

Instructions for Handling Test Materials and Recording Results

Receipt and Storage

- On receipt of the test material, store at the temperature given on the label for each sample until ready to test.
- The test material should be analysed in accordance with the deadlines provided.

Basic Chemical Testing

Sample 1A – pH

Material supplied: 1 x 60mL buffer solution

Determine the pH of the solution provided. Results are to be reported to 2 decimal places.

Sample 1B – Acid/Base Titration

Material supplied: 1 x 60mL acid solution

Titrate 10mL \pm 0.04mL of the solution provided using 0.1M NaOH (sodium hydroxide) to an end point equivalent to a pH value of 8.75 using either:

- a. A calibrated pH meter (i.e. potentiometrically)
- b. A suitable indicator solution (e.g. phenolphthalein)

Report your result as the titre standardised to 0.1M sodium hydroxide, e.g. a titre of 25mL using 0.09M sodium hydroxide equals $25 \times 0.09/0.1 = 22.50\text{mL}$. Results are to be reported to 2 decimal places.

Sample 1C – Other Basic Titration

Range: 8.5 – 9.5g/L

Material supplied: 1 x 125mL solution of sodium chloride solution

Titrate 25mL \pm 0.04mL of the solution provided using 0.1M silver nitrate to an accurate end point determined by:

- Potentiometry using a calibrated pH meter (according to BP Appendix VIII, Ph Eur 2.2.20)
- Use of a suitable indicator solution (e.g. according to the equivalent USP method)

Sample 1D – Density

Material supplied: 1 x 60mL oil sample

Determine the density of the material provided at 20°C. Results are to be reported as g/cm³ to 3 decimal places.

Sample 1E – Refractive Index

Material supplied: 1 x 60mL sugar solution

Determine the refractive index of the solution provided. The refractive index should be measured at 20 \pm 0.5°C. Results are to be reported to 4 decimal places.

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Sample 1F – Melting point

Range: 150 – 200°C
Material supplied: 1 x 2g of test material

Grind the sample in a mortar and pestle to obtain a fine powder. Dry the finely powdered substance in vacuo and over anhydrous silica gel for 24 hours. Raise the temperature of the bath or melting-point apparatus to about 10°C below the melting point range and then adjust the rate of heating to about 1°C/min. Record the temperature at which the last particle passes into the liquid phase.

Advanced Chemical Testing

Sample 2A – HPLC

Materials supplied: 1 x 0.5g of test material, labelled Sample A
1 x 0.5g of Aspirin reference standard (99%)
1 x 0.5g of Salicylic Acid reference standard (99%)

HPLC Mobile Phase:
(2:400:600 v/v), Phosphoric Acid : Acetonitrile : Deionised water

Phosphoric Acid: H₃PO₄, CAS 7664-38-2, analytical reagent grade of commerce containing not less than 84% w/w of H₃PO₄ and about 15.7M in strength.

Test solution preparation:

Accurately weigh 0.2g of Sample A and dissolve in Acetonitrile with the aid of ultrasound and dilute to 100mL with the same solvent. Dilute 1 volume of this solution to 20 volumes with the same solvent. Prior to injection, filter an aliquot thorough a syringe-tip filter (0.45µm pore) in order to remove insoluble excipients.

Standard solution preparation:

Create 2 separate standard solutions at the following concentrations.

Aspirin reference standard: 0.01% w/v in the mobile phase

Salicylic Acid reference standard: 0.001% w/v in the mobile phase

Chromatographic system:

Mode: LC

Detector: UV 237nm

Column: 4.6mm x 250mm; 5µm packing C18 RP silica gel

Column temperature: 25°C

Flow rate: 1 mL/min

Injection volume: 10µL

Analysis:

Analyse the two calibration solutions and compare with the test solution using the chromatographic system described above.

Calculate the content of aspirin and salicylic acid in the unknown sample A using the declared content of aspirin and salicylic acid in the provided reference standards.

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Sample 2E – Residual Solvents

Materials supplied: 1 x 1mL spiking solution
1 x 2g residual solvents sample

Dissolve 0.200g of the Residual Solvents sample in dimethylformamide (DMF) and transfer to a headspace vial. Add 0.100mL of the spiking solution to the vial, using a syringe with a needle diameter of 0.8mm or smaller and a minimum 70mm length, and dilute to 20.0mL with DMF.

Analyse the sample solution by your routine headspace chromatography method, quantify the residual solvents present in the sample and report the results on PORTAL in the format specified in the Pharmassure scheme description for sample 2E Residual Solvents.

Note: The concentration range given in the Pharmassure scheme description should be used as a guide. Occasionally, the detected concentration of parameters in a test material may exceed the maximum concentration limit stated in the scheme description.

Sample 6D - LOD

Range: 5 – 15%(w/w)
Material supplied: 1 x 5g of test material

Determine on 1.000g by drying in an oven at 105°C to constant weight.

Sample 6E – FTIR

Materials supplied: 1 x 0.5g Methyl Cinnamate reference standard (99%)
1 x 0.5g Ethyl Cinnamate reference standard (99%)
1 x 0.5g Cinnamic Acid reference standard (99%)
1 x 0.5g Unknown Sample A – base chemical Cinnamic Acid
1 x 0.5g Unknown Sample B – base chemical Cinnamic Acid

Using the reference standards provided, determine whether the unknown samples A and B contain an impurity and identify the impurity present in the sample(s). Report the identity of the impurity if present and the wavenumbers for two key peaks in the IR spectrum between approximately 1200 and 1400cm⁻¹ for the sample(s) which contain the impurity. Please add the wavenumber(s) as a comment against the analyte.

Example method of analysis

Examine solids dispersed in a suitable liquid (mull) or in a solid (halide disc), as appropriate. If prescribed in the monograph, make a film of a molten mass between 2 plates transparent to infrared radiation.

A. Mull

Triturate a small quantity of the substance to be examined with the minimum quantity of *liquid paraffin R* or other suitable liquid; 5-10 mg of the substance to be examined is usually sufficient to make an adequate mull using one drop of *liquid paraffin R*. Compress the mull between 2 plates transparent to infrared radiation.

B. Disc

Triturate 1-2 mg of the substance to be examined with 300-400 mg, unless otherwise specified, of finely powdered and dried *potassium bromide R* or *potassium chloride R*. These quantities are usually sufficient to give a disc of 10-15 mm diameter and a spectrum of suitable intensity. If the substance is a hydrochloride, it is recommended to use *potassium chloride R*. Carefully grind the mixture, spread it uniformly in a suitable die, and submit it to a pressure of about 800 MPa (8 t·cm⁻²). For substances that are unstable under normal

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atmospheric conditions or are hygroscopic, the disc is pressed in vacuo. Several factors may cause the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium or an insufficient reduction of particle size. A disc is rejected if visual examination shows lack of uniform transparency or when transmittance at about 2000 cm^{-1} ($5\text{ }\mu\text{m}$) in the absence of a specific absorption band is less than 60 per cent without compensation, unless otherwise prescribed.

Prepare the substance to be examined and the reference substance by the same procedure and record the spectra between $4000\text{-}650\text{ cm}^{-1}$ ($2.5\text{-}15.4\text{ }\mu\text{m}$) under the same operational conditions. The transmission minima (absorption maxima) in the spectrum obtained with the substance to be examined correspond in position and relative size to those in the spectrum obtained with the reference standards.

When the spectra recorded in the solid state show differences in the positions of the transmission minima (absorption maxima), treat the substance to be examined and the reference substance in the same manner so that they crystallise or are produced in the same form, or proceed as prescribed in the monograph, then record the spectra.

Sample 6F – Karl Fischer

Range: 1 – 10%(w/w)
Material supplied: 1 x 3g of test material

Determine moisture of 0.25g of test material, using methanol and formamide (2:1 v/v) as the solvent.

Sample 8A – Low level Conductivity

Material supplied: 1 x 100mL solution

After calibrating the apparatus with a certified reference material solution, rinse the conductivity cell several times with *distilled water R* and at least twice with the aqueous solution to be examined. Carry out successive measurements as described in the monograph at 25°C .

Sample 11 – Endotoxins in Solution

Material supplied: 1 x 4mL solution

- The sample should be stored in the freezer (at -20°C) on arrival.
- Ensure that the sample is thoroughly defrosted at room temperature before use.
- Mix the sample for at least 10 minutes by using a vortex intermittently before subsampling.
- Test using your routine lab methods and report the results in EU/mL.

Sample 14 – Elements in Herbal Supplements

Material supplied: 1 x 50g powdered herbal supplement

Analyse the material using your routine laboratory methods.

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Sample 16 – Cannabidiols in Supplements

Material supplied: 1 x 10mL oil

Analyse the material using your routine laboratory methods.

Please add a comment to your result detailing the extraction method used by your laboratory. Examples include but are not limited to the below:

SPE followed by LC-MS-MS

SPE followed by HPLC

Liquid-liquid extraction followed by LC-MS-MS

Microbiology Samples (all sample types)

Receipt and Storage

- On receipt of the test material, store refrigerated at 2-8°C until ready to test.
- The test material should be analysed in accordance with the deadlines provided.

Sample Details

- The test material represents a simulated pharmaceutical sample, which may or may not contain the target organism(s), at a range of inoculum levels
- The test material is supplied as a freeze-dried test material contained within a glass vial
- Occasionally test materials contain high numbers of the target organisms, therefore it may be necessary to perform dilutions. Further guidance on levels is available in the PHARMASSURE Scheme Description.

Resuscitation of samples 3, 4A & B and 5 (with exception of Identification test only-, see Level 3 below)

- Prepare 100ml sterile deionised water or suitable alternative microbiological diluent
- Aseptically remove cap and rubber stopper from the vial and resuscitate the freeze-dried test material by adding approximately 10ml of the bulk diluent.
- Replace the stopper and shake to dissolve then add this concentrate to the bulk diluent
- Repeat this procedure two or three times to ensure all the freeze-dried test material is recovered from the vial and added to the bulk diluent.
- Leave the diluted test material to stand for a minimum of 60 minutes but no longer than 90 minutes. Immediately before testing, mix the sample by gently inverting.
- This final 100ml represents a 'neat' sample.

Sample 3 - Microbiology Low-level Enumeration and Identification

For both Enumeration and Identification Test

- Perform membrane filtration on the entire 100ml test material and record the total number of colonies present. It is recommended to use non-selective agar and incubate at mesophilic temperature range.
- Report result as cfu/100ml as the 100ml test material represents a 'neat' sample.
- Identify the colonies present using your normal laboratory procedures.

Identification Test only

- If you do not wish to carry out enumeration on this sample, simply add 1ml of sterile diluent to the test material, leave to resuscitate for a minimum of 60 minutes but no longer than 90 minutes, and then inoculate a sample of the test material onto your selected media. Identify the colonies present using your normal laboratory procedures.

Samples 4A & 4B - Microbiology Enumeration

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Testing

- Test for the target organism(s) using your routine laboratory method(s).
- Tests for sample 4A include total aerobic microbial count, total bacterial count, detection and/or enumeration of *Staphylococcus aureus*, detection and/or enumeration of *Escherichia coli*, and detection and/or enumeration of bile-tolerant gram-negative bacteria.
- Tests for sample 4B include detection of *Pseudomonas aeruginosa*, detection and/or enumeration of *Candida albicans*, total yeast and mould count, enumeration of yeast, enumeration of mould and detection of *Burkholderia cepacia*.
- Participants are not required to enter results for each parameter, only for those tests performed on a routine basis.
- Report results as cfu/ml
- The reconstituted test material (100ml) should be treated as the 'neat' pharmaceutical sample.

Sample 5 - Sterility Testing

Testing

- The sterility test comprises a set of 5 different samples to test for sterility using your routine laboratory method.
- The reconstituted test material should be treated as the 'neat' pharmaceutical sample.
- Report results as 'Sterile' or 'Not sterile'

Sample 9 – Detection of Salmonella

Testing

- Test for the target organism using your routine laboratory method(s).
- Prepare 10ml sterile deionised water or suitable alternative microbiological diluent
- Aseptically remove cap and rubber stopper from the vial and resuscitate the freeze-dried test material by adding the entire 10ml diluent.
- Replace the stopper and shake to dissolve.
- Leave the diluted test material to stand for a minimum of 60 minutes but no longer than 90 minutes. Immediately before testing, mix the sample by gently inverting.
- This final 10ml represents a 'neat' sample.

Sample 10 – Microbial testing of medicinal herbs

Testing

- Prepare 90ml of diluent as stipulated by your test method.
- From this volume take 10ml and add it to the vial after aseptically removing cap and rubber stopper.
- Replace the vial stopper and shake to dissolve.
- Leave the test material to resuscitate at room temperature for a minimum of 60 minutes, but no longer than 90 minutes.
- Resuscitate the matrix with the remaining amount of diluent prepared in step 1.
- After the 60 to 90 minute period add the vial contents prepared in step 2 to the matrix preparation from step 5 back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial. The test material is now ready to test using your routine laboratory methods.
- Remember to take the initial dilution factor (1:10) into account when calculating results, which should be recorded as cfu/g.

Precautions to be taken with microbiology samples

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- Test materials contain viable micro-organisms and are supplied on the understanding that the purchaser has suitably competent and qualified personnel to handle them safely. Test materials must only be opened in a laboratory by qualified personnel.
- Test materials may contain micro-organisms classed as Hazard Group 2 according to the Advisory Committee on Dangerous Pathogens (HSE 1998- ISBN0717610381) and should be handled accordingly.
- In case of accidental spillage, contain the spillage and alert nearby personnel. Decontaminate the spillage with suitable disinfectant. Clean using absorbent disposable paper or tissue.
- For further safety information concerning the samples a safety data sheet should be obtained from the scheme provider.

Recording Results

- All results should be submitted using PORTAL
- Please go to <https://portal.lgcstandards.com>
- Login using your Lab ID, username and password.

- A PORTAL user guide can be downloaded from the help section.

If you need any help at all please do not hesitate to contact our support team using the details below or your local LGC representative.

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