

DRAFT PROPOSAL FOR AMENDMENTS(Uploaded on 23rd October 2020)

The following Draft proposal for Amendments are placed for stakeholders comments, if any. The comments may be sent to the Indian Pharmacopoeia Commission through email id lab.ipc@gov.in

2.3.34. Oxygen-Flask Method, Page 149

Insert before 2.3.35. Peroxide Value

For Selenium

Diaminonaphthalene solution. Dissolve 0.1 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylamine hydrochloride in 0.1M hydrochloric acid and dilute to 100 ml with the same solvent (*Note- Use freshly prepared solution*).

Test solution. Burn the specified quantity of the substance under examination in the prescribed manner using 25 ml of dilute nitric acid (1 in 30) as the absorbing liquid. When the process is complete, add a few ml of water in the cup, loosen the stopper, and rinse the stopper, the specimen holder, and the sides of the flask with water. Transfer the solution with the aid of about 20 ml of water to a 150-ml beaker, and heat gently to the boiling temperature. Boil for 10 minutes, and allow the solution to cool to room temperature.

Reference solution. Dissolve 40 mg of metallic selenium in 100 ml of dilute nitric acid (1 in 2) in a 1000-ml volumetric flask, gently heat on a water-bath if necessary, dilute to volume with water. Dilute 5.0 ml of the solution to 200.0 ml with water (1 ml contains 1 µg of selenium (1 ppm)). To 6.0 ml of the solution into a 150-ml beaker, add 25 ml of dilute nitric acid (1 in 30) and 25 ml of water.

Treat the reference solution, the test solution and the reagent blank consisting 25 ml of dilute nitric acid (1 in 30) and 25 ml of water, at the same time and in the same manner, using ammonium hydroxide solution (1 in 2) adjusted to pH 2.0. Dilute with water to 60 ml and transfer to a low-actinic separator with the aid of 10 ml of water, adding 10 ml of water for rinsing to the separator. Add 0.2 g of hydroxylamine hydrochloride, and swirl to dissolve, immediately add 5.0 ml of diaminonaphthalene solution, insert the stopper, and swirl to mix. Allow the solution to stand at room temperature for 100 minutes. Add 5.0 ml of cyclohexane, shake vigorously for 2 minutes, and allow the layers to separate. Discard the aqueous layer, and centrifuge the cyclohexane extract to remove any dispersed water. Determine the absorbance of the cyclohexane extracts of the test solution and the reference solution in a 1-cm cell at the wavelength of maximum absorbance at about 380 nm (2.4.7), using the cyclohexane extract of the reagent as the blank, and compare the absorbances. The absorbance of the test solution is not more than that of the reference solution where a 0.2 g test substance has been taken, or is not more than one-half that of the reference solution where a 0.1 g test substance has been taken.

Note- Clear combustion of the test material is an important factor in conducting the test. For compounds that burn poorly and produce soot, the addition of magnesium oxide usually results in more thorough combustion and reduces ash formation. Where the need to add magnesium oxide has been identified, it is specified in the individual monograph.

2.4.42. Inductively Coupled Plasma-Mass Spectrometry. Page 287

Title

Change to: **2.4.42. Inductively Coupled Plasma Spectrometry**

Insert after Title

Inductively Coupled Plasma Spectrometry is a plasma-based instrumental technique, which is useful for pharmaceutical analyses. The inductively coupled plasma (ICP) is a high-temperature excitation source that desolvates, vaporizes, and atomizes aerosol samples and ionizes the resulting atoms. The excited analyte ions and atoms can then subsequently be detected by observing their emission lines, a method termed inductively coupled plasma-atomic emission spectroscopy (ICP-AES; also referred to as inductively coupled plasma optical emission spectroscopy), or the excited or ground state ions can be determined by a technique known as inductively coupled plasma-mass spectrometry (ICP-MS). ICP-AES and ICP-MS may be used for either

single- or multi-element analysis and used for either sequential or simultaneous analyses with good sensitivity over an extended linear range.

Inductively Coupled Plasma-Mass Spectrometry

Insert at the end

Inductively Coupled Plasma-Atomic Emission Spectrometry

Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) is an atomic emission spectrometry method that uses an inductively coupled plasma (ICP) as the excitation source. An ICP is a highly ionised inert gas (usually argon) with equal numbers of electrons and ions sustained by a radio-frequency (RF) field. The high temperature reached in the plasma successively desolvates, vaporises, excites - atomic emission spectrometry (AES) detection - and ionises - mass spectrometry (MS) detection - atoms from the sample. Detection limits are, generally, in the lower nanogram (ICP-MS) to microgram (ICP-AES) per litre range.

The plasma is formed by a tangential stream of support gas through a 'torch', i.e. a system consisting of 3 concentric quartz tubes. A metal coil (the load coil) surrounds the top end of the torch and is connected to a radio-frequency (RF) generator. Power (usually 700-1500 watt) is applied through the coil and an oscillating magnetic field corresponding to the frequency of the generator (in most cases 27 megahertz, 40 megahertz) is formed. The plasma forms when the support gas is made conductive by exposing it to an electric discharge, which produces seed electrons and ions. Inside the induced magnetic field, the charged particles (electrons and ions) are forced to flow in a closed annular path. As they meet resistance to their flow, heating takes place producing additional ionisation. The process occurs almost instantaneously, and the plasma expands to its full strength and dimensions. The radio-frequency oscillation of the power applied through the coil causes radio-frequency electric and magnetic fields to be set up in the area at the top of the torch. When a spark (produced by a Tesla tube or some other seeding device) is applied to the support gas flowing through the torch, some electrons are stripped from the support gas atoms. These electrons are then caught up in the magnetic field and accelerated. Adding energy to the electrons by the use of a coil is known as inductive coupling. These high-energy electrons in turn collide with other support-gas atoms, stripping off still more electrons. The collisional ionisation of the support gas continues in a chain reaction, breaking down the gas into a physical plasma consisting of support-gas atoms, electrons and support-gas ions. The plasma is then sustained within the torch and load coil as radio-frequency energy is continually transferred to it through the inductive coupling process.

The ICP appears as an intense, very bright, plume-shaped plasma. At the base the plasma is toroidal, and this is referred to as the induction region (IR), i.e. the region in which the inductive energy transfer from the load coil to the plasma takes place. The sample is introduced through the induction region into the centre of the plasma.

Apparatus

ICP-AES consists of sample-introduction system with peristaltic pump for delivering the solution at constant flow rate into a nebuliser, radio-frequency (RF) generator, plasma torch, transfer optics focussing the image of the plasma at the entrance slit of the spectrometer, radial viewing is better for difficult matrices (alkalis, organics), whereas axial viewing gives more intensity and better detection limits in simple matrices, wavelength dispersive devices consisting of diffraction gratings, prisms, filters or interferometers, detectors converting radiant energy into electrical energy, data-acquisition unit.

Interference

Interference is anything that causes the signal from an analyte in a sample to be different from the signal for the same concentration of that analyte in a calibration solution. The well-known chemical interference that is encountered in flame atomic absorption spectrometry is usually weak in ICP-AES. In rare cases where interference occurs, it may be necessary to increase the RF power or to reduce the inner support-gas flow to eliminate it. The interference in ICP-AES can be of spectral origin or even the result of high concentrations of certain elements or matrix compounds. Physical interference (due to differences in viscosity and surface tension of the sample and calibration standards) can be minimised by dilution of the sample, matrix matching and use of internal standards or through application of the method of standard additions.

Another type of interference occasionally encountered in ICP-AES is the so-called 'easily ionised elements (EIEs) effect'. The EIEs are those elements that are ionised much more easily, for example alkaline metals and alkaline earths. In samples that contain high concentrations of EIEs (more than 0.1 per cent), suppression or enhancement of emission signals is likely to occur.

Spectral interference

This may be due to other lines or shifts in background intensity. These lines may correspond to argon (observed above 300 nm), OH bands due to the decomposition of water (at about 300 nm), NO bands due to the interaction of the plasma with the ambient air (between 200 nm and 300 nm), and other elements in the sample, especially those present at high concentrations. The interference falls into 4 different categories: simple background shift, sloping background shift, direct spectral overlap, and complex background shift.

Absorption interference

This arises when part of the emission from an analyte is absorbed before it reaches the detector. This effect is observed particularly when the concentration of a strongly emitting element is so high that the atoms or ions of that element that are in the lower energy state of transition absorb significant amounts of the radiation emitted by the relevant excited species. This effect, known as self-absorption, determines the upper end of the linear working range for a given emission line.

Multicomponent spectral fitting

Multiple emission-line determinations are commonly used to overcome problems with spectral interferences. A better, more accurate method for performing spectral interference corrections is to use the information obtained with advanced detector systems through multicomponent spectral fitting. This quantifies not only the interference, but also the background contribution from the matrix, thereby creating a correction formula. Multicomponent spectral fitting utilises a multiple linear-squares model based on the analysis of pure analyte, the matrix and the blank, creating an interference-corrected mathematical model. This permits the determination of the analyte emission in a complex matrix with improved detection limits and accuracy.

Procedure**Sample preparation and sample introduction**

The basic goal for the sample preparation is to ensure that the analyte concentration falls within the working range of the instrument through dilution or preconcentration, and that the sample-containing solution can be nebulised in a reproducible manner.

Several sample-introduction systems tolerate high acid concentrations, but the use of sulfuric and phosphoric acids can contribute to background emission observed in the ICP spectra. Therefore, nitric and hydrochloric acids are preferable. The availability of hydrofluoric acid-resistant (for example perfluoroalkoxy polymer) sample-introduction systems and torches also allows the use of hydrofluoric acid. In selecting a sample-introduction method, the requirements for sensitivity, stability, speed, sample size, corrosion resistance and resistance to clogging have to be considered. The use of a cross-flow nebuliser combined with a spray chamber and torch is suitable for most requirements. The peristaltic pumps used for ICP-AES usually deliver the standard and sample solutions at a rate of 1 ml per minute or less.

In the case of organic solvents being used, the introduction of oxygen must be considered to avoid organic layers.

Operating conditions

The standard operating conditions prescribed by the manufacturer are to be followed. Usually, different sets of operating conditions are used for aqueous solutions and for organic solvents. Suitable operating parameters are to be properly chosen as wavelength selection, support-gas flow rates (outer, intermediate and inner tubes of the torch), RF power, viewing position (radial or axial), pump speed, conditions for the detector (gain/voltage for photomultiplier tube detectors, others for array detectors) and integration time (time set to measure the emission intensity at each wavelength).

Control of instrument performance**System suitability**

The following tests may be carried out with a multi-element control solution to ensure the adequate performance of the ICP-AES system:

Energy transfer (generator, torch, plasma); measurement of the ratio Mg II (280.270 nm)/Mg I (285.213 nm) may be used, sample transfer, by checking nebuliser efficiency and stability; resolution (optical system), by

measuring peak widths at half height, for example As (189.042 nm), Mn (257.610 nm), Cu (324.754 nm) or Ba (455.403 nm), analytical performance, by calculating detection limits of selected elements over the wavelength range.

Method validation

Satisfactory performance of methods prescribed in monographs is verified at suitable time intervals.

Linearity

Prepare and analyse not fewer than 4 reference solutions over the calibration range plus a blank. Perform not fewer than 5 replicates. The calibration curve is calculated by least-square regression from all measured data of the calibration test. The regression curve, the means, the measured data and the confidence interval of the calibration curve are plotted. The operating method is valid when the correlation coefficient is at least 0.99, the residuals of each calibration level are randomly distributed around the calibration curve. Calculate the mean and relative standard deviation for the lowest and for the highest calibration level. When the ratio of the estimated standard deviations of the lowest and the highest calibration level is less than 0.5 or more than 2.0, a more precise estimation of the calibration curve may be obtained using weighted linear regression. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed. If the means compared to the calibration curve show a deviation from linearity, two-dimensional linear regression is used.

Accuracy

Verify the accuracy preferably by using a certified reference material (CRM). Where this is not possible, perform a test for recovery.

Recovery

For assay determinations a recovery of 90 per cent to 110 per cent is to be obtained. The test is not valid if recovery, for example for trace-element determination, is outside of the range 80 per cent to 120 per cent of the theoretical value. Recovery may be determined on a suitable reference solution (matrix solution) spiked with a known quantity of analyte (concentration range that is relevant to the samples to be determined).

Repeatability

The repeatability is not greater than 3 per cent for an assay and not greater than 5 per cent for an impurity test.

Limit of quantification

Verify that the limit of quantification (for example, determined using the 10σ approach) is below the value to be measured.

Parenteral Preparations. Page 1113

Powders for injection. Page 1116

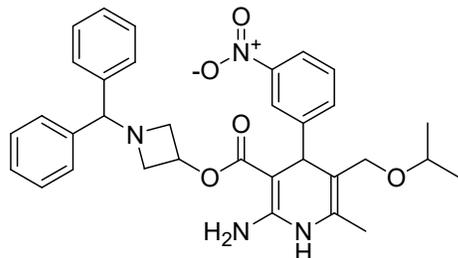
Uniformity of content. Line 4

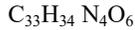
Change from : 50 mg

to : 40 mg

Azelnidipine. Page 1304

Change to: **Azelnidipine**





Mol. Wt. 582.7

Azelnidipine is 3-[1-(Diphenylmethyl)azetidin-3-yl] 5-(1-methylethyl)(4RS)- 2-amino -6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Azelnidipine contains not less than 99.0 per cent and not more than 101.0 per cent of $\text{C}_{33}\text{H}_{34}\text{N}_4\text{O}_6$, calculated on the dried basis.

Description. A light yellow to yellow crystalline powder. It shows polymorphism.

Identification

A. Determine by infrared adsorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azelnidipine RS* or with the reference spectrum of azelnidipine.

B. When examined in the range from 200 nm to 400 nm (2.4.7), a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maxima at the same wavelength as that of *azelnidipine RS* prepared in the same manner.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *acetonitrile* and 20 volumes of *water*.

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 μm),
- column temperature 40 $^{\circ}$,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 3.0 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 5.5 with *orthophosphoric acid*, 45 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 μl .

Inject reference solution. The test is not valid unless the column efficiency is not less than 15000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0.

Inject reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak for test solution. The area of any secondary peak eluting at a relative retention time of about 0.5 is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent), the area of any secondary peak eluting at a relative retention time of about 1.42 is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (0.7 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying under vacuum at 70 $^{\circ}$ for 5 hours.

Assay. Determine by liquid chromatography (2.4.14)

Solvent mixture. 50 volumes of *water* and 50 volumes of *acetonitrile*.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.002 per cent w/v solution of *azelnidipine RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.03M *potassium dihydrogen orthophosphate* in water,
- B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
12	30	70
20	30	70
25	80	20
30	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{34}N_4O_6$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Calcium Pantothenate. Page 1462

Identification

Change to: **Identification**

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *calcium pantothenate RS* or with the reference spectrum of calcium pantothenate.

B. Specific optical rotation. (see Test)

C. Gives reaction (A) of calcium salts (2.3.1).

Chlorthalidone. Page 1603

Related substances

Change to: **Related substances.** Determine by liquid chromatography (2.4.14).

Solvent mixture. 2 volumes of a 0.2 per cent w/v solution of *sodium hydroxide*, 48 volumes of mobile phase B and 50 volumes of mobile phase A.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.0001 per cent w/v solution of *chlorthalidone RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica gel (5µm),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.32 g of *ammonium phosphate* in 900 ml of *water*, adjusted to pH 5.5 with *dilute phosphoric acid* and dilute to 1000 ml with *water*,
B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
16	65	35
21	50	50
35	50	50
45	65	35

Name	Relative retention time
Chlorthalidoneimpurity B ¹	0.7
Chlorthalidoneimpurity J ²	0.9
Chlorthalidone(retention time: about 7 minutes)	1.0
Chlorthalidoneimpurity G ³	6.0

¹2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid,

²impurity of unknown structure with a relative retention of about 0.9,

³(3RS)-3-(3,4-dichlorophenyl)-3-hydroxy-2,3-dihydro-1H-isindol-1-one.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to impurity B is not more than 7 times the area of principal peak in the chromatogram obtained with the reference solution (0.7 per cent), the area of any peak due to impurity J is not more than 3 times the area of principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak due to impurity G is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 12 times the area of the principal peak in the chromatogram with the reference solution(1.2 per cent).

Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

FramycetinSulphate. Page 2129

Para 2, line 2 and 3

Change **from:** 630 µg of neomycin Bper mg

to: 630 IU of neomycin B per mg

Assay.lines 2 and 3

Change **from:**µg of neomycin B per mg

to: IU of neomycin B per mg

Lamivudine, Nevirapine and Zidovudine Paediatric Dispersible Tablets

Page 2385

Related substances

Change to: **Related substances.** Determine by liquid chromatography (2.4.14)

Solvent mixture. 60 volumes of *water* and 40 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Lamivudine, in the solvent mixture and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution (a). A solution containing 0.0001 per cent w/v of *lamivudine diastereomer RS*, 0.001 per cent w/v of *zidovudine impurity B RS*, 0.05 per cent w/v of *lamivudine RS* and 0.1 per cent w/v of *zidovudine RS* in the solvent mixture.

Reference solution (b). A solution containing 0.03 per cent w/v of *lamivudine RS*, 0.04 per cent w/v of *nevirapine RS* and 0.04 per cent w/v of *zidovudine RS* in the solvent mixture.

Reference solution (c). Dissolve 4 mg of *nevirapine impurity A RS* in 40 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with *water*.

Reference solution (d). Dilute 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Prontosil C18H),
- sample temperature: 5°,
- mobile phase: A. a mixture of 100 volumes of 0.23 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.5 with *orthophosphoric acid* and 0.2 volume of *methanol*,
B. *acetonitrile*,
- flow rate: 1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 266 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	99	1
10	99	1
20	90	10
25	85	15
42	70	30
55	70	30
57	99	1
65	99	1

Name	Relative retention time	Correction factor
<i>Lamivudine impurities</i>		
Carboxylic acid impurity ¹	0.23	2.33*
Lamivudine diastereomer ²	0.57	1.43*
<i>Nevirapine impurities</i>		
Nevirapine impurity A ³	1.45	---
Nevirapine impurity B ⁴	1.22	0.83#
Nevirapine impurity C ⁵	1.67	1.03#
<i>Zidovudine impurities</i>		
Zidovudine impurity A ⁶	0.76	0.86°
Zidovudine impurity B ⁷	1.02	1.06°
Zidovudine impurity C ⁸	0.39	0.86°
Thymidine ⁹	0.66	0.93°
Zidovudinethreo isomer ¹⁰	0.96	1.06°

Zidovudine thymidine adduct¹¹ 1.28 0.98°

* Correction factor with respect to Lamivudine

#Correction factor with respect to Nevirapine impurity A

° Correction factor with respect to Zidovudine

¹ 4-amino-2-oxo-pyrimidinyl-1,3-oxathiolane-2-carboxylic acid,² (2*S-cis*)-(-)-1-[(2*R*, 5*R*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine,³ 5,11-dihydro-6*H*-11-ethyl-4-methyl-dipyrido(3,2-*b*:2',3'-*e*)(1,4)diazepin-6-one,⁴ (5,11-dihydro-4-methyl-6*H*-dipyrido(3,2-*b*:2',3'-*e*)(1,4)diazepin-6-one),⁵ 5,11-dihydro-4-methyl-6*H*-11-propyl-dipyrido(3,2-*b*:2',3'-*e*)(1,4)diazepin-6-one,⁶ 3'-azido-3'-deoxy-3'-Azido-3'-deoxythymidine; stavudine,⁷ 3'-chloro-3'-deoxythymidine,⁸ 2,4-dihydroxy-5-methyl pyrimidine; thymine,⁹ 1-(2-deoxy-β-D-ribofuranosyl)-5-methyl uracil,¹⁰ 1-(3-azido-2,3-dideoxy-β-D-threo-pentafuranosyl)-thymidine,¹¹ 1-(3-(3-(3-azido-2,3-dideoxy-pentofuranosyl))-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1-yl)-2,3-dideoxypentofuranosyl)-5-methylpyrimidine-2,4-dione.

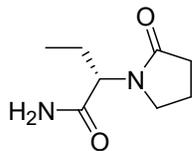
The retention time of lamivudine, zidovudine, nevirapine and nevirapine impurity A peaks are about 18.5 minutes, 31 minutes, 40.6 minutes and 45 minutes respectively.

Inject reference solution (a) and (d). The test is not valid unless the resolution between the peaks due to lamivudine diastereomer and lamivudine is not less than 1.5 and between zidovudine and zidovudine impurity B is not less than 1.5 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent, for the peaks due to lamivudine, nevirapine related compound A and zidovudine in the chromatogram obtained with reference solution (d).

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to carboxylic acid impurity, lamivudine diastereomer, each of, is not more than 1.66 times the area of the peak due to lamivudine in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to nevirapine impurity A, nevirapine impurity B and nevirapine impurity C, each of, is not more than 1.25 times the area of peak due to nevirapine impurity A in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to zidovudine impurity A (stavudine), zidovudine impurity B, thymidine, zidovudine threo isomers and zidovudine thymidine adduct, each of, is not more than 1.25 times the area of the peak due to zidovudine in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to zidovudine impurity C (thymine) is not more than 2.5 times the area of the peak due to zidovudine in the chromatogram obtained with reference solution (d) (1.0 per cent), the area of any other secondary peak is not more than 1.66 times the area of the peak due to lamivudine in the chromatogram obtained with reference solution (d) (0.5 per cent). The sum of all the impurities is not more than 2.5 per cent.

Levetiracetam. Page 2407

Change to: **Levetiracetam**

C₈H₁₄N₂O₂

Mol. Wt. 170.2

Levetiracetam is 1-Pyrrolidineacetamide, α-ethyl-2-oxo-, (α*S*).

Levetiracetam contains not less than 98.0 per cent and not more than 102.0 per cent of C₈H₁₄N₂O₂ calculated on the anhydrous and solvent-free basis.

Category. Antiepileptic.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levetiracetamRS* or with the reference spectrum of levetiracetam.

B. In the test for enantiomeric purity, the principal peak in the chromatogram obtained with reference solution (c) corresponds to the levetiracetam S-enantiomer peak in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 125 mg of the substance under examination in mobile phase (a) and dilute to 25.0 ml with mobile phase (a).

Reference solution. A 0.0005 per cent w/v solution of *levetiracetamRS* in mobile phase (a).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3µm),
- mobile phase: A. a mixture of 95 volumes of buffer solution prepared by dissolving 2.7 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 5.5 with 2 per cent w/v solution of *potassium hydroxide* and 5 volumes of *acetonitrile*,

B. *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
20	71	29
25	100	0
28	100	0

Name	Relative retention time	Correction factor
Levetiracetam impurity C ¹	0.37	-
Levetiracetam acid ²	0.62	0.83
Levetiracetam	1.0	-
Levetiracetam impurity A ³	1.25	2.85

¹Pyridin-2-ol¹ (Not included in the total impurities limit).

²(S)-2-(2-oxopyrrolidin-1-yl) butanoic acid.

³(S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide,

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam impurity C is not more than 0.25 times the area of the principal peak in a chromatogram obtained with the reference solution (0.025 per cent), the area of any peak corresponding to levetiracetam acid is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak corresponding to levetiracetam impurity A and any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent) and the sum of areas of all the secondary peaks including impurity B obtained from the test for Levetiracetam impurity B is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.4 per cent). Ignore any peak with a relative retention time of 0.19 or less.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance underexamination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.005 per cent w/v solution of *levetiracetamRS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *levetiracetamracemic mixture RS* in the mobile phase.

Reference solution (c). Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, amylose tris-3,5-dimethylphenylcarbamate (AD-H) bonded to porous silica(5µm),
- mobile phase: a mixture of 80 volumes of *n-hexane* and 20 volumes of *dehydrated alcohol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

The relative retention time with respect to levetiracetam S-enantiomer for levetiracetam R-enantiomer is about 0.55.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to levetiracetam R-enantiomer and levetiracetam S-enantiomer is not less than 4.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to levetiracetam R-enantiomer (impurity D) is not more than 1.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

Levetiracetam impurity B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance underexamination in the mobile phase and dilute to 100.0 ml with mobile phase.

Reference solution (a). A 0.2 per cent w/v solution of *levetiracetam impurity B RS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, octadecylsilane bonded to porous silica(5µm),
- mobile phase: a mixture of 85 volumes of the buffer solution prepared by dissolving 1.22 g of *sodium 1-decanesulfonate* in 1000 ml of *water*, add 1.3 ml of *orthophosphoric acid*, adjusted to pH 3.0 with 20 per cent w/v solution of *potassium hydroxide* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 50 µl, for system suitability 10 µl.

The retention time for levetiracetam impurity B is about 9 minutes.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 3.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam impurity B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 2 g complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 0.1 g of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.01 cent w/v solution of *levetiracetam* RS in the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{14}N_2O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Marbofloxacin Injection. Page 4256

Assay. Chromatographic system, line 11

Change **from:** 5 ml per minute,
to: 1.2 ml per minute,

Montelukast and Levocetirizine Hydrochloride Tablets. Page 2633

Related substances

Change **to: Related substances.** Determine by liquid chromatography (2.4.14).

Solvent mixture. 15 volumes of mobile phase A and 85 volumes of *methanol*.

NOTE — Carry out the test protected from light and prepare solution immediately before use.

Test solution. Disperse a quantity of powdered tablets containing 25 mg of Levocetirizine in 30 ml of solvent mixture with the aid of ultrasound for 5 minutes, and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.005 per cent w/v of *montelukast* sulphoxide RS and *montelukast styrene* RS in the solvent mixture.

Reference solution (b). A solution containing 0.053 per cent w/v of *montelukast sodium* RS and 0.025 per cent w/v of *levocetirizine hydrochloride* RS in the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (c). Dilute 5.0 ml of reference solution (a) and reference solution (b) to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- column temperature: 40°,
- sample temperature: 8°
- mobile phase: A. a 0.06 per cent w/v solution of *ammonium acetate* in *water*, adjusted to pH 5.5 with *glacial acetic acid*,
B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	48	52
15	48	52
38	15	85
55	15	85
60	48	52
65	48	52

Name	Relative retention time
Levocetirizine	0.24
Montelukastsulphoxide impurity	0.89
Montelukast (Retention time about 35 minutes)	1.0
Montelukast styrene impurity	1.12

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to montelukastsulphoxide is not more than the area of the peak due to montelukastsulphoxide in the chromatogram obtained with reference solution (c) (2.0 per cent), the area of any peak corresponding to montelukast styrene is not more than 0.5 times the area of the peak due to montelukast styrene in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of the peak due to montelukast in the chromatogram obtained reference solution (c) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the peak due to montelukast in the chromatogram obtained with reference solution (c) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak due to montelukast in the chromatogram obtained with reference solution (c) (0.05 per cent) and the peak due to levocetirizine.

Paracetamol Tablets. Page 2858

Related substances. Chromatographic system, line 6

Change **from:** 1.15 g

to: 0.115 g

Sulfasalazine Gastro-resistant Tablets. Page 4516

Salicylic acid and Sulfapyridine. Chromatographic system

Change **to:** Use chromatographic system as described under Related substances with the following modifications

– spectrophotometer set at 300 nm,

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
12	70	30
15	30	70
25	30	70
27	70	30
35	70	30

Tranexamic Acid Injection. Page 3416

Assay

Change **to: Assay.** Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection containing 0.1 g of Tranexamic Acid to 100.0 ml with water.

Reference solution. A 0.1 per cent w/v solution of *tranexamic acid*RS in water.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature : 35°,
- mobile phase: a mixture of 40 volumes of *methanol* and 60 volumes of buffer solution prepared by dissolving 11g of *monobasic sodium phosphate* in 500 ml of *water*, add 5 ml of *triethylamine*, add 1.4 g of *sodium lauryl sulphate*, adjusted to pH 2.5 with 10 per cent w/v solution of *orthophosphoric acid* and dilute to 600 ml with *water*,

DRAFT PROPOSAL FOR AMENDMENTS

- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 μ l.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{15}NO_2$.