Work Plan (Time scheduled of the work)

Total expected duration of work is approx. 2 year. Back up of work detail are given bellow.

First six month: -

Second six month: -
Development and optimise antioxidants sample preparations for LC/UV, GC/MS, LC/MS in ready to eat food.

Third six month: -
Development and optimise sample preparations of CAP for LCMS/MS in three different food products 1) raw and processed honey 2) milk and milk product 3) Meat and meat product

Four six month: - Developed methods were validated on following ground.

- Specificity & selectivity
- Linearity and range
- Limit of Detection/Limit of Quantitation
- Recovery (Accuracy)
- Repeatability (Precision)
- Reproducibility
- Ruggedness
Methodology

Before starting new measurements, it is important to investigate whether there is any other such kind of study or development activities going on in the region. It may be global programmes, regional programmes, or national monitoring activities. Another source of information is the reports from the recent international journal.

The process to determine the concentrations of the Analyte in a sample include a series of events as outlined in Figure 4.1.

![Figure 4.1](image)

**Figure 4.1**: Different stages of the process to determine analyte concentrations.

Some of the major operations in the process will be briefly discussed in this chapter.

A. **Sampling**

The aim of any sampling activity is to obtain a sample that can serve the objective of the study. In this activity it is considered indispensable to ensure the representativeness and integrity of the sample during the entire sampling process. The analyte, matrix,
sampling site, time or frequency, and conditions should be determined depending on
the objective of the sampling. Although it may be too expensive to get full accreditation
for sampling, Quality Assurance and Quality Control (QA/QC) procedures for sampling
should be put in place.

B. Extraction and clean-up

Sample preparation prior to chromatographic separation there are three major
objectives
1. The dissolution of analyte in suitable solvent
2. Removal of interfering compound as possible
3. Preconcentration of the analyte

The appropriately prepared sample can be extracted by any one of a number of
techniques.

I. Liquid-Liquid Extraction

Liquid-liquid extraction is useful for separating analytes from interferences by
partitioning the sample between two immiscible liquids or phases. One phase in LLE
often is aqueous and second phase an organic solvent and it is follow like dissolve like
rule. The technique is simple, rapid and has relatively small cost factor per sample when
compared to others.

II. Solid phase extraction:

Solid phase extraction is the most important technique used in sample pretreatment for
HPLC. SPE occur between a solid phase and a liquid phase. It is easier to obtain a higher
recovery of analyte. The sorbent is C18-silica, primary secondary amine (PSA), florisil,
alumina etc., and SPE assembles both LLE and reversed phase HPLC in its separation
characteristics.

III. Precipitation method

Protein precipitation is the simple method of extraction as compared to the LLE and
SPE. This can be carried out by using the suitable organic solvents which has good
solubility of the analyte and protein precipitating properties. Acetonitrile is the first
choice of solvent for protein precipitation due to its complete precipitation of proteins
and methanol is the second choice of organic precipitant provided the solubility of the
analyte in these solvents.
C. **Analysis**

Today the separation has been improved by the use of capillary columns and the selectivity by the use of mass spectrometric detectors (MS). Based on the availability of commonly used instruments for the determination of Antioxidants/Drugs (Chloramphenicol), different types of instrumental analysis can be proposed.

i. **General conditions to initiate HPLC method development**

Method development starts with literature survey of the molecule in which we find the nature of the molecule its pKa, solubility, molecular weight etc. Either isocratic or gradient mode may be used to determine the initial conditions of the separation. Optimization can be started only after a reasonable chromatogram has been obtained. A reasonable chromatogram means that near symmetrical peaks and a good separation.

Basic Parameters for chromatographic optimization are given bellow.

1) Selection of Mobile phase
2) Role of pH
3) Role of Buffer
4) Selection of Column:
5) Role of temperature
6) Role of Flow rate
7) Selection of Internal Standard

It is anticipated that improved analytical methods will be developed over the life of the study, and the project should be structured so that these improved techniques can be adopted. There is a need to improve the accuracy and lower the costs of these analyses. Emerging procedures with low environmental impact may become more widely available and accepted. It will be necessary to consider comparability as new methods are developed. This could be achieved by analysis of archived samples and direct comparison of new and old methods.

ii. **General conditions to initiate mass spectrometric method development**

Mass spectrometry is based on slightly different principles to the other spectroscopic methods. Basic parameter need to optimizes are as follow

**Ionization**

1. Electro Spray Ionization (ESI)
2. Atmospheric Pressure Chemical Ionization (APCI)
Ion preparation

1) MS Operating Modes
2) MS/MS Operating Modes
   a. The Daughter (Product) Ion Spectrum
   b. The Parent (Precursor) Ion Spectrum
   c. MRM: Multiple Reaction Monitoring

Table 4.2 Requirements for the instrumental analysis of Antioxidants/Drugs (Chloramphenicol).

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Infrastructure needs</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic sample extraction and clean-up</td>
<td>Nitrogen/air conditioning/ power/personnel specifically trained to operate and troubleshoot equipment problems</td>
<td>Antioxidant Std, Sodium Sulphate, Sodium chloride etc.</td>
</tr>
<tr>
<td>equipment, capillary GC/ECD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic sample extraction and clean-up</td>
<td>Organic Solvent/air conditioning/ power/personnel specifically trained to operate and troubleshoot equipment problems</td>
<td>Antioxidant Std, Sodium Sulphate, Sodium chloride etc.</td>
</tr>
<tr>
<td>equipment, LC/UV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample extraction and clean-up equipment</td>
<td>Helium/air conditioning/ consistent power/ personnel specifically trained to operate and trouble-shoot equipment problems</td>
<td>Antioxidant Std, Sodium Sulphate, Sodium chloride etc.</td>
</tr>
<tr>
<td>capillary GC/MS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample extraction and clean-up equipment</td>
<td>Nitrogen /air conditioning/ organic solvent (methanol, Acetonitril) consistent power/high operational costs /personnel specifically trained to operate and troubleshoot complicated instrumentation</td>
<td>Antioxidant Std, Chloroamphenicol, Sodium Sulphate, Sodium chloride etc.</td>
</tr>
<tr>
<td>capillary GC/MS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample extraction and clean-up equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC/MS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Data treatment

There are a number of parameters that have to be reported together with the analytical results. These include the efficiency of the extraction and clean-up, and the blank values,
but the results should not be compensated for these parameters. The uncertainty of the results should also be at least estimated, but preferably determined, using results from inter- or intralaboratory comparisons.

E. **Organization of quality control**

The organization of the quality control needs special attention in the study. Many of the recommendations mentioned above deal with QA/QC measures, but to be able to compare results from different labs and regions (and also for the same laboratory over time) there is a need for overarching activities.

**Main parameter of method validation consideration**

<table>
<thead>
<tr>
<th>Molecular Structure</th>
<th>Synthetic Route</th>
<th>Sample Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>· PKa</td>
<td>· (Especially last 2 steps)</td>
<td>· API</td>
</tr>
<tr>
<td>· Functional Groups</td>
<td>· Process Intermediates</td>
<td>· Stressed</td>
</tr>
<tr>
<td>· Hydrophobicity</td>
<td>· Reaction By-Products</td>
<td>· Precursors</td>
</tr>
<tr>
<td>· Chirality</td>
<td>· Reagents, Catalysts, etc</td>
<td>· Isomers</td>
</tr>
<tr>
<td>· Counterion</td>
<td>· Isomers</td>
<td>· Rxn By-Products</td>
</tr>
</tbody>
</table>

**Method Development: - Some Factors to Consider**

<table>
<thead>
<tr>
<th>Methods Screen</th>
<th>Secondary Purity Evaluation Technique</th>
<th>Detection Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>· RPLC Starting Point</td>
<td>· TLC, Normal Phase HPLC, CE, GC ...</td>
<td>· UV, EC, Conductivity, RI, LS, MS, Fluorescence,</td>
</tr>
<tr>
<td>· Purity Assessment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>