



Report on

Two Days Hands on Training (Workshop)

On

“Analytical Method Development on High Performance Liquid Chromatography (HPLC)”

09-10 October 2017

Organized by

RUSA Centre for Herbo Medicinal Studies

**Swami Ramanand Teerth Marathwada University,
Nanded (Maharashtra)**

About Hands on Training (Workshop):

High performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for analysis of many drugs in both dosage form and biological fluids. The rapid growth of HPLC has facilitated by development of reliable, moderately precise instrumentation and efficient columns.

Analytic method development, validation, and transfer are key elements of any pharmaceutical development program. Method development is a continuous process that progresses in parallel with the evolution of the drug product. HPLC is a potent analytical tool, allowing for the separation, identification and quantification of drug substances. It also presents many challenges, from the correct use of instrumentation, to analysing results, to the fulfillment of the regulatory requirements of method validation.

Key feature of Training: Over two days, this hands on training will offer participants the opportunity to learn the important aspects of HPLC theory and method development, including troubleshooting and participants will examine quantitation and method validation.

Eligibility: All graduate, Post graduates students, Research Scholars, Faculty and Industry Persons who are interested and willing to participate.

Lodging and Boarding: Lodging shall be provided on request by the participant to University at campus faculty house/guest house at minimum cost.	 																	
Important dates Last date of receipt of application form : 06 th October 2017 Confirmation & selection (by email only) : 06 th October 2017	Rashtriya Uchchatar Shiksha Abhiyan (RUSA) Government of Maharashtra and Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded-431606 Organizes Two Days Hands on Training (Workshop) On “Analytical Method Development on High Performance Liquid Chromatography (HPLC)” 09-10 October 2017 Venue RUSA Centre for Herbo Medicinal Studies Swami Ramanand Teerth Marathwada University, Vishnupuri Nanded-431606 (Maharashtra)																	
Organizing Committee: <table><tr><td>Chief Patron Prof (Dr.) Pandit Vidyasagar Hon'ble Vice Chancellor, S.R.T.M. University, Nanded</td><td>Patron Dr. G. N. Shinde Hon'ble Pro-Vice Chancellor, S.R.T.M. University, Nanded</td></tr></table> Co-Patrons: <table><tr><td>Prof. (Dr.) R. M. Mulani Registrar</td><td>Dr. G. B. Katalakute Finance and Accounts Officer</td></tr><tr><td>Prof. (Dr.) V. N. Laturkar Director, IQAC & NAAC</td><td>Dr. R. N. Sarode Director, BEE</td></tr></table> Conveners Prof. (Dr.) S. J. Wadher (Convener, RUSA Centre, SRTMU, Nanded) Contact: +919420683967 Prof. (Dr.) C. N. Khobragade (PI-RUSA Project) Contact: +919890796404 Co-conveners: <table><tr><td>Prof. R. S. Khairnar Director, School of Physical Sciences</td><td>Dr. S. R. Butle School of Pharmacy</td></tr></table> Committee Members: <table><tr><td>Mr. R. V. Kshirsagar</td><td>Dr. Krishna Chaitanya</td><td>Mr. S. L. Patwekar</td></tr><tr><td>Dr. Sachin Narangale</td><td>Dr. Rahul Pinjari</td><td>Mr. R. S. Moon</td></tr><tr><td>Dr. G. B. Zore</td><td>Dr. B. S. Surwase</td><td>Dr. S. S. Pekamwar</td></tr></table>	Chief Patron Prof (Dr.) Pandit Vidyasagar Hon'ble Vice Chancellor, S.R.T.M. University, Nanded	Patron Dr. G. N. Shinde Hon'ble Pro-Vice Chancellor, S.R.T.M. University, Nanded	Prof. (Dr.) R. M. Mulani Registrar	Dr. G. B. Katalakute Finance and Accounts Officer	Prof. (Dr.) V. N. Laturkar Director, IQAC & NAAC	Dr. R. N. Sarode Director, BEE	Prof. R. S. Khairnar Director, School of Physical Sciences	Dr. S. R. Butle School of Pharmacy	Mr. R. V. Kshirsagar	Dr. Krishna Chaitanya	Mr. S. L. Patwekar	Dr. Sachin Narangale	Dr. Rahul Pinjari	Mr. R. S. Moon	Dr. G. B. Zore	Dr. B. S. Surwase	Dr. S. S. Pekamwar	
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About SRTM University

The Swami Ramanand Teerth Marathwada University, Nanded established in 1994, although situated in rural area is fastest growing University. The university has been awarded 'A' grade for its third cycle of reaccreditation by NAAC, Bangalore. This University is catering to the educational needs of Marathwada Region by providing quality education. It has beautiful campus spread over 550 acres of land and has holistic and huge infrastructure. About 44 PG courses are running through 14 different Schools. The university is an affiliating university with 392 affiliated colleges including two autonomous colleges. The University campuses offer 08 UG, 44 PG, 11 Certificate and 08 Diploma courses through school system.

RUSA Centre for Herbo - Medicinal Studies

As a part of RUSA program, SRTM University, Nanded received the funds for the development of RUSA Centre for "Herbo-Medicinal Studies". A well equipped laboratory has been established in RUSA centre in the year 2016 for Technology Transfer. The collaborative project emphasizes on isolation of actives/phytochemical from the medicinal plants. Under the Central Facility scheme, researchers/industrialists/academia can use the resources present in the Centre or avail an experts help on chargeable basis. Currently, Centre is working especially for the isolation of active constituents from the herbs through advanced equipments available like 'Preparative HPLC' and 'Flash System'. Other developmental activities related to research can also be completed here.

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Eligibility: All graduate, Post graduates students, Research Scholars, Faculty and Industry Persons who are interested and willing to participate.

Resource Persons

The course will be conducted by experts from Universities, Research Institutions, and Industries.

Registration:

Total participants are restricted to 15 only. Applications will be accepted on first come first served basis. Registration will be accepted with registration form fully endorsed by head/principal of Institute/colleges or Industry. Registration fees: Rs. 800/- for students and Rs.1000/- for faculty/others in the form of DD in favor of The Finance and Accounts Officer, SRTM University, Nanded payable at Nanded. Or Through NEFT Account No.: 500104000028422, Account Name: Finance and Accounts Officer, SRTM University, Nanded, Bank Name: IDBI, Nanded and IFSC : IBKL0000500.

Submission of Application form

Participants should send hard copy of filled application form along with details of payment through NEFT to Prof. S. J. Wadher, School of Pharmacy, SRTM University, Nanded and scanned copy of application form on email: sjwadher@gmail.com, on or before 6th October 2017.

Important Schedule: Inauguration on 09th October 2017 at RUSA Centre for Herbo Medicinal Studies at 10.00 am

	Rashtriya Uchchatar Shiksha Abhiyan (RUSA) Government of Maharashtra and Swami Ramanand Teerth Marathwada University RUSA Centre for Herbo Medicinal Studies Vishnupuri, Nanded-431606 Two Days Hands on Training (Workshop) On "Analytical Method Development on High Performance Liquid Chromatography (HPLC)"	
09-10 October 2017 Registration Form (Please fill in Block letters)		
Name of Delegate	:	_____
Position/Designation	:	_____
Higher Qualification	:	_____ Gender _____
College/ Institute	:	_____
Mailing Address	:	_____
Town/City	:	_____ Pin code _____
D.D./ NEFT details	:	_____
Phone	:	_____
Mobile	:	_____
E-mail	:	_____
Accommodation	:	☐ Required ☐ Not Required
Date:	Signature of Applicant _____	
Recommendation of Head/Principal of Institute/Industry		
Dr./Mr./Ms. _____ is employed/working as Faculty/Student in _____ He/She is sponsored to attend 02 days Hands on Training (Workshop).		
Place:	Date:	Signature & Seal Principal / HOD of Industry

Inauguration:

Two days' workshop was inaugurated by Hon'ble Vice Chancellor Prof. Pandit Vidyasagar sir. Dr. R. M. Mulani, Registrar, Dr. G. B. Katalakute, Finance and Accounts Officer, Convener Dr. S. J. Wadher, Dr. C. N. Khobragade and Dr. R. S. Khairnar were present for the inauguration.

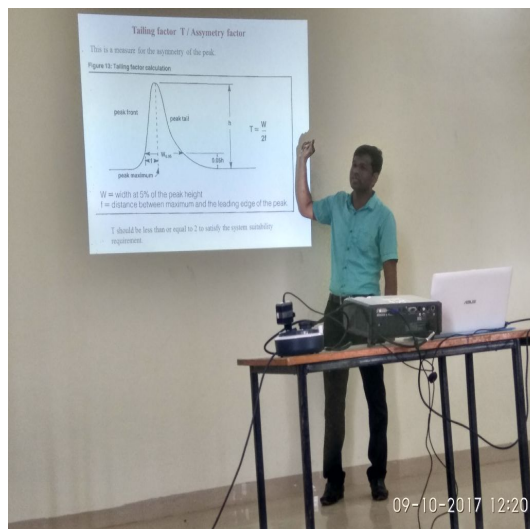


Inauguration at Hon'ble Vice Chancellor Prof. Pandit Vidyasagar sir

Twenty eight (28) students registered for workshop hands on training. Dr. Santosh Chhajad, Associate Professor, Mumbai Education Trust College of Pharmacy, Nashik and Mr. Ram Sakhare, Assistant Professor, School of Pharmacy, SRTM University delivered talk on Basic of HPLC system and application of HPLC in Pharma field.



Lecture by Dr. Santosh Chhajad



Lecture by Mr. Ram Sakhre

In the training workshop on HPLC following work has been assigned to each students

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMOXICILLIN TRIHYDRATE AND METRONIDAZOLE IN BULK AND IN-HOUSE TABLET

Materials and method

Pharmaceutically pure sample of amoxicillin trihydrate and metronidazole was obtained from Aarati drugs ltd. Tarapur as gift samples. All other chemicals (water, acetonitrile, methanol) used in the analysis were HPLC grade.

Instrumentation

Chromatographic separation was performed on HPLC system (model Shimadzu SCL – 10), C18 column (250mm × 4.6mm; 5 μm), UV Detector, equipped with a solvent delivery pump, sample injector and column thermostats. Lab solution (version 1.25) software was applied for data collecting and processing. Mobile phase: water: acetonitrile: methanol in the ratio of 70:20:10 (v/v/v).

Selection of analytical wavelength

Accurately weighed 10 mg AMT and MET were transferred into 100 ml volumetric flasks separately and dissolved in mobile phase. This solution was sonicated for 20 minute and volume was made up to mark with mobile phase. Pipetted out 2 ml of stock solution and

diluted to 10 ml to get a concentration of 20 µg/ml of AMT and MET. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. The overlain spectra showed iso-absorptive point at 237.3 nm and this wavelength was selected for further detection.

Preparation of standard solution

Accurately weighed 100 mg of AMT and 80 mg of MET working standard was transferred into 100 ml volumetric flask and dissolved in mobile phase. This solution was sonicated for 20 minute, then volume was made up to mark with mobile phase to get concentration of 1000 µg/ml for AMT and 800 µg/ml for MET. From the standard stock solution, mixed standard solution was prepared to contain 20 µg/ml of AMT and 16 µg/ml of MET.

Optimized chromatographic conditions

Mobile phase : Water: ACN: Methanol (70:20:10 v/v/v)

Column : C18 column

Detector wavelength : 237.3 nm

Injection volume : 20 µl

Flow rate : 1.4 ml /min

Run time : 15 minutes

System Suitability test

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed, mixed standard solution of AMT and MET was injected into HPLC system and their system suitability parameters were recorded. Validation of the method

Method was validated according to ICH guidelines for linearity, accuracy, precision, LOD, LOQ, robustness and specificity.

Linearity

Linearity was studied by preparing standard solution at different concentration levels. Standard stock solution of AMT and MET was further diluted to get concentration in the range of 20-100 µg/ml for AMT and 16-80 µg/ml for MET. Each dilution of both the drugs was injected into the HPLC system and peak areas were determined. Standard calibration curves were constructed by plotting peak areas versus concentrations of drug and the regression equations were calculated Accuracy:

The accuracy of the proposed method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding a known amount of standard drug to pre-analyzed sample. The resulting solutions were then reanalyzed by proposed method. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at three replicate of three concentrations levels.

Precision:

Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation). The precision was determined at different parameter like repeatability, intermediate precision (intra-day, inter-day). Repeatability was determined by analyzing AMT (100 µg/ml) and MET (80 µg/ml) three times. Intraday precision was determined by analyzing same concentration of solution for three times within the day and Interday precision was determined daily for three days. Then % RSD was calculated and it was within limit (less than 2 %). **Robustness:**

The robustness of the method was determined as a measure of the analytical method capability to be unaffected by small variations in method parameters and the different variants such as variation in flow rate by ± 0.2 ml/minute; variation in wavelength by ± 2 nm were selected. At these changed conditions, the standard and sample solutions were injected. The system suitability was evaluated in each varied condition.

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD is the lowest concentration of analyte in sample that can be detected but not necessarily quantified. LOQ is the lowest concentration of analyte in sample that can be quantitatively determined with precision and accuracy. LOD and LOQ was calculated from linear curve using following formulae

$$\text{LOD} = 3.3 \sigma/\text{slope}, \quad \text{LOQ} = 10 \sigma/\text{slope}$$

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

Specificity:

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both amoxicillin trihydrate and metronidazole from impurities.

Analysis of in-house tablet

Due to unavailability of tablet containing amoxicillin trihydrate and metronidazole in local Indian market, in-house tablets were prepared via direct compression technique using commonly used excipients containing 250 mg of amoxicillin trihydrate and 200 mg of metronidazole.

Twenty tablets were taken and their average weight was determined, crushed to fine powder; powder equal to 50 mg of AMT and 40 mg MET was taken in 100 ml volumetric flask. Then 80 ml of mobile phase was added and the flask was sonicated for about 20 minute to solubilize the drug present in tablet, after that solution was filtered through Whatman filter paper No. 41. The filtrate was collected and the volume was made up to the mark with mobile phase. Further dilution was done in 10 ml volumetric flask to get final concentration of 20 µg/ml of AMT and 16 µg/ml of MET. The standard and sample solution was injected into HPLC system and peak areas were measured. The content of AMT and MET was calculated by using following formula.

$$\text{Amount of drug (mg)} = A_t/A_s \times D_s/D_t \times W_s/W_t \times A \quad \dots\dots\dots (i)$$

$$\% \text{ Estimation} = A_t/A_s \times W_s/W_t \times \text{Avg. wt (A)}/\text{Label claim} \times 100 \quad \dots\dots\dots (ii)$$

Where,

A_t = Area count for sample solution

As = Area count for standard solution

Ds = Dilution factor for standard

Dt = Dilution factor for sample

Ws = Weight of standard (mg)

Wt = Weight of sample (mg)

A = Average weight of mixture

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF SIMVASTATIN IN BULK AND TABLET DOSAGE FORM

MATERIALS & METHODS

Pure simvastatin was procured as a gift sample from IPCA laboratory, Mumbai India. The marketed pharmaceutical tablet dosage form of simvastatin SIMVAS 10 by Micro Labs, India was purchased from local market, and used within its shelf life period. Acetonitrile, methanol and water was used in the method development of HPLC-grade. 0.1 N HCl, 0.1 N (NaOH), 30% H₂O₂ prepared in lab. Buffer materials and all other chemicals were of AR grade was purchased from Merck Specialties Private Limited.

The chromatographic system model SHIMADZU SCL-10 VP equipped with UV-Visible detector controlled by LC solution (version 1.25) software and column enable C18 (250×4.5mm) was used as a stationary phase.

Chromatographic conditions

The chromatographic conditions were performed using following:

Mobile phase: Acetonitrile: phosphate buffer (pH4.5) (25:75 v/v)

Run time: 20 min

Wavelength of scanning: 238 nm

Diluent: Mixture of 400 ml phosphate buffer (pH 4.5) and 600 ml acetonitrile in the ratio of 40:60% v/v was taken.

Preparation of standard solution

A standard solution was prepared by transferring 50 mg of simvastatin in 100 ml of volumetric flask. To it diluent was added and sonicated for 20 min to dissolve completely, and then volume was made up to the mark with diluent to get concentration 1000 µg/ml. It was filtered through Whatman filter paper No. 41. It was further diluted to get concentration of 10 µg/ml.

Selection of mobile phase and chromatographic conditions

Trials were carried out using different solvent combinations including methanol, water, acetonitrile in various proportions. Acetonitrile : phosphate buffer 25:75 v/v (pH: 4.5 adjusted with O-phosphoric acid) was selected as optimum mobile phase with in a run time of 20 min and filtered by a millipore vacuum filter system equipped with 0.45 µm high vacuum filter, which gave resolution and sharp peak for the drug. Other chromatographic conditions like run length, sample injection volume, flow rate, detection wavelength, was optimized to give Rt and symmetrical peak shape for the drug.

Selection of detection wavelength

The standard solution was scanned over the range of 200-400 nm and the spectrum was obtained.

Analysis of marketed formulation

Twenty tablets (SIMVAS 10 labelled to contain 10mg of simvastatin) were weighed accurately, average weight was determined and finely powdered. A quantity of powder equivalent to 10 mg of simvastatin was weighed and transferred to a 200 ml volumetric flask to it 140 ml of diluent was added and sonicated for 30 min with intermediate shaking. It was diluted up to the mark with diluent. It was further diluted to get concentration of 10 µg/ml of simvastatin. The analytical procedure was repeated six times for the powder sample.

VALIDATION PARAMETERS

The HPLC method was validated on accuracy, precision, LOD, LOQ, linearity, range and robustness as per ICH guidelines ¹⁷.

Accuracy

To check the accuracy of proposed method was determined by performing recovery studies by standard addition method in which pre-analyzed sample of tablet powder was taken and known amount of pure was added at three different levels (80%, 100% and 120%) and the % recovery of simvastatin ranging from 98.70 to 102.6 % was calculated.

Precisions

This parameter was evaluated by carrying out six independent test samples. RSD (%) of six assay values obtained which was calculated. The system precision and method precision was carried out by analysing the sample in different days. The RSD (%) values for method precision and system precision were less than 2% indicating high degree of precision of developed method.

Linearity and range

Five different concentrations ranging from 50, 75, 100, 125 and 150 µg/ml of simvastatin was prepared in 100 ml volumetric flask. The peak areas of those solutions were measured at 238 nm.

Specificity

This method was carried out by injecting the working standard, blank and stressed samples into the chromatograph to check the co-elution; at the retention time of simvastatin peak.

Limit of Detection

The limit of detection (LOD) for simvastatin was determined from standard deviation of the response and slope of the curve by using equation 1.

$$LOD = \sigma/S \times 3.3 \text{ ----- (1)}$$

Limit of Quantitation

Limit of quantitation (LOQ) for simvastatin was determined from standard deviation of the response and the slope of curve by using equation 2.

$$LOQ = \sigma/S \times 5 \text{ ----- (2)}$$

Robustness

Robustness was carried out by changed in chromatographic conditions such as changed in wavelength of detection (±2nm), flow rate (±0.1ml/min), pH (±0.5) & acetonitrile content in mobile phase (±2%).

System suitability parameters

The system suitability test parameters was carried out to evaluate resolution and reproducibility of the system for the performed analysis, using five replicate injections of reference solution containing 100 µg/ml of simvastatin. The parameters like resolution, retention time, theoretical plates and tailing factor were measured.



Participants of Workshop



Hon'ble Vice Chancellor Prof. Pandit Vidyasagar with Participants of workshop



**Valedictory program and certificate distribution at the Hand of Hon'ble Vice Prof.
Pandit Vidyasagar**

