

# Manual

Accompanying the GPHF-Minilab™

Supplement 2015

Volume II

## THIN LAYER CHROMATOGRAPHIC TESTS



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Merck Darmstadt · Germany



**USAID**  
FROM THE AMERICAN PEOPLE



PROMOTING THE QUALITY OF MEDICINES

# A Concise Quality Control Guide on Essential Drugs and other Medicines

## **SUPPLEMENT 2015 TO VOLUME II ON THIN LAYER CHROMATOGRAPHIC TESTS**

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### **About the GPHF-Minilab™ Project**

Counterfeit medicines proliferation constitutes serious health hazards. The international police organisation Interpol estimates that a disturbing proportion of ten to thirty percent of all drugs offered in developing countries are either counterfeit or of deficient quality already. Fighting falsified medicines will ensure that decades of investments in healthcare are not undone through lack of vigilance.

To prevent counterfeit and extreme poor anti-infective medicines infiltrating drug supply organisations and priority disease programmes in malaria, TB and HIV/AIDS endemic countries, the Global Pharma Health Fund (GPHF) in Frankfurt, a charity maintained exclusively by Merck Darmstadt · Germany, set out to develop and supply at low cost the GPHF-Minilab™, a mini-laboratory for rapid drug quality verification and counterfeit medicines detection.

Since many years, GPHF-Minilabs are acting as a first-line defence against counterfeit and substandard quality medicines threatening the health of millions of people living in developing nations. Overall, more than 680 Minilabs have been supplied to 90 countries across the African, Asian-Pacific and Latin American region already.

Main implementation partners are national health and medicines regulatory authorities together with the World Health Organization and the U.S. Pharmacopeia's Promoting the Quality of Medicines programme. Joint drug quality monitoring projects run by Interpol in South East Asia and East Africa triggered off the seizure of millions of counterfeit antimalarial pills without any active principles in the recent years.

The unchanged need for non-sophisticated and affordable drug quality monitoring in low-income countries forms the driving force behind the development of new GPHF-Minilab™ test protocols today. The need for more testing emphasises the important collaboration with our US based implementing partners. For more patient safety and better health in developing countries, other parties are invited to join in.

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## 6.80 Atenolol

### Primary Screening via Physical Inspection and Disintegration Test

#### I. PHYSICAL INSPECTION

Search for deficiencies on labelling, 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 tablet or capsule usually contains 25, 50 or 100 mg of atenolol.

#### II. DISINTEGRATION TEST

All quick release atenolol tablets and 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 a thin layer chromatographic test.

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

#### I. PRINCIPLE

Atenolol is extracted from tablets and capsules with methanol and determined by TLC with reference to an appropriate secondary standard.

#### II. EQUIPMENT AND REAGENTS

- 1) Pestle
- 2) Aluminium foil
- 3) Funnel
- 4) Label tape
- 5) Marker pen
- 6) Pencil and ruler
- 7) 10-ml vials
- 8) Set of straight pipettes (1 to 25 ml)
- 9) Set of laboratory glass bottles (25 to 100 ml)
- 10) Merck TLC aluminium plates pre-coated with silica gel 60 F<sub>254</sub> size 5x10 cm
- 11) Glass microcapillaries (2-µl filling capacity)
- 12) TLC developing chamber (500-ml jar)
- 13) Hot plate
- 14) Filter paper
- 15) Pair of scissors
- 16) Pair of tweezers
- 17) UV light of 254 nm
- 18) Iodine chamber
- 19) TLC dipping chamber (250-ml beaker)
- 20) Ninhydrin
- 21) Methanol
- 22) Ammonia solution 25%
- 23) Reference standard, for example atenolol 50 mg tablets

### III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic drug product for reference purposes, for example, tablets containing 50 mg of atenolol. Wrap up one reference tablet into aluminium foil and crush it down to a fine powder using a pestle. Carefully empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solids with 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 supernatant liquid. The solution obtained should contain 5 mg of total atenolol per ml and be labelled as '*Atenolol Stock Standard Solution*'. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

The stock standard solution requires no further dilution. It already represents the final working concentration of 5 mg of total atenolol per ml. Just for more convenient handling, some of the supernatant liquid may want to be transferred into a 10-ml vial.

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

### 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 4 mg of total drug per ml and be labelled as '*Atenolol Working Standard Solution 80%*'.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of atenolol 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 PRODUCT CLAIMING TO CONTAIN 25 MG OF ATENOLOL PER UNIT

Take one whole tablet or capsule from an appropriate drug product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 5 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 supernatant liquid.

#### 50 MG OF ATENOLOL PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 10 ml of methanol using a straight pipette and a 25-ml laboratory glass bottle. Continue to work as above.

#### 100 MG OF ATENOLOL PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 20 ml of methanol using a straight pipette and a 40-ml laboratory glass bottle. Continue to work as above.

All stock sample solutions produced should finally contain 5 mg of total atenolol per ml and be labelled as '*Atenolol Stock Sample Solution*'. Freshly prepare these solutions for each test. Continue to work with the clear or hazy supernatant liquids.

## VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Atenolol stock sample solutions require no further dilution. They already represent the final working concentration of 5 mg of atenolol per ml. If prepared from a high quality product, the sample solution should match the concentration of atenolol of the higher working standard solution produced above.

## VIII. SPOTTING

Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2  $\mu$ l of each test and standard solution 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 diameters 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 20 ml of methanol and 0.2 ml of concentrated ammonia solution into the jar being used as 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 vapour. 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 first under UV light of 254 nm using the battery-driven lamp supplied. Then, expose the plate to iodine vapour for about one minute. Use the iodine staining for both, atenolol identification and quantification purposes.

Further verification of drug identity and content can be achieved when immersing the iodine plate in ninhydrin staining solution. However, the staining result will be more pronounced when using a freshly developed plate without prior contact to iodine. For the staining, weigh in 3 g of ninhydrin (about 10 times a well-filled spatula) and dissolve in a mix of a 150 ml of methanol and 30 ml of glacial acetic acid. Submerge the iodine plate into the staining solution using a pair of tweezers. Instantly remove the plate again from the solution and let all surplus liquid run down onto paper tissue. Wipe off residual liquid from the back of the plate and continue to dry off all staining solution at full level of the hot plate supplied. During heating, all atenolol spots are gradually becoming visible at daylight after about one minute. Again, use this method of detection for both, atenolol identification and quantification purposes. The ninhydrin staining process is illustrated on page 26 of the main manual issued 2008. Note that skin contaminated with ninhydrin solution will be stained as well. However, this is not dangerous to health and the violet spots will disappear after about a day or two.

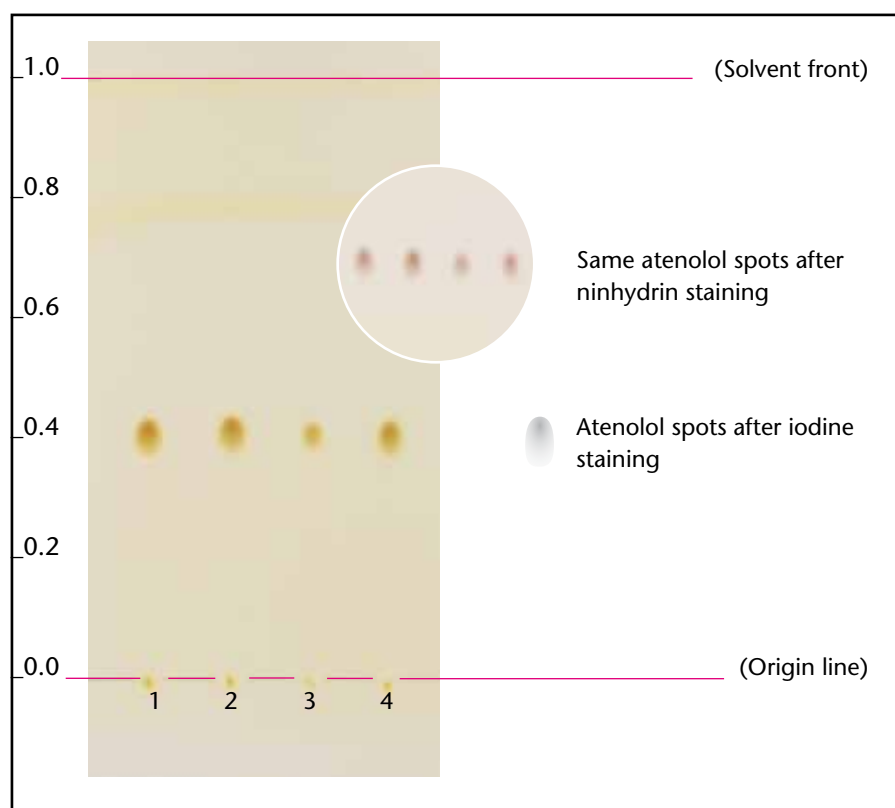
## XI. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

Run No.1:  
Upper working standard  
representing 100% of total atenolol

Run No.2:  
A product of good quality with  
acceptable atenolol content

Run No.3:  
A product of poor quality with  
unacceptable low atenolol content

Run No.4:  
Lower working standard  
representing 80% of total atenolol



## XII. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.41 indicates the presence of atenolol in the test solution. However, atenolol performs weak here.

## XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all atenolol spots already observed at UV-light of 254 nm are now turning deep orange brown. Atenolol performs strong here. Additional strong spots generated by the test solution would point at other drugs or atenolol degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principle spot from the test solution may also indicate a poor atenolol content and no spot at all complete atenolol absence. Still observe the plate when iodine evaporates already. Spots reflecting poor quality products will disappear first gradually followed by the reference spots representing a drug content of an 80 and 100 percent, respectively. Auxiliary agents incorporated in the different tablet or capsule formulations might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

## XIV. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHYDRIN STAINING

When exposing the iodine plate to ninhydrin and heat, all atenolol spots already observed during iodine staining are now turning reddish brown or even deep purple if a freshly developed plate without previous contact to iodine is used. Again, a smaller principle spot from the test solution will indicate a poor atenolol content and no spot complete atenolol absence.

## XV. RESULTS & ACTIONS TO BE TAKEN

The atenolol spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. 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. For documentation purposes, take a picture of the reading with a digital camera turning off the flash first.

# Genuine or Fake?



Fighting Counterfeit Medicines · Protecting People's Life



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