Name of the Content Creator: Dr. Nisha Sharma

Email: nishasharma@csjmu.ac.in

SUBJECT: BP-705P

INTRUMENTAL METHODS OF ANALYSIS

SOP OF FLUROMETER

SOP OF FLAME PHOTOMETER

SOP OF FTIR

SOP OF NEPHELO-TURBIDITYMETER

Email of Registrar: registrarcsjmuknp@gmail.com

Institute Name: University Institute of Pharmacy

University Name: C.S.J.M. University Campus, Kanpur

UNIVERSITY INSTITUTE OF PHARMACY, C.S.J.M. UNIVERSITY, KANPUR

STANDARD OPERATING PROCEDURE: - Make and Model: E L I C O FLUOROMETER

MODEL:

PURCHASE DATE: **EFFECTIVE DATE:**

SOP NO: UIP-P/INST.LAB/ **PREPARED BY DR. NISHA SHARMA**

PROCEDURE:

1. Connect A.C. power pack to the main unit by inserting the cable socket into the panel behind the main unit. Hook the power pack to 230V AC mains. Switch on the stabilizer.
2. Switch ON the power pack unit.
3. Switch ON the mercury lamp
4. Allow 15 min for warming the mercury lamp.
5. Insert the primary filter (366nm peak) which looks dark violet in the slot between big lens & sample hole. i.e. in the path of incident light.
6. Switch ON detector for activating the main unit (detector & amplifier) & observe digital display.
7. Select the range say 2ppm max. concentration of quinine sulphate range with sensitivity switch.
8. Take 0.1 N H_2SO_4 as blank solution in a tube provided & insert in the sample holder.
9. Adjust “Zero” by using the controls coarse & fine.
10. Replace blank tube with 1ppm quinine sulphate solution & set 100.0 by means of GAIN control & light iris on digital display. OR take 2ppm solution & set 199.0 on digital display in same manner.
11. Take unknown solution & read its concentration in the range selected. If the concentration is more than 1.999 ppm change to higher range & repeat steps 7,8 9 & 10. Whenever the range is changed set ZERO with BLANK solution & set 100.0 with the solution of appropriate concentration is need to follow.
12. For short periods of non-use, the instrument need not be switched off the mercury lamp, since the lamp takes time for cooling & restart. You may switch off the detector part of the unit.

Note: Never remove the primary filter when the lamp is glowing. If it is necessary to change, you switch off DETECTOR part of the unit & then filter can be changed safely.
Ensure all test tubes are clean devoid of any finger prints.

Preparing quinine sulphate $C_{20}H_{24}N_2O_2)_2\,H_2SO_4\,2H_2O$, molar mass 782.97

1ppm quinine sulphate

2ppm quinine sulphate

UNIVERSITY INSTITUTE OF PHARMACY, C.S.J.M. UNIVERSITY, KANPUR

STANDARD OPERATING PROCEDURE: - Make and Model: E l i c o FLAME PHOTOMETER

MODEL:	**Unit 1 (Flame photometer) + Unit 2 (Air compressor)**
PURCHASE DATE:	**EFFECTIVE DATE:**
SOP NO: UIP-P/INST.LAB/	**PREPARED BY DR. NISHA SHARMA**

1. Prepare the standard solutions well in advance. The substance should be dried at 180°C for 1 hour. (635mg/250ml=1000ppm for NaCl, then diluting 1ml to 10 ml to get 100ppm) (477mg/250ml-1000ppm (KCl); 624mg/250ml $CaCO_3$, dissolve in 1:1 HCl minimum qty, 1000ppm).
2. Prepare different dilutions from the 100ppm stock solution. For better readings concentration of less than 40ppm should be taken.
3. Connect the Unit 1 with the air compressor unit provided with the pipe. Switch on the air compressor unit.
4. Maintain the air pressure reading between 0.4-0.6 kg/cm^2 in unit 2.
5. Connect the LPG Gas cylinder with the pipe provided to Unit 1 at the back of the unit and maintain the gas flow slowly rotating the gas knob anticlockwise and keep on pressing the auto ignitor knob provided at the back of main unit (glitters brick red) to lit the flame.
6. Put the Chimney at the position provided.
7. Select the desired filter.
8. Take the double distilled water in the sample holder. Keep on the mixing chamber platform.
9. Connect the plastic capillary tubing with the fine metal capillary. Dip the capillary tube in the sample.
10. Sample will be aspirated and color of flame may change depending upon the sample.
11. Allow the water to be aspirated for cleaning of the capillary tube.
12. Adjust the blank reading to Zero with Zero knob.
13. Remove the blank replace with the standard solution of higher concentration and adjust the reading with calibration knob to the ppm of solution.
14. Clean the capillary tube with double distilled water and take the reading of another concentrations to get a calibration curve.
15. After each reading of the sample, the capillary should be washed with double distilled water.
16. After every 20-30 minutes of operation, the instrument should be re-calibrated by repeating the operation of calibration.

PRECAUTIONS:

1. Air compressor should always be switched on first, then only the LPG gas should be ignited.
2. Flame should not be too high, should form a cone of flame, can be viewed from the window provided. If too high and not maintained, the flame may burn the unit and other parts.
3. Never try to look from the top of the igniting chamber while the flame is burning (harmful for eyes). Always view from the window provided.
4. Always use clean glass apparatus and double distilled water.

Accessories: 5 sample holders, 3 plastic capillaries, 1 chimney, Unit -1, Unit-2 , gas pipe, drain pipe, air inlet and outlet pipe.

UNIVERSITY INSTITUTE OF PHARMACY, C.S.J.M. UNIVERSITY, KANPUR

STANDARD OPERATING PROCEDURE: - DIGITAL TURBIDITY METER

Make and Model: MODEL: EI-331 **PURCHASE DATE: 7.11.2006**

SOP NO: UIP-P/INST.LAB/ **EFFECTIVE DATE: 6.6.2018**

PREPARED BY DR. NISHA SHARMA

Range switch: Two position selector, 200 and 1000 NTU range. Resolution of NTU is 1 NTU.

Set Zero: To set zero display when the test tube containing distilled water is inserted and lid is closed.

Calibrate: This control is 10 turn pot for calibration of the instrument with standard solution in the light path.

ON/OFF switch: The instrument must be switched off when not in use.

Calibration:

Turbidity free water: It is difficult to obtain, however pass the distilled water through a membrane filter with a precision size holes of 0.2mm, rinse the collecting flask with filtered water twice and discard 200ml of filtered water.

Stock Solution turbidity Suspension:

1. Solution 1: Dissolve 1.000g hydrazine sulphate (caution: carcinogen, avoid inhalation, ingestion, skin contact) in distilled water and dilute to 100ml in a volumetric flask.
2. Solution 2: Dissolve 10g hexamine LR grade in distilled water and dilute to 100ml in a volumetric flask.
3. In a 100ml volumetric flask, mix 12.5ml sol. 1 and 12.5ml sol. 2. Stand for 24 hours at 25±3°C. Dilute to mark and mix. The turbidity of suspension is 1000NTU.
4. Prepare the solutions and suspensions weekly.

Standard turbidity Suspension:

Dilutions of other conc. can be prepared from this 400 NTU solution by following method.

Solution:	1000NTU	Distilled water
400NTU	40ML	60ML
200NTU	20ML	80ML
100NTU	10ML	90ML

Calibration:

1. Switch ON. Wait.
2. Select appropriate range depending on expected turbidity
3. Set Zero with turbidity free water using a blank solution & adjust 000 with the set Zero knob. The CAL control should be moved 5 turns clockwise from 0 positions.
4. Take standard suspension prepared. For 0-200NTU use 100 NTU solution and for higher range use 400 NTU solution.
5. Take the measurements and set display to the value of the standard suspension with the calibration knob.
6. Now the instrument is ready to take measurements of any unknown solution.

OPERATION:

1. Allow sufficient warm up period after switching ON the instrument.
2. Take the test tube containing distilled water or blank solution in the test tube holder and close the test tube holder lid. Make sure that the mark on the test tube coincides with the mark on the panel.
3. Select the required range for measurement.
4. Adjust the display to 000 by adjusting “set zero” knob.
5. Remove the test tube containing distilled water & insert another test tube containing standard solution (say 400 NTU). Place it in the test tube holder.
6. Take the measurement of the solution suspension & adjust the ‘Calculate’ knob so that the display reads the selected standard solution value.
7. Again check the display zero with the test tube containing distilled water.
8. Now the instrument is ready to take measurement of any unknown suspension.
   Note: Ensure that the appropriate range is selected.

UNIVERSITY INSTITUTE OF PHARMACY, C.S.J.M. UNIVERSITY, KANPUR

STANDARD OPERATING PROCEDURE: - FTIR- IR-Affinity-1S Make and Model: MODEL: EI-331

PURCHASE DATE: 31.3.2014 **EFFECTIVE DATE: 6.6.2018**

SOP NO: UIP-P/INST.LAB/ **PREPARED BY DR. NISHA SHARMA**

Demonstrated By Mr. Nahar Singh 09313324177

PRECAUTIONS

1. Check the silica gel beads, always should be Blue. If pink colored, Replace with fresh packets of silica (4 packs)
2. Dehumidifier should be switched on daily in the morning whether the instrument is in use or not and while leaving in evening switch off the dehumidifier by directly unplugging the instrument at the back. Humidity should be between 22-25°C. In any case it should not go beyond 55°C.
3. Do not shift the instrument. Do not try to take rest on instrument.
4. Keep the NaCl discs always in desiccator when not in use.

Liquid sample NaCl discs: Demountable OR Mountable (fixed thickness)

For volatile/organic samples. For aqueous samples: KBr S5 holder, or ZnSe window

PRECAUTIONS:

1. Make sure no water is present in the sample, otherwise it will dissolve the NaCl discs.
2. After working, the discs are cleaned by washing with CCL_4 /$CHCl_3$.
3. Keep in desiccator always.
4. Don't touch the discs with fingers in the center. Touch only the edges of discs.
5. Place the discs over each other symmetrically to overlap them and tighten in the plate supplied.
6. Don't over tight the discs otherwise they may break.

PROCEDURE

1. Switch on the instrument
2. Go to LABSOLUTIONS –IR on desktop. Double click the ikon. Parameters viz. Spectrum, Quantification, Photometric etc. are displayed on the window opened. Option Post run is used if we have any saved data.
3. In between the window alert showing that the instrument may be damaged may appear. (No problem)
4. When the software window opens, Initializing of the instrument starts automatically (status seen on the left hand side of the window). Last message is Success. Initialization takes place for diagnostics. 3 green colors on the right hand side of the window indicating humidity, lamp, laser are displayed.
5. Place the plate in the sample holder space in the instrument with any sample. (Tare kind)
6. Then go to background option done in respect to air, NaCl, KBr. i.e. without sample. And carry out sample scan. Monitor option used for maintenance . Select the folder and save it in D/E drive and not C drive. Connect the sample, Select Sample ID. Go to back ground option. Scan click OK. Go to measurement mode. Upto 45 scans are recommended by shimadzu. But 25-35 scans can be selected. Under Resolution option 4 to 1 can be selected, but best is 4. RANGE for liquid sampling (NaCl) is 600 onwards.
7. Then place the sample. Repeat the same.
8. Validation of the instrument is done as per EP/JP Pharmacopoeia. EP has less tolerance.
9. The disc should have no air gap when other disc is placed over the sample. Just like cover slip. Measurement mode selected is % Transmission. Apodization selected is Happgeneral. Select no. of scans, resolution to 4, range from 600 to 4700. With KBr disc range is 350, with KRs range is 250.
10. Select the peak table, Under manipulation select peak pick. Threshold. 0.1 transmittance 75%. Transmission can be 100%, 102-103%.
11. Calculate – click OK. In Comment column typing can be done. Go to print preview. Go to search and library for matching. Select the type of compound whether organic or any other as per the list provided.
12. Remove the sample plate, unscrew it and safely remove the plate and demount the discs. Clean them with the help of Chloroform. Do not wipe with any type of paper. Let it dry on its own and place in desiccator.

FOR DETERMINATION OF PURITY OF SAMPLE

1. We have IR data 2 and IR data 3. Go to manipulation option, select purity option.
2. Get the data purity graph. View data, drag the data, calculate.
3. We get purity index (No %). Print template is selected from the file to enable the wave no. values at the corresponding peaks.
4. Under file option, when clicked two data I and data P are visible. Data I selected when the table is to be seen on right side and graph parallel to table on left side. Generally data P option selected where in 1st graph appears in printout and later below graph table with values is seen.

Demountable cell is used for less volatile samples.

For highly volatile and quantitative analysis mountable plate is used (fixed thickness 0.1mm). Insert the sample with the help of syringe from one end till the sample appears from the other end, taking care that sample does not spills out from the end. Cap both the ends. Place the cell into the sample holder.

PROCEDURE FOR VALIDATION OF INSTRUMENT (FTIR) (done every month)

Precaution: The polystyrene film card supplied should be kept safely. Should not be allowed to scratched.

1. Go to software ikon, at the desktop.
2. Select Macro option. Select EP option.
3. Run the option. A window showing Lab sol. IR appears with USERID and PW options.
4. Under user id type Admin. Do not disturb the password option. Click OK.
5. EP window appears showing LOAD, Measurement and Background option.
6. Click only Measurement, Not background.
7. Left side window – shows succeed. 3 green rectangular boxes on left side.
8. Window appears with Options
9. Instrument name (do not change), Sr. no. 21965100179 (do not change), Temp, sample name (polystyrene), Humidity, Inspected by, no. of scans 45.
10. In between window alarming about that instrument may be damaged may appear. Click OK.
11. Carry out the scan in air and then with polystyrene film. Set polystyrene film into the sample compartment. Click OK.
12. In EP absorbance peaks are observed. In JP transmittance peaks are observed. Take the print.
13. Validation completed at the end. Note the date somewhere in diary till next validation.

Pellet Press Sampling (for Powdered samples)

3 sample holder 1 Ellen key Sample holder window Die (with small pin hole) and punch First place the die with a pin point facing down Fill the sample in the sample holder Place on the die Place the punch over the sample Check the pressure is zero Tighten the vertical screw Then tighten the horizontal screw so that the pressure is about 1.7 ton. Leave for about 2 minutes Ratio of sample to KBr 1:300 The pellet formed should be as transparent as possible The sample pellet should be washed with acetone/chloroform. Always keep KBr, sample holder other attachment in Dessicator.	 Use gloves while handing as the moisture from the hands may give wrong results. To open the block, remove the bottom holder first by rotating lightly, then the sample pellet from the punch by slight rotation.

Place the pellet holder window in the Instrument such that the 2 knobs on holder are facing away from the IR lamp path.

Start the Labsolution software, as discussed previously

Place the KBr pellet, Start scan, black areas represent the moisture peaks.

Go to Instrument, initialization for the preliminary scan. Initialization succeeded. 3 Green blocks on right side.

BKG scan. (for KBr background)

Go to Measurement. Window opens. Go to measurement mode. Select Absorbance/transmittance. Usually 45 scans (can be 22-45 scans also), Resolution of 2-4 depends on Pharmacopoeia. Range of 400-4000/cm. BKG scan. Save the datas always in drive D not in C.

Peaks at 2400 or 600 indicates that of CO_2.

Then place the sample pellet. Take sample scan. Peak table below the graph.

To differentiate the CO_2 peaks and sample peaks at same wave frequency, Go to manipulation, Go to atmospheric correction, click Ok.

Peak pick table, go to manipulation, peak pick, window opens, select threshold can be 45 or more. Noise level 0.100, if 0.00 peaks will be very near.

Peak ratio: add peak, delete peak etc.

Purity index: Click Purity, window opens, Intensity can be changed. Take standard peak, and sample peak, drag the standard peak over the other one. Send to source to reference. Calculate. Purity is shown in purity index.