13. SUMMARY AND CONCLUSION

I. Isolation of seven biomarkers was successfully carried out. The identification of the isolated compounds was confirmed by TLC, HPTLC fingerprints, HPLC profile and spectral studies by comparing with the respective standards. The markers were isolated from different medicinal plants mentioned as below:

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Marker compound</th>
<th>Botanical source</th>
<th>Part used</th>
<th>Technique of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Withaferin A</td>
<td><em>Withania Somnifera</em></td>
<td>Roots</td>
<td>Column chromatography</td>
</tr>
<tr>
<td>2.</td>
<td>Beta-sitosterol-D-glucoside</td>
<td><em>Momordica charantia</em></td>
<td>Fruits</td>
<td>Column chromatography</td>
</tr>
<tr>
<td>3.</td>
<td>Bacoside A</td>
<td><em>Bacopa Monnieri</em></td>
<td>Whole plant</td>
<td>Preparative Thin layer chromatography</td>
</tr>
<tr>
<td>4.</td>
<td>Conessine</td>
<td><em>Holarrhena Antidisenterica</em></td>
<td>Stem bark</td>
<td>Column chromatography</td>
</tr>
<tr>
<td>5.</td>
<td>Kaempferol</td>
<td><em>Phyllanthus niruri</em></td>
<td>Whole plant</td>
<td>Column chromatography</td>
</tr>
<tr>
<td>6.</td>
<td>Rutin</td>
<td><em>Phyllanthus niruri</em></td>
<td>Whole plant</td>
<td>Column chromatography</td>
</tr>
<tr>
<td>7.</td>
<td>Quercetin</td>
<td><em>Phyllanthus niruri</em></td>
<td>Whole plant</td>
<td>Column chromatography</td>
</tr>
</tbody>
</table>

II. HPLC/HPTLC methods were developed and validated as per ICH guidelines using the isolated biomarker compounds. Simple, reproducible, rapid methods were developed and validated so that herbal manufacturers can readily use for standardization of their plant-based products. These methods can be used for standardization of plant-based products such as crude plant material, extracts, finished products etc. The method can be employed for routine in-process quality control and assessment of quality of finished products. The developed HPTLC and TLC fingerprints can also be used for chemo profiling for seasonal variation of the raw materials.
Following methods were developed and validated:

1. HPLC method for Withaferin A
2. HPTLC method for Beta-sitosterol-D-glucoside
3. HPTLC method for Bacoside A
4. HPTLC method for Conessine
5. HPTLC method for simultaneous estimation of Withaferin A and Beta-sitosterol-D-glucoside
6. HPTLC method for simultaneous estimation of Kaempferol, Rutin and Quercetin

III. Marker based standardization of different commercial formulations were carried out using the validated methods. The formulations were of different types such as Modern dosage form (Tablets, Capsules), Traditional medicine such as Ayurvedic (Vati, Churna, Ghutika, Arishta) and homeopathic (Tincture).

Following plant based medicines were successfully standardized:

1. Seven formulations containing Ashwgandha
2. Two extracts of Karela
3. Seven formulations containing Brahmi
4. Three products containing Kutja
5. Two formulations containing Aprajita

IV. Some plant-based products contain medicinal plants which exhibit biological activities due to presence more than one biomarker. Similarly some plant-based formulations contain more than one medicinal plant or extracts
Standardization of Some Plant-Based Formulations by Modern Analytical Techniques

(Polyherbal). For analysis of such herbal products a single analytical method which can detect and quantify simultaneously more than one marker compounds becomes more accurate. The standardization of herbal products by such methods is more convenient, less time consuming and thus highly acceptable for routine analysis in industries. The same method can also be employed for estimation of content of single marker compound in respective formulations.

Following are the two methods for simultaneous estimation of more than one marker compounds

1. Simultaneous estimation of Withaferin A and Beta-sitosterol-D-glucoside by HPTLC method was successfully employed for four different formulations containing Ashwgandha

2. Simultaneous estimation of Kaempferol, Rutin and Quercetin by HPTLC method was successfully employed for three herbal products of Bhuiamla and Amla.

V. Applicability of the developed and validated methods was extended to analyse forced degradation studies of three marker compounds. The stability indicating assays are important to determine the shelf life of the products. It also helps to determine the storage conditions by knowing the process of degradation. It is carried out by performing forced degradation studies.

The stability indicating studies were carried out for following biomarkers:

1. Rutin
2. Quercetin
3. Conessine

The developed and validated methods were found to be stability indicating for
rutin, quercetin and conessine.

VI. It is very important to develop an analytical method for pharmacokinetic studies for plant based medicine. There are very few analytical method developed, validated and applied for pharmacokinetics studies of constituent/s of plant origin.

An attempt was made to apply the developed and validated HPTLC method for conessine for pharmacokinetics studies of Kutjavati and Kutjarishta.

These types of studies using sophisticated techniques are important for global acceptance and to bring the herbal medicines at par to allopathic medicines.