

### **3.1. Rationale**

Few HPLC methods are available for the analysis of active pharmaceutical ingredients (API) of herbal origin namely hypericin and khellin but these methods required lengthy run times and complicated gradient elution systems using solvent mixtures. Further other methods like, UV-Vis and fluorescence spectroscopic methods are insufficient to determine an accurate amount of hypericin and khellin due to interference from other constituents in the extracts. Similarly, the analysis of these API using non-aqueous capillary electrophoresis, cyclic voltametry also show low resolution owing to poor reproducibility. Keeping these things in mind, novel HPLC methods were developed and validated for the quantification of these API of herbal origin. Many HPTLC methods were used earlier for the estimation of herbal API's namely strychnine, brucine, vincristine, vinblastine, podophyllotoxin, etoposide but these methods are tedious, lengthy and less sensitive and provides very less amount of these API's. Therefore in the present investigation, novel HPTLC methods were developed and validated for the quantification of these API of herbal origin.

### **3.2. Objectives**

The main objectives of the study are as follows:

- Development of sensitive, specific, reproducible, very economic and laboratory friendly HPTLC methods for the quantification of strychnine and brucine, vincristine and vinblastine, podophyllotoxin and etoposide.
- Development of sensitive, specific, reproducible, very economic and laboratory friendly HPLC methods for the quantification of khellin and hypericin.
- Validation of the above mentioned HPTLC and HPLC methods as per ICH guidelines.