrate/anhydrous</td>
<td>5.5/5.0 g</td>
</tr>
<tr>
<td>Yeast extract (water-soluble)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Pancreatic digest of casein</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Sodium thioglycollate</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Thioglycollic acid</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>Resazurin sodium solution (1 g/l of resazurin sodium), freshly prepared</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Water R</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

pH after sterilization 7.0 to 7.2.

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with the water R and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, add sodium hydroxide (1 mol/l) VS so that, after sterilization, the solution will have a pH of 7.0 to 7.2. If filtration is necessary heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution, mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2 °C and 25 °C in a sterile, airtight container. If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink colour disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30–35 °C.

For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20–25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

**Alternative thioglycollate medium**

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution, sterilize as directed above. The pH after sterilization is 7.0 to 7.2. Heat in a water-bath prior to use and incubate at 30–35 °C under anaerobic conditions.
**Soya-bean casein digest medium**

Pancreatic digest of casein
Papaic digest of soya-bean meal
Sodium chloride
Dipotassium hydrogen phosphate
Glucose monohydrate/anhydrous
Water R

17.0 g
3.0 g
5.0 g
2.5 g
2.5/2.3 g
1 000 ml

pH after sterilization 7.1 to 7.5.

Dissolve the solids in water R, warming slightly to effect solution. Cool the solution to room temperature. Add sodium hydroxide (1 mol/l) VS, if necessary, so that after sterilization the solution will have a pH of 7.1 to 7.5. Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2 °C and 25 °C in a sterile well-closed container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soya-bean casein digest medium is to be incubated at 20–25 °C.

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

**Sterility.** Incubate portions of the media for 14 days. No growth of microorganisms occurs.

**Growth promotion test of aerobes, anaerobes and fungi**

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of microorganisms are indicated in Table 1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: *Clostridium sporogenes, Pseudomonas aeruginosa, Staphylococcus aureus*. Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: *Aspergillus brasiliensis, Bacillus subtilis, Candida albicans*. Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the microorganisms occurs.
Table 1. Strains of the test microorganisms suitable for use in the Growth Promotion test and the Method Suitability test

<table>
<thead>
<tr>
<th>Aerobic bacteria</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anaerobic bacterium</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium sporogenes</em></td>
<td>ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus brasiliensis</em></td>
<td>ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method suitability test**

Carry out a test as described below under Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

**Membrane filtration.** After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

**Direct inoculation.** After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the medium.

In both cases use the same microorganisms as those described above under Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of microorganisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the
conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

This method suitability is performed:

• when the test for sterility has to be carried out on a new product;
• whenever there is a change in the experimental conditions of the test.

The method suitability may be performed simultaneously with the Test for sterility of the product to be examined.

**Test for sterility of the product to be examined**
The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

**Membrane filtration.** Use membrane filters having a nominal pore size not greater than 0.45 µm whose effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g. for antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

**Aqueous solutions.** If appropriate, transfer a small quantity of a suitable, sterile diluent such as a 1 g/l neutral solution of meat or casein peptone pH 7.0 to 7.4 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralising substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of five times 100 ml per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media.
Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

Table 2. Minimum quantity to be used for each medium

<table>
<thead>
<tr>
<th>Quantity per container</th>
<th>Minimum quantity to be used for each medium unless otherwise justified and authorized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquids</strong></td>
<td></td>
</tr>
<tr>
<td>• less than 1 ml</td>
<td>The whole contents of each container</td>
</tr>
<tr>
<td>• 1-40 ml</td>
<td>Half the contents of each container but not less than 1 ml</td>
</tr>
<tr>
<td>• greater than 40 ml and not greater than 100 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>• greater than 100 ml</td>
<td>10 per cent of the contents of the container but not less than 20 ml</td>
</tr>
<tr>
<td><strong>Antibiotic liquids</strong></td>
<td>1 ml</td>
</tr>
<tr>
<td><strong>Insoluble preparations, creams and ointments to be suspended or emulsified</strong></td>
<td>Use the contents of each container to provide not less than 200 mg</td>
</tr>
<tr>
<td><strong>Solids</strong></td>
<td></td>
</tr>
<tr>
<td>• less than 50 mg</td>
<td>The whole contents of each container</td>
</tr>
<tr>
<td>• 50 mg or more but less than 300 mg</td>
<td>Half the contents of each container but not less than 50 mg</td>
</tr>
<tr>
<td>• 300 mg – 5 g</td>
<td>150 mg</td>
</tr>
<tr>
<td>• greater than 5 g</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

**Soluble solids.** Use for each medium not less than the quantity prescribed in Table 2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injections R, sodium chloride (9 g/l) TS or peptone (1 g/l) TS1 and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

**Oils and oily solutions.** Use for each medium not less than the quantity of the product prescribed in Table 2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate R shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 ml of a suitable sterile solution such as peptone (1 g/l) TS1 containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability test, for example polysorbate 80 at a concentration of 10 g/l. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.
Ointments and creams. Use for each medium not less than the quantities of the product prescribed in Table 2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent in isopropyl myristate R as described above, by heating, if necessary, to not more than 40 °C. In exceptional cases it may be necessary to heat to not more than 44 °C. Filter as rapidly as possible and proceed as described above for oils and oily solutions.

Direct inoculation of the culture medium. Transfer the quantity of the preparation to be examined prescribed in Table 2 directly into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed.

If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

Oily liquids. Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g/l.

Ointments and creams. Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as peptone (1 g/l) TS1. Transfer the diluted product to a medium not containing an emulsifying agent.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic microorganisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

Observation and interèretation of results
At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 ml) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:

a) the data of the microbiological monitoring of the sterility testing facility show a fault;

b) a review of the testing procedure used during the test in question reveals a fault;

c) microbial growth is found in the negative controls;
d) after determination of the identity of the microorganisms isolated from the test, the

growth of this species or these species may be ascribed unequivocally to faults with

respect to the material and/or the technique used in conducting the sterility test proce-
dure.

If the test is declared to be invalid it is repeated with the same number of units as in

the original test.

If no evidence of microbial growth is found in the repeat test the product examined

complies with the test for sterility. If microbial growth is found in the repeat test the

product examined does not comply with the test for sterility.

Table 3. Minimum number of items to be tested

<table>
<thead>
<tr>
<th>Number of items in the batch*</th>
<th>Minimum number of items to be tested for each medium, unless otherwise justified and authorized**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parenteral preparations</strong></td>
<td></td>
</tr>
<tr>
<td>Not more than 100 containers</td>
<td>10 per cent or 4 containers whichever is the greater</td>
</tr>
<tr>
<td>More than 100 but not more than 500 containers</td>
<td>10 containers</td>
</tr>
<tr>
<td>More than 500 containers</td>
<td>2 per cent or 20 containers (10 containers for large-volume parenterals) whichever is the less</td>
</tr>
<tr>
<td><strong>Ophthalmic and other non-injectable preparation</strong></td>
<td></td>
</tr>
<tr>
<td>Not more than 200 containers</td>
<td>5 per cent or 2 containers whichever is the greater</td>
</tr>
<tr>
<td>More than 200 containers</td>
<td>10 containers</td>
</tr>
<tr>
<td>If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use</td>
<td></td>
</tr>
<tr>
<td><strong>Bulk solid products</strong></td>
<td></td>
</tr>
<tr>
<td>Up to 4 containers</td>
<td>Each container</td>
</tr>
<tr>
<td>More than 4 containers but not more than 50 containers</td>
<td>20 per cent or 4 containers whichever is the greater</td>
</tr>
<tr>
<td>More than 50 containers</td>
<td>2 per cent or 10 containers whichever is the greater</td>
</tr>
</tbody>
</table>

*If the batch size is not known, use the maximum number of items prescribed

**If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.
Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 2 diluting where necessary to about 100 ml with a suitable sterile solution, such as peptone (1 g/l) TS1.

When using the technique of direct inoculation of media, use the quantities shown in Table 2, unless otherwise justified and authorized. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

Minimum number of items to be tested

The minimum number of items to be tested in relation to the size of the batch is given in Table 3.

* * * * *

New reagent to be added to Ph.Int.

Isopropyl myristate R. Description: A clear, colourless, oily liquid.

Miscibility: Immiscible with water, miscible with ethanol, with fatty oils, with liquid paraffin. Relative density: about 0.853

Annex 1

Consequential changes to monographs

A search of the Ph.Int. indicates that there are 30 API monographs which invoke method 3.2.1; many of these are antibiotic APIs which also invoke method 3.2.2. The following change would be necessary in, for example, the monograph for Ampicillin sodium:

Sterility. Complies with 3.2.2 Sterility testing of antibiotics, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure and using the sampling plan described under 3.2.1 Test for sterility of non-injectable preparations.

Change to read:

Sterility. Complies with 3.2 Test for sterility, applying either the membrane filtration test procedure with added penicillinase TS or the direct test procedure.

The other API monographs invoking method 3.2.1 include those that are used in ophthalmic dosage forms. The following change would be necessary in, for example, the monograph for Timolol maleate:

Sterility. Complies with 3.2.1 Test for sterility of non-injectable preparations.

Change to read:

Sterility. Complies with 3.2 Test for sterility.
A search of the Ph.Int. indicates that, in addition to the antibiotic API monographs that invoke both methods 3.2.1 and 3.2.2, there are some antibiotic monographs (API and dosage forms) that invoke only method 3.2.2. The following change would be necessary in, for example, the monograph for Clindamycin phosphate:

**Sterility.** Complies with 3.2.2 Sterility testing of antibiotics, applying the membrane filtration test procedure and using a solution in water containing 150 mg of clindamycin phosphate per ml.

Change to read:

**Sterility.** Complies with 3.2. Test for sterility, applying the membrane filtration test procedure and using a solution in water containing 150 mg of clindamycin phosphate per ml.

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**Chewable albendazole tablets**

Draft proposal for the International Pharmacopoeia (September 2011). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail mendyc@who.int. All working documents are available for comment at http://www.who.int/medicines/publications/pharmacopoeia/mono_dev/en/index.html

**Category.** Anthelminthic.

**Storage.** Chewable Albendazole tablets should be kept in a tightly closed container.

**Labelling.** The designation on the container should state that the tablets may be chewed, swallowed whole or crushed and mixed with food or liquid, and the tablets should be crushed before giving to a young child.

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

**Requirements**

Comply with the monograph for «Tablets».

Definition. Chewable albendazole tablets contain not less than 90.0% and not more than 110.0% of the amount of Albendazole (C12H15N3O2S) stated on the label.

**Identity tests**

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

A. 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 dichloromethane R, 7 volumes of glacial acetic acid R, and 3 volumes of ether R as the mobile phase. Apply separately to the plate 5 μl each of the following two solutions in a mixture of 9 volumes of dichloromethane R, and 1 volume of glacial acetic acid R. For solution (A) shake a quantity of the powdered tablets containing about 25 mg of Albendazole with
25 ml, filter and use the filtrate. For solution (B) use 1.0 mg of albendazole RS per ml. After removing the plate from the chromatographic chamber, allow the plate to dry in a current of warm air, and examine the chromatogram under ultraviolet light (254 nm).

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

[Note from Secretariat: addition of a system suitability criteria is under investigation.]

B. 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 obtained with solution (3).

C. See the test described under Assay, method B. The absorption spectrum (1.6) of solution (1), when observed between 220 and 340 nm, exhibits a maximum at 308 nm and a minimum at 281 nm.

D. Dissolve a quantity of powdered tablets containing about 0.2 g of Albendazole in 30 ml of ethanol R by warming in a water bath. Filter and evaporate the filtrate to dryness. Ignite about 0.1 g of the residue, fumes are evolved, staining lead acetate paper R black.

E. See the test described under Identity test D. Dissolve about 0.1 g of the residue in warmed dilute sulfuric acid R, add potassium iodobismuthate TS, a reddish-brown precipitate is produced.

**Related substances**

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

Prepare the following solutions.

Solvent mixture: dilute 1 volume of sulfuric acid R with 99 volumes of methanol R. For solution (1), transfer a quantity of the powdered tablets containing about 25.0 mg of Albendazole in 5 ml of the solvent mixture and dilute to 50.0 ml with methanol R.

For solution (2), dilute 1 volume of solution (1) with 100 volumes of methanol R.

Inject separately 20 µl each of solutions (1) and (2). Record the chromatogram for about 45 minutes (retention time of albendazole: about 22 minutes).

In the chromatogram obtained with solution (1), the area of any peak, other than the peak due to albendazole, is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (0.75%). The sum of the areas of all peaks, other than the peak due to albendazole, is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak 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 × 4.6 mm) packed with octadecylsilyl base-deactivated silica gel for chromatography R (5 μm). (Inertsil ODS-SP has been found suitable.)

As the mobile phase, use a solution prepared as follows: dissolve 1.25 g of monobasic ammonium phosphate R in 1000 ml of water R, mix, and filter through a 0.45 μm membrane. Make adjustments if necessary. Mix 400 ml of this solution with 600 ml of methanol R.

Prepare the following solutions.

Solvent mixture: dilute 1 volume of sulfuric acid R with 99 volumes of methanol R.

For solution (1) weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder, containing about 100 mg of Albendazole, to a 50 ml volumetric flask. Add 5 ml of the solvent mixture and 20 ml of methanol R, and shake to dissolve for about 15 minutes. Dilute to volume with methanol R, mix and filter, discarding the first 15 ml of the filtrate. Dilute 5.0 ml of this solution to 50.0 ml with methanol R and mix. For solution (2), transfer 25.0 mg of albendazole RS to a 25 ml volumetric flask, add 2 ml of the solvent mixture and 20 ml of methanol R, and shake to dissolve. Dilute to volume with methanol Re and mix. For solution (3), dilute 2 ml of solution (2) to 10.0 ml with methanol R and mix.

Operate with a flow rate of 1 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Inject separately 20 μl each of solutions (1) and (3). The peak due to albendazole is eluted at the following retention time: about 22 minutes. The test is not valid unless, in the chromatogram obtain with solution (3), the number of theoretical plates of the column is not less than 5000, calculated with reference to the peak due to albendazole.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (3), and calculate the content of albendazole (C12H15N3O2S) in the tablets.

B. Weigh and powder 20 tablets. Transfer a quantity of the powder containing about 20 mg of Albendazole, accurately weighed, to a 50ml volumetric flask, add 30 ml of hydrochloric acid/methanol (0.01 mol/l) VS, shake for 15 minutes and dilute to volume with the same solvent. Mix and filter this solution, discarding the first 10 ml of the filtrate. Transfer 1.0 ml of the subsequent filtrate to a 50 ml volumetric flask and dilute to volume with sodium hydroxide (~4 g/l) TS. Measure the absorbance of the resulting solution at the maximum at about 308 nm. Calculate the content of Albendazole (C12H15N3O2S), using the absorptivity value of 74.2.

[Note from Secretariat: reagents to be confirmed.]
New reagents to be added to Ph.Int.

Sodium hydroxide (~4 g/l) TS: A solution of sodium hydroxide R containing about 4 g/l of NaOH (approximately 0.1 mol/l).

Monobasic ammonium phosphate R. Monoammonium Phosphate; Ammonium Dihydrogen Phosphate. NH4H2PO4.

A commercial reagent of suitable grade.

Artenimolum
Artenimol

Draft proposal for the International Pharmacopoeia (August 2011). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail mendyc@who.int. All working documents are available for comment at http://www.who.int/medicines/publications/pharmacopoeia/mono_dev/en/index.html

[Note from Secretariat: The current monograph is under review in the context of the general revision of artemisinin derivatives. This revised draft only takes account of the changes proposed as regards to the correction of the information related to stereochemistry. Therefore, it might be subject to further changes on other aspects of the monograph.)

Artenimol is the International Nonproprietary Name (INN) for this substance. However, the trivial name «dihydroartemisinin» is also in common use.

C15H24O5

Relative molecular mass. 284.4


[Note from Secretariat: a systematic name following IUPAC rules has been added as an alternative for the main component.]

Other names. «Dihydroartemisinin», β-dihydroartemisinin.
Description. Colourless needles or a white or almost white, crystalline powder. Solubility. Practically insoluble in water; slightly soluble in acetonitrile R, ethanol (~750 g/l) TS and dichloromethane R.

Category. Antimalarial drug.

Storage. Artenimol should be kept in a well-closed container, protected from light. Additional information. In solution, Artenimol (10S-epimer) and 10-epi-artenimol (10R-epimer) are in slow equilibrium.

Requirements

Definition. Artenimol contains not less than 97.0% and not more than the equivalent of 102.0% of C15H24O5 using Assay method A, and not less than 98.0% and not more than the equivalent of 102.0% of C15H24O5 using Assay method B, both calculated with reference to the dried substance.

Identity tests

Either test A alone 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 artenimol RS or with the reference spectrum of artenimol.

B. See the test described below under «Related substances test B». The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

C. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

D. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, and 4 drops of starch TS; a violet colour is immediately produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 10.0 mg/g.

Related substances

Either test A or test B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica
gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 μm). As the mobile phase for gradient elution, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water for the first 17 minutes; then run a gradient, which should reach 100% acetonitrile within 13 minutes.

Prepare the following solutions in methanol R with sonication. For solution (1) use 10 mg of Artenimol per ml and for solution (2) use 50 μg of Artenimol per ml.

For the system suitability test prepare solution (3) by dissolving 1.0 mg of artemisinin RS per ml and 1.0 mg of artemimol RS per ml in methanol R with sonication.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

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

The test is not valid unless the relative retention of artemimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses for artemimol and 10-epi-artenimol obtained in the chromatograms from solutions (1) and (2), and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution (1), the area of any peak, other than the two principal peaks, is not greater than that obtained with solution (2) (0.5%). Not more than one peak is greater than half the area of the two principal peaks obtained with solution (2) (0.25%). The sum of the areas of all the peaks, other than the two principal peaks, is not greater than twice the area of the two principal peaks obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the two principal peaks in the chromatogram obtained with solution (2).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 ml of each of the following 5 solutions in toluene R containing (A) 10 mg of Artenimol per ml, (B) 0.05 mg of Artenimol per ml, (C) 0.025 mg of Artenimol per ml, (D) 0.10 mg of Artenimol per ml, and (E) 0.10 mg of artemimol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. 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 B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

**Assay**

Either method A or method B may be applied.

Prepare fresh solutions and perform the tests without delay.

A. Determine by 1.14.4 High-performance liquid chromatography, using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 μm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water R.
Prepare the following solutions in the mobile phase: solution (1) 1.0 mg of Artenimol per ml, and solution (2) 1.0 mg of artemimol RS per ml.

For the system suitability test prepare solution (3) containing 1.0 mg of artemisinin RS per ml and 1.0 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water R.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

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

The test is not valid unless the relative retention of artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses for artemimol and 10-epi-artenimol obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of C15H22O5 with reference to the dried substance.

B. Dissolve about 0.05 g of Artenimol, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50 ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.

Measure the absorbance of a 1 cm layer at the maximum at about 292 nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of C15H22O5 in the substance being tested by comparison with artemimol RS, similarly and concurrently examined, and with reference to the dried substance.

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**Medroxyprogesterone injection**

Draft proposal for the International Pharmacopoeia (September 2011). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail mendyc@who.int. All working documents are available for comment at http://www.who.int/medicines/publications/pharmacopoeia/mono_dev/en/index.html

**Category.** Contraceptive.

**Storage.** Medroxyprogesterone injection should be protected from light. On standing, solid matter may separate; it should be redissolved by heating before use.

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 150 mg/ml in 1-ml vial.
Requirements

Complies with the monograph for “Parenteral preparations”.

Definition. Medroxyprogesterone injection is a sterile aqueous suspension of Medroxyprogesterone acetate. It contains not less than 90.0% and not more than 110.0% of the amount of Medroxyprogesterone acetate \((\text{C}_{24}\text{H}_{34}\text{O}_{4})\) stated on the label.

Identity tests

A. Centrifuge a volume of the injection to be examined containing 50 mg of Medroxyprogesterone acetate. Decant the supernatant liquid and wash the residue with two quantities of 15 ml of water R, discarding the water washings. Dissolve the combined residues in 10 ml of dichloromethane R. Evaporate to dryness on a water-bath and continue drying at 105 °C for 3 hours. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from medroxyprogesterone acetate RS or with the reference spectrum of medroxyprogesterone acetate.

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 R5 as the coating substance and a mixture of 10 volumes of dichloromethane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μl of each of the following two solutions in dichloromethane R. For solution (A) dilute a suitable volume of the injection to be examined to obtain a concentration of 5 mg of Medroxyprogesterone acetate per ml. For solution (B) use 5 mg of medroxyprogesterone acetate RS per ml. After removing the plate from the chromatographic chamber, heat it at 120°C for 30 minutes, spray with a mixture of equal volumes of sulfuric acid R and ethanol (−750 g/l) TS, and heat further at 120°C for 10 minutes. Allow the plate to cool, and examine the chromatogram in ultraviolet light (365 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 examine the chromatogram in daylight.

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

[Note from Secretariat: addition of a system suitability criteria under investigation.]

C. Centrifuge a volume of injection to be examined containing 30 mg of Medroxyprogesterone acetate. Decant the supernatant liquid, dissolve the residue in 5 ml of sulfuric acid R, and introduce 5 ml of ethanol (−750 g/l) TS to form an upper layer; a bluish violet ring is formed at the interface of the two layers.

pH. pH of the injection, 3.0-7.0.
**Medroxyprogesterone acetate impurity F**

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 10 volumes of tetrahydrofuran R, 45 volumes of tert-butyl methyl ether R and 45 volumes of hexane R as the mobile phase. Apply separately to the plate 10 ml of each of the following two solutions in dichloromethane R. For solution (A) dilute a suitable volume of the injection to be examined to obtain a concentration of 20 mg of Medroxyprogesterone acetate per ml.

For solution (B) use 20 mg of medroxyprogesterone acetate RS and 0.1 mg of medroxyprogesterone acetate impurity F per ml. Develop the chromatogram for a distance of about 10 cm. Allow it to dry in air and carry out a second development in the same direction using a freshly prepared mobile phase. After removing the plate from the chromatographic chamber, heat it at 100°C to 105 °C for 30 minutes, spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120 °C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (365 nm).

In the chromatogram obtained with solution A, any spot due to medroxyprogesterone acetate impurity F with an Rf value higher than the principal spot, is not more intense than the corresponding spot in the chromatogram obtained with solution B (0.5%).

**[Note from Secretariat]**:

- addition of a system suitability criteria under investigation
- reagents 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.

Prepare the following solutions with the mobile phase. For solution (1) dilute a suitable volume of injection to be examined to obtain a concentration of 0.4 mg of Medroxyprogesterone acetate per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 4 μg of Medroxyprogesterone acetate per ml. For solution (3), use 20 μg of medroxyprogesterone acetate RS and 50 μg of megestrol acetate RS per ml. For solution (4), use 3.65 μg of methyl hydroxybenzoate R and 0.4 μg of propyl hydroxybenzoate R per ml.

Inject 20 µl of solution (3). Record the chromatogram for about 30 minutes. The following peaks are eluted in the order: megestrol acetate (retention time about 12 minutes), medroxyprogesterone acetate (retention time about 14 minutes). The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to medroxyprogesterone acetate and to megestrol acetate is at least 3.3.

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

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 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). Disregard the two peaks due to methyl hydroxybenzoate and propyl hydroxybenzoate in the chromatogram obtained with solution (4).

Assay

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 μm). (Phenomenex Prodigy ODS3 is suitable.)

As the mobile phase, use a solution prepared as follows: mix 100 volumes of tetrahydrofuran R, 350 volumes of acetonitrile R and 500 volumes of water R, and filter through a 0.45-μm filter.

Prepare the following solutions in the mobile phase. For solution (1) dilute a suitable volume of the injection to be examined to obtain a concentration of 40 μg of Medroxyprogesterone acetate per ml. For solution (2) dissolve 10 mg of medroxyprogesterone acetate RS in 50 ml and mix. Dilute 5 ml of this solution to 20 ml with the mobile phase. For solution (3) use 20 μg of medroxyprogesterone acetate RS per ml and 50 μg of megestrol acetate RS per ml.

Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 241 nm.

Inject 20 μl each of solutions (1) and (2). The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to medroxyprogesterone acetate and to megestrol acetate is at least 3.3.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of medroxyprogesterone acetate (C\textsubscript{24}H\textsubscript{34}O\textsubscript{4}).

[Note from Secretariat: proposal to add a list of impurities, either at the end of this monograph or preferably, in a revised text for the API monograph; the impurities would then be designated within this text in replacing the chemical names (ex. megestrol acetate) by the corresponding assigned names (impurity A, B C, etc.).]

Ritonavir tablets

Draft proposal for the International Pharmacopoeia (August 2011). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail mendyc@who.int. All working documents are available for comment at http://www.who.int/medicines/publications/pharmacopoeia/mono_dev/en/index.html
Category. Antiretroviral (Protease Inhibitor).

Storage. Ritonavir tablets should be stored in a tightly closed container, at temperatures not exceeding 30 °C.

Additional information. Strength in the current WHO Model List of Essential Medicines: 25 mg, 100 mg.

Requirements

Comply with the monograph for “Tablets”.

Definition. Ritonavir tablets contain not less than 90.0% and not more than 110.0% of the amount of ritonavir \( \text{C}_{37}\text{H}_{48}\text{N}_{6}\text{O}_{5}\text{S}_{2} \) stated on the label.

Identity tests

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

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia \( \text{(~260 g/l)} \) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions in methanol R. For solution (A) shake a quantity of the powdered tablets containing 25 mg of Ritonavir with 5 ml and filter. Add 0.5 ml of ammonia \( \text{(~260 g/l)} \) TS to 2 ml of the filtrate and shake. For solution (B) use 2 ml of a 5 mg/ml solution of ritonavir RS. Add 0.5 ml of ammonia \( \text{(~260 g/l)} \) TS and shake. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

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

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Spray lightly with basic potassium permanganate \( \text{(~5 g/l)} \) TS and examine the chromatogram in daylight.

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

B. See the test described under Assay. 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).

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 \( \text{(0.1 mol/l)} \) VS, and rotating the paddle at 100 revolutions per minute. At 60 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool down to room temperature.

Determine the content of ritonavir \( \text{C}_{37}\text{H}_{48}\text{N}_{6}\text{O}_{5}\text{S}_{2} \) in the medium by 1.14.4 High-performance liquid chromatography, using the conditions described under Assay and a suitable solution of ritonavir RS as a reference solution.
For each of the six tablets tested, calculate the total amount of ritonavir (C_{37}H_{48}N_{6}O_{5}S_{2}) in the medium. 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 releases less than 60%.

**Related substances**

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

Prepare the following solutions using a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B as diluent. For solution (1) shake a quantity of powdered tablets containing 25 mg of Ritonavir with 50 ml of diluent, filter and use the clear filtrate. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 0.5 µg of Ritonavir per ml.

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

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

Maintain the column temperature at 35 °C.

Inject 20 µl of solution (3). The test is not valid unless the resolution between the principal peak (retention time about 22 minutes) and the peak with a relative retention of about 0.8 is at least 2.0. The test is also not valid unless the resolution between the principal peak and the peak with a relative retention of about 1.5 is at least 6.5. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

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 principal peak, is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the area of not more than two such peaks is greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%); the area of not more than four 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 ten times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%). Disregard any peak with a relative retention time less than about 0.5 in the chromatogram obtained with solution (3).

**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 C18 has been found suitable.)
Use the following conditions for gradient elution:

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

Mobile phase B: 70 volumes of acetonitrile R, 28 volumes of sodium phosphate buffer pH 4.0 and 2 volumes of water R.

Prepare the sodium phosphate buffer pH 4.0 by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate R and 1.88 g of sodium hexanesulfonate R in 800 ml of water R, adjust the pH to 4.0 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 ml with water R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>20–30</td>
<td>70 to 0</td>
<td>30 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–40</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>40–45</td>
<td>70</td>
<td>30</td>
<td>Isocratic re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions using a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B as diluent. For solution (1) shake a quantity of powdered tablets containing 25 mg of Ritonavir with 50 ml of the diluent, filter and use the clear filtrate. For solution (2) use 0.5 mg of ritonavir RS per ml of diluent.

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

Maintain the column temperature at 35 °C.

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

Inject 20 µl of solution (3). The test is not valid unless the resolution between the principal peak (retention time about 22 minutes) and the peak with a relative retention of about 0.8 is at least 2.0. The test is also not valid unless the resolution between the principal peak and the peak with a relative retention of about 1.5 is at least 6.5.

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 ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the tablets.