

## LEVAMISOLE TABLETS

### Draft proposal for *The International Pharmacopoeia*

(September 2010)

**REVISED DRAFT FOR COMMENT**

This document was provided by a quality control expert and was discussed at the recent WHO consultation on specifications for medicines and quality control laboratory issues. Previous comments received have been incorporated into this revised draft. Should you have any comments, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: [kopps@who.int](mailto:kopps@who.int) with a copy to Ms C. Mendy [mendyc@who.int](mailto:mendyc@who.int) by 26 October 2010.

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**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.364**  
*International Pharmacopoeia monograph on Levamisole tablets*

	<b>Date</b>
Preparation of first draft by laboratory	April-May 2010
Discussion at consultation on specifications for medicines and quality control laboratory issues	10-12 May 2010
Draft monograph mailed out for comments	July 2010
Collation of comments	August 2010
Revised draft discussed during video-/teleconference on specifications for medicines	25 August 2010
Revised draft mailed out for comments	September 2010
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	18-22 October 2010
Further action as necessary	

**Draft proposal for *The International Pharmacopoeia***  
**(September 2010)**

**LEVAMISOLE TABLETS**

**Category.** Anthelmintic.

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

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

**Additional information.** Strengths in the current WHO Model list of essential medicines: 50 mg, 150 mg. Strengths in the current WHO Model list of essential medicines for children: 50 mg, 150 mg

**Requirements**

Comply with the monograph for "Tablets".

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

**Identity tests**

- Either tests A, B and D or tests A, C and D may be applied.
- A. Shake a quantity of the powdered tablets containing the equivalent of about 450 mg of levamisole with 30 ml of water R, filter. Wash the filter with 20 ml of water R and add the washings to the filtrate. To the combined filtrate add ammonia (~100 g/l) TS to make it alkaline and extract with two quantities, each of 25 ml and 15 ml, of dichloromethane R. Combine the dichloromethane extracts and evaporate to dryness. Add 0.5 ml of hydrochloride acid (~420 g/l) TS, heat on a water-bath to dryness. Dissolve the residue in 10 ml of hydrochloric acid (0.1 mol/l) VS. The optical rotation of the resulting solution is not less than  $-5^\circ$ .
- B. See the test described below under Related substances using ultraviolet light (254 nm) to examine the chromatogram. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution D.

- C. See the test described below under Assay, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).
- D. Shake a quantity of the powdered tablets containing the equivalent of about 100 mg of levamisole with 40 ml of water R and filter. The filtrate yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

### Related substances

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

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

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

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

### Assay

- Either method A or method B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (3 µm)<sup>1</sup>.

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

Mobile phase A: acetonitrile R

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

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<sup>1</sup> Phenomenex Gemini C18 has been found suitable.

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

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

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

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

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of levamisole (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>S) in the tablets.

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

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

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Revised draft for comment