



INSTRUMENTS, APPARATUS AND TOOLS USED FOR RESEARCH

Pharmacognosy Department Faculty of Pharmacy, Assiut University Assiut, Egypt.

MICROSCOPES



Type: Leica Microsystems (EC3)

(Heerbrugg, Switzerland)

Description:

The Leica EC3 is an affordable high speed digital camera (3.1 megapixel) combined with Leica microscopes. The system incudes LAS EZ software, a perfect solution for performing a variety of imaging tasks such as annotations, calibrations and image measurements. Windows XP, Windows Vista and Windows 7 are used as operating system for EC3.

- Switch on the microscope's light source.
- Rotate the nosepiece to the objective power (4-40× magnification).
- Place a microscope slide on the stage under the slide clips.
- Adjust the small fine focus knob until the specimen image has clear and sharp contrast.
- Scan the slide (right to left and top to bottom) to see all the specimen.
- Using LAS EZ software for imaging the elements of the plant powders or the layers of the plant organs in the TS (transverse section).

MICROSCOPES





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MPLC



Type: MPLC (Micro Pump KP-7)

(Medium Pressure Liquid Chromatography)

(Kusano Kagakukikai Work CO., Tokyo, Japan)

Description:

MPLC is one of analytical technique used for isolation of the natural products under pressure. MPLC is commonly used now beside or in combination with other common preparative tools (Open column chromatography, Flash chromatography or HPLC). It utilizes two columns; a pre-column containing thermally deactivated silica and a main column of activated silica as a stationary phase (or any other packed materials). A solvent or mixture of solvents used as a mobile phase. A pump of 40 bar maximum is used for controlling the flow rate of the solvents. The eluates are collected manually or by fraction collector and the compounds are identified by TLC.

- Packing the column with stationary phase (Dry or wet filling).
- Solvent selection of the eluent system is a crucial point in development and optimization of MPLC. The selection of solvents occurs by TLC.
- Column preparation and regeneration before sample loading (Washing successively with methanol, ethyl acetate and hexane for Silica columns).
- Choice of the optimum flow rate (3-16 mL/min) for elution process (\downarrow flow rate $\rightarrow \uparrow$ the separation efficiency & \uparrow the separation time).
- Sample introduction (Injection of a small volume of concentrated solution is usually preferred).
- Collection of the eluates are performed manually or by fraction collector.

FLASH CHROMATOGRAPHY

Type: Compi-Flash Retrieve®

(Flash Chromatography)

(Teledyne ISCO, Nebraska, USA)

Description:

Flash Chromatography is a rapid form of preparative column chromatography based on optimized pre-packed columns through which is pumped solvent at a high flow rate (2-10 mL/min). It includes components of HPLC systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent.

- A plastic column with usually silica gel is used as a stationary phase (disposal).
- Selection of the eluent system occurs by TLC.
- Column preparation before sample loading (Washing successively with methanol, ethyl acetate and hexane for Silica columns).
- The flow rate used for elution process is 2-10 mL/min.
- Loading sample amount is 0.01-100 g
- Collection of the eluates are performed by fraction collector.

SPECTROPHOTOMETER

Type: JENWAY Spectrophotometer 6300 (single-beam)

(Bibby Scientific, Staffordshire, UK)

Description:

The 6300 spectrophotometer is a visible spectrophotometer covering a wavelength range from 320nm to 1000nm, with measurement modes for absorbance, % transmittance and concentration. Model 6300 is supplied with a 10mm cuvette holder.

How to use it!

• Instrument calibration: The calibration must be performed at the same wavelength at which the sample will be measured. Insert a cuvette containing the blank (clear) solution into the sample chamber and close the instrument lid. Press the CAL key. The instrument will perform a zero % transmission calibration followed by a 0.000 Absorbance calibration. An internal shutter is automatically activated to perform the zero % setting and this part of the routine is therefore independent of the solution in the light path.

How to use it! (continued)

- Sample measurement: Once the calibration has been performed a sample can be measured. Remove the cuvette containing the blank solution and place a cuvette containing the sample to be measured in the sample holder. Close the instrument lid and the absorbance result will be shown on the screen.
- Subsequent samples can be measured in the same way. If the wavelength is adjusted between sample measurements then the instrument must be calibrated again before more samples can be measured

ROTARY MICROTOME

Type: Rotary Microtome

(Micro-Tec, Bloomberg, Germany)

Description:

The tool used to cut extremely thin slices (sections) of plant tissues for microscopical examination. Microtome use steel blades to prepare plant tissues for light microscopy. The steel blades are typically fixed in horizontal position.

- Paraffin block of the plant organ is fixed in sample holder.
- Use the roller to adjust the required thickness of the section (3-5 μ m).
- Through the motion of the sample holder by flywheel, the sample is cutted by the blade.
- The paraffin sections containing TS of the plant organ are transferred into water bath to expand it.
- The TS of the plant organ is picked up on glass slide and placed into clearing agent (xylene) to remove the paraffin from the TS.
- The TS of the plant organ is hydrated and stained for microscopical examination.

V&CUUM PUMP

Type: Gast High Capacity Vacuum Pump (Gast manufacturing INC., Mich., USA)

Description:

Gast vacuum pump is air moving product for pressure and vacuum generation.

- The pump is connected to the device (Rota-vapor).
- The pressure is adjusted according to the boiling point of the solvent.
- The rota-vapor system is closed when the pump switched on.
- The pump is turned off after finishing the distillation of the solvent. 22

HPLC UNIT

Type: High Performance Liquid Chromatography (KNAUER).

Description:

HPLC is an advanced form of LC that used to separate the components of a mixture.

HPLC consists of the following components:

- a. Manual injector.
- b. HPLC Pump k-500, KNAUER.
- c. HPLC column, Phenomenex.
- d. UV detector K-2500, KNAUER.
- e. Data system (recorder), KNAUER.

How to use it!

After setting all components of HPLC.

- 1. The filtered sample is dissolved in the mobile phase and loaded manually into injector.
- 2. The degassed mobile phase (single solvent or mixture of solvents) is prepared and placed in its position that connected to the inlet of the pump.
- 3. The pressure is adjusted according to the used method.
- 4. When the UV-detector respond to different component, the recorder transform the signal into a peak to obtain the chromatogram.

SONICATOR

Type: Sonicator Ultrasonic CREST.

Description:

Sonication is the act of applying sound energy to agitate particles in a sample, for various purposes.

- 1. Fill the sonicator with water till the effective level.
- 2. Turn on the power of the device.
- 3. Put the sample need to be dissolved in a suitable closed vial and immerse it within the water till complete dissolution of the sample.

TISSUE CULTURE UNIT:

Type: Tissue culture unit.

Description:

Tissue culture is the growth of tissues or cells separate from the organism. This is typically facilitated via use of a liquid, semi-solid, or solid groth medium, such as broth or agar. With the more specific term plant tissue culture being used for plants. The term "tissue culture" was coined by American pathologist <u>Montrose Thomas Burrows</u>.

Consists of:

- Laminar flow work station.
- Cooled incubator with timed cycling and illumination.

• Other accessories:

- 1. Freezer.
- 2. Automatic multi-program dish washer.
- 3. Digital pH meter.
- 4. Centrifuge.

UV LAMP

Type: UV hand lamp, Vilber Lourmat, France (6 W, 365 nm tube, power: 12 W).

Description:

Used to detect and visualize the spots in TLC.

How to use it:

Turn on the power of the device and switch between long and short wave lengths for visualization.

FREEZE DRYER:

Description:

Freeze-drying or lyophilisation is an effective way of drying materials without harming them. It makes use of the physical phenomenon of sublimation, which involves the direct transition between the solid state and the gaseous state without passing through the liquid phase. To achieve this, the frozen product is dried under vacuum, without being allowed to thaw out. The process is suitable for a wide range of applications.

How to use it:

Before loading a new product, it is important to remove any water from the previous batch that remains in the ice condenser chamber. When this has been done the outlet valve and the ventilation valve are closed. The product should only form a layer of 1–2 cm, because if it is too thick this will have a detrimental effect on the drying time.