A Concise Quality Control Guide on Essential Drugs and Other Medicines

# Manual

Accompanying the GPHF-Minilab®

**Extension 2007**
**More Antivirals**

## Special Edition on Oseltamivir
## Thin Layer Chromatography

GPHF
German Pharma Health Fund e.V.

USAID
FROM THE AMERICAN PEOPLE

USP
U.S. Pharmacopeia
*The Standard of Quality*℠

A Global Assistance Initiative of Research-based Pharmaceutical Companies in Germany
and the United States Pharmacopeia Drug Quality and Information Program

## WHY HAS THE GPHF-MINILAB BEEN DEVELOPED?

On the consumer level, counterfeit money or fake watches, jewelry, and software may still be detected with the naked eye. This does not apply to medicines where the final consumer usually cannot distinguish between good and bad medicines. Due to this and to the widespread danger of counterfeit medicines, quality control in the distribution systems of developing countries has taken on a broader role today. If adherence to good medicine manufacturing and trading practices cannot be assumed, a greater number of samples must be tested in order to maintain an appropriate assurance of drug quality and a high level of patient protection. Yet at the same time, pharmacopeial analyses have become more and more expensive and only a few centers of excellence in some countries are currently available to perform them. The development and use of simple tests can bridge the capacity gap in drug quality testing in low-income countries and facilitate a balance between the need to increase the extent of drug testing on the one hand, and the need to contain costs on the other.


Fake or genuine

## WHAT DOES A MINILAB CONTAIN?

GPHF-Minilabs contain the essential lab ware and chemicals, as well as authentic tablets and capsules for reference purposes. Supplies include sufficient quantities in order to perform about 1000 assays while ensuring that the total material costs for one test run do not exceed two Euros. Two suitcases contain the essential components - a full range of glassware for sample extraction, preparation, pipetting and spotting, high performance chromatographic plates, developing and detection chambers, UV lamps with different wavelengths, a hot plate, a spirit lamp, test tubes, caliper rules and storage containers. Even pens and pencils are included. If needed, a digital pocket balance can easily be added. Of particular importance is a full collection of authentic secondary reference standards for more than forty active ingredients and a set of manuals providing simple operation procedures. Written in a non-scientific format and rich in illustrations, the manuals read more like a cooking recipe than an instruction booklet; they are also available in French and Spanish.


GPHF-Minilab® TLC Kit

## WHAT IS THE MINILAB CAPABLE OF DOING

The GPHF-Minilab takes the basic drug testing scheme published by the World Health Organization (WHO) some thirty years ago into the 21st century. New test methods have been introduced, and supplied are not only operation manuals printed in different languages, but also a complete range of lab ware, starter kit chemicals and reference standards - all suitably packed for global shipment by air. Now, counterfeit medicines containing wrong, too little, or no ingredients at all can be identified instantly anywhere in the world. Results obtained by a set of physical and chemical screening tests must match the product label claims for, at least, drug identity and content. If they do not match or results are inconclusive, then the appropriate batches can be frozen for further investigation. GPHF-Minilab tests cannot replace extensive testing on questions of drug release, chemical purity, or microbial burden; those and detailed forensic testing for court actions must be referred to a comprehensive drug quality control laboratory that employs legally accepted methods. The GPHF-Minilab has been developed for rapid drug quality verification and counterfeit medicines detection only.

Counterfeit Antimalarial Pills (right) and Genuine Product (left)

## WHO IS USING MINILABS?

Vertical priority health programs frequently use GPHF-Minilabs to monitor the quality of medicines in malaria-, TB- and AIDS-endemic countries. In Tanzania, GPHF-Minilabs are already used as a first-line defense to protect the country's communities against the infiltration of substandard quality and counterfeit medicines. Major support comes from the World Health Organization (WHO), the United States Agency for International Development (USAID) and the U.S.Pharmacopeia Drug Quality and Information Program.

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# Oseltamivir

## Primary Screening via Visual Inspection & Disintegration Test

### I. VISUAL INSPECTION

Search for deficiencies on labeling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual. Write down all product particulars using the *Reporting Form* as a guide. Each capsule usually contains 98.5 mg of oseltamivir phosphate, equivalent to 75 mg of oseltamivir free base.

### II. DISINTEGRATION TEST

All quick-release oseltamivir capsules must pass the disintegration test as described in the opening chapters on general methods and operations of the main manual. They should disintegrate in water at 37 ˚C in less than 30 minutes. It is a major defect if a drug product does not pass this test.

### III. RESULTS & ACTIONS TO BE TAKEN

Drug products from unusually cheap sources, drug products with missing or incorrect accompanying documents and drug products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels written in a foreign language should be subjected to an identity test.

## Verification of Drug Identity and Content via Thin Layer Chromatography

### I. PRINCIPLE

Oseltamivir is extracted from capsules or tablets with methanol and determined by TLC with reference to an authentic secondary standard.

### II. EQUIPMENT AND REAGENTS

**1)** Pestle
**2)** Aluminium foil
**3)** Laboratory glass bottles with a filling capacity of 25 to 100 ml
**4)** Funnel
**5)** Set of straight pipettes (1 to 25 ml)
**6)** 10-ml vials
**7)** Label tape
**8)** Marker pen
**9)** Pencil
**10)** Merck TLC aluminium plates pre-coated with silica gel 60 F254, size 5x10 cm
**11)** Glass microcapillaries of 2-µl filling capacity
**12)** Hot plate
**13)** TLC developing chamber (jar)
**14)** Filter paper
**15)** Pair of scissors
**16)** UV light of 254 nm
**17)** Methanol
**18)** Ethylacetate
**19)** Toluene
**20)** Ammonia solution 25%
**21)** Oseltamivir 75 mg reference capsules

## III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic drug product for reference purposes, for example, a capsule containing 75 mg of oseltamivir. Carefully open and empty the capsule over a 25-ml laboratory glass bottle, adding the cap and body shells into the bottle last. Add 10 ml of *methanol* using a straight pipette, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to sit for an additional five minutes until undissolved residues settle below the hazy supernatant liquid. The solution obtained should contain 7.5 mg of total drug per ml and be labeled as *'Oseltamivir Stock Standard Solution.'* Freshly prepare this solution for each test.

## IV. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)

The oseltamivir stock standard solution requires no further dilution. It already represents the final working concentration of 7.5 mg of total drug per ml.

This working standard solution represents a drug product of good quality containing 100 % of oseltamivir.

## V. PREPARATION OF THE WORKING STANDARD SOLUTION 80% (LOWER WORKING LIMIT)

Pipette 4 ml of the stock standard solution into a 10-ml vial and add 1 ml of *methanol*. Close and shake the vial. The solution obtained should contain 6.0 mg of total drug per ml and be labeled as *'Oseltamivir Working Standard Solution 80%.'*

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of oseltamivir as stated on the product's label. In the current investigation, this drug level represents the lower acceptable limit for a given product.

## VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 75 MG OSELTAMIVIR PER UNIT

The preparation of a stock sample solution requires a whole tablet or capsule from an appropriate drug product sampled in the field. Oseltamivir is extracted completely from the sample using the same procedure as for the authentic reference standard: tablets are wrapped up into aluminium foil and crushed down to a fine powder prior to transfer into a 25-ml laboratory glass bottle. Powder obtained from a capsule should be transferred directly into the laboratory glass bottle, putting the empty cap and body shells into the bottle last. Add 10 ml of *methanol* using a straight pipette, close the bottle, and shake for about three minutes until most of the solids are dissolved. Allow the solution to sit for an additional five minutes until any undissolved residue settles below the hazy supernatant liquid. The final solution should contain 7.5 mg of total drug/ml and be labeled as *'Oseltamivir Stock Sample Solution.'* Freshly prepare this solution for each test.

## VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Oseltamivir stock sample solutions require no further dilution. They already represent the final working concentration of 7.5 mg of total drug per ml.

## VIII. SPOTTING

Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2 µl of each test and standard solution prepared as shown in the picture opposite using the microcapillary pipettes supplied.

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameter never should. Different intensities are due to residual amounts of tablet and capsule excipients or different drug concentrations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

## IX. DEVELOPMENT

Pipette 8 ml of *methanol*, 6 ml of *ethylacetate,* 4 ml of *toluene* and 2 ml of *concentrated ammonia solution* into the jar being used as a TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes, thus ensuring saturation of the chamber with solvent vapor. Carefully place the loaded TLC plate into the jar. Close the jar and develop the chromatoplate until the solvent front has moved about three-quarters of the length of the plate, the developing time being about 15 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate using a hot plate if necessary.

## X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm with the battery-driven lamp supplied. Use this method of detection for both, identification and quantification purposes.

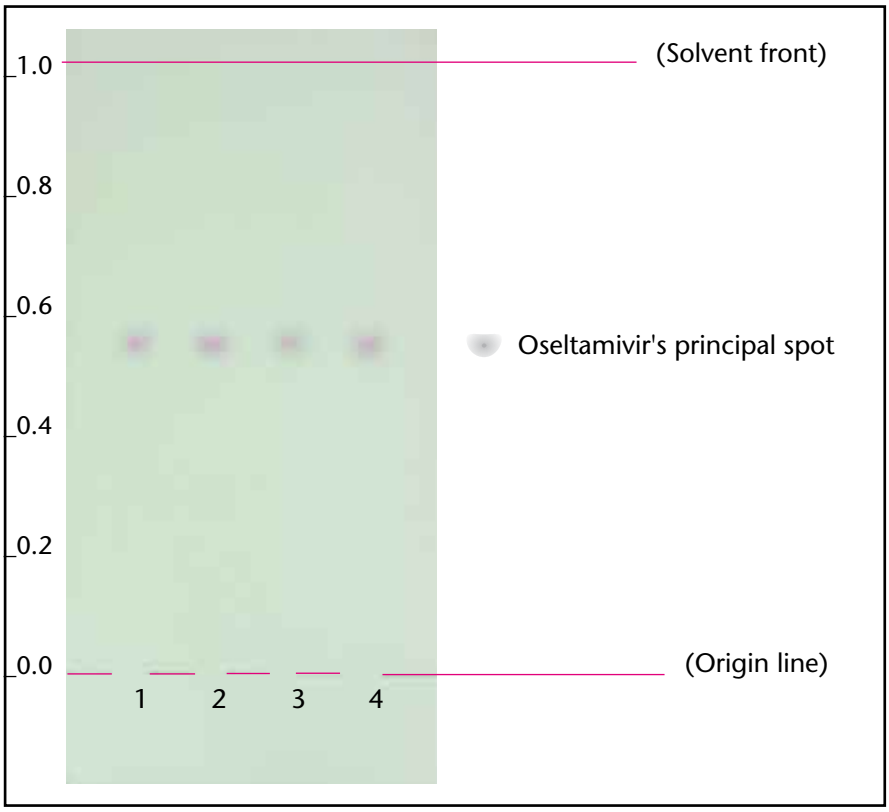
## XI. CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

Run No.1:
Oseltamivir's upper working limit representing 100% of total drug.

Run No.2:
A drug product of good quality.

Run No.3:
A drug product of poor quality.

Run No.4:
Oseltamivir's lower working limit representing 80% of total drug.




## XII. OBSERVATIONS MADE AT 254 NM

The presence of oseltamivir is indicated by a blue-violet spot at a travel distance of about 0.57. Additional strong spots generated by the test solution indicate drug degradation especially when associated with a smaller principal spot. Some fainter spots emerging near or on the origin line of the chromatoplate are normally caused by auxiliary agents incorporated in the different tablet and capsule formulations. The existence of relabeled aciclovir products would be indicated by a very strong blue-violet spot at a travel distance of about 0.23.

## XIII. RESULTS & ACTIONS TO BE TAKEN

The principal spot in the chromatogram obtained with the test solution must correspond in terms of color, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solutions. If this is not achieved, repeat the run from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken.