A Concise Quality Control Guide On Essential Drugs And Other Medicines

Manual

Accompanying The GPHF-Minilab®

Extension 2003
Antiretrovirals

Third Supplement To Volume II
Thin Layer Chromatography

GPHF
German Pharma Health Fund e.V.

An Initiative of Research Based Pharmaceutical Companies in Germany

7.34 Nevirapine - Incl. fixed combinations with lamivudine and stavudine

Primary Screening via Visual Inspection & Disintegration Test

I. VISUAL 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 usually contains 200 mg of nevirapine. Fixed combination products with other antiretrovirals, for example lamivudine and stavudine are more and more common. Furthermore, suspensions for oral administration are available usually containing 10 mg of nevirapine per millilitre.

II. DISINTEGRATION TEST

All quick release nevirapine tablets 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 chromatograpic assay.

Verification of Identity and Drug Content via Thin Layer Chromatograpy

I. PRINCIPLE

Nevirapine suspensions are diluted and tablets or capsules are extracted with acidified water and determined by TLC with reference to an authentic secondary reference standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Spatula
3) Aluminium foil
4) Laboratory glass bottles with a filling capacity of 25 to 100 ml
5) Funnel
6) Set of straight pipettes (1 to 25 ml)
7) 10-ml vials
8) Label tape
9) Marker pen
10) Pencil
11) Merck TLC aluminium plates pre-coated with silica gel 60 F254, size 5x10 cm
12) Glass microcapillaries of 2-µl filling capacity
13) Hot plate
14) TLC developing chamber (jar)
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV lamp of 254 nm
19) Iodine chamber
20) pH indicator test paper
21) Hydrochloric acid solution 36%
22) Water
23) Methanol
24) Ethylacetate
25) Toluene
26) Authentic reference standard, for example nevirapine 200 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION	The preparation of a stock standard solution requires an authentic product for reference purposes, for example a tablet containing 200 mg of nevirapine. Wrap up the tablet in aluminium foil and crush it down to a fine powder using a pestle. Empty the foil into a 50-ml laboratory glass bottle and wash down all residual solids with 40 ml of water using a straight pipette. Acidify below pH 3 with three drops of concentrated hydrochloric acid using a transfer pipette and verify acidity with the pH indicator test paper supplied. Close the bottle and shake for about three minutes till most of the solids are dissolved. Leave the solution to sit for further five minutes and allow the undissolved residues to settle at the bottom. The solution obtained should contain 5 mg of total drug per ml and be labelled as *'Nevirapine Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the hazy supernatant liquid.
IV. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)	Pipette 1 ml of the stock standard solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial. The solution obtained should contain 1.25 mg of total drug per ml and be labelled as *'Nevirapine Working Standard Solution 100%'*. This higher working standard solution represents a drug product of good quality containing 100 % of total nevirapine.
V. PREPARATION OF THE WORKING STANDARD SOLUTION 80% (LOWER WORKING LIMIT)	Pipette 1 ml of the stock standard solution into a 10-ml vial and add 4 ml of methanol. Close and shake the vial. The solution obtained should contain 1.00 mg of total drug per ml and be labelled as *'Nevirapine Working Standard Solution 80%'*. This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of nevirapine 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 SOLID DOSAGE FORMS CLAIMING A POTENCY OF 200 MG OF NEVIRAPINE PER UNIT	Take one whole tablet or capsule from an appropriate drug product sampled in the field. Tablets are wrapped up into aluminium foil and crushed down to a fine powder prior to transfering into 50-ml laboratory glass bottle. Powder obtained from a capsule should be put directly into the laboratory glass bottle adding finally the empty cap and body shells as well. Then, add 40 ml of water using a straight pipette, acidify below pH 3 with three drops of concentrated hydrochloric acid and verify the acidity with the pH indicator test paper supplied. Close the bottle and shake for about three minutes till most of the solids are dissolved. Allow the solution to sit for further five minutes until the undissolved residue settles below the hazy supernatant liquid.
LIQUID DOSAGE FORMS CLAIMING A POTENCY OF 10 MG OF NEVIRAPINE PER ML	Open one sample container, transfer 4 ml of the suspension presented into a 10-ml laboratory glass bottle and dilute with 4 ml of water using each time straight pipettes of appropriate size. Acidify below pH 3 with one drop of concentrated hydrochloric acid using a transfer pipette and verify the acidity with the pH indicator test paper supplied. Close the bottle and shake for about three minutes till most of the solids are dissolved. Allow the solution to sit for a further five minutes until the undissolved residue settles below the clear or hazy supernatant liquid. All solutions finally obtained should contain 5 mg of total drug per ml and be labelled as *'Nevirapine Stock Sample Solution*'. Freshly prepare these solutions for each test. Continue to work with the hazy supernatant liquids or clear to hazy dilutions obtained.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 1 ml of the stock sample solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial and label it as *'Nevirapine Working Sample Solution'*.

The expected concentration of total drug in this solution is 1.25 mg/ml and should match the concentration of nevirapine 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 µl 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 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 a homogeneous spotting is not achieved first time.

IX. DEVELOPMENT

Pipette 11 ml of ethylacetate, 5 ml of methanol and 4 ml of toluene 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 again 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 with UV light of 254 nm using the battery-driven fluorescent lamp supplied. Use this method of detection for quantification purposes. Further verification of drug identity and content can be achieved when observing the same plate in daylight after iodine staining.

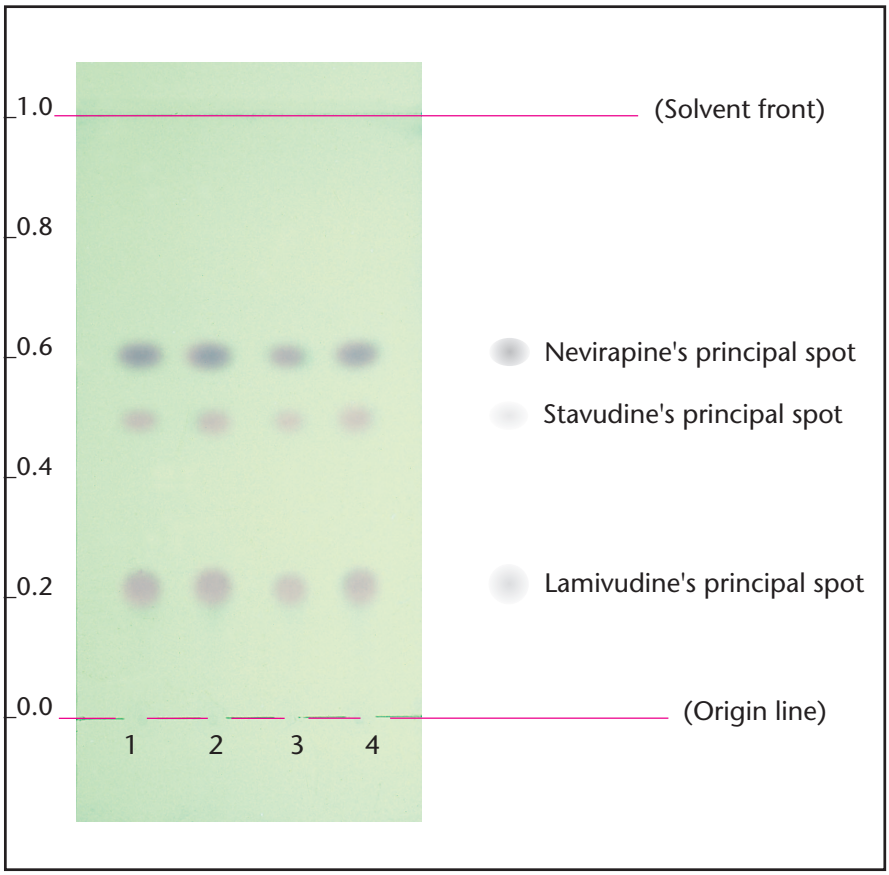
XI. CHROMATOPLATE OBSERVED AT 254 NM

Run No.1:
A standard solution representing 100% of total nevirapine, stavudine and lamivudine as upper working limit.

Run No.2:
A fixed triple combination product of good quality.

Run No.3:
A fixed triple combination product of poor quality.

Run No.4:
A standard solution representing 80% of total nevirapine, stavudine and lamivudine as lower working limit.



XII. OBSERVATIONS MADE AT 254 NM

The presence of nevirapine is indicated by a strong blue-violet spot at a travel distance of about 0.60 when the chromatoplate is observed with the UV lamp supplied. In antiretrovirals where nevirapine is presented in a fixed combination with stavudine and lamivudine, a second and third principal spot can be observed at travel distances of about 0.48 and 0.21 respectively. 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 different product formulations.

XIII. OBSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, several weak orange-brown spots are generated matching the pattern of spots already observed on the plate exposed to UV light of 254 nm.

XIV. RESULTS & ACTIONS TO BE TAKEN

The principal spot/spots 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 with a second sample from scratch. Reject the batch if the drug content can't be verified in a third run. For a second opinion, refer additional samples to a fully equipped drug control laboratory. Retain samples and put the batch on quarantine till a final decision on rejection or release has been taken.