PHARMASSURE

Pharmaceutical Products Proficiency Testing Scheme

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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 ± 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 x 0.09/0.1 = 22.50mL. Results are to be reported to 2 decimal places.

Sample 1C – Other Basic Titration

Titre Range: 10 – 30mL
Magnesium Range: 2000 – 7500mg/L
Materials supplied: 1 x 60mL magnesium sulfate solution

Pipette 10mL of the supplied solution into a 500mL conical flask and dilute to 300mL with deionised water. Add 10 mL of ammonium chloride buffer solution pH 10.0 and about 50mg of mordant black 11 triturate. Heat to about 40°C then titrate at this temperature with 0.1M sodium EDTA until the colour changes from violet to full blue. Report your result as the titre of the sodium EDTA solution and the concentration of the magnesium in the solution.

N.B. 1mL of 0.1M sodium EDTA is equivalent to 2.431mg of Mg

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/cm^3 to 3 decimal places.

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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±0.5°C. Results are to be reported to 4 decimal places.

Sample 1F – Melting point

Range: 200 – 250ºC
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 2 tablets, labelled as Sample A
1 x 0.2g of Acetaminophen reference standard (100%)

HPLC Mobile Phase:
(35 : 65 v/v) Methanol : Deionised water, adjusted to pH 3.1 with 10% orthophosphoric acid.

Test solution preparation:
Crush one tablet to a fine powder using a mortar and pestle. Dissolve in 50mL of mobile phase in a 100mL volumetric flask, using a sonicator if required. Fill the 100mL volumetric flask to volume and invert to thoroughly mix. Some particulate matter from the tablet may not fully dissolve.
Perform a 1 in 100 dilution using mobile phase and then filter an amount of the solution into an HPLC vial through a 0.45µm pore syringe filter.

Standard solution preparation:
Acetaminophen stock concentration: 1mg/mL in mobile phase
Weigh out 100mg of reference standard into a 100mL volumetric flask and fill to volume with mobile phase. Invert to thoroughly mix.
Create 3 calibration solutions using the stock solution at 25, 50 and 100 µg/mL. Use blank mobile phase for a zero calibration point if desired.

Chromatographic system:
Mode: LC
Detector: UV 235nm
Column: 4.6mm x 150mm; 5µm packing C18 RP silica gel
Column temperature: ambient
Flow rate: 1.8 mL/min
Injection volume: 10µL

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Analysis:
Analyse the three calibration solutions and compare with the test solution using the chromatographic system described above.

Calculate the content of acetaminophen in the unknown tablet A in mg using the declared content of acetaminophen in the provided reference standards. Report as the amount of active ingredient to one decimal place along with the retention time of the main peak in minutes to two decimal places.

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 6J – Advanced Titration

Material supplied: 1 x 1g test material

Dissolve 0.320g in 20mL of dilute hydrochloric acid and dilute to 100mL with deionised water. Using 20mL of the solution, carry out the complexometric titration of magnesium.

Introduce the prescribed solution into a 500mL conical flask and dilute to 300mL with deionised water. Add 10mL of ammonium chloride buffer solution pH 10.0 and about 50mg of mordant black 11 triturate. Heat to about 40°C then titrate at this temperature with 0.1M sodium edetate until the colour changes from violet to full blue.

Dilute Hydrochloric Acid: Dilute 20g of hydrochloric acid to 100mL with deionised water. The final solution contains 7.3% w/v of HCl.

Ammonium chloride buffer solution pH 10.0: Dissolve 5.4g of ammonium chloride in 20mL of deionised water, add 35mL of 10M ammonia and dilute to 100mL with deionised water.

Mordant Black 11 Triturate: A mixture of 1 part of mordant black 11 with 99 parts of sodium chloride. The solution complies with the following test. Sensitivity to magnesium - Dissolve 50mg in 100mL of deionised water; a brownish violet colour is produced. Add 0.3mL of 6M ammonia; the colour changes to blue. Add 0.1mL of a 1% w/v solution of magnesium sulfate; the colour changes to violet. Store in an airtight container protected from light.

Disodium Edetate: Disodium dihydrogen ethylenediaminetetra-acetate, dihydrate; sodium edetate; $C_{10}H_{14}N_2Na_2O_8, 2H_2O$ = 372.2 (CAS 6381-92-6). Analytical reagent grade of commerce

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1mL of 0.1M sodium edetate is equivalent to 2.431mg of Mg.
Report as concentration of magnesium (%w/w to 2 decimal places).

Sample 6K – NMR

Material supplied: 1 x 1g Sample A
1 x 1g Sample B

Dissolve each substance to be examined as prescribed and filter; the solution must be clear. Use a chemical shift internal reference compound, which, unless otherwise prescribed, is a solution containing 0.5% (v/v) to 1.0% (v/v) of tetramethylsilane (TMS) in deuterated organic solvents or 5g/L to 10g/L of sodium tetradeuteriodimethylsilapentanoate acid (TSP) in deuterium oxide.

Take an aliquot of the test solution and record the NMR spectrum using appropriate conditions for the instrument to be used.

The list of possible chemicals in each sample is listed on Portal. Identify the chemical (or chemicals) present in each test material, report “Detected” against the relevant chemicals and quantify in (%w/w) to 2 decimal places.

Sample 8B – Particulate Determination

Material supplied: 1 x 125mL test solution
Count Range: 0 – 5000 particles per millilitre

Method 1. Light obscuration particle count test

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of particle-free water R and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 min or sonicating.

Remove 4 portions, each of not less than 5mL, and count the number of particles equal to or greater than 10µm and 25µm. Disregard the result obtained for the first portion and calculate the mean number of particles for the preparation to be examined.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per millilitre equal to or greater than 10µm and does not exceed 3 per millilitre equal to or greater than 25µm.

Method 2. Microscopic particle count test

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per millilitre equal to or greater than 10µm and does not exceed 2 per millilitre equal to or greater than 25µm.

Calculate and report the number of particles present in the sample (number of particles per millilitre) and record your result as ‘pass’; indicating that the preparation complies with the test; or ‘fail’; indicating that the sample is does not comply with the test.

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Sample 19 – Phytochemical Identification

Material supplied: 1 x 1g powdered supplement

Analyse the material using your routine laboratory methods. Report whether the sample provided is Gingko Biloba or not.
Report as True (the provided sample is Gingko Biloba) or False (the provided sample is not Gingko Biloba).

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.

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Samples 4A & 4B - Microbiology Enumeration

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.

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Precautions to be taken with microbiology samples

- 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.
Tel: +44(0)161 762 2500
Email: axiopt@lgcgroup.com