NIRMA UNIVERSITY

INSTITUTE OF PHARMACY

ICMR Sponsored

National Workshop on

Modern Instrumental Techniques in

Herbal Drug Research

10th -11th April 2012

PRACTICAL HANDBOOK

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Organized By:

Institute of Pharmacy

Nirma University

Sarkhej-Gandhinagar Highway,

QUALITY STANDARDS OF INDIAN MEDICINAL PLANTS FOR AYURVEDIC OR HERBAL DRUG INDUSTRY

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BIODATA

Dr. Neeraj Tandon is Deputy Director General (Senior Grade) / Scientist F Indian Council of Medical Research, Ansari Nagar, New Delhi. She has completed her Ph. D in Biosciences and Msc. First Class, Delhi University, Delhi. She is having total 29 years of experience in field of research, She has been awarded WHO fellowship on Medicinal Plants Information systems at Department of Medical Chemistry college of Pharmacy, University of Illinois, Chicago. She is also a Coordinator, Editor and Collaborative Participant as part of all the volumes of Quality Standards of Indian Medicinal Plants and also Phytochemical Reference Standards of Selected Indian Medicinal plants. She has also written several monographs on Medicinal Plants of India Volume II. 1987; Reviews on Indian Medicinal Plants Vol- 1,2,3,4,8,9; Perspectives of Indian Medicinal Plants in the management of Liver Disorders 2007.

ABSTRACT

The growing worldwide interest in medicinal plants and fast expanding global market in the last two decades has necessitated for quality ensured herbal drugs. Further, the increase in the incidence of toxicity reported due to the indiscriminate use of herbal preparations available over the counter and as food supplements make it important to ensure their safety and efficacy. Standardization of the presumed active approach, as only in few cases doses the drug-activity depends upon a single component. In fact, it is the outcome of synergistic effect of several active compounds and accompanying inert substances. It is thus reasonable to use the naturally occurring wholesome medicinal plant material for standardization. A number of factors such as age and sign of the plant, time of its collection, method of drying, garbling, storage etc. influence the proportion of various components in the plant materials. The analytical limits therefore, cannot be as precise as for single pure synthetic drug. In spite of these and other inherent difficulties, quality standards for the medicinal plants used in India are necessary for the drugs and formulations produced from them to be of adequate quality, safety and efficacy for their wider acceptance.

DEVELOPMENT OF STANDARDIZED POLYHERBAL FORMULATIONS: AN OVERVIEW

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BIODATA

Dr. PRATEEK PATEL is DGM - R&D / QC-QA, Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat. He has completed his B. Sc, M. Sc and Ph. D from Mumbai University. He is having more than 15 years of experience including 10 years of Managerial level experience. He has also worked with Pharmanza Herbal Pvt. Ltd., Anand, Gujarat as Manager R&D / QC-QA); SGS India Pvt. Ltd., (LSS), Chennai as Assistant Manager Bioanalysis, Therapeutic Drug Monitoring Laboratory as Manager Bioanalytical Projects, Therapeutic Drug Monitoring Laboratory, Mumbai, Quality Manager, Therapeutic Drug Monitoring Laboratory, Mumbai, Analyst & Sr. Analysts. He is life member of professional bodies like IPA and CSI. He is having 4 international and 8 national publications on his credit. He has handled many research projects in various areas like Nutraceutical products; Development, Validation & Transfer of analytical & bioanalytical methods, Laboratory & Production Equipment, Mfg. Process, Cleaning & Sanitization; Novel drug delivery systems, Method development, in various herbal any poly herbal formulations in various categories of dosage form (Tablets, Capsules, Syrups, Oils, Creams, Ointments, etc.) & its evaluation (toxicity, preclinical & clinical).

PERSPECTIVES IN PHYTOMEDICINE: A FEW CASE STUDIES

Prof. (Dr.) S.H. MISHRA
M.S. University, Vadodara

BIODATA

Prof. (Dr.) S. H. MISHRA Professor, Pharmacy Department, Faculty of Technology & Engineering, The Maharaja Sayajirao University Of Baroda. Sir has completed his B. Pharm (1969), M.Pharm (1972), Ph.D (1980) from Sagar University. Sir is having total 43 years of experience in teaching and research. Sir has started his career as Manufacturing Chemist, M/s. Venus Laboratoires, Indore (M.P.). Before joining M. S. University sir has worked as assistant professor with Govt. of M.P Education Department. Sir is having broad area of expertise in herbal drug research. Prof. Mishra has guided more than 25 Ph. D students and more than 50 M. Pharm students. Sir is having more than 100 research paper on his credit and awarded IDMA Best Research Paper award in 1996. He has completed various research projects sponsored by UGC, AICTE, MHRD etc. worth Rs. more than 1 crore. Sir is also life member of various professional bodies like IPA, APTI, ISP. Sir was also a chairman of board of Studies on Pharmacy University of Indore and M.S. University. He has also served as member various committees like Staff Selection Committee of M.S.University, Research Degree Committee of University of Indore, Education Regulation Committee Pharmacy Council of India, Peer Committee RID AICTE, PG Approval Committee AICTE etc.

HPTLC: AN ANALYTICAL TOOL FOR HERBAL ANALYSIS

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BIODATA

Chintan Shah is currently working as Application Specialist at Anchrom Enterprise (I) Pvt Ltd. Mumbai. He has completed his Diploma In clinical Research from Catalyst clinical services Pvt ltd. Delhi in 2007; Bachelor of Pharmacy from Rajiv Gandhi University Health Science Bangalore in 2008; M. Pharma (Quality Assurance) in 2011 from KLE University Belgaum. He has done Advance Certificate Program in Project Management from Catalyst clinical services Pvt ltd. Delhi. and Advanced PG Diploma in Drug Regulatory Affairs in 2011 from Raaj Global Pharma Regulatory Affairs Consultancy Analyzing various sample like synthetic and herbal drugs, Biological, Dyes and food samples. He is presently involved in method development, Parameters of validation, standardization of herbals, degradation studies, handling HPTLC and its latest Automation like Gradient HPTLC, AMD2, ADC2, and ATS4. He has writen several national and international papers and also delivered lectures as resource person. He is reviewer of International Journal of Pharmaceutical and Applied Science. He has also done project approved by US FDA on the data of UV spectroscopy, HPTLC, RP- HPLC of "Analytical Method Development and Validation of Cinacalcet hydrochloride".

ABSTRACT

HPTLC over many years has become an indispensable tool in phytopharmaceutical R&D as well as in quality laboratory. This reflects the high significance of this technique for phytochem industry. Numerous samples can be run in a single analysis there by dramatically reducing analytical time for a series of samples. It allows viewing different wavelengths of light providing a more complete profile of the samples. In recent years, the HPTLC technique has been improved to incorporate the following features e.g. sample application, development mode, detection mode & documentation. HPTLC can be run in a highly standardized way, a very important aspect for method used in phytoindustry. Traditionally TLC plays central role in the identification of medicinal plants and derived products. It is part of corresponding quality monographs of most pharmacopeias. In recent years there is an international trend to modernized and improve TLC methods which often date back to the 1970s.The typical analytical task performed by HPTLC are, identification of Raw Materials and products detection of adulteration, stability test, quantification of marker compounds, finger printing analysis of herbal sample, etc.

HANDLING OF INSTRUMENTS

SUPER CRITICAL FLUID EXTRACTION

Introduction:

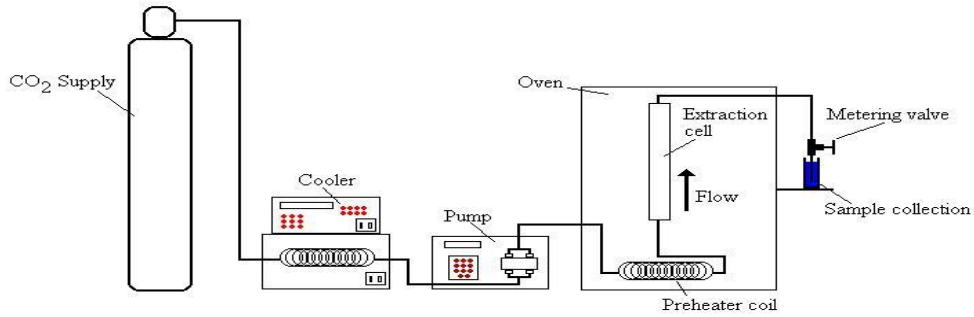
- Super Critical Fluid Extraction is the process of separating one component (the extractant) from another (the matrix) using supercritical fluid as the extracting solvent.
- Super critical fluid works on the basis of critical temperature and critical pressure. The temperature above which the substance can no longer exist as a liquid, no matter how much pressure is applied is called supercritical temperature.
- Likewise, there is a pressure above which the substance can no longer exist as a gas no matter how high the temperature is raised and called supercritical pressure respectively. Super critical fluids are neither true liquids nor true gas. The most widely used solvent in SFC is CO_2.
- There are two types of SFC, packed column super critical fluid chromatography (pSFC), and capillary column super critical fluid chromatography (cSFC) based on the type of column used

Principle:

A solid stationary phase and a supercritical mobile phase are used and the main mechanism of separation is adsorption. When a mixture of components is dissolved in mobile phase is introduced in to the column, the individual components move with different rates depending on their relative affinities. The compound with lesser affinity towards the stationary phase moves faster and hence eluted out of the column first. The one with greater affinity towards the stationary phase moves slower down the column and hence it is eluted later. Thus the components are separated. The type of interaction between the stationary phase and solute is reversible in nature

Instrumentation:

The main component of SFC includes pump, injector, oven, column, pressure control device and detector. The basic instrumental configuration required for any analytical supercritical fluid system is minimally defined by the pressure region



Cool circulator (JASCO MODEL CH-201)

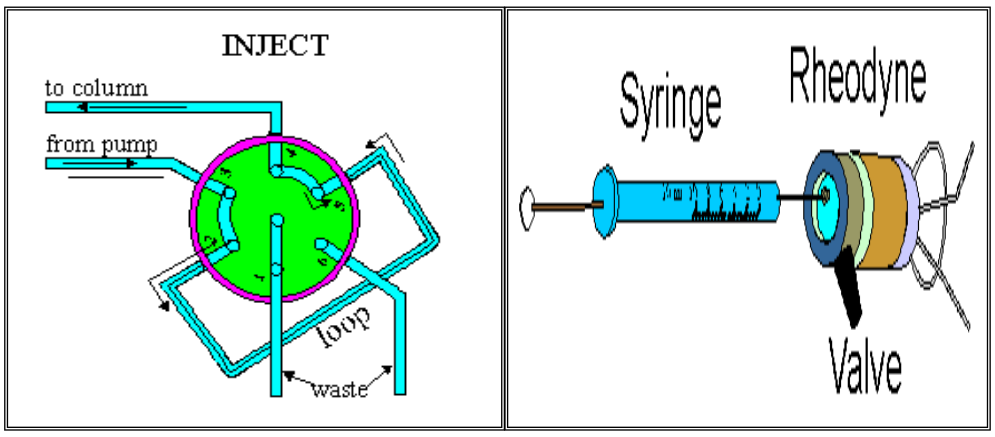
This device is responsible for cooling of carbon dioxide gas and converting it in to liquid phase when delivered to the pump. The temperature is maintained at about -11ºC, liquefying temperature of carbon dioxide.

Pump and mixer (JASCO MODEL PU-980)

There must be a pump to initially pressurize the system and to maintain supercritical pressure under dynamic operating conditions. The chromatograph for cSFC usually contains a single syringe pump (Delivery rate: 1-80µLmin^{-1}) while that of pSFC contains high pressure reciprocating pump (delivery rate: 0.001-10 mLmin^{-1}) .

Sample injector (Rheodyne model 7125 with 20µl fixed loop)

The amount of sample that can be introduced depends on the type of SFC. In cSFC the injection volume is about 0.5 nL while in pSFC, it is 5-20 µL So, an injection valve with split/splitless mechanism is necessary to obtain reproducible injections.



Column and Oven (JASCO model CO-965)

The columns for cSFC are typically made from fused silica with diameter of 0.025-0.1 mm and length of 1-35 m. The columns for pSFC are made up of stainless steel and having internal diameter 0.5-4.6 mm and length 3-25 cm. The column temperature of cSFC is maintained through GC-type ovens, while in pSFC the column temperature is maintained in a specific range.

Stationary phases used in SFC

Stationary phases used in cSFC are similar to GC. The most commonly used stationary phase is polysiloxanes. (0.1- 3µm diameter). The stationary phases for pSFC are similar to those of HPLC (3-10 µm diameter).

Mobile phases used in SFC

The mobile phase in SFC is the most influential parameter governing solute retention on the column. The most widely used mobile phase for supercritical fluid chromatography is carbon dioxide because it is an excellent solvent for a variety of organic molecules. The critical parameters CO_2 are 31^0C and 72.8 atm. The advantages of CO_2 over other solvents are its low toxicity, cost effectiveness and compatibility with most detectors. Generally, the addition of low percentages (1-20 %) of polar modifiers like methanol into CO_2 is an efficient means to enhance the solvent strength of mobile phase.

Back pressure regulator (JASCO MODEL 880-81)

This is a device, which is used to maintain desired pressure in the column by a pressure-adjustable diaphragm or controlled nozzle so that the same column-outlet pressure is maintained irrespective of the mobile phase pump flow rate. It keeps the mobile phase supercritical throughout the separation to prevent clogging. The pressure restrictor is placed either after the detector or at the end of the column.

Detector (JASCO MODEL UV 975)

A variety of detectors can be used in SFC, but the most critical step in selecting a detector is the nature of the analytes, flow rate and composition of the mobile phase. Detectors used in cSFC are Flame Ionization Detector (FID), Thermal Ionization Detector (TID), Flame Photometer Detector (FPD), Nitrogen-Phosphorous Detector (NPD), Electron Capture Detector (ECD), and Evaporative Light Scattering Detector (ELSD). In case of pSFC the choice of detector is usually UV-VISIBLE Detector and Fluorescence

Aim: To extract volatile oil from Cinnamon bark powder by SFE

Requirements:

Sample, pump, column oven, injector, back pressure regulator, detector, sample collector, CO_2 gas, methanol, Cinnamon bark powder

Procedure:

- Fill methanol (2-2.5 L) in cool circulator.
- Switch ON the cool circulator.
- Adjust temperature -11ºC by up and down arrow keys.
- Once the required temperature is achieved, switch ON the carbon dioxide and modifier pump. Keep the valve for carbon dioxide and methanol closed.
- Place the required material in extraction vessel. Arrange necessary connections.
- Switch ON the column oven and adjust the required temperature by pressing the EDIT button and using up and down arrows.
- Turn ON the UV visible detector.
- Adjust the required wavelength by pressing digit “2” and entering the required wavelength from the keypad. Then press ENTER.
- Switch ON the backpressure regulator. Adjust the required backpressure and temperature.
- When all the parameters are set, then turn on the gas supply from carbon dioxide cylinder.
- Turn ON the pump by pressing pump button on keypad.
- Adjust the required flow of CO2 and modifier by pressing digit “2” from keypad. Enter the required flow rate with the help of keypad and then press ENTER.
- Switch ON the computer.
- Keep the rheodyne loop injector into LOAD position.
- Inject the sample into the injector and turn the injector into INJECT position.
- Allow the chromatogram to run for specific time period.
- Chromatogram gets saved automatically in the software and later can be opened to get the details.
- For using SFE, replace the extracting vessel provided with the instrument and if not required then bypass UV detector.

- After completion of work, turn off the gas supply and give wash to the column with mobile phase for half an hour.
- Turn OFF the instrument.

Applications in herbal drug research:

- Decaffeination of coffee and tea
- Deodorization of oils and fats
- Extraction of vegetable oil from seeds and grains
- Extraction of essential oils from plants
- For determination of constituents such as chlorophyll and its derivative, carotenoids, tocopherols , vitamins and phenolics
- For the analysis of pesticide residues in canned foods, fruits and vegetables wherein pyrethroids, herbicides, fungicides and carbamates are present
- Denicotinization of tobacco
- Separation of lanolin from wool
- Flavour/fragrance extraction
- Identification of gingerol and shogoal in ginger

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

CAMAG HPTLC

Introduction:

Chromatography is a dynamic method of separation of molecular mixtures, where the separation process involves differential distribution of molecules between two immiscible phases stationary phase and mobile phase. The heart of a chromatographic system is column where the molecules (or ions) undergoing separation are being continuously redistributed between the mobile phase and the stationary phase.

The modern use of TLC has seen a strong move in the direction of plate scanning and video imaging as a means of providing sensitive and reliably accurate results and a more permanent record of the chromatogram.

Principle:

When solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others.

The fundamental parameter in HPTLC is the retardation factor R_f:

$$\mathbf{R_f = Zs / (Z_f - Zo)}$$

Where,

Z_f: Distance traveled by the solvent front from the point of application.

Zs: Distance traveled by the solute front from the point of application.

Zo: Distance between the point of application of solvent and solute.

Note that the efficiency of a planar system is not constant, but depends on the distance that the solute has traveled, or its retention and R_f value.

Instrumentation:

The HPTLC system consisted of a CAMAG Linomat V semiautomatic spotting device with nitrogen flow, a CAMAG 100 µL applicator syringe (Hamilton, Bonaduz, Schweiz), and a CAMAG (10 cm × 10 cm) twin-trough chamber with a stainless steel lid. Densitometry was carried out with a CAMAG TLC Scanner 3 supported by the winCATS software (Version 1.4.2.8121).

Standard operating procedure:

Spotter: Linomat V:

- Turn the power switch of the Linomat V to the ON position.
- Adjust the pressure of Nitrogen gas to 5 bar.
- Enter the required parameter for spotting in winCATs software.
- Fill the syringe with required volume of solution and place it into holder.
- Press “execute” key in software tab and subsequently press “ENTER” button on the instrument.
- After completion of operation a long beep will be sounded.
- Remove the syringe and wash with methanol.

Development of Plate:

- Prepare the appropriate volume of developing solvent.
- Pour solvent into chamber.
- Tilt chamber to the side (about 45 [degrees]) so that solvent volume in both troughs equalizes.
- Set chamber on bench, replace the lid and let chamber equilibrate as per optimized time.
- Mark the desired developing distance with a pencil on the edge of the plate.
- Slide off the lid to the side.
- Insert the plate into the front trough.
- Replace lid.
- Develop plate to the mark.
- Open lid, remove plate.

TLC Scanner III:

- Turn the power switch of the TLC Scanner III to the ON position.

- Place the developed TLC plate on the stage.
- Press “ILLUM” button and load the pod.
- Determine the X and Y position of the track to be scanned on the TLC plate.
- Enter the X and Y position along with the distance between two tracks (if multiple bands are applied) and other required parameters in the software menu.
- Press “execute” key in software tab.

CAMAG Linomat-5 sample applicator

CAMAG Scanner 4

Steps invloved for HPTLC

- Activate the precoated HPTLC plate in Hot air oven at 110^{o}c for 30 min.
- Prepare the standard and samples to be analyzed by dissolving in appropriate solvent.
- Rinse the Hamilton Syringe 3-5 times with methanol and fill it with the sample and place on syringe holder.
- Place the activated plate on the Linomat sample applicator platform in appropriate direction.
- Apply the require volume and width of band of sample by giving the command to Linomat sample application in Win cats software.
- After application of sample, remove the plate from sample applicator and view under UV light to confirm proper spotting.
- Meanwhile prepare the mobile phase developed for the analysis by suitably mixing the solvents of appropriate volume and transfer it to the developing chamber (size 10x10 cm). Allow the chamber to saturate for 10 – 15 minutes.
- Place the spotted plate in to the saturated chamber for development with mobile phase. (it should be noted that the spots of samples applied should be above the level of mobile phase).
- Develop the plate with mobile phase up to the distance of at least 75 % of the plate height and measure the distance travelled by solvent front.
- Dry the developed plate using hot air dryer appropriately.

- Observe the developed plate for migration of spots in UV Chamber at 254nm and 366nm.
- If the spots are not visualizing, there is need for derivatization with suitable spraying reagent for visibility of spots.
- For identification and quantification, mount the plate in appropriate direction in CAMAG TLC Scanner.
- Select most appropriate slit dimensions for complete and accurate scanning
- Measure the responses of all the developed spots having same Rf value as that of standard obtained due to densitometric scanning.

Experimental:

Aim: To estimate Rutin in *Tephrosia purpurea* powder and Asiatic acid in *Shorea robusta* gum resin

Requirements:

Chemicals and reagents: Standard Rutin, *Tephrosia purpurea* powder, Standard Asiatic acid, *Shorea robusta*, ethyl acetate, formic acid, methanol, glacial acetic acid, toluene, chloroform

Glassware: Conical flask, beaker, pipette, petridish, volumetric flask (10 ml)

Procedure:

(A) For rutin estimation:

Preparation of standard solution:

10 mg of rutin was weighed and dissolved in 2 ml methanol and the volume was made up to 10 ml. (1000 ppm)

From the above solution, 1 ml of solution was pipetted out and the volume was made up to 10 ml in volumetric flask. (100 ppm)

Preparation of sample (Extract):

5 gm of *Tephrosia purpurea* powder was weighed and extracted with methanol (50×3 ml) for 1 hour under reflux. The extracts were combined and filtered using Whatmann filter paper. The filtrate was concentrated to 10 ml volume.

Further dilution was done by taking 1 ml of extract in 10 ml volumetric flask and volume was made with methanol. The sample and standard solution thus prepared was used further for chromatographic analysis.

(B) <u>For asiatic acid estimation:</u>

Preparation of standard solution:

10 mg of Asiatic acid was weighed and dissolved in 2 ml methanol and the volume was made up to 10 ml. (1000 ppm)

From the above solution, 1 ml of solution was pipette out and the volume was made up to 10 ml in volumetric flask. (100 ppm)

From the above solution, 2 ml of solution was pipette out and the volume was made up to 10 ml in volumetric flask. (20 ppm)

Preparation of sample (Extract):

5 gm of *Shorea robusta* powder was weighed and extracted with methanol (50×3 ml) for 1 hour under reflux. The extracts were combined and filtered using Whatmann filter paper. The filtrate was concentrated to 10 ml volume.

Further dilution was done by taking 1 ml of extract in 10 ml volumetric flask and volume was made with methanol. The sample and standard solution thus prepared was used further for chromatographic analysis.

Chromatographic conditions:

(A) <u>For Rutin estimation:</u>

Stationary Phase: Precoated TLC plate, silica gel 60 F_{254}

Mobile Phase: Toluene: ethyl acetate: formic acid: toluene: methanol: glacial acetic acid (6:2:2:1:0.25) Sample Band width: 5mm

Chamber saturation time: 15 min.

Plate development distance: 80 mm

Detection of Spots: by CAMAG TLC Scanner

Scanning Slit Dimensions: 4 X 0.20 mm

Scanning Wavelength: 254 nm

(B) For Asiatic acid estimation:

Stationary phase: Precoated TLC plates, silica gel 60 F_{254} (10×10 cm)

Mobile phase: Chloroform: Methanol (9:1)

Sample Band width: 5 mm

Chamber saturation time: 20 min.

Plate development distance: 80 mm

Spraying Reagent: Anisaldehyde - H_2SO_4

Detection of Spots: by CAMAG TLC Scanner

Scanning Slit Dimensions: 4 X 0.20 mm

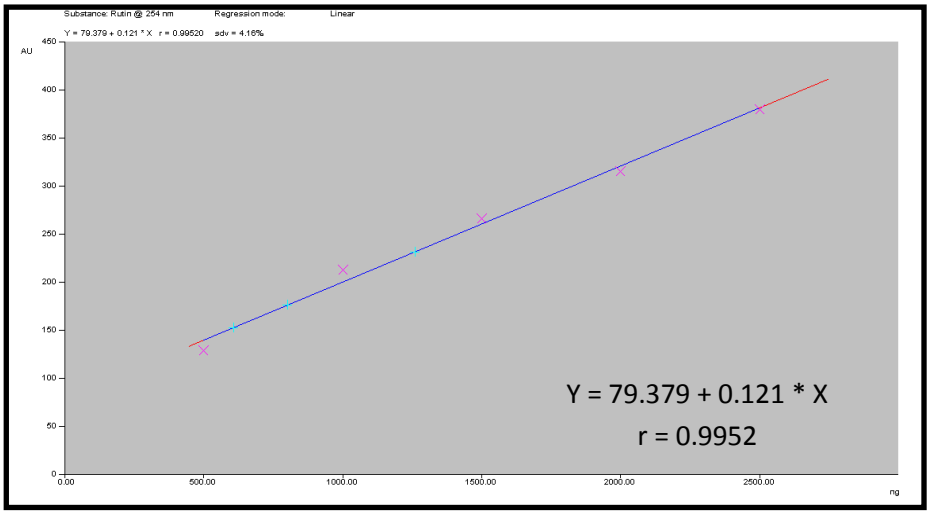
Scanning Wavelength: 615 nm

Results:

a) Rutin estimation in *Tephrosia purpurea*

Track Fraction	Vial Height	Rf X(calc)	Amount	X(calc)	Remarks	Area	Amount	Sample ID
1	1	0.46	500.00 ng	128.84	--	4948.83	--	Rutin
2	1	0.46	1000.00 ng	212.84	--	8901.41	--	Rutin
3	1	0.45	1.500 µg	266.5	--	11461.32	--	Rutin
4	1	0.45	2.000 µg	315.03	--	13799.8	--	Rutin
5	1	0.45	2.500 µg	379.78	--	15747.47	--	Rutin
6	2	0.45	--	231.75	**1.261 µg**	16990.09	**2.636 µg**	Extract
7	2	0.44	--	176.31	**802.31 ng**	14623.84	**2.189 µg**	Extract
8	2	0.43	--	152.69	**606.84 ng**	14058.69	**2.083 µg**	Extract

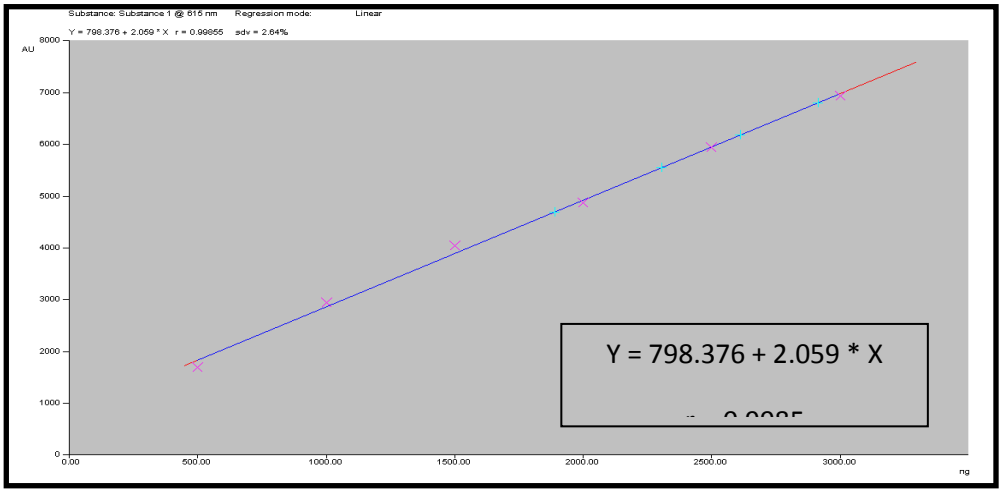
Linearity Curve for Rutin



B) Asiatic acid estimation in *Shorea robusta*

Track Fraction	Vial No.	R_f X(calc)	Amount	X(calc)	Remark	Area	Amount	Sample ID
1	1	0.34	500.00 ng	94.82	--	1690.96	--	Asiatic acid
2	1	0.34	1000.00 ng	171.19	--	2943.49	--	Asiatic acid
3	1	0.35	1.500 µg	232.04	--	4038.65	--	Asiatic acid
4	1	0.35	2.000 µg	276.23	--	4860.49	--	Asiatic acid
5	1	0.34	2.500 µg	317.08	--	5940.59	--	Asiatic acid
6	1	0.34	3.000 µg	362.78	--	6934.46	--	Asiatic acid
7	2	0.34	--	260.55	**1.925 µg**	4688.27	**1.889 µg**	10 mg ext SR
8	2	0.34	--	295.42	**2.260 µg**	5543.88	**2.305 µg**	10 mg ext SR
9	2	0.34	--	325.49	**2.549 µg**	6174.42	**2.611 µg**	10 mg ext SR
10	2	0.35	--	343.62	**2.723 µg**	6796.62	**2.913 µg**	10 mg ext SR

Linearity Curve for Asiatic acid



Applications in herbal drug research:

- Standardization of medicinal plant extracts
- Standardization of herbal formulations
- Estimation of phytoconstituents in crude drugs
- Fingerprint for identification of medicinal plants
- Identification of pesticides and mycotoxins in herbs and health foods
- For analysis of lipid biomedical examination
- Analysis of alkaloids – Cinchona alkaloids , Rauwolfia alkaloids , Ephedra alkaloids

IWORX

Introduction:

IWORX is data acquisition system used for measurement of various Cardiovascular, respiratory and gastro-intestinal parameters. Different transducers and probes are available for measurement of these parameters. For measurement of blood pressure and heart rate, pressure transducer is used which is attached to the acquisition system.

iWorx LabScribe2 is a data recording and analysis software, developed over 5 years. *LabScribe2* software is the result of more than 70 years of collective experience in data recording.

There are some pre-requisite system requirements as follows:

- The software requires windows (win98 through Vista) or a Macintosh (OSX 10.3 or later) computer.
- Pentium III level computer with at least 256 megabytes of RAM and at least 50 megabytes of free space on your hard drive is required.
- The preferred processor is a Pentium IV or dual core processor with 1 Gigabytes of RAM. The faster the processor, smoother the screen scrolling, however, all data is recorded regardless of the screen scrolling display.

The management of recorded data is divided into 3 steps:

1) Recording the data onto the computer
2) Navigating the recorded data to find particular areas of interest, and
3) Outputting the data either in printed or analyzed form.

Standard operating procedure:

- Connect the amplifier to the computer using USB cable.
- Connect the power cable of amplifier and transducer with mains. Connect the transducer & the amplifier with each other using green colored to banana for earthing.
- Connect transducer channel with respective amplifier channel (in analog inputs only, do not connect in digital output) using connecting cable.
- Switch on the mains. Switch on amplifier and transducer power supply switch. Green colored light will appear when switch is on.
- Switch on the computer system.
- Connect the transducer (as per the experiment protocol eg. Pressure transducer for Blood pressure recording.) to the 8 pins near to the channel selected in step no. 3.
- Open LabScribe-2 software. When all the systems are on, a message indicating hardware found IWX 118:2008 1-3 will appear on screen. Click OK.
- Open the setting menu and load the settings as per the requirement of the experiment.
- Now to record the data press record button and to stop the recording , press stop button from LabScribe-2 software.
- Analyze the data using the software and save the file with proper name and close the software.
- Shut down the computer system

Aim: To record hemodynamic parameters in rats by carotid artery canulation.

Requirements: Ketamine, Diazepam, Heparinized saline, canulas, bull dog clamp, scissors, forceps, hemostatic forceps, thread

Procedure:

- The rat is anaesthetized by Ketamine (100 mg/kg, i.p.) + Diazepam (7 mg/kg, i.m.).
- The rat is placed on thermodynamic board maintaining body temperature at 37 ± 1 °C during the experiment
- The ventral cervical area is shaved and the skin is swabbed with surgical scrub.
- A 1-1.5 cm skin incision is made over the intersection of the omohyoid and sternohyoid muscles.

- The sternohyoid muscle is separated by blunt dissection to locate the trachea and carotid artery.
- A small V-shaped cut is made into the trachea and it is cannulated to maintain respiratory rate.
- The carotid artery behind the trachea was exposed.
- The vagus nerve is carefully separated from the carotid artery.
- Supporting the carotid artery using forceps, two pieces of equal length of sterile silk suture are passed beneath the carotid artery.
- In reference to the heart, the more distal silk suture is tied tightly to occlude blood flowing from the head region.
- Near to the heart, bull dog clamp is placed on the artery to stop blood flowing from the heart.
- A small cut is made using microscissors into the carotid artery between the two ligatures. A catheter filled with heparinized saline solution is inserted into the carotid artery. (Note: the catheter is attached to the transducer and syringe containing heparinized saline with help of 'T')
- The catheter is tied along with the artery with the silk sutures.
- The bull-dog clamp is removed and heparinized saline in injected.
- The recordings are noted down.

Applications in herbal drug research:

- It is basically used for recording of Blood pressure.
- The hypotensive/ Antihypertensive effect of any herbal extract can be measured by using *iWorx.*
- The comparison of the hypotensive activity of a Standard with test drug can also be made.
- The effect of any herbal drug on cardiac functioning can be determined.
- Study of the effect of herbal drugs on cardiac dysfunction associated with other diseases can be carried out.

PHARMACOLOGICAL SCREENING FOR WOUND HEALING ACTIVITY

Introduction:

- "Wound is defined as the disruption of the cellular and discontinuity of a tissue". Wound can be produced by physical, chemical, thermal, microbial or immunological exposure to the tissue.
- The mechanism of healing of wound is integrated cellular and biochemical events leading to formation of structural and functional integrity with gaining the strength of injured tissue.
- Various activities play an important role in wound healing process.
    - Anti-inflammatory activity
    - Antioxidant effect
    - Antimicrobial activity
    - Analgesic activity

The phases that characterize wound healing include:

- Haemostasis,
- Inflammation,
- Cellular migration and proliferation,
- Protein synthesis and wound contraction
- Remodeling

Screening Models

In-vitro model

In-vitro assays are useful in wound healing research for determining the possible effectiveness of various treatments, particularly antimicrobial and healing enhancing agents. Wound closure studies have been conducted on single cell monolayer systems. The principle *in vitro* technique for studying the skin penetration evolves the use of variety of diffusion cells in which animal or human skin is fastened to a holder and the passage of compounds from epidermal surface to a fluid bath is measured.

***In-vivo* model**

1) Excised wound or Open Wound method
2) Incised or Sutured Skin Wound method
3) Dead space wound method
4) Musculoperitoneal Wound
5) Burn wounds

Aim: To evaluate the wound healing activity

Requirements:

- Wistar rats of either sex
- Anaesthesia- chlorhydrate
- Digital tensiometer
- Surgical thread (No. 000) and curved needle (No.11).
- IPA(Isopropyl Alcohol)
- Herbal extract

Procedure:

Preparation of plant extract

Boil the air-dried aerial parts of plant *Centella asiatica* and the powder of *Curcuma longa* rhizomes in purified water for 2 hours. Filter the solution through a coarse sieve twice to obtain the final extract.

Preparation of gel formulation

- Take hot water in a beaker and add carbopol 934 keep on magnetic stirrer
- Then add plasticizer PG
- Then add the herbal extract and mix
- Then finally maintain pH by adding Triethanolamine.
- Add Propylparaben as preservative

Grouping of animals:

Group No.	Group	No. of Animals
1	Induced Control untreated	6
2	Induced Control treated with Standard (Povidone-iodine) (1gm/day, topically)	6
3	Induced Control treated with ointment Base (1gm/day, topically)	6
4	Induced Control treated with test formulation	6

Incised or sutured skin wound:

- Anaesthetized the Wistar rats of either sex
- Make one longitudinal paravertebral incision of 2-3cm length through the skin and cutaneous muscle at a distance of about 1cm from the midline on each side of the depilated back.
- Suture the parted skin, after the incision, 1cm apart using a surgical thread (No. 000) and curved needle (No.11).
- Leave the wound, after stitching, undressed.
- Apply the test and standard formulations daily up to 9 days.
- Remove the suture on 9^{th}postoperative day.
- Measure the skin breaking strength of the 10th day old wound by digital Tensiometer

Excised wound or open wound:

- Shave the back of each animal and wash with spirit for operation.
- Excise the 300 mm^2 area, with sharp knife and scissors, under ether anesthesia.
- Apply gel by topical application daily.
- On 9th and 17th day sacrifice the wounded animals and perform biochemical estimation of granulation tissue and histological examination.

Parameter to be determined:

- Histopathological studies
- Hydroxyproline and collagen content estimation
- Protein estimation(Lowry method)
- Tensile strength (Incised model)
- Area measurements by Tracing by graph paper(Excised wound)

Histopathological studies:

- Sacrifice one rat from each groups of excision wound model on 9^{th} and 17^{th} day
- Remove the tissues from the wound site of the individual animal
- Fix the samples separately in 10% formalin for 24hrs and dehydrate and clear through ethanol-xylene series of solution and embed in paraffin wax (melting point 56°C).
- Cut serial sections of 10µm and stain with Haematoxylin and Eosin
- Examine the sections under light microscope for epithelisation, formation of granulation tissue, fibrogenesis and new blood vessel formation and then take photomicrographs.

Hydroxyproline and collagen content estimation:

- Estimate the Hydroxyproline content and collagen content in excision wound model on 9^{th} day and 17^{th} day. A Hydrolyse the known weight of the fresh wound tissue by 6 N HCl in sealed tubes at 110°C for 16 h.
- Evaporate the hydrolyzed samples near to dryness over a boiling water bath to remove acid
- Dissolve the residue in distilled water and made up to a known volume.
- Mix 1 ml of the clear filtrate with 1 ml of freshly prepared chloramine-T solution and allow to stand for 20 min. Then mix with 1 ml of 3.15 M perchloric acid and incubate for 5 min.
- Finally, add 1 ml of freshly prepared p-dimethylaminobenzaldehyde, mix well and place on a water bath at 60°C for 20 min.
- Measure the absorbance at 560 nm.
- Calculate the total collagen content in the tissue by multiplying the hydroxyproline content by the factor 7.46.
- Hydroxyproline and collagen content estimation is done to check the formation of collagen and hydroxyproline formed after the wound healing, and in directly we come to know the effect of our formulation.

Tensile strength (Incised model)

$$\text{Tensile Strength } (N/cm^2) = \frac{\text{Tensile strength (in Kg)} \times 9.8}{\text{Width (cm)} \times \text{thickness (cm)}}$$

TEXTURE ANALYZER

Introduction :

The Texture Analyser is a self-contained bench-top instrument providing laboratory precision, even on the production floor. It is designed to operate as a standalone unit, with a PC running the TexturePro™ software package for enhanced analytical power.

Utilizing simple 'Compression' or 'Tension' forces, we are able to imitate almost all conditions imposed during the manufacture or handling of a wide range of Pharmaceuticals, foods, industrial materials and personal care products. Such measures provide a 'real life' insight into the physical properties of a product, often invaluable in maintaining consistent quality manufacture whilst minimizing product waste in production. Initial R & D trials set the specifications for at-line Quality Control in full-scale production, enabling a proactive manufacturing approach with optimized process control.

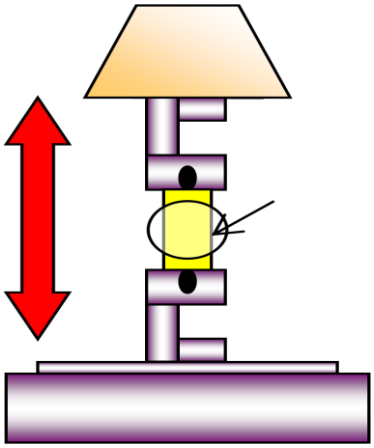
Introduction to TexturePro™

TexturePro™ is a completely new concept in texture analysis data collection and parameter calculation. This unique software system harnesses the power of Microsoft Excel to calculate a full range of referenced texture parameters through an easy to use menu driven programme. Operating directly within Excel, our unique parameter-defined selection option eliminates any requirement for the incorporation of complicated macros. Users benefit from the full power of Excel statistical and data analysis functions, whilst generation of professional reports, presentations and general project management are fully facilitated through working in a single software package.

TexturePro™ stores data within single project files with batch differentiation to optimize project management. Data generated through product evaluation can be overlaid graphically to identify trends between samples, and view critical parameters. The calculation of results can be tailored to meet your specific requirements through user defined functions. The storage of data files permits unlimited re-analysis capabilities in case you forget to record that all important parameter.

Aim : To study the tensile strength of given film formulation**.**

Requirement : Polymeric Film Sample (2 cm X 7 cm) and Dual Grip Jig



Procedure :

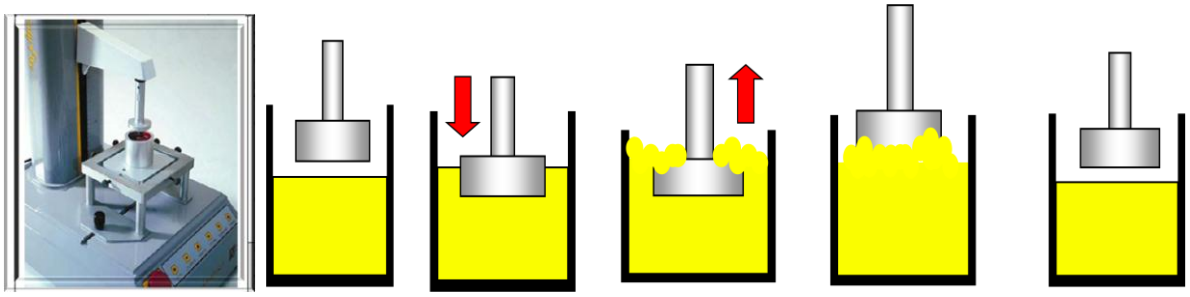
- Cut the polymeric film in to require size and attach Dual Grip Jig assembly on Texture Analyzer.
- Fix the film on the Dual Grip Jig by fixing the constant length between the two jig.
- Set the process parameters as TENSION.
- Depending upon the elongation of film, set the target value in distance.
- Select the test results as peak load (force required to break).
- Run the test by clicking PLAY button and generate the REPORT.
- Calculate the tensile strength based on dimension of the film.
- Obtain the graph of Load Vs Distance and manually determine the "Elongation Length at Break". Calculate the % elongation based on original length of film (between the grips)
- Repeat the different batches with same test parameter and compare the results**.**

Results :

- Peak Load (Force at Break) : _____
- Tensile Strength : _____
- Elongation at Break : _____
- % Elongation : _____

Aim: To study the Adhesiveness of given Gel formulation**.**

Requirement : Gel Sample (40 mL) and Disc Probe



Procedure :

- Fill the beaker with Gel sample and attach the Disc Probe on Texture Analyzer.
- Set the parameter at COMPRESSION.
- Select the Back off enable and return at Test Speed Options.
- Target value should be based on Volume of Gel sample, up to which the Disc Probe should enter inside the Gel sample and then return back.
- Select the Result parameter as Peak Load, Negative Peak Load and Adhesiveness.
- Run the Test by clicking PLAY button.
- Generate the REPORT and obtain the graph of Load Vs Distance.
- Peak Load represents the Gel strength, negative Peak Load represents the Adhesive Force and Area Under the Curve (AUC) of Negative Peak Load vs Distance represents Adhesiveness.
- Repeat the different batches with same test parameter and compare the results.

Results :

- Peak Load (Gel Strength) : _____
- Negative Peak Load (Adhesive Force) : _____
- AUC of Negative Peak Load (Adhesiveness) : _____

Applications of Texture Analyzer:

Applications in Medical device products:

Syringe and Hypodermic Needle Sharpness, Extrusion Force (e.g. syringes), Metering Valve Performance (e.g. metered dose inhalers), Material Strength (e.g. catheters, tubing), Tensile Strength (e.g. bandages, medical gloves, sutures, stents, adhesives), Compression Fatigue (e.g. breast implants), Puncture Resistance and Relaxation (e.g. breast implants), Simultaneous Force and Voltage of Membrane Switches, Tack of materials to adhere/bond medical devices to body

Applications in Cosmetic products:

Hardness, Break strength, bend strength, Compaction strength, Container lid opening force, Actuation force of pumps and sprays, Consistency of moisturizing cream and paste, Friction of cosmetic applications and exfoliators, Spreadability of creams and face mask formula, Stickiness and curing of nail lacquers, Tube extrusion energy and behaviours

Applications in Adhesive products:

Adhesiveness, Quick stick, Tackiness, Cohesiveness, Shear strength and creep, Stringiness, Extensibility, Film and tensile strength, 180 degree peel strength, 90 degree peel strength, Floating peel strength

Applications in Gel products:

Firmness, Relaxation, Swelling, Adhesiveness, Tack, Stickiness, Cohesiveness, Rupture/burst

Applications in Film products:

Film Relaxation and Creep Testing, Film Brittleness Testing, Film Burst Testing, Film Adhesion and Cohesion Testing, Film Fatigue Testing, Film Swelling & Disintegration Testing, Film Conductivity, Film To Film Adhesion, Film To Tablet Adhesion

Applications in Powder products:

caking and bridging, cohesion, speed sensitivity, granule attrition, powder compaction, powder relaxation, dusting, powder rest-flow transition, friability, flow/dust relationships, surface friction and surface finishes, granulation, aggregation, end-point determination

IMMOBILIZATION

Introduction:

Immobilization is a process of confining or entrapping the cells, enzyme or molecules in a distinct polymeric matrix, wherein the substrates are continuously converted to products which is released in surrounding medium without affecting biosynthetic capacity.

Methods of immobilization:

- **Adsorption:** In this the cells evidently get adhered to the surface of a carrier on account of the spectacular combination of hydrophobic effects and the formation of several salt linkages per cell molecule.

  Example of adsorbents : Chitosan, Cepharose, gelatin, etc.

- **Covalent bonding:** In this the cells are attached to the preformed carrier matrix. The actual strength of the bond happens to be quite strong and hence there is no lose of cells during usage. The formation of covalent bonds takes place particularly with side-chains of amino acids. This method causes lose in cell activity due to involvement of specific active site in the process of immobilization.

  Example: CarboxyMethylCellulose, Glutaraldehyde , Methacrylate polymer, etc

- **Entrapment:** This refers to the phenomenon whereby the cells are either held or entrapped within the appropriate matrix. It would be regarded as putting the cells virtually in the molecular cage. Entrapment is based upon the coupling of cells specific to the lattice of polymer matrix or enclosing them in semi-permeable membranes, to check and prevent the release of proteins but permitting the adequate diffusion of substrates and products.

  Examples of matrix – Sodium alginate, Calcium alginate, Cellulose acetate, etc.

- **Encapsulation:** In this method, the enzyme molecules invariably taken up in an aqueous medium, may be strategically confined very much with in a semi permeable membrane that ideally permits an almost absolute free movement of the enzymes in either direction to the products and substrates but fails to allow their migration and escape.

  Examples: Cellulose acetate, Ethyl cellulose, Eudragit, Sodium alginate etc.

Advantages:

- Immobilized cells can be separated conveniently from the reaction system involved**.**
- They may be used predominantly in continuous production systems.
- Some immobilized cells exhibit thermo stability.
- Immobilized enzymes increase the biosynthetic potential and production.
- They maybe employed at a much higher concentration range in comparison to the corresponding free cells.

Disadvantages :

- Costlier
- Immobilization of cells invariably affects the stability and/or activity adversely.
- Practical utilization of the immobilized enzymes may not prove to be of any use or advantageous when one of the substrate is found to be insoluble.
- Certain immobilization protocols do offer a good number of serious problems with respect to the diffusion of ensuing substrate to have an access to the corresponding enzymes.

Aim: To perform the immobilization of yeast cells.

Requirements:

a) Glasswares and equipments: beakers, conical flasks, petri dishes, syringe (20 ml), glass rod, autoclave, laminar hood, incubator.

b) Chemicals: Distilled water, sugar, potassium nitrate, potassium dihydrogen phosphate, calcium chloride solution (0 .2 M), sodium alginate solution (4%), yeast granules

Procedure:

Step *(i) Preparation of media*

Sr. No.	INGREDIENTS	QUANTITY
1	Sugar	120 g
2	KNO_3	0.5 g
3	$K_2H_2PO_4$	0.5 g
4	Distilled water	Upto 500 ml

ii) Preparation of solutions:

0.2 M $CaCl_2$ solution - 5.8808 g of $CaCl_2$ was dissolved in 200 ml distilled water

4% sodium alginate solutions was prepared

iii)Sterilization:

All the glass wares were autoclaved and were transferred into laminar hood in a tray.

iv) Regeneration of yeast cells:

- 2 gms of yeast granules were weighed and mixed with warm water till a paste like consistency was obtained in a petri/porcelain dish.

v) Preparation of beads:

- The yeast paste was transferred into 4% sodium alginate solution and was mixed well with the glass rod.
- With the help of sterilized syringe (20 ml), slurry was transferred drop-wise in the 0.2 M $CaCl_2$ solution and was allowed to stand for some time to enable formation of beads.
- The excess $CaCl_2$ solution was decanted and the beads were washed with sterile water to remove traces $CaCl_2$ solution completely.
- The beads were transferred to the media in conical flasks and were incubated for 7 days under 12 hours light-dark condition at 20 rpm.
- After 7 days, the media was subjected to distillation and 12 ml of distillate was collected and alcohol content was determined with the help of 10 ml specific gravity bottle.

Observation and calculations :

- Weight of empty specific gravity bottle : (W1) – 20.80 g
- Weight of empty specific gravity bottle + distilled water : (W2) – 31.58 g
- Weight of empty specific gravity bottle + distillate : (W3) – 31.51 g

- Specific gravity of distillate = $\frac{W3 - W1}{W2 - W1} = 0.9935$

Results: Good , shiny and elegant immobilized beads were obtained with 0.2 M $CaCl_2$ which showed production of alcohol

Immobilized beads

Applications:

- In Biotransformation: Biotransformation is a process through which functional groups of organic compounds are modified by living cells.

  Eg. immobilized cells of *Papaver somniferum* reduces codeinone giving codeine.

  Immobilized cells of *Digitalis lanata* hydrolyses ß- methyl digitoxin to ß-methyl digoxin
- For biosynthesis of secondary metabolites like alkaloids, terpenoids etc.
- Alcoholic fermentation
- Production of antibiotics and vaccines
- Production of fermented beverages and foods
- Production of single cell proteins

ATOMIC ABSORPTION SPECTROPHOTOMETER

Introduction:

Atomic absorption spectrometers are used for environmental analysis, including the monitoring of air pollution, water contamination, analysis of heavy metals in pharmaceutical and food products, crude drugs and herbal formulations, the analysis of metallic materials and the analysis of trace metals in livings organism.

Atomic absorption spectrometry is mostly performed using the flame method. Nearly all inorganic elements can be measured using atomic absorption spectrophotometry.

Principle:

Organic metals in sample are digested by wet digestion or dry digestion or high pressure microwave digestion and determine the amount of heavy metals, i.e. Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) by using Graphite furnace atomic absorption spectrophotometer (GF-AAS) and flow injection analysis system- atomic absorption spectrophotometer (FIAS-AAS). The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer-Lambert Law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometer (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using Beer-Lambert Law.

Atomic absorption spectrophotometer Shimadzu AAS-6300

Aim:- To determine heavy metal (Pb) content in crude herbal drug using atomic absorption spectrophotometer.

Procedure:

1) Preparation of Standard solutions of lead (Pb)

Stock solution of lead nitrate (Pb $(NO_3)2$)1000ppm

- Dissolve 1.5890gm of $Pb(NO_3)_2$ in 100ml distilled water and makeup the volume upto 1 litre by distilled water.
- Prepare solutions of 1ppm, 3ppm, 6ppm, and 9ppm from stock solution.

2) Sample preparation

Prepare the reagent blank as in sample preparation but without adding the solution. Sample preparation can be carried out by following method:

Dry ashing:

- Accurately weigh 2.5g sample into a silica dish and add 3ml of 50% w/v magnesium nitrate.
- Dry on the water bath and ash the residue first in the heating mantle until no more fumes of nitrous oxide evolve and then incinerate in muffle furnace at 500^oC for 3 hours.
- Cool, add 25ml 6M hydrochloric acid, filter into a 50 ml volumetric flask and dilute to volume with distilled water.

3) Sample injection

A) Calibration curve: Inject standard calibration solutions into the FIAS-AAS at the specified condition. Plot the response versus concentration of each standard solution.

B) Inject sample solution into FIAS-AAS. Record the response and concentration of Pb in sample solution and then calculate ug/gm of Pb in sample.

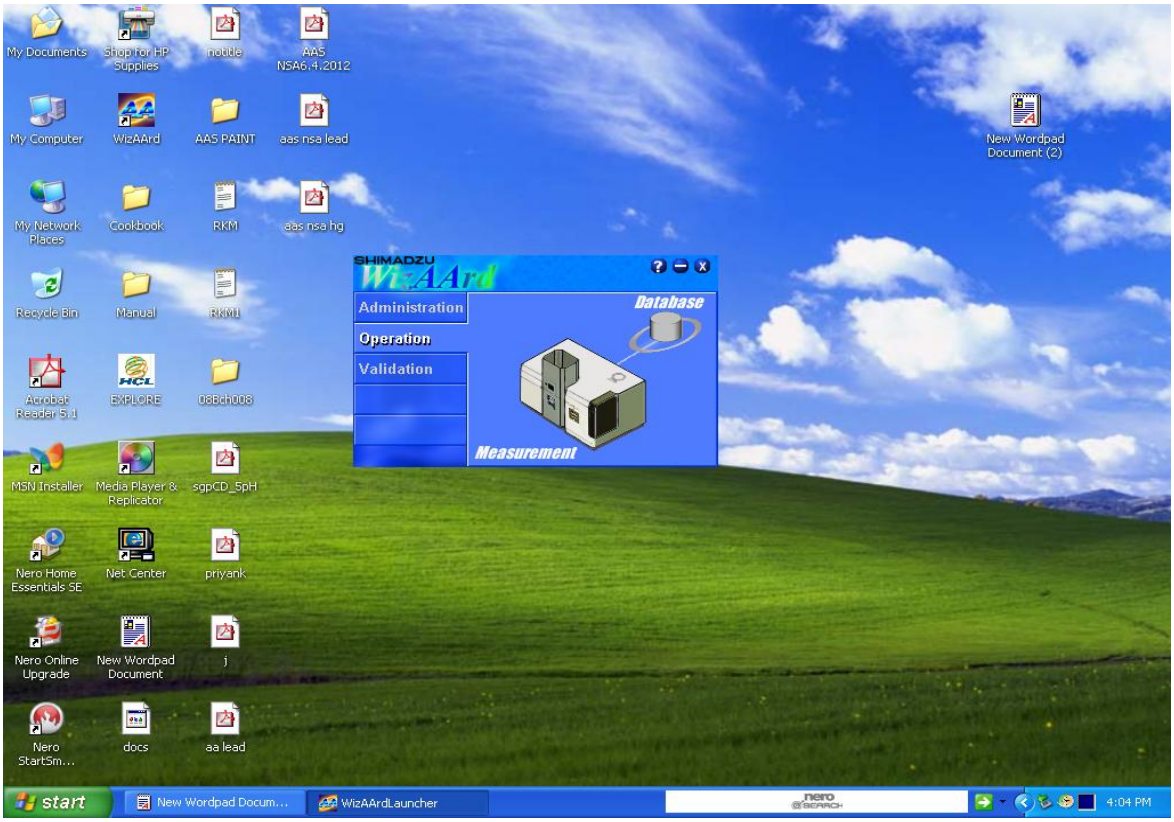
Permissible limits of heavy metal as per A.P.I.

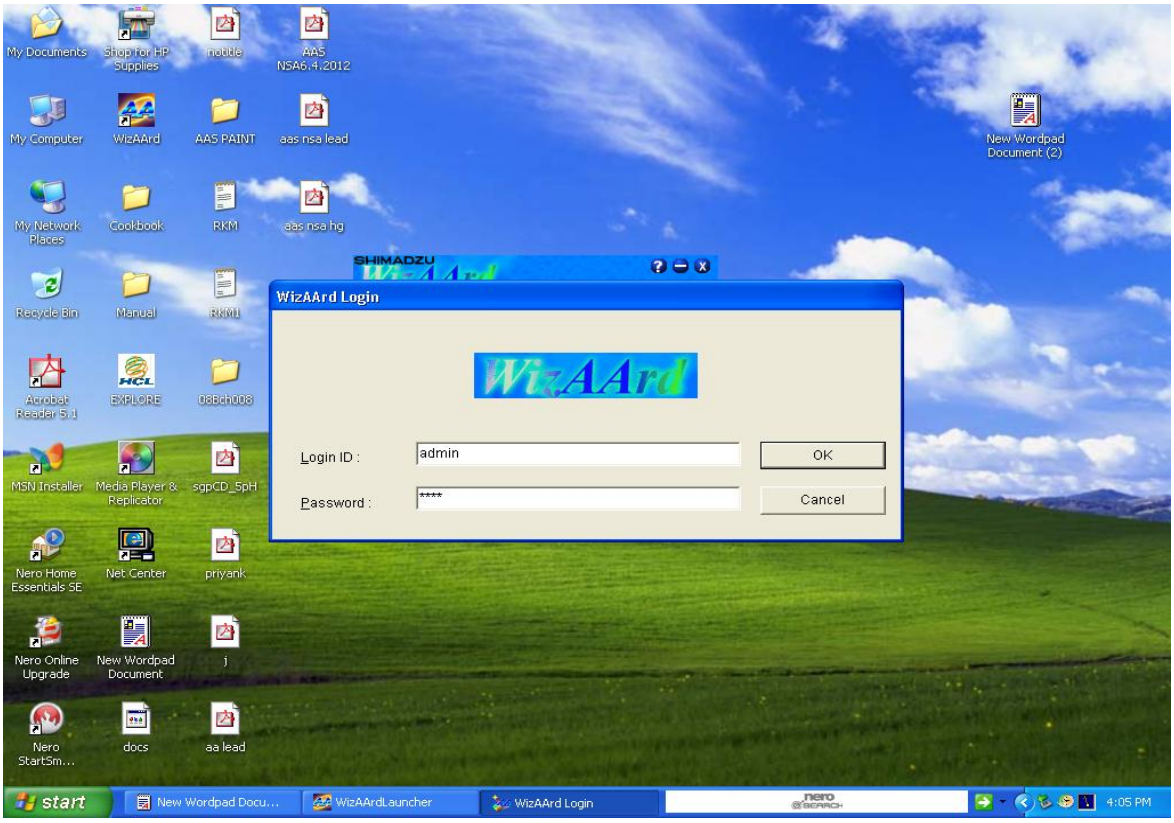
Metal	**Limit**
Lead	10 ppm
Mercury	1 ppm
Cadmium	0.3 ppm
Arsenic	3 ppm

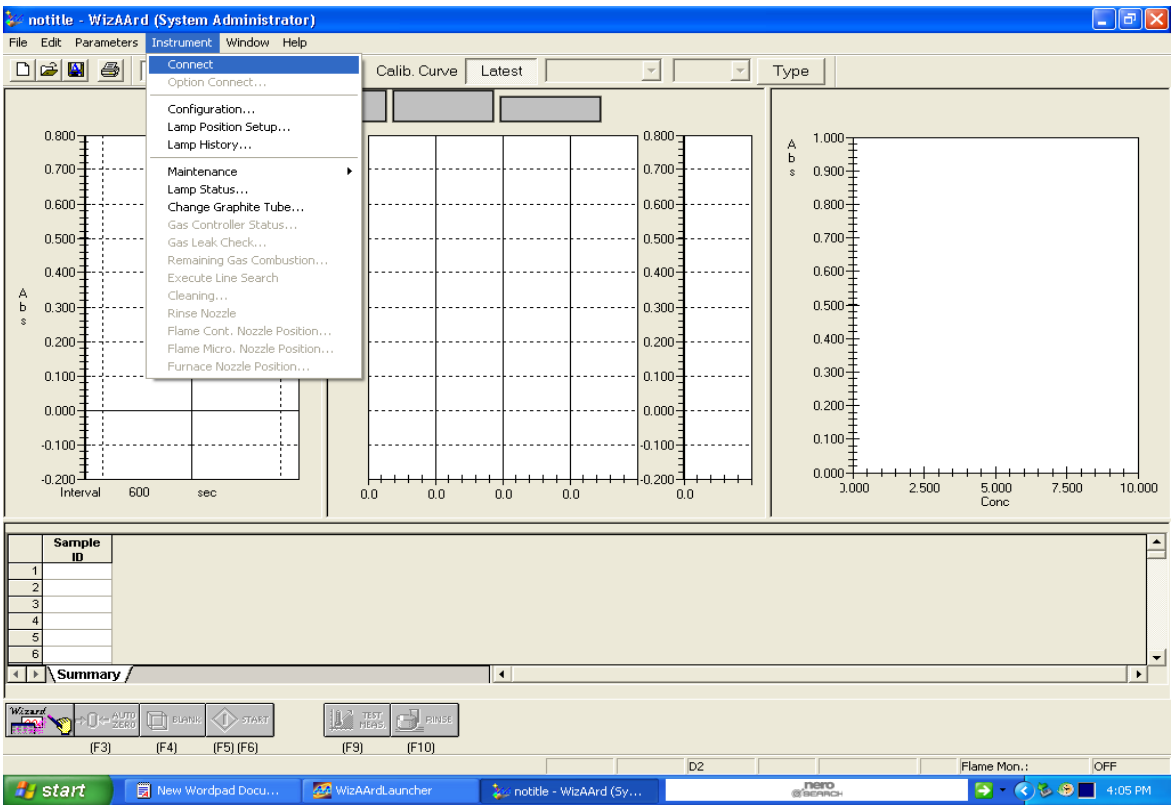
Applications of Atomic Absorption Spectrophotometer:

- Water analysis for Ca, Mg, Fe, Se, Al, Ba content
- Food analysis
- Analysis of animal feedstuffs for Fe, Cu, Mn, Cr, Se, Zn
- Analysis of additives in lubricating oils and greases for Ba, Ca, Na, Li, Zn, Mg
- Analysis of soils
- Clinical analysis of blood samples, whole blood, plasma, serum for Ca, Li, Mg, Na, Fe
- Analysis of herbal drugs and herbal formulations for heavy metal content

SOP-Atomic Absorption Spectrophotometer



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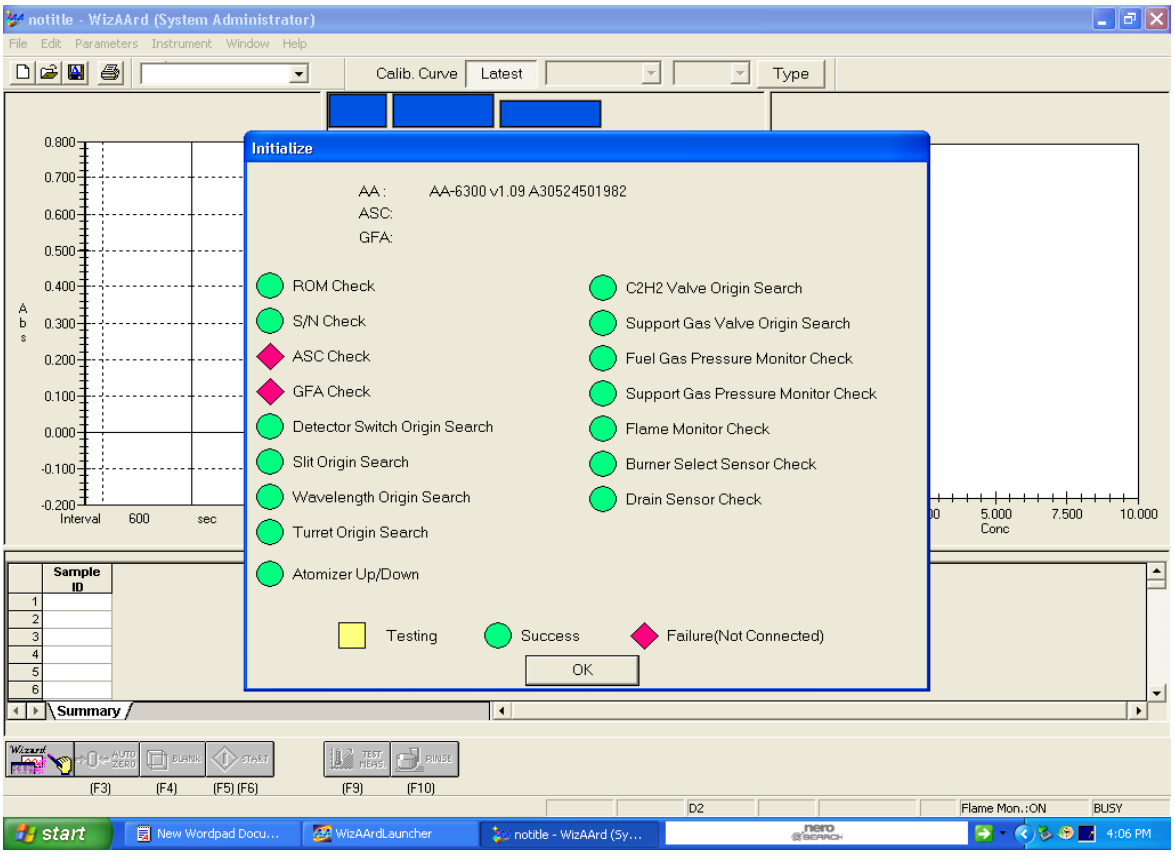
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GFA:
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Slit Origin Search
Wavelength Origin Search
Turret Origin Search
Atomizer Up/Down
C2H2 Valve Origin Search
Support Gas Valve Origin Search
Fuel Gas Pressure Monitor Check
Support Gas Pressure Monitor Check
Flame Monitor Check
Burner Select Sensor Check
Drain Sensor Check
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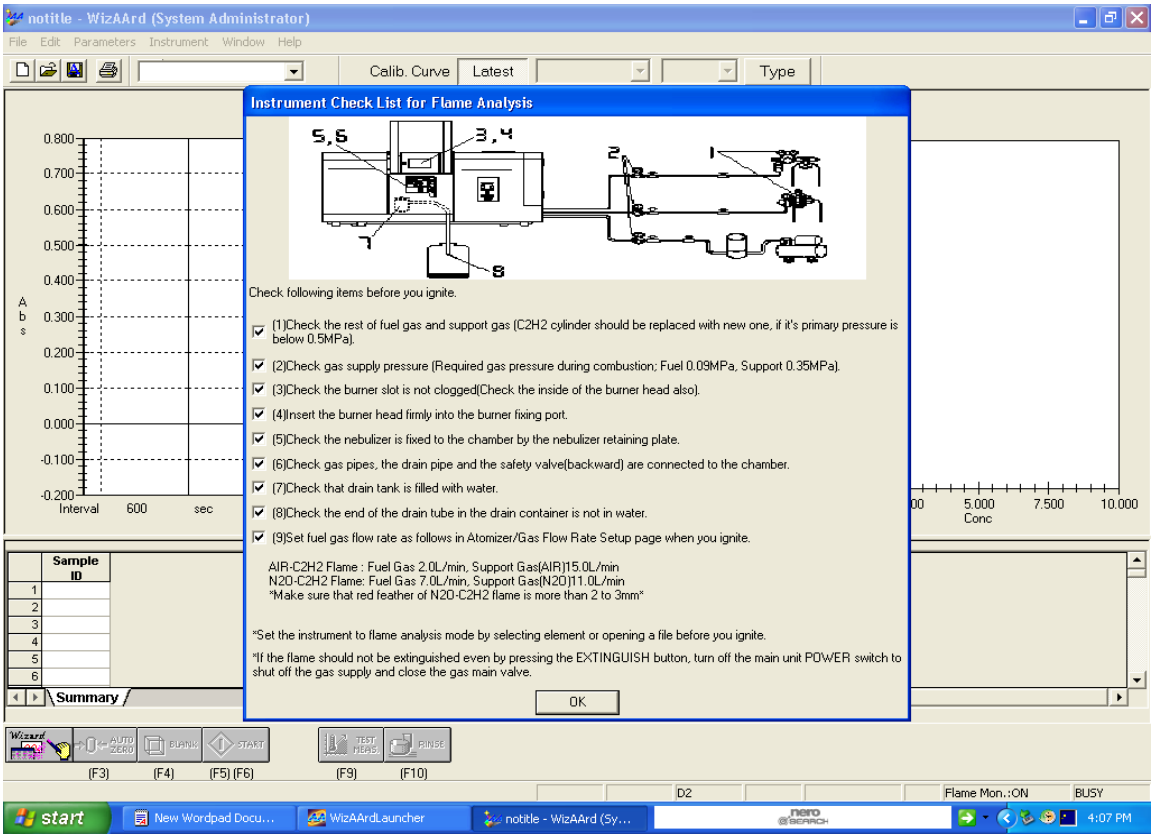
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8
Check following items before you ignite.
(1)Check the rest of fuel gas and support gas (C2H2 cylinder should be replaced with new one, if it's primary pressure is below 0.5MPa).
(2)Check gas supply pressure (Required gas pressure during combustion; Fuel 0.09MPa, Support 0.35MPa).
(3)Check the burner slot is not clogged(Check the inside of the burner head also).
(4)Insert the burner head firmly into the burner fixing port.
(5)Check the nebulizer is fixed to the chamber by the nebulizer retaining plate.
(6)Check gas pipes, the drain pipe and the safety valve(backward) are connected to the chamber.
(7)Check that drain tank is filled with water.
(8)Check the end of the drain tube in the drain container is not in water.
(9)Set fuel gas flow rate as follows in Atomizer/Gas Flow Rate Setup page when you ignite.
AIR-C2H2 Flame : Fuel Gas 2.0L/min, Support Gas(AIR)15.0L/min
N2O-C2H2 Flame: Fuel Gas 7.0L/min, Support Gas(N2O)11.0L/min
Make sure that red feather of N2O-C2H2 flame is more than 2 to 3mm
*Set the instrument to flame analysis mode by selecting element or opening a file before you ignite.
*If the flame should not be extinguished even by pressing the EXTINGUISH button, turn off the main unit POWER switch to shut off the gas supply and close the gas main valve.
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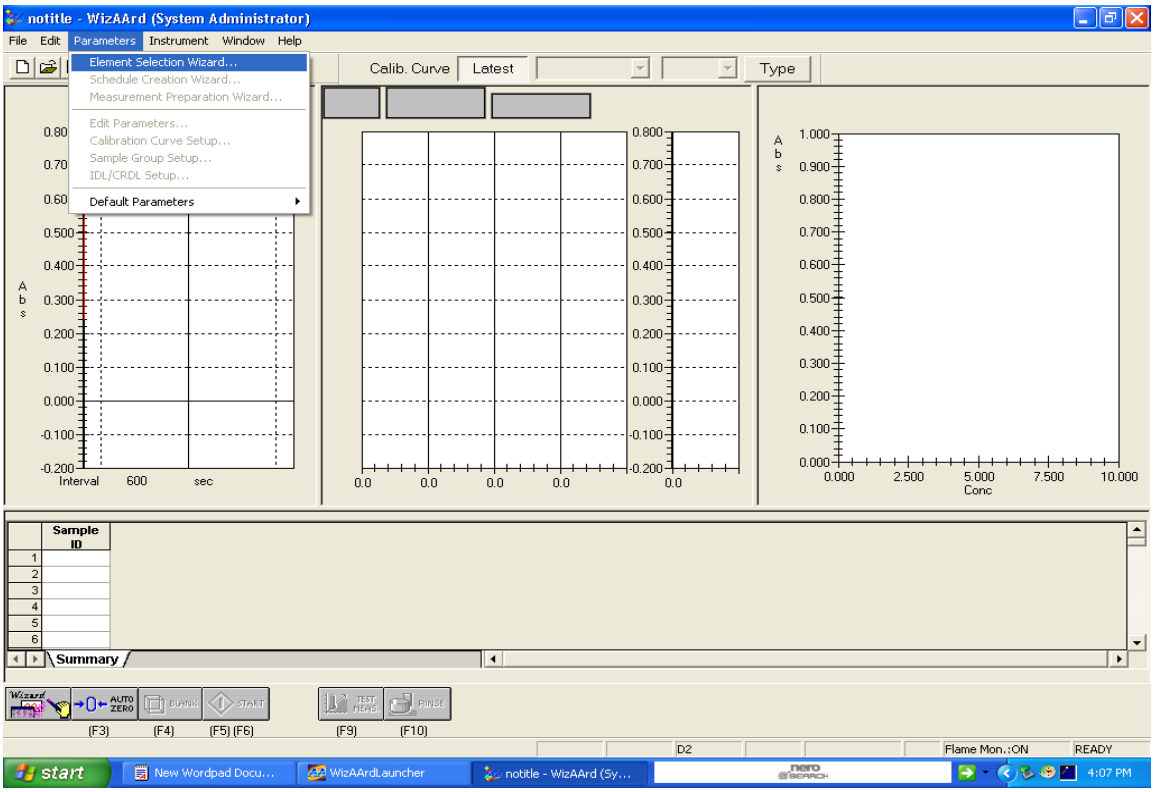
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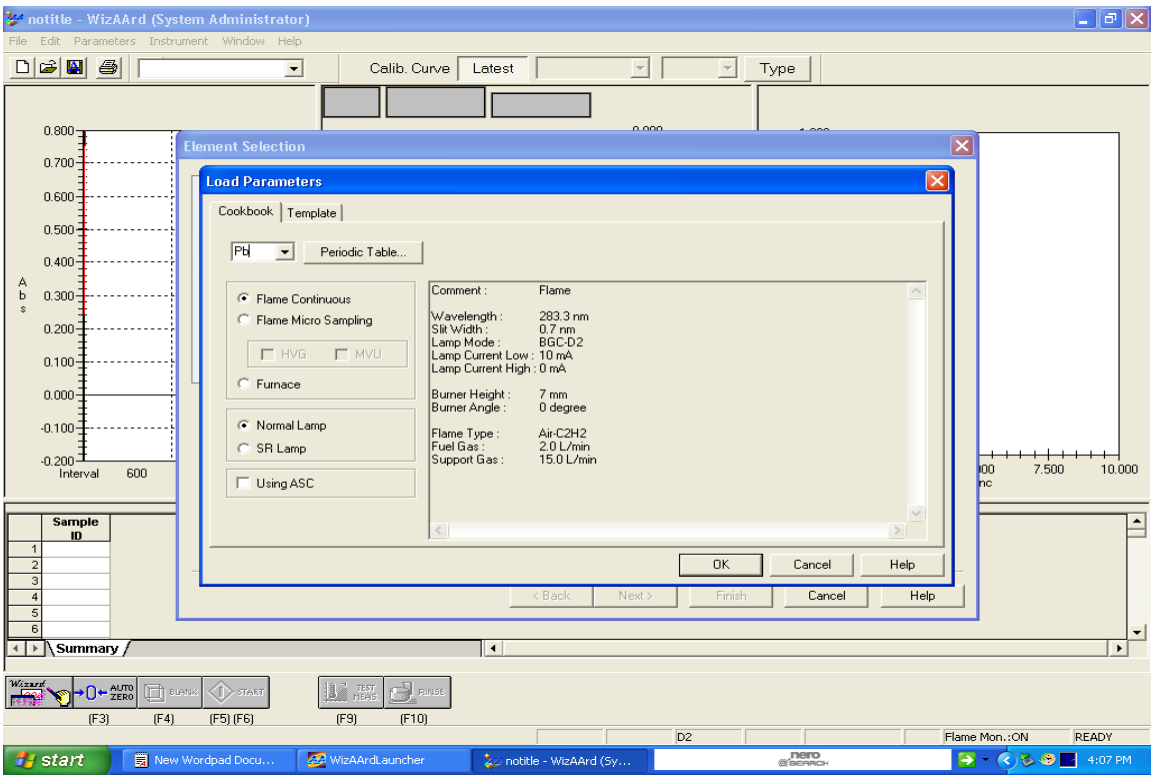
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Lamp Mode : BGC-D2
Lamp Current Low : 10 mA
Lamp Current High : 0 mA
Burner Height : 7 mm
Burner Angle : 0 degree
Flame Type : Air-C2H2
Fuel Gas : 2.0 L/min
Support Gas : 15.0 L/min
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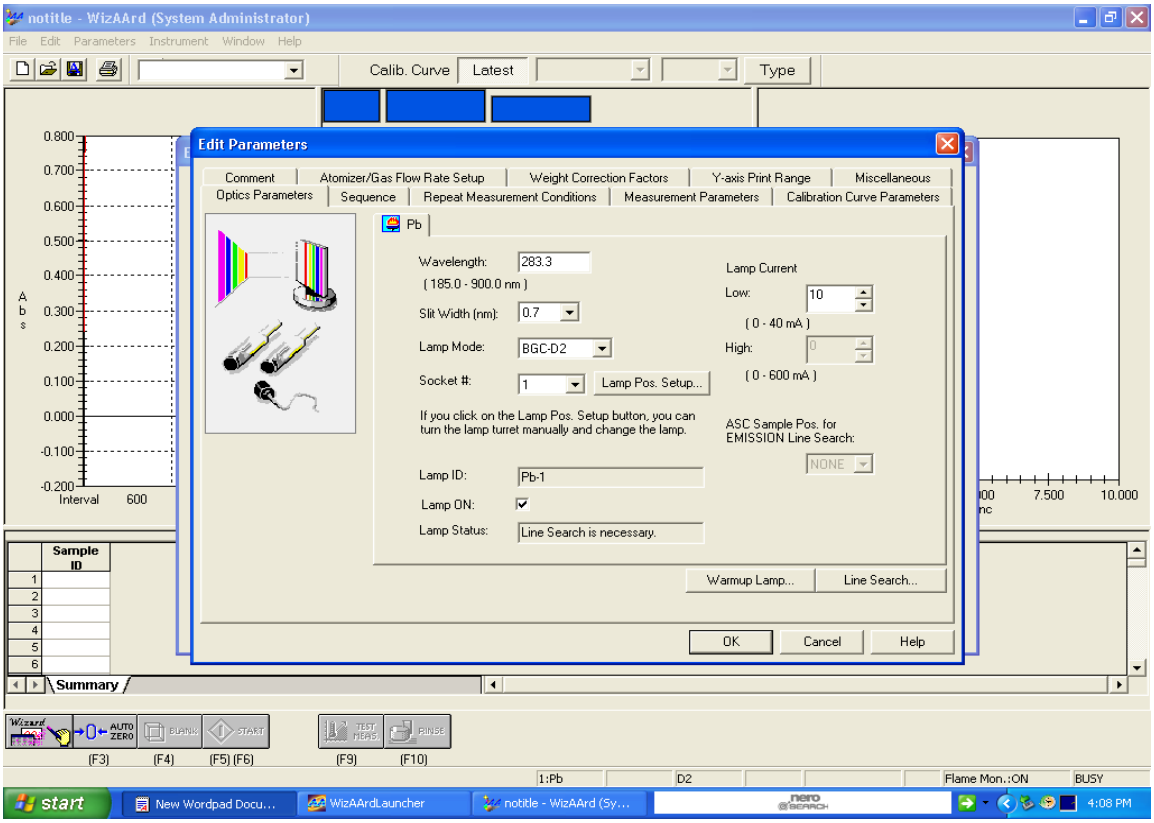
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File Edit Parameters Instrument Window Help
Calib. Curve
Latest
Type
Edit Parameters
Comment
Atomizer/Gas Flow Rate Setup
Weight Correction Factors
Y-axis Print Range
Miscellaneous
Optics Parameters
Sequence
Repeat Measurement Conditions
Measurement Parameters
Calibration Curve Parameters
Pb
Wavelength: 283.3
(185.0 - 900.0 nm)
Slit Width (nm): 0.7
Lamp Mode: BGC-D2
Socket #: 1
Lamp Pos. Setup...
If you click on the Lamp Pos. Setup button, you can turn the lamp turret manually and change the lamp.
Lamp Current
Low: 10
(0 - 40 mA)
High: 0
(0 - 600 mA)
ASC Sample Pos. for EMISSION Line Search:
NONE
Lamp ID: Pb-1
Lamp ON:
Lamp Status: Line Search is necessary.
Warmup Lamp...
Line Search...
OK
Cancel
Help
Abs
Interval 600
Sample ID
Summary
(F3) (F4) (F5) (F6) (F9) (F10)
1:Pb
D2
Flame Mon.:ON
BUSY
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WizAArdLauncher
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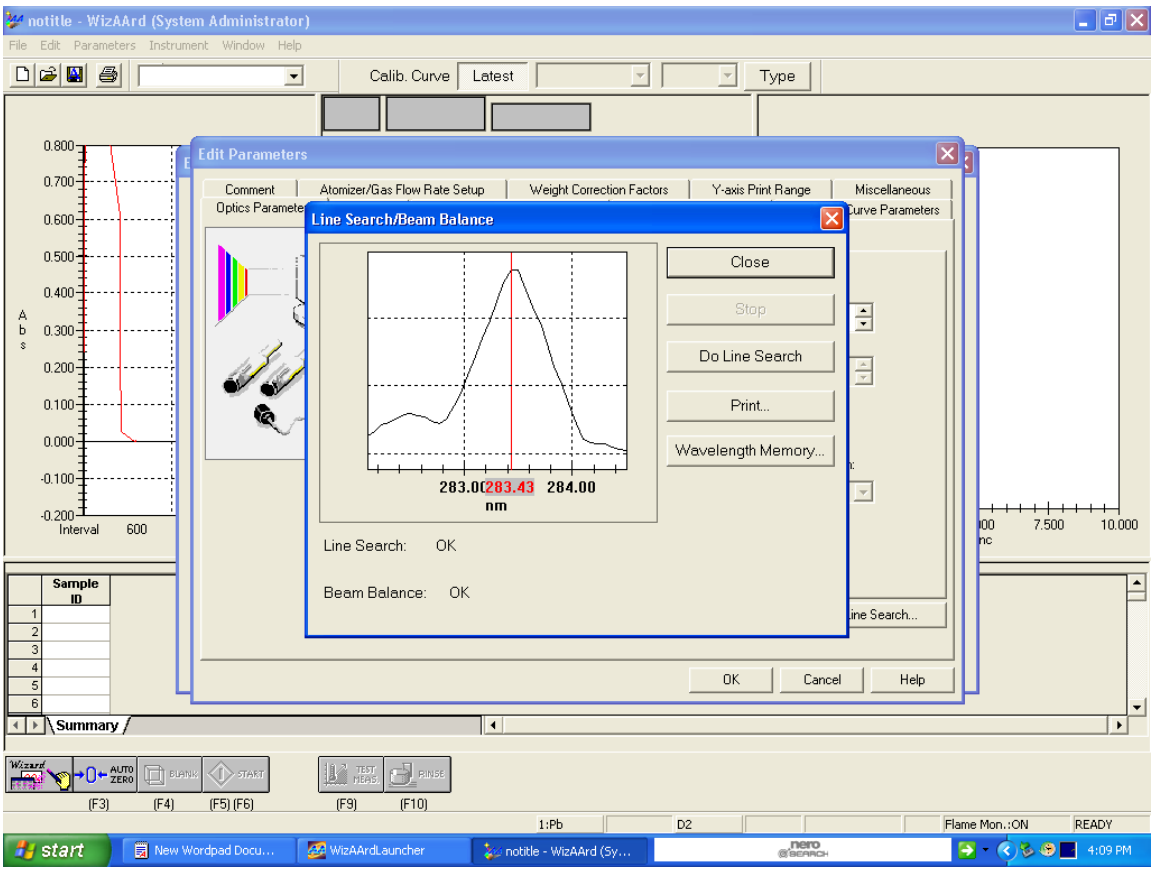
4:08 PM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File Edit Parameters Instrument Window Help
Calib. Curve
Latest
Type
Edit Parameters
Comment
Atomizer/Gas Flow Rate Setup
Weight Correction Factors
Y-axis Print Range
Miscellaneous
Optics Parameter
Curve Parameters
Line Search/Beam Balance
Close
Stop
Do Line Search
Print...
Wavelength Memory...
283.0(283.43 284.00
nm
Line Search: OK
Beam Balance: OK
OK
Cancel
Help
Sample ID
Summary
Abs
Interval 600
7.500 10.000
(F3) (F4) (F5) (F6) (F9) (F10)
1:Pb D2 Flame Mon.:ON READY
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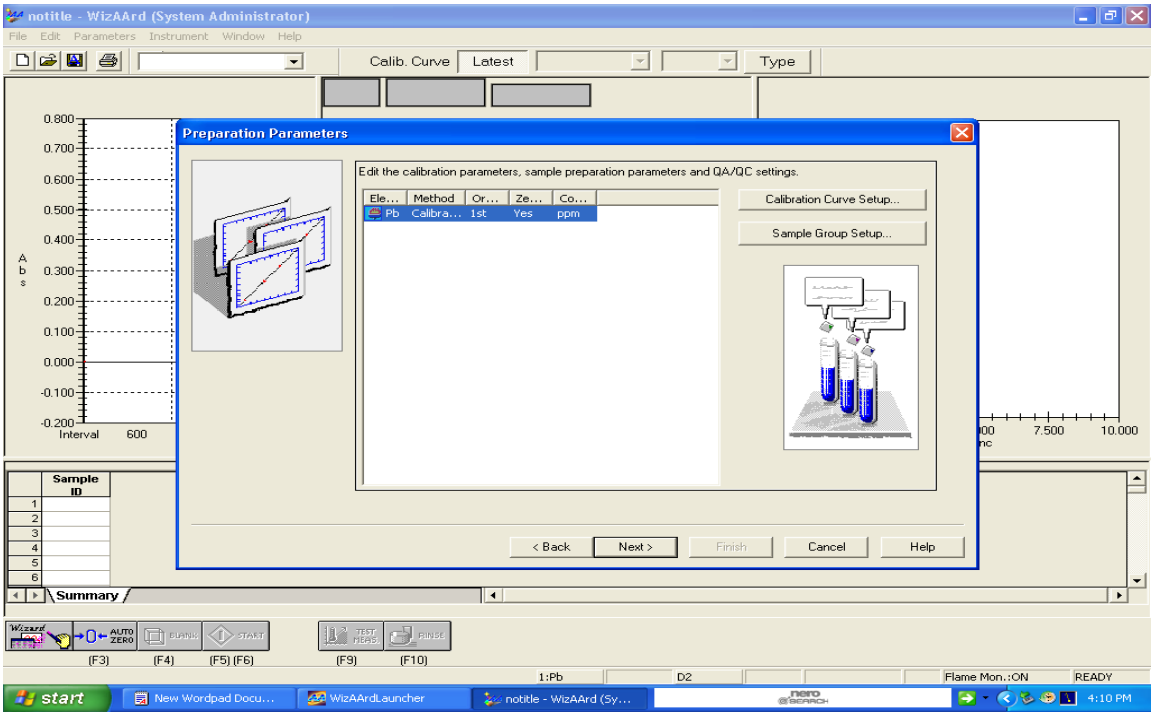
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File Edit Parameters Instrument Window Help
Calib. Curve
Latest
Type
Preparation Parameters
Edit the calibration parameters, sample preparation parameters and QA/QC settings.
Ele... Method Or... Ze... Co...
Pb Calibra... 1st Yes ppm
Calibration Curve Setup...
Sample Group Setup...
< Back
Next >
Finish
Cancel
Help
Sample ID
Summary
Abs
Interval 600
7.500 10.000
(F3) (F4) (F5) (F6) (F9) (F10)
1:Pb D2 Flame Mon.:ON READY
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WizAArdLauncher
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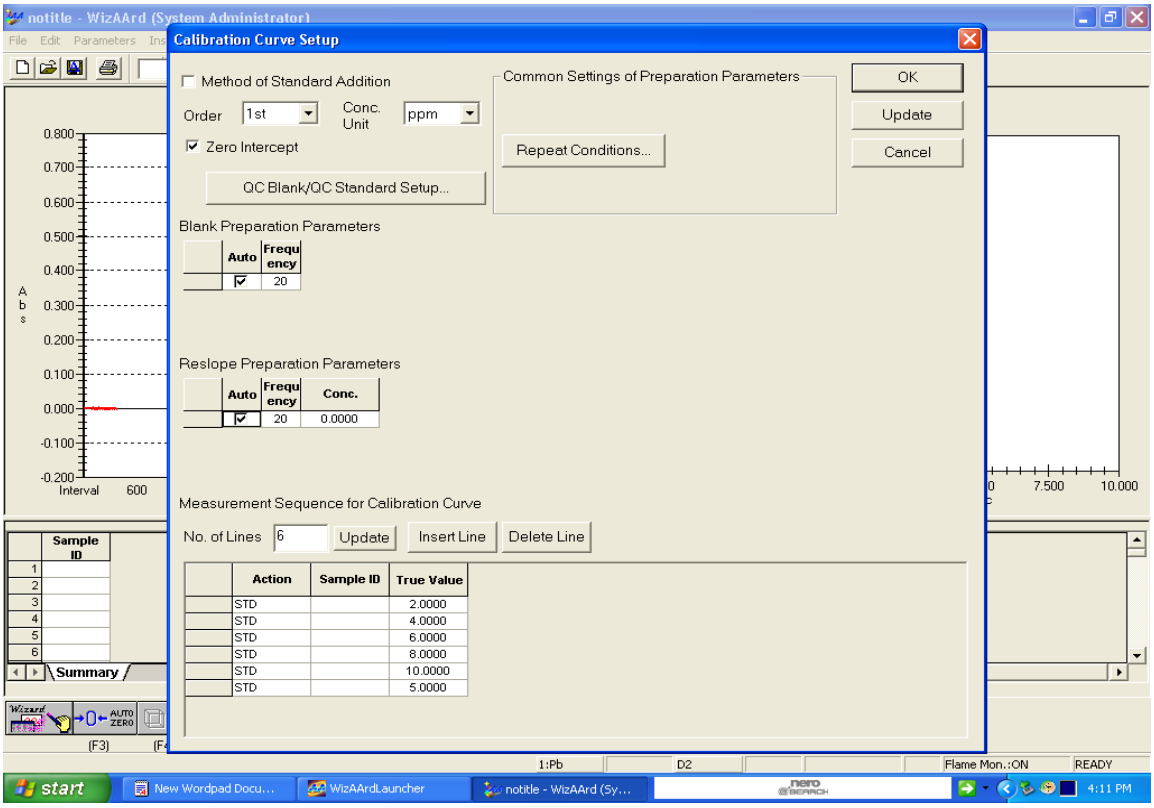
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Calibration Curve Setup
Method of Standard Addition
Order
1st
Conc. Unit
ppm
Zero Intercept
QC Blank/QC Standard Setup...
Common Settings of Preparation Parameters
Repeat Conditions...
OK
Update
Cancel
Blank Preparation Parameters
Auto
Frequency
20
Reslope Preparation Parameters
Auto
Frequency
Conc.
20
0.0000
Measurement Sequence for Calibration Curve
No. of Lines
6
Update
Insert Line
Delete Line
Action
Sample ID
True Value
STD 2.0000
STD 4.0000
STD 6.0000
STD 8.0000
STD 10.0000
STD 5.0000
Sample ID
Summary
Interval
600
7.500
10.000
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start
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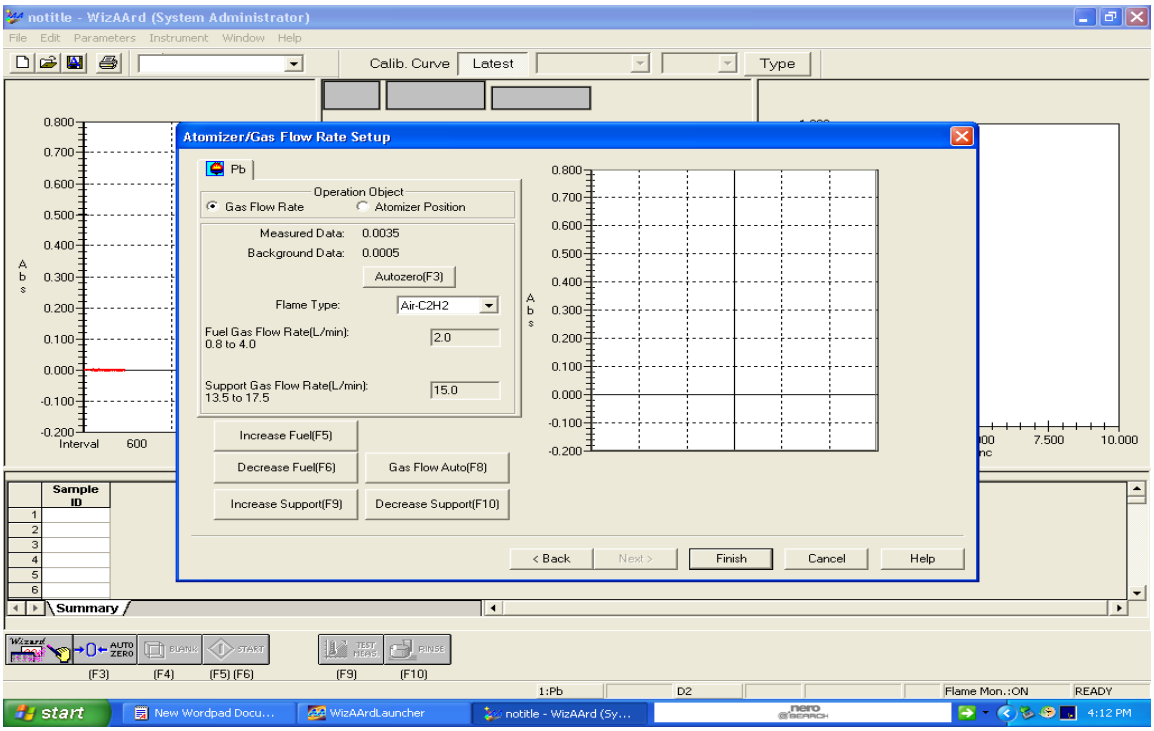
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File Edit Parameters Instrument Window Help
Calib. Curve
Latest
Type
Atomizer/Gas Flow Rate Setup
Pb
Operation Object
Gas Flow Rate
Atomizer Position
Measured Data: 0.0035
Background Data: 0.0005
Autozero(F3)
Flame Type:
Air-C2H2
Fuel Gas Flow Rate(L/min): 0.8 to 4.0
2.0
Support Gas Flow Rate(L/min): 13.5 to 17.5
15.0
Increase Fuel(F5)
Decrease Fuel(F6)
Gas Flow Auto(F8)
Increase Support(F9)
Decrease Support(F10)
< Back
Next >
Finish
Cancel
Help
Sample ID
Summary
Interval
600
7.500
10.000
(F3)
(F4)
(F5) (F6)
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(F10)
1:Pb
D2
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READY
start
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WizAArdLauncher
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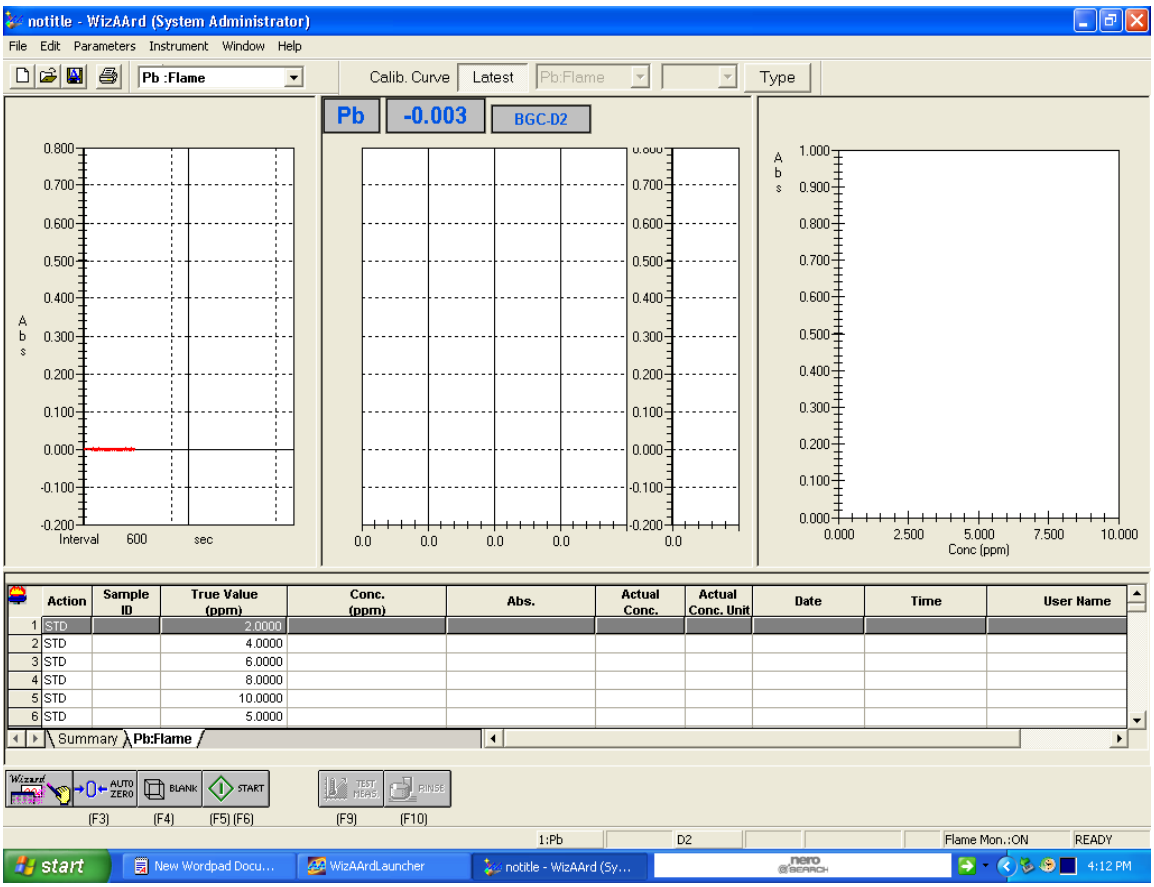
4:12 PM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File Edit Parameters Instrument Window Help
Pb :Flame
Calib. Curve
Latest
Pb:Flame
Type
Pb
-0.003
BGC-D2
Abs
Interval 600 sec
Conc (ppm)
Action Sample ID True Value (ppm) Conc. (ppm) Abs. Actual Conc. Actual Conc. Unit Date Time User Name
1 STD 2.0000
2 STD 4.0000
3 STD 6.0000
4 STD 8.0000
5 STD 10.0000
6 STD 5.0000
Summary Pb:Flame
AUTO ZERO
BLANK
START
(F3) (F4) (F5) (F6) (F9) (F10)
1:Pb D2 Flame Mon.:ON READY
start
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WizAArdLauncher
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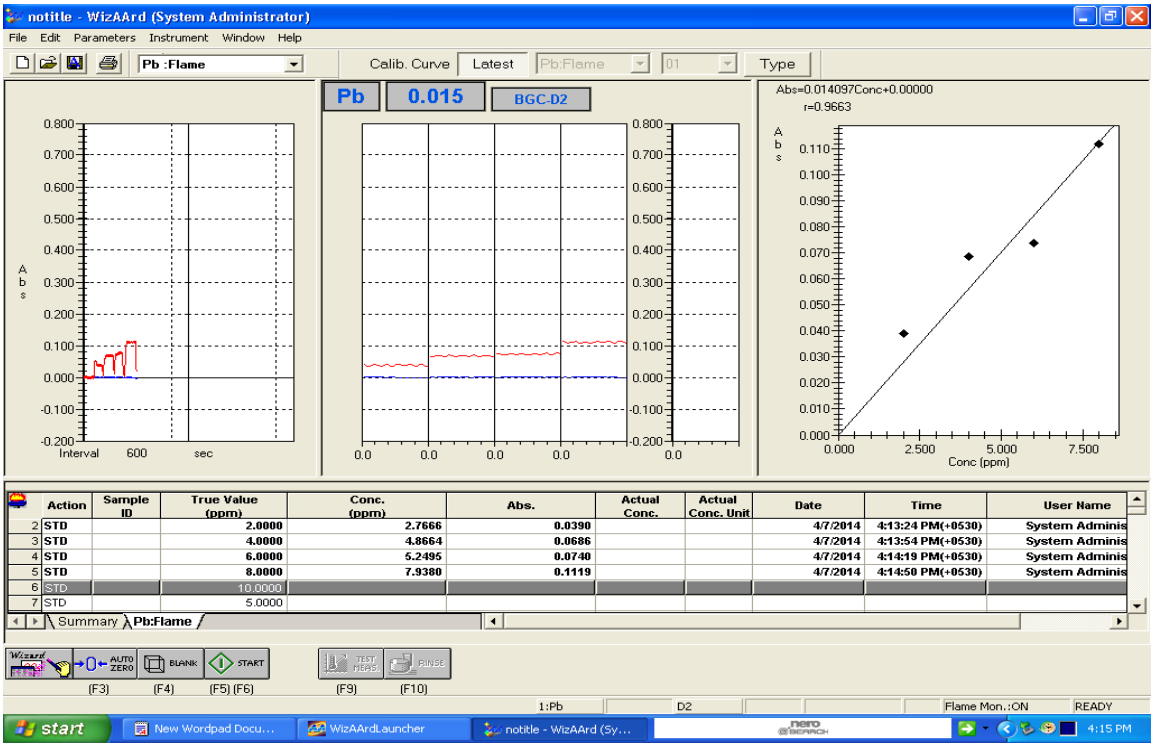
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File Edit Parameters Instrument Window Help
Pb :Flame
Calib. Curve
Latest
Pb:Flame
01
Type
Pb
0.015
BGC-D2
Abs=0.014097Conc+0.00000
r=0.9663
Abs
Interval 600 sec
Conc (ppm)
Action Sample ID True Value (ppm) Conc. (ppm) Abs. Actual Conc. Actual Conc. Unit Date Time User Name
2 STD 2.0000 2.7666 0.0390 4/7/2014 4:13:24 PM(+0530) System Adminis
3 STD 4.0000 4.8664 0.0686 4/7/2014 4:13:54 PM(+0530) System Adminis
4 STD 6.0000 5.2495 0.0740 4/7/2014 4:14:19 PM(+0530) System Adminis
5 STD 8.0000 7.9380 0.1119 4/7/2014 4:14:50 PM(+0530) System Adminis
6 STD 10.0000
7 STD 5.0000
Summary Pb:Flame
AUTO ZERO
BLANK
START
(F3) (F4) (F5) (F6) (F9) (F10)
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COMBIFLASH

Introduction:

Preparative HPLC has long been a mainstay in organic chemistry labs for purifying compounds on reverse phase.

New Flash chromatography systems with higher pressures and flow rates, such as the CombiFlash Rf, allow the use C18 columns with reduced particle size to give results approaching those of preparative HPLC systems.

CombiFlash Rf system can be changed easily between normal and reverse phase solvent systems with little more than a flush of the system with a solvent miscible to both solvent systems used.

HPLC systems are generally configured only to run reverse phase C18 chromatography. CombiFlash Medium Pressure Liquid Chromatography (MPLC) systems allow a variety of loading techniques.

Samples can be dissolved then adsorbed on a variety of materials prior to loading or injected as a liquid.

The main advantage of MPLC injection mechanisms is the high sample recovery. The compounds are easily transferred to the column during the sample run. Liquid loading is also an option on flash systems.

The sample is injected directly onto the column or through the valve on CombiFlash systems with a syringe. By washing, and applying the wash to the column, nearly 100% of the compound is transferred for purification.

MPLC systems do not require that solvent be filtered or degassed. The pumps are robust and are designed to tolerate a small amount of particulates in the solvent.

Air bubbles do not cause MPLC systems to lose their prime.

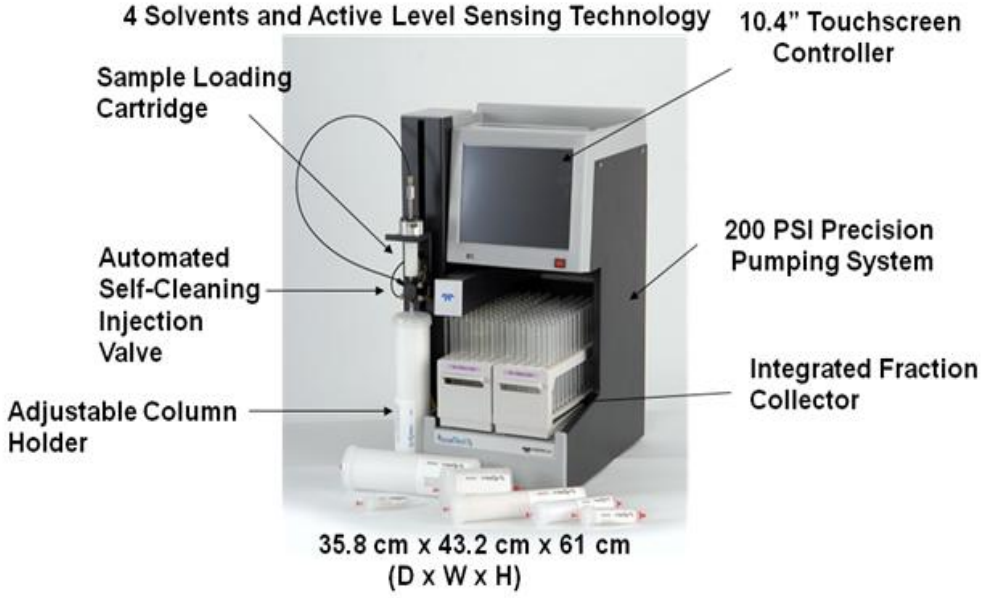
Since solvents do not need filtering, there is less time required to start purification.

Teledyne Isco has made advances in automated chromatography again with the CombiFlash Rf system. The CombiFlash Rf uses RFID (Radio Frequency Identification) technology to automate setting the parameters for purification runs and fraction collection.

The CombiFlash Rf has built-in solvent and waste management that prevent columns from drying, and messy waste overfills. A new automated valve lets you load samples and walk away – and it's self-cleaning to wash away materials that can contaminate the next run.

The CombiFlash Rf improves productivity whether you separate synthetic compounds, natural products, peptides, polymers, or other chemical separations.

The CombiFlash Rf can be relied on to perform all types of flash chromatography purifications under normal and reversed phase conditions.



Pumping System:

- Dual pumps are used in the Rf for greater accuracy and reproducibility of the gradient.
- Competing designs only use single pumps and rely on proportioning valves to create the gradient using less accurate low-pressure mixing.
- The Rf utilizes high-pressure mixing rather than low-pressure mixing, eliminating gradient variations due to solvent viscosity and elevation.
- Solvent bottles can be placed below the system for added convenience. Competing systems must have the bottles placed above the system for pumping accuracy.

RFID Columns

Combi*Flash Rf* scans the RFID label on each column and automatically loads column parameters including:

- Silica type (silica, alumina, C18, etc.)
- Flow rate
- Equilibration volume
- Run length
- Back-pressure rating
- Wavelengths
- Peak detection settings
- Column history (lot no., date, column usage, last solvents used)
- Column Sizes from 4 g – 330 g
- Sample sizes from 5 mg – 30 g
- 200 PSI Pressure Rating
- Higher flow rates
- Compatible with a broad range of solvent viscosities
- Able to withstand on-column compound crystallization

Automatic sample injection

Proprietary automatic 6-port injection valve simplifies operation by:

- Automatic column equilibration.
- Automatic in-line sample introduction without the use of loops or other devices.
- Wide-bore self-cleaning valve to minimize clogging, extend valve life, and minimize cross-contamination**.**

Sample introduction

1. Dry Loading
    - Pre-adsorption of sample onto silica for low solubility samples
2. Wet “dry loading”
    - Pipetting sample onto pre-packed solid load cartridge.
3. Liquid Injection
    - Through valve allows for column equilibration.
4. Liquid injection directly onto column
    - Equilibration is skipped and sample is run through dry column (for speed).

Features:

- Space saving design is smaller than other flash chromatography systems on the market, freeing scarce hood space for other uses.
- Solvent and waste management prevents columns from drying and waste containers from hazardous overflows.
- Large touch screen 10.5" (26.7 cm) puts the most used controls on a single, easy to read display.
- Large fraction capacity allows longer runs without changing racks. You don't need to divert time from other activities to watch your purification.
- RFID (Radio Frequency Identification) identifies columns and loads the appropriate method for the best separation. With reusable bonded-phase columns, the system tracks the number of runs on the column as well as the last solvent used, so you know whether you need to flush the column.
- RFID identification of fraction collection racks sets the maximum fraction size to prevent overflows.
- Injection valve features wide bore, straight plumbing, and self-cleaning to prevent clogs that cause down-time.
- No waiting to access data - networking allows lets you connect to the CombiFlash® Rf to monitor and remotely control your experiment, analyze data, and print to network printers.
- Rapid column air purge prevents solvent from being discarded with the used column. It is better for the environment, safer in the lab, and eliminates messy column removal.
- TLC Rf to Gradient calculator determines optimal separation parameters while minimizing run time.
- Easy scale-up with built-in method-scaling means you won’t need to re-develop your method. You save time when you increase reaction size. You can even scale up to a 1500g column run on a Companion XL.
- Back pressures to 200 psi for improved separations.
- One-year parts and labor warranty.
- Large fraction capacity allows longer runs without changing racks
- You don't need to divert time from other activities to watch your purification
- Easy scale-up with built-in method-scaling means you won’t need to re-develop your method
- You save time when you increase reaction size
- You can even scale up to a 1500g column run on a Companion XL

- 200 psi MPLC pressure capabilities for versatile use, including Reversed Phase
- Precision, high pressure binary gradient formation
- Automated, self-cleaning injection valve
- Active waste level-sensing prevents overflow conditions
- Active inlet level-sensing ensures adequate solvent for purification
- Quarternary solvent source with automated switching during the run
- UV and UV-Vis PDA detector options with all wavelength collection capability
- Scale up easily from mg to multigram purification with the click of a button
- External input for alternative detection technology

Applications in herbal drug research:

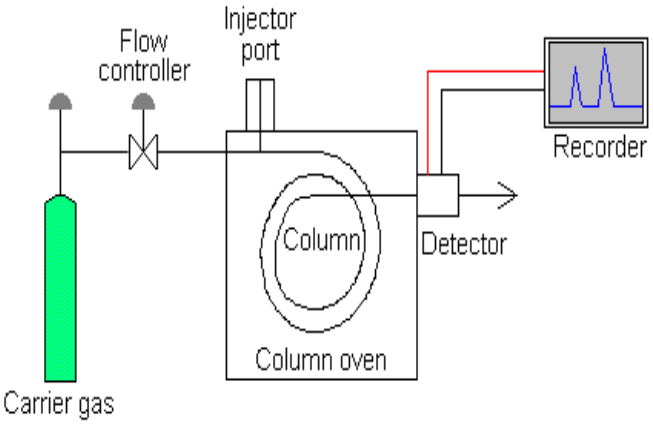
- In isolation of various herbal extracts containing peptides and polymers
- In purification of various extracts of compounds of natural origin
- In organic synthesis & Purification
-

GAS CHROMATOGRAPHY

Introduction:

The gas chromatograph makes it possible to separate the volatile components of a very small sample and to determine the amount of each component present. The essentials required for the method are an injection port through which samples are loaded, a "column" on which the components are separated, a regulated flow of a carrier gas (often helium) which carries the sample through the instrument, a detector, and a data processor. In gas chromatography, the temperature of the injection port, column, and detector are controlled by thermostatic heaters.

Gas chromatograph



Schematic diagram of GC

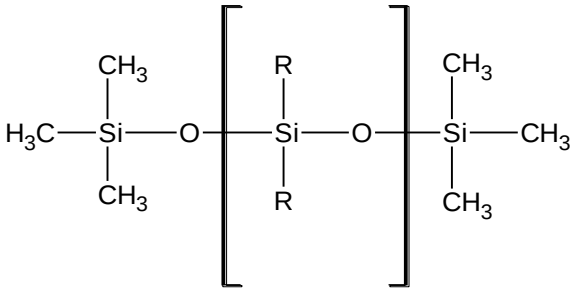
Injection port

The sample to be analyzed is loaded at the injection port via a hypodermic syringe. The injection port is heated in order to volatilize the sample. Once in the gas phase, the sample is carried onto the column by the carrier gas, typically helium. The carrier gas is also called the mobile phase. Gas chromatographs are very sensitive instruments. Typically samples of one micro liter or less are injected on the column. These volumes can be further reduced by using what is called a split injection system in which a controlled fraction of the injected sample is carried away by a gas stream before entering the column.

Column

The column is where the components of the sample are separated. The column contains the stationary phase. Gas chromatography columns are of two types—packed and capillary. Capillary columns are those in which the stationary phase is coated on the interior walls of a tubular column with a small inner diameter.

The stationary phase in column is generally a polysiloxane material. The basic structure of the polymeric molecules is shown below, where n indicates a variable number of repeating units and R indicates an organic functional group. In columns, 5% of the "R's" are methyl groups (-CH_3) and 95% of the "R's" are phenyl groups (-C_6H_5)n



This polymeric liquid has a high boiling point that prevents it from evaporating off the column during the experiment.

The components in the sample get separated on the column because they take different amounts of time to travel through the column depending on how strongly they interact with the stationary phase. As the components move into the column from the injection port they dissolve in the stationary phase and are retained. Upon re-vaporization into the mobile phase they are carried further down the column. This process is repeated many times as the components migrate through the column. Components that interact more strongly with the stationary phase spend proportionally less time in the mobile phase and therefore move through the column more slowly. Normally the column is chosen such that its polarity matches that of the sample. When this is the case, the interaction and elution times can be rationalized according to Raoult's law and the relationship between vapor pressure and enthalpy of vaporization. The rule of thumb is that retention times correlate with boiling points

As described above, the rate at which compounds move through the column depends on the nature of the interaction between the compound and the stationary phase. Other variables that affect this rate are column temperature and carrier gas flow rate. In this experiment, you will be provided a set of initial column conditions to analyze your samples. Based on the results of your first run, you will then vary the column temperature in order to achieve good separation of the peaks in the shortest possible time. One should avoid experimental conditions that lead to excessively long elution times. Not only do you waste valuable resources (your time and chart paper) but broadening of the peaks and loss of resolution will become evident when the elution times are too long. This broadening is an inevitable consequence of diffusion. The theory of diffusion shows that the width

of a peak is roughly proportional to the square root of elution time. Thus the optimum conditions are those that result in complete separation of the peaks in the shortest possible time.

Detector

If the column conditions are chosen correctly, the components in the sample will exit the column and flow past the detector one at a time. There are several different types of detectors common to gas chromatography instruments. The choice of detector is determined by the general class of compounds being analyzed and the sensitivity required. Our gas chromatographs are equipped with flame ionization detectors (FIDs)—the most widely used detectors for organic samples. FIDs use an air/hydrogen flame to pyrolyze the effluent sample. The pyrolysis of the compounds in the flame creates ions. A voltage is applied across the flame and the resulting flow of ions is detected as a current. The number of ions produced, and therefore the resulting current, depends on the flame conditions and the identity of the molecule in question. (As a rough approximation, the current is proportional to the number of reduced carbons in the molecule.) In other words, the detector shows a different response to each compound. For this reason, separate calibrations must be performed for each compound analyzed.

Integrating recorder

The output of the detector (converted from current to voltage) is sent to an integrating recorder that plots, stores, and analyzes the.

GC – in essential oil testing:

When using Gas Chromatography to test an essential oil, a tiny sample of the oil is injected into the apparatus which contains a very thin coiled silica tube called a 'capillary column'. This capillary column may measure up to 100 meters in length and is coated on the inside with a material that has an affinity to different chemicals at different temperatures. The column is housed within a temperature regulated oven and is programmed to steadily increase in temperature over a period of time in a very precise manner.

When the sample of oil is injected into the column it immediately vaporises, and an inert carrier gas (usually hydrogen or helium) moves the vapour along the column to a detector called a Flame Ionisation Detector which is situated at the end of the column.

Applications in herbal drug research:

- Analysis of essential oils
- Separation of terpenoids from volatile oils

DRUG	COLUMN	GAS	DETECTOR
Clove oil	capillary column	Nitrogen	FID
Carvone	capillary column	Nitrogen	FID
Menthol	capillary column	Nitrogen	FID
Limonene	capillary column	Nitrogen	FID
Myrcene	capillary column	Nitrogen	FID
Camphor	capillary column	Nitrogen	FID
Capsaicin	capillary column	Nitrogen	FID