



U.S. PHARMACOPEIA  
DRUG QUALITY AND  
INFORMATION PROGRAM



# SIMPLE, RAPID BASIC TESTS FOR EVALUATION OF QUALITY OF OSELTAMIVIR

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## ABSTRACT

Since its emergence in Southeast Asia in mid-2003, Avian Influenza (AI) has spread across Asia, into some countries of Europe and Eurasia, and to Africa. Due to the rapid spread of the virus, there has been an increasing concern that it could potentially develop into a global human pandemic.

Osetamivir phosphate is an antiviral drug that is effective against avian influenza caused by the H5N1 virus, which will be the principle medicine for reducing the morbidity and mortality it causes. Due to high demand and long manufacturing lead times, stockpiling osetamivir has been a central strategy of many country government plans for preparedness against an AI pandemic. The true shelf-life of osetamivir medicines is uncertain but is estimated to be between three and five years, depending on formulation and

storage conditions. Therefore, all stockpiled and circulated osetamivir products should be tested periodically to ensure that their quality meets pharmacopeial requirements.

To ensure that only good quality medicines reach priority public health programs, the Global Pharma Health Fund (GPHF) and U.S. Pharmacopeia Drug Quality and Information (USP DQI) program, with the generous support from the U.S. Agency for International Development (USAID), developed a simple and affordable screening method for osetamivir phosphate. This presentation describes three basic analytical techniques—physical/visual examination, simplified disintegration, and thin layer chromatography (TLC)—validated at the U.S. Pharmacopeia Research and Development Laboratory that can be used in non-laboratory settings, e.g., medicines warehouses, hospitals, field locations, and surveillance sites.

## METHODS

### VISUAL INSPECTION:

Visually examining for deficiencies on labeling, packaging and dosage form:

- Missing or incorrect accompanying documents;
- Packaging with incomplete, damaged, missing labels or with indelible print;
- Container seal broken;
- Defective dosage forms e.g. tablet cracked, broken, crushed, sticky, non-uniform coloration.

Visual and physical inspection of pharmaceutical products is the first step in any drug quality control testing.

A label of a finished dosage form should at least contain:

1. The name of the drug product;
2. The name of each active pharmaceutical ingredient (API), with international non-proprietary or generic name;
3. The drug's strength or content of each active ingredient;
4. The dosage form and the number of unit doses in the container or package;
5. The batch or lot number;
6. The date of manufacture and expiration date;
7. Storage conditions and handling precautions as necessary;
8. The name and address of the manufacturer, company, or person responsible for placing the product on the market; and
9. Instructions for use.



Each capsule usually contains 98.5 mg of osetamivir phosphate, equivalent to 75 mg of osetamivir free base.

### DISINTEGRATION TEST

Disintegration is defined as that state in which no residue of the tablets and capsules, except fragments of undissolved coating, remains in the test solution.

#### Procedure:

1. Place one tablet or capsule into a 100-150 mL wide neck bottle containing 100 mL water at close to 37± 2° C.
2. Stir or shake the liquid by swirling the bottle periodically.
3. Read and record the time (in minute) when disintegration is complete.
4. Repeat the test on a five (5) additional tablets or capsules.

#### Test evaluation:

- The batch **passes** disintegration test if all six tablets or capsules disintegrate within 30 minutes. Should one (1) tablet or capsule fail to disintegrate, repeat the entire test cycle.
- The batch **fails** disintegration test if one of the additional tablets or capsules fail again in the second run.



**All quick-release osetamivir capsules must pass the disintegration test. 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.**

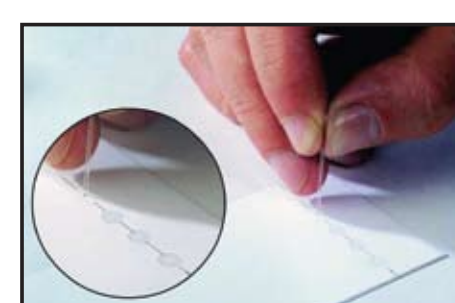
### THIN LAYER CHROMATOGRAPHY (TLC):

#### Experimental:

- TLC plates:** Merck TLC aluminum plates pre-coated with silica gel 60 F254, size 5x10 cm
- Developing solvent:** 8 ml of methanol, 6 ml of ethylacetate, 4 ml of toluene and 2 ml of concentrated ammonia solution
- Detection:** UV light of 254 nm
- Reference Standard (RS):** Osetamivir 75 mg reference capsules
- Standard solution 100% (upper working limit):** 7.5 mg/ml of RS in methanol
- Standard solution 80% (lower working limit):** 6.0 mg/ml of RS in methanol
- Working Sample Solution:** Osetamivir is extracted from capsules or tablets sampled in the field with 10 ml of methanol
- Spotting:** 2 µl of each test and standard solution



Working solutions



Spotting



TLC development



Detection

### CHROMATOGRAPHY:

#### Run No.1:

Osetamivir's upper working limit representing 100% of total drug.

#### Run No.2:

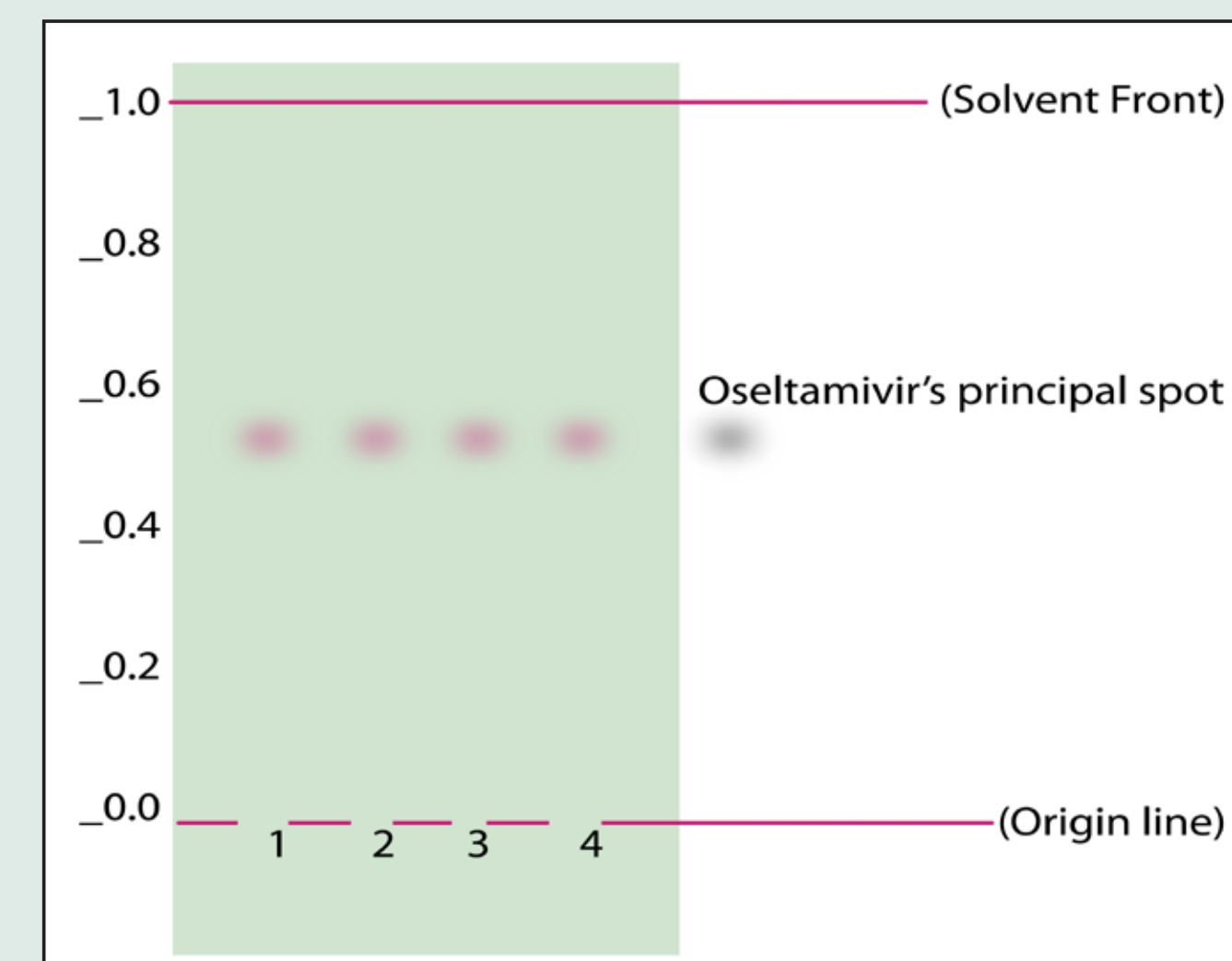
A drug product of good quality.

#### Run No.3:

A drug product of poor quality.

#### Run No.4:

Osetamivir's lower working limit representing 80% of total drug.



### OBSERVATIONS:

- The presence of osetamivir 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;
- The existence of relabeled aciclovir products would be indicated by a very strong blue-violet spot travel distance of about 0.23.

### 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.

## DISCUSSION

Counterfeit and substandard medicines are global concerns that contribute to poor treatment outcomes and may cause drug resistance and even death. Counterfeits are present in all regions, but developing countries bear the brunt of the problem. Therefore, the development and use of simple and affordable analytical techniques for rapid drug quality verification and detection of counterfeit medicines is essential in low-income countries. GPHF has developed the Minilab® for analyzing the authenticity of a wide range of essential drugs relatively simply and inexpensively compared to other analytical techniques currently used for checking the quality of medicines.

Osetamivir phosphate is one of the main drugs currently being stockpiled by international government agencies for use as the first line of defense against outbreaks of pandemic influenza. The true shelf life of osetamivir products is uncertain—somewhere between three and five years—depending on the formulation and storage conditions. Consequently, the quality of all stockpiled and circulated osetamivir products should be tested periodically.

To ensure that only good quality medicines reach priority public health programs, the GPHF and USP DQI program developed and validated a simple and affordable screening method for osetamivir phosphate. The method can be used in non-laboratory settings, e.g., medicines warehouses, hospitals, field locations, and surveillance sites.

## CONCLUSION

Simple and rapid basic tests (visual examination, simple disintegration, and TLC) for evaluating the quality of osetamivir products were developed and validated. The Testing Manual accompanying the GPHF-Minilab is published. The developed tests will allow public health officials to verify the quality of osetamivir products.

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