

Analytical Method Development

Case Study

Domain: Natural Preparations

Type: Method Development

Revolutionizing research in Naturals through analytical method development used for identification and characterization of active components

Challenge

- **Sample Natural Extracts:**
 - Sample Preparation suitable for Mass spectrometric analysis
 - Evaluation of appropriate Sample Concentration from Crude samples with very low purity level
- **Method Development:**
 - Developing a LC/ MS-MS method for estimation of mass value of major known and unknown components in the Natural Products from complex matrix (thousands) of compounds
- **Identification and Characterization of Peaks:**
 - Identification and Elucidation of molecular structures for major known and unknown components present in Natural Products.

Expertise

- A group of specialists proficient in analytical techniques
- Expertise in Development and Validation of analytical methods for drug discovery, clinical research, characterization and impurities profiling in natural, synthetic and APIs
- Strong hand on skills in the use of these drug studies, such as- HPLC, GC, LCMS and LCMS/ MS

Solutions

- Preparation of sample depending upon its solubility in different solvents and the concentrations suitable for MS sensitivity.
- Development of HPLC method to separate the components from the natural products.
- Adoption of HPLC method in line with LC/MS-MS method.
- Detection of the mass values of parent and fragmented molecules in the Herbal products.
- Validation for the equivalence of area percentage of major peaks.

“Ensuring the quality of Natural products by Qualitative and Quantitative elucidation of unknown components”

Evaluating Requirements:

The customer is located in Maharashtra (a state located on the western coast of India famous for its rich vegetation) is a leading manufacturer and supplier of high quality natural products, oils and extracts used in cosmetics, fine fragrances, natural flavors and natural remedies.

The complete characterization of unknowns is an important requirement for global markets. Intense regular communications with the client helped us to understand their specific requirements and constraints. This strategy of Bio-Analytical Technologies™ (B.A.T.) evolved an appropriate methodology for unique identification and characterization of core components in their natural products using LCMS.

Solution:

Major steps towards the solution:

- Method development on LCMS-MS using C18 column.
- Separation and Identification of unknown major peaks.
- Qualitative resemblance analysis of area percentage of unknown major peaks to evaluate percentage of purity present in the compound.
- A thorough study for identification and elucidation of molecular structures of the major unknown peaks, using the data-base available in-house and in the public domain.

The Team:

B.A.T. team comprises of people with Multidisciplinary skills. The current project required experts from different streams. The Skilled Analysts and Domain Experts from Biotechnology Group worked rigorously in the process of method development leading to the required solution.

The client valued and appreciated our efforts, commitment, competence and vast pool of experience and expertise that our organization brings on the table.

Methodology:

An imperative aspect of this project was the assessment of Qualitative characteristics of the natural preparations. The entire process from requirement analysis to the final delivery was handled cautiously and efficiently. This process comprised of five important stages and which followed meticulous work pattern for the team.

The deliverables were targeted with following steps:

Stage 1- Prerequisites:

- Instrumentation- LC, LC column/s, MS, Scan type
- Appropriate Method of Analysis and Validation of Method
- Skilled domain group for interpretation of results.



- Shimadzu - HPLC
- C18 Column
- Analyst 1.4.2 s/w
- API - MS 4000 Q Trap
- Q1 scan of MS
- Analytical expertise

Stage 2- Method Designing:

- This important stage involved the Designing of analytical method.
- Initially the analysis of natural products was carried out using syringe pump.
- Subsequently, scan type and other parameters were decided and implemented in the method using LC.

Stage 3- Interpretation of Results:

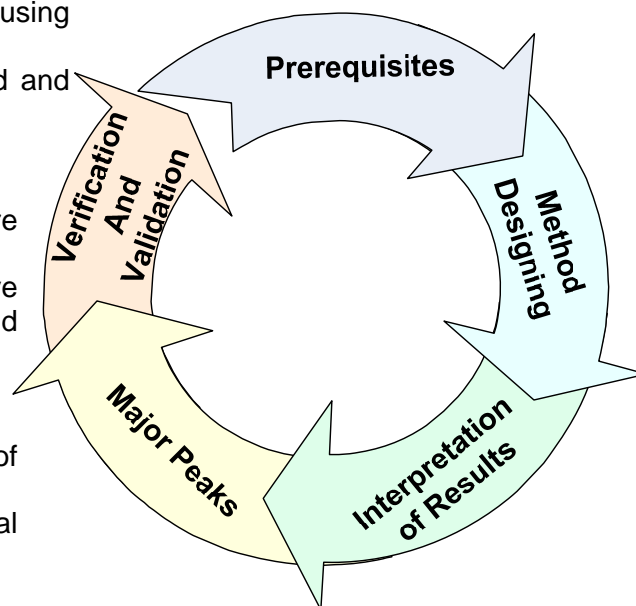
- Major peaks of known key components of chromatograms were qualitatively assessed.
- Area percentage ratio was calculated and its qualitative resemblance with standard components was compared and analyzed.

Stage 4- Major Peaks

- Natural preparations are well known for their wide range of peaks, known as well as unknown.
- Major unknown peaks were assessed for their names, chemical structure using web search.

Stage 5- Verification and Validation:

- Method and result validation and verification was done with Standard-Sample comparison technique.

**Benefits:**

- B.A.T. explored and attained another milestone to perform exceptionally well in the Naturals segment having global market with main focus on Identification and Characterization for their known-unknown key components.
- This project has added immense value to the client's product development portfolio and the analytical data presented by the team with multiple skills was proved to be highly profitable to the customer.
- Our third win approach was accomplished as the natural extractions yielded an increase in purity by almost 15% to 25% for the key components.
- The ability to deploy qualified resources and complete the project in the stipulated time was extremely crucial to the client's product launch.