A simple, sensitive and specific RP-HPLC method has been developed for the quantification of hypericin in *Hypericum perforatum*. The method involved evaluation of hypericin after resolving it by RP-HPLC with UV detector at 590 nm using acetonitrile: methanol: 10 mM ammonium acetate (pH 5.0) in the ratio 54: 36: 10 (v/v/v) as mobile phase. The method was validated as per the ICH guidelines for linearity, precision (inter-day, intra-day and inter-system), robustness, accuracy, LOD and LOQ. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 10-80 µg mL^{-1} for hypericin. The % RSD value for method precision was found to be 0.36-1.35% for hypericin. Accuracy of the method was checked by recovery studies conducted at three different levels and the average percentage recovery was found to be 100.13%. The proposed method for the quantification of hypericin was found to be simple, precise, specific, sensitive and accurate and the method can be used for the routine analysis of hypericin for quality control of raw material of *H. perforatum,* several Unani and Ayurvedic formulations containing it as an ingredient.

Developed RP-HPLC method for the quantification of khellin was simple, selective and specific. The marker compound was quantified in the seeds of *Ammi visnaga*. The method involved evaluation of khellin after resolving it by RP-HPLC with UV detection at 247 nm using methanol: water (75: 25, v/v) as the mobile phase. The method was validated for precision (inter-day, intra-day, inter-system), robustness, accuracy, LOD and LOQ. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 10 µg mL^{-1} to 80 µg $mL^{-1}$for khellin. The % RSD value for method precision was found to be 0.63-1.97% for khellin. Accuracy of the method was checked by recovery studies conducted at three different levels and the average percentage recovery was found to be 100.53%

for khellin. The RP-HPLC method for the quantification of khellin was found to be simple, precise, specific, sensitive, accurate and the proposed method can be used for routine analysis and quality control of raw material of *Ammi visnaga* and several Unani and Ayurvedic formulations containing it as an ingredient.

A thin layer chromatography densitometric method has been developed for the simultaneous quantification of vincristine and vinblastine in the leaves of *Catharanthus roseus*. The method involved simultaneous estimation of vincristine and vinblastine after resolving it by High Performance TLC on silica gel plate with toluene-methanol-diethylamine (8.75: 0.75: 0.5, v/v/v) as the mobile phase. The method was validated as per the ICH guidelines for precision (inter-day, intra-day, inter-system), robustness, accuracy, LOD and LOQ. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 100 ng $spot^{-1}$ to 4000 ng $spot^{-1}$for vincristine and 200 ng $spot^{-1}$ to 4000 ng $spot^{-1}$ for vinblastine. The method precision was found to be 0.77-1.78 (%RSD) and 1.24-2.13 (% RSD) for vincristine and vinblastine, respectively. Accuracy of the method was checked by recovery study conducted at three different levels and the average percentage recovery was found to be 100.21 % for vincristine and 99.99 % for vinblastine, respectively. The HPTLC method for the simultaneous quantification of vincristine and vinblastine was found to be simple, precise, specific, sensitive and accurate and can be used for routine analysis and quality control of raw material of *C. roseus.* and several Unani and Ayurvedic formulations containing as an ingredient.

A very simple, precise, sensitive and specific thin layer chromatography densitometric method has been developed for the quantification of Podophyllotoxin and Etoposide. Podophyllotoxin was quantified in the roots of *Podophyllum hexandrum* whereas Etoposide was quantified in a marketed formulation. The method involved densitometric evaluation of both Podophyllotoxin

and Etoposide after resolving it by High Performance TLC on silica gel plate with dichloromethane-methanol-formic acid (9.5: 0.5: 0.5, v/v/v) as the mobile phase. The method was validated for precision (inter-day, intra-day, inter-system), robustness, accuracy, LOD and LOQ. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 150 ng $spot^{-1}$ to 2400 ng $spot^{-1}$ for Podophyllotoxin and 200 ng/spot to 2000 ng/spot for Etoposide. Instrumental precision was found to be 1.03-1.80 (%RSD) and 0.79-1.99 (%RSD) for Podophyllotoxin and Etoposide, respectively. Accuracy of the method was checked by recovery studies conducted at three different levels and the average percentage recovery was found to be 100.6 % for Podophyllotoxin and 100.4 % for Etoposide, respectively. The HPTLC method for the quantification of podophyllotoxin and etoposide was found to be simple, precise, specific, sensitive and accurate and it can be used for routine quality control of raw material of *Podophyllum hexandrum* and several Unani and Ayurvedic formulations containing as an ingredient.

The strychnine and brucine were quantified by a laboratory friendly, cheap, easy method in the seeds of *Strychnos nux-vomica* using HPTLC. The method involved simultaneous estimation of strychnine and brucine after resolving it by HPTLC on silica gel plate with chloroform-methanol-formic acid (8.5: 1.5: 0.4 v/v/v) as the mobile phase. The method was validated as per the ICH guidelines for precision (inter-day, intra-day, inter-system), robustness, accuracy, LOD and LOQ. The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 50-1000 ng $spot^{-1}$ for strychnine and 100 - 1000 ng $spot^{-1}$ for brucine. The method precision was found to be 0.58-2.47 (% RSD) and 0.36-2.22 (% RSD) for strychnine and brucine, respectively. Accuracy of the method was checked by recovery studies conducted at three different concentration levels and the average percentage

recovery was found to be 100.75% for strychnine and 100.52% for brucine, respectively. The HPTLC method for the simultaneous quantification of strychnine and brucine was found to be simple, precise, specific, sensitive and accurate and can be used for routine analysis and quality control of raw material of *S. nux-vomica* and several Unani and Ayurvedic formulations containing as an ingredient.

HPLC methods were developed and validated as per ICH guidelines for the determination of hypericin and khellin. These method were found to be simple, rapid, accurate, specific and robust for the analysis of hypericin and khellin in crude drugs and can be adopted by any laboratory for the quality control of crude drugs and formulations that contains hypericin and khellin as an active markers.

HPTLC methods were developed and validated as per ICH guidelines for the determination of vincristine and vinblastine (simultaneously), podophyllotoxin (individually) and etoposide (individually), strychnine and brucine (simultaneously). These methods were found to be very simple, rapid, accurate, specific and robust for the analysis of vincristine and vinblastine , podophyllotoxin and etoposide, strychnine and brucine in crude drugs and can be used for the quality control of crude drugs and formulations that contains vincristine and vinblastine, podophyllotoxin and etoposide, strychnine and brucine as active markers, in several herbal drug industries even in those also who do not have any sophisticated equipments but simple TLC. Hence the proposed validated and applied analytical methods are important from point of view that these are economic, simple, less time consuming, robust, precise, accurate with good range of linearity and using very simple extraction procedures and can be applied in the Indian Herbal Pharmaceutical Companies, which do not have sophisticated equipments.