KFRI Research Report No. 533

Capability Development in Instrumental Methods of Analysis

Final Report of Project No. KFRI 512/2006

E.M.Muralidharan

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Kerala Forest Research Institute,
(An Institution of Kerala State Council for Science, Techno logy and Environment)
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Summary of Project

1. Title of the Project	:	**Capability Development in Instrumental Methods of Analysis**
2. Principal Investigators	:	Dr. M. Balagopalan Dr. E.M.Muralidharan
3. Date of commencement	:	April 2006
4. Date of completion	:	March 2014
5. Funding agency	:	Plan Grants, KFRI

Contents

Background

The Central Instrumentation Unit (CIU) was established in 2006 with an objective of providing a central facility of latest and advanced analytical instruments for all the researchers in KFRI. CIU is located in the Silver Jubilee Block of KFRI . The CIU has imparted training on the use and maintenance of sophisticated instruments to students and researchers with the involvement of the service engineers of the instruments. The CIU also offers the facilities to researchers from other organizations on payment basis.

The CIU is housed in a separate wing on the -1 floor of the Silver Jubilee Block of KFRI. The Unit is provided with separate cubicles to facilitate the segeregation of the equipment for convenience of use as well as to provide airconditioning, wherever necessary. A sample preparation room where all samples are stored and preparations of the samples are done is also available. The Instrumentation Laboratory room that has more work area was partitioned into 6 cabins in 2013. Continuous Flow Analyzer and CHNS Analyzer are placed in separate cabins to the left of the Instrumentation Laboratory and all the heat generating equipments like water bath, oven etc are placed to the right side of the Instrumentation Laboratory in separate cabins.

The CIU now functions under a Scientist –In Charge and the day to day work is done with the help of a research Fellow trained in handling of sophisticated instruments. The Central Instrumentation Unit has the potential to become an advanced sophisticated instrument facility that can be utilized by researchers within KFRI and outside. Further additions to the available instruments is envisaged to achieve this goal. Support from the Kerala State Science, Technology and Environment and Department of Science and Technology under various available schemes will be explored.

The sophisticated instrument facility in the CIU includes the following :

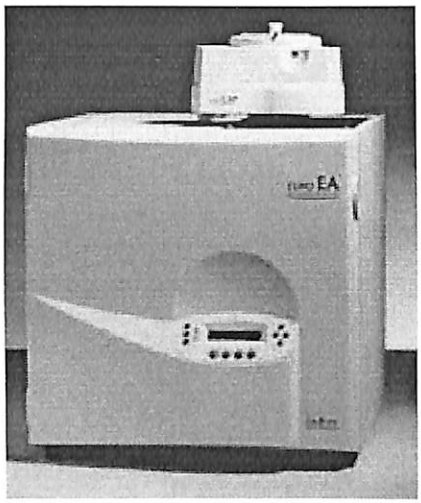
- Gas Chromatography Mass Spectrometer (Shimadzu GCMS QP 2010)
- Gas Chromatography ECD/FID (Shimadzu GC 17A)
- High performance Liquid Chromatograph (Shimadzu SCL-10Avp)
- Continuous Flow analyzer (NH_3/Total N , Ortho/Total Phosphate , Nitrate/ Nitrite) Skalar
- CHNS Analyzer (Eurovector)
- Particle Size Analyzer (Ankersmid)
- Soil CO_2 Analyzer (ADC)
- Muffle furnace
- UV –Vis Spectrophotometer (XP2001 Xplorer)
- Flame Photometer BWB-1

In addition, other basic equipment for sample preparation available are

- Shaker - Infors HT
- Double Distiller and Single Distiller
- Deionizer

- Vacuum pumps
- Electronic balance
- High purity water - Milli-Q- system
- Soxhelt Extraction mantle
- Hot air oven
- Weighing machine
- Stirrer (Rotek)
- Water bath
- Oven (Labline)
- Stirrer magnetic
- Microwave oven

CHNS ELEMENTAL ANALYZER



Principle: The CHNS (O) Analyzer find utility in determining the percent of Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen of organic compounds, which are generally combustible at 1800° C based on the principle of "Dumas method" which involves the complete and instantaneous oxidation of the sample by "flash combustion". Gases are detected as peaks using Supersensitive Thermal Conductivity Detector (TCD) and results are presented as percent content of each element. Solid, Viscous and Liquid samples can be analysed in the instrument. The samples are held inside a tin capsule for CHNS mode and silver capsule for Oxygen mode.

Max Furnace Temperature: 1100°C
TurboFlash Combustion Temperature: 1800 °C

Instrument Performances:

- Simultaneous Determination of the 4 elements (CHNS)
- Less than 6 minutes total run time for CHNS and 2.5 minutes for O
- K factor or Linear Regression selection of calibration procedure
- Full automation using Callidus, the most advanced dedicated software to Elemental Analysis

Specifications : Cat. No. Euro EA 3000
Elemental Analyzer for simultaneous analysis CHNS and O

Accessories : Auto sampler (80 positions)
Callidus, Standard Parts, Tool Kit Bag, Bubble Flow meter

Configuration Kit for CHNS, Configuration Kit for O

Price : US$ 25,420/- (24700 + 750 for CIF) /Rs 1, 07,889

Consumables:

CHNS Reactor (200 samples per reactor)
Tin Capsule
High Purity Helium & Oxygen gas
Reference Standard

Analysis Done:

Number of Samples (October 2012 –October 2013): **898**
The instrument was mainly used for doing soil and plant analysis by Soil Science and Physiology Department. Of the 898 samples done during the last year, 110 samples were external samples.

GAS CHROMATOGRAPH

Principle:

Gas chromatography (GC), is used for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, identification of a compound or separating the different components of a mixture. In gas chromatography, the *mobile phase* is a carrier gas, usually an inert gas such as helium or nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Instrument Performances:

- Testing the purity of a particular substance
- Separation and determination of the relative amounts of the different components in a mixture
- Identification and quantification of a compound that vaporizes below 300 °C and is thermo stable
- Quality assurance of products
- Measurement of toxic substances in soil, air or water
- Measures picomoles of a substance in a 1ml liquid sample or parts per billion concentrations in gaseous samples

Columns:

Column Phase	*Composition*	*Temp*	*General Use of Phase*	*Equivalents*
DB-1	100 % Dimethyl polysiloxane	50-325°C	Most frequently used phase with excellent Thermal stability Low Selectivity separates compounds based on the boiling point ***Applications :*** Amines, hydrocarbons, pesticides, PCBs, phenols, sulfur compounds, flavors and fragrances	SE-30,OV-1, OV-101, SPB-1, BP-1, HP-1 , ULTRA -1, RTx-1,AT-1, CPSil -5
DB - 5	5% Phenyl 95%	50-325°C	Similar to methyl polysiloxane but slightly more selective due to phenyl content	SE-54,OV-23,SPB-5, BP-

	dimethylpoly siloxane		Excellent Thermal stability ***Applications :*** Semi volatiles, alkaloids, drugs, FAMEs, halogenated compounds, pesticides, herbicides	5, HP-5,ULTRA -2, RTx-5, CPSil-8
BPX-70	70% Cyanopropyl Polysilpheny lenesiloxane	50-250°C	Ideal for cis/trans isomer separation Low bleed ***Applications :*** Fatty acid methyl esters, carbohydrates	DB-23, Rtx-2330, SP-2330, CP-Sil 88, SP2380, HP-23

Detectors:

Electron capture detector (ECD)

Electron capture detector (ECD) uses a radioactive beta particle (e.g. 63Ni, 3H) source for detecting atoms and molecules in a gas through the attachment of electrons via electron capture ionization.

Capable of high sensitivity detection of electrophilic compounds containing halogen elements (CI, Br, F, I). There may also be some sensitivity for compounds not containing halogen elements.

Detection of molecules containing electronegative elements and functional groups like halogens , carbonyl, nitriles, nitrates , nitro groups, and organometalics

- Pesticides
- PCBs
- Insecticides (organochlorine & synthetic pyrethroid)

Selectivity	*Sensitivity*	*Gases*	*Temperature*
Halogens , Nitrates & Conjuated Carbonyls	0.1-10 pg (Halogens) 1-100 pg(Nitrates) 0.1-1 ng (Carbonyls)	N_2	300-400°C

Flame Ionization detector (FID)

The sample is heated to extremely high temperatures, which causes the organic species to ionize. The signal that is measured by FID is proportional to the concentration of the ionized species, which will be proportional to the concentration of the organic species itself.

Sensitive for compounds containing carbon i.e. organic / hydrocarbon containing compounds but relative insensitive to a few small molecules e.g. N2, NOx, H2S, CO, CO2, H2O.

Determination of VOC's and Air monitoring

Biochemical compounds such as proteins, nucleotides, and pharmaceuticals can be studied using FID

Selectivity	*Sensitivity*	*Gases*	*Temperature*
C-H	0.1-10 ng	Combustion: H_2 & Air Make up : He	250 -300 °C

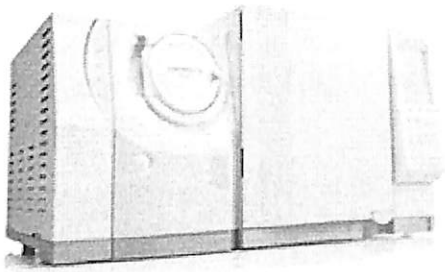
Mass spectrometer (MS)

The Ion Source ionizes the sample and the ions (cations) are sorted and separated according to their mass and charge by the analyzer and finally the detector measures the separated ions and displays the mass spectrum which shows the relative abundance of fragments with each m/z ratio.

Identification of the compounds based on their fragmentation pattern.

Selectivity	*Sensitivity*	*Gases*	*Temperature*
Any compound that fragments within the selected mass range	1-10 ng Full Scan 1-10 pg SIM)	None (Vacuum)	250 -300 °C (Transfer Line) 150-250°C (Source)

Gas Chromatograph – Mass Spectrometer



Specifications:

Make	: Shimadzu
Model number	: QP2010
Sr. no	: C70384300095
Software Name	: GCMS solution Version 2.40

Price :
Purchase Order No : KFRI/Plan 05-06 /Mass Spectrometer dated 26-12-06
Installation : Service Engineer from Toshvin Analytical Pvt Ltd demonstrated the instrument operation with GCMS Solution software on 29/11/06

GC

Specifications:

Make	: Shimadzu
Model number	: GC 17 A AFW
Sr. no	: C11124105413SA
Software Name	: GC solution

Price :
Purchase Order No : KFRI/Plan 03-04 /
Installation : Service Engineer from Toshvin Analytical Pvt Ltd installed and demonstrated the instrument operation with GC Solution software on 17/10/05. Demonstration of ECD was given on 07/06/06. **FID**

Analysis: The main analysis being done using GC includes

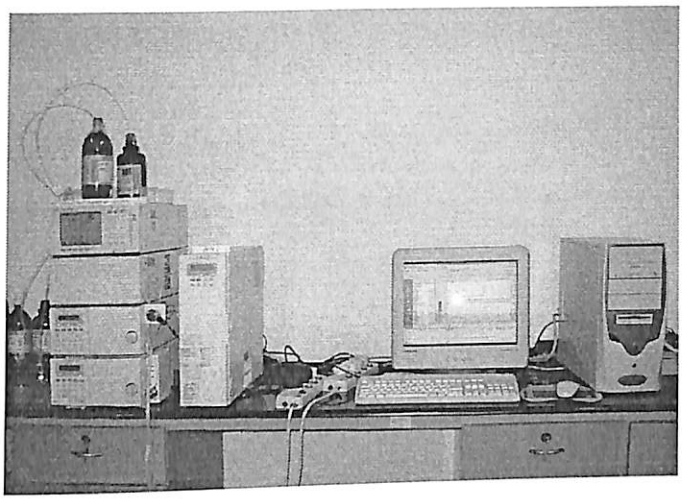
- Profiling and quantification of Cinnamon oil
- Identification and Quantification of pesticides in soil and plants
- Identification of microbial FAME in soil
- Identification and Quantification of fatty acids in seeds
- Profiling of unknown samples

Number of Samples done (October 2012 –October 2013) : **430**
The instrument was used by various departments as NTFP, Entomology, Genetics, and Physiology of KFRI.

Consumables:

- High Purity Gases (Helium, Nitrogen, Zero Air, Hydrogen)
- Spares (Septum, Ferrule, Rotary Pump Oil etc)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



Principle:

High-performance liquid chromatography is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol) and is referred to as a "mobile phase".

Instrument Performances:

Columns:

Column Phase	*Composition*	*General use of Phase*
Luna 5u C18 (2) 100A (Reverse Phase)	Most commonly used phase Octadecyl silane ligands are bound to the silica surface C18 columns will definitely be more "hydrophobic rather than the	Separation of hydrophobic compounds as flavanoids, saponins, terpenoids *Mobile phase* : Acetonitrile , Methanol, Isopropyl alcohol

	C8 columns.	
Luna 5u C8 (2)100 A (Reverse Phase)	Octyl silane ligands are bound to the silica surface Less hydrophobic retention than C18 but comparable selectivity	Separation of very hydrophobic proteins and peptides and large non polar organic compounds as phenolic compounds toluene , pyridine *Mobile phase* : Acetonitrile , Methanol, Isopropyl alcohol
Luna 5u Silica (2) 100A (Normal Phase)	Porous silica particles or a monolithic silica rod	Separation of Polar compounds as fat soluble vitamins , fatty acids and lycopenes *Mobile Phase* : n- hexane , Tetrahydrofuran

Detectors:

Photodiode array detector (PDA) A photodiode is a type of photodetector capable of converting light into either current or voltage, depending upon the mode of operation. D2 lamp - 190-370 nm W lamp - 370-800 nm For 190-800 nm select both.	Simultaneous measurement in all wavelengths from ultraviolet to visible Determination of the correct wavelengths (maximum absorbance) in one run Peak purity analysis 3D data presentation and spectral analysis with spectral library search for positive peak identification
RF 20A Spectrofluorometric detector Spectrofluorometric detector detects fluorescence emitted in the direction orthogonal to the exciting light. Typically, fluorescence sensitivity is 10 -1000 times higher than that of the UV detector for strong UV absorbing materials. A Xenon lamp has long-life (2000 hrs) and provides light output from 190-1200 nm.	High Sensitivity, Selectivity and Repeatability Suitable for analytes with little or no UV absorbance Suitable for trace analysis and for samples containing high levels of impurities. Analysis of aflatoxins, carbamates, vitamins, polyaromatic hydrocarbons and amino acids.

Specifications:

Make	: Shimadzu
Model number	: SCL-10Avp
Sr. no	: 891
Software Name	: LC Solution

Price :

Purchase Order No : KFRI/Plan Fund/04-05/130

Installation: The equipment was temporarily installed in the Soil Chemistry Laboratory on 28/05/2004 by a service engineer from Spinco Laboratory Pvt Ltd. Demonstration of the LC 10ATvp (Binary system) with PDA detector and LC Solution software was done by Mr B. Dinesh, Service Engineer , Spinco Biotech on 28/05/04. The instrument was later reinstalled in the Instrumentation lab on 21/07/05. Installed the RF 20A spectrofluorometric detector on 02/06/2010.

Analysis:

Number of Samples done (October 2012 –October 2013): **210** injections

The main analysis done with HPLC includes

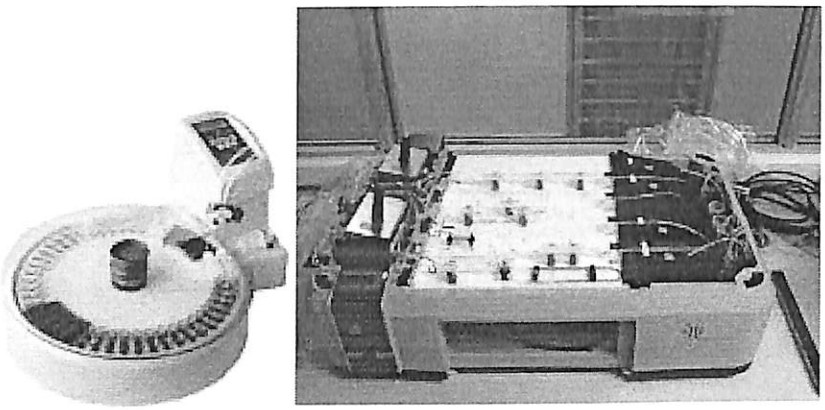
- Quantification of Camptothecin alkaloid
- Analysis of Cinnamon samples and std (Eugenol, Cinnamaldehyde & Cinnamylacetate)
- Analysis of Iturin and surfactin antibiotic standards and samples
- Analysis of Embellin content in callus tissues

It has been used by fellows from Pathology, Biotechnology and Genetics department.

Consumables:

HPLC Grade Solvents (Methanol, Water, Acetonitrile, Isopropanol)
Guard Column Catridge
Syringe Filters and membranes for sample preparation

CONTINUOUS FLOW ANALYZER



Principle:

Continuous Flow Analyzer is based on the Segmented Flow Analysis (SFA). Automated segmented flow analysis is a continuous flow method in which a stream of reagents and samples segmented with air bubbles is pumped through a manifold to undergo treatments such as mixing heating, dialysis etc. before entering a flow cell to be detected. It is used mainly for soil, plant, water and fertilizer samples. The main parts of the analyzer system are:

- Auto sampler
- Chemistry unit with pump, rinsing valves, and chemistry modules
- Detectors
- Data handling

Instrument Performances:

- Speed of Analysis (approximately 3 minutes)
- Correlation between the analyzer and the accurately done manual analysis will be 0.9999
- Cost per analysis is very low compared to manual analysis
- Air segmentation eliminates cross contamination
- 5 chemistry modules with built in robust dosing pump and air injection system makes it possible to monitor 16 analytical measurements on a single sample simultaneously.

Specifications:

Make : Skalar
Model number : Skalar San++
Chemistry Unit: SA 5000
Auto sampler : SA 1100
Interface : SA 8505 SFA

Sr. no : 891
Software Name : Flow Access
Price : Rs 30, 62,181.00 / 39648.00 Euro

Purchase Order No : KFRI/P/537/09-10/P-271 dated 01/10/2009

Installation: The installation and demonstration of Skalar autoanalyser was done by a service engineer from Agaram Industries on 17/09/2009.

Analysis:

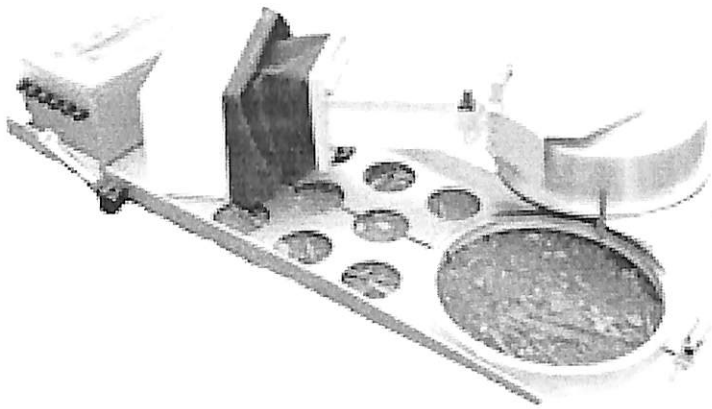
Number of Samples done (October 2012 –October 2013): **408**

Total Nitrogen is the main analysis done and it is used mainly by the Soil Science Department. The samples include plant digest, soil digest and compost digest.

Modules Available:

- Ammonia /Total Nitrogen
- Ortho/ Total phosphate
- Nitrate/Nitrite

SOIL CO_2 EXCHANGE SYSTEM



Principle:

The Automatic CO_2 Exchange System (ACE) is used to measure CO_2 exchange in soil (soil respiration). Soil flux is expressed as Net CO_2 Exchange Rate (NCER) in µmol m^{-2} sec^{-1}. The amount of gas exchange taking place is frequently used as an indicator of microbial soil activity and so is used to characterize the "health" of that soil. Understanding soil flux and its relationship with other sources and sinks within the carbon cycle are currently subject to increasing scientific scrutiny in relation to global climatic change.

Instrument Performance:

- ACE Stations are available in either a closed system or an open system configuration.
- The delta CO_2 is determined from the difference between the reference gas entering the chamber and the analysis gas within the chamber at the end of the experiment.
- The motorized arm containing an infra-red gas analyzer (IRGA) closes during the periodic measurements and the recorded measurements consists of the soil CO_2, ambient CO_2, PAR (Photosynthetic Active Radiation) and up to 6 temperature and 4 soil moisture readings

Specifications:

Make : Skalar
Model No : ACE Automated CO_2 Exchange Station

Chemistry Unit: SA 5000
Auto sampler : SA 1100
Interface : SA 8505 SFA
Sr. no : 32750
Software Name : Flow Access

Price : GBP 7120 / Rs. 6, 06,350.00

Purchase Order No : KFRI/P-537/09-10/P-699 dated 25/03/2010

Installation: The installation of ACE Automated CO_2 Exchange Station was done by a service engineer from Concord Scientific Instruments on 08/01/2010. The demonstration of Open Zero mode was given on the same day .The battery along with the charger was supplied free of cost.