6. SUMMARY

Plants under study; daruhaldar, haldar, kali mori, vavding, ashwagandha, kaucha beej, gokharu and jethimadh were purchased from local market of Ahmedabad and were authenticated by the botanist, Dr. Mukesh Prajapati, H.N.S.B. Science College, Himmatnagar. Organoleptic character of all the plants were compared with the characters prescribed in the Ayurvedic Pharmacopeia of India and Indian herbal Pharmacopeia. Voucher specimens of the plants parts were deposited in Sunrise Remedies Pvt. Ltd.

Physicochemical parameters like extractive values, ash values, moisture content, pH and microbial contamination showed that commercial powders and extracts had lower water soluble and alcohol soluble extractives than the laboratory prepared. Total ash values were found higher in all the powders prepared in laboratory except P. nigrum and M. pruriens. Total ash values were found higher in commercial extract of P. nigrum, M. pruriens and W. somnifera. The powder and extracts prepared in laboratory from the plant parts had shown lower acid insoluble ash values than the purchased from local supplier. The powder and extracts prepared in laboratory had shown lower moisture content than the commercial powder and extracts. The pH value of all powders and extracts were 3.75 to 7.14 indicating all powders and extracts were palatable for oral administration. Commercial and laboratory prepared powders and extracts showed bacterial and fungal count less than prescribed limit 300CFU and 100CFU respectively.

Preliminary phytochemical screening showed that alkaloids were present in all the drugs except T. terristris. Glycosides were present in C. longa, G. glabra and W. somnifera whereas absent in B. aristata, P. nigrum, E. ribes, T. terristris and M. pruriens. Flavonoids were present in C. longa, G. glabra and W. somnifera whereas absent in B. aristata, P. nigrum, E. ribes, T. terristris and M. pruriens. B. aristata, C. longa, P. nigrum, E. ribes, G. glabra and W. somnifera showed presence of tannins while tannins were absent in T. terristris and M. pruriens. Triterpenoids and steroids were absent in B. aristata, P. nigrum and E. ribes whereas present in C. longa, T. terristris, M. pruriens, G. glabra and W. somnifera. Phenolic compounds were present in B. aristata, C. longa, P. nigrum, E. ribes and W. somnifera whereas absent in T. terristris, M. pruriens and G. glabra. Carbohydrates were present in B. aristata,
C. longa, P. nigrum, E. ribes and M. pruriens while absent in T. terristris, G. glabra and W. somnifera. Amino acids were present in C. longa, P. nigrum, E. ribes and M. pruriens whereas absent in B. aristata, T. terristris, G. glabra and W. somnifera. Fixed oils and fats were absent in all the drugs except fruit drug P. nigrum and E. ribes.

TLC study of the laboratory prepared and commercial powders and extracts showed spots at same Rf of all reference standards and were consistent with standard literature.

Wavelength for individual drugs carried out by scanning the drug solution in the UV range 200-400nm were berberine at 266nm, curcumin at 425nm, embelin at 292nm, glycyrrhetenic acid at 252nm, l-dopa at 280nm, diosgenin at 205nm, and withaferin A at 227nm and overlay spectra for simultaneous estimation of berberine and glycyrrhetenic acid at 230nm, berberine and curcumin at 425nm and embelin with piperine at 292nm.

The developed method for estimation of berberine at 266nm in the range 5-80µg/ml showed regression equation \( y=156.5x + 288.1 \) and correlation coefficient 0.999. LOD and LOQ were 1.31µg/ml and 4.39µg/ml with accuracy 98.96%. Intraday-interday precision was 0.82%, 0.80% respectively. Theoretical plates were 5748 (more than 2000) with asymmetry 1.10371 (≤2). Validation parameters were within the acceptance range from the prescribed standard indicating developed method was simple, linear, accurate, sensitive and precise and hence was suitable for routine analysis of berberine in bulk, herbs and herbal formulation.

The developed method for estimation of curcumin at 425nm in the range 2-12µg/ml showed regression equation \( y = 1103x + 319 \) with correlation coefficient 0.995. LOD and LOQ were 0.18µg/ml and 0.62µg/ml respectively with accuracy 97.59%. Intraday-interday precision was 0.80%, 0.84% respectively. Theoretical plates were 4814 (more than 2000) with asymmetry 1.00871 (≤2). Validation parameters were within the acceptance range from the prescribed standard indicating developed method was simple, linear, sensitive, precise and accurate and hence was suitable for routine analysis of curcumin in bulk, herbs and herbal formulation.

The developed method for estimation of embelin at 292nm gave regression equation \( y=1048x-703.6 \) and correlation coefficient 0.999 in the range 5-80µg/ml. LOD and
LOQ were 1.19 µg/ml and 3.97 µg/ml with accuracy 97.78%. Intraday-interday precision was 0.95%, 1.13% respectively. Retention time was 6.715 min (less than 10 min) with theoretical plates 15876 (more than 2000) and asymmetry 1.1077 (≤2). Validation parameters were within the acceptance range from the prescribed standard indicating developed method was simple, sensitive, precise and accurate, hence was suitable for routine analysis of embelin in herbs and herbal formulation.

The developed method for estimation of piperine at 343 nm in the range 4-32 µg/ml showed regression equation \( y = 1310x + 566.2 \) and correlation coefficient 0.999. LOD and LOQ were 0.43 µg/ml and 1.46 µg/ml with accuracy 95.36%. Intraday-interday precision was 1.01%, 1.23% respectively. Theoretical plates were 9547 (more than 2000) with asymmetry 1.0734 (≤2) indicating developed method was simple, sensitive, accurate and precise hence was suitable for routine analysis of piperine in herbs and herbal formulation.

The method for estimation of diosgenin developed at 205 nm in the range 10-80 µg/ml showed regression equation \( Y = 18018x - 32694 \) and correlation coefficient 0.998. LOD and LOQ were 1.637 µg/ml and 5.457 µg/ml with accuracy 95.82%. Intraday-interday precision was 1.18%, 1.07% respectively. Theoretical plates were 4183 (more than 2000) and asymmetry 1.15100 (≤2). All the validation parameters were within the acceptance range from the prescribed standard indicating method was simple, sensitive, accurate and precise; hence the method was appropriate for routine analysis of diosgenin.

The developed method for estimation of l-dopa at 280 nm in the range 10-80 µg/ml showed regression equation \( y = 318.3x + 1119 \) and correlation coefficient 0.997. LOD and LOQ were 1.80 µg/ml and 6.01 µg/ml with the accuracy 98.83%. Intraday precision was 0.77% and interday precision was 0.83%. Theoretical plates were 12989 (more than 2000) and asymmetry 1.41972 (≤2). Validation parameters were within the acceptance range indicating method was simple, sensitive, accurate and precise; hence the method was suitable for routine estimation of l-dopa.

The developed method for estimation of withaferin A at 227 nm in the range 5-30 µg/ml showed regression equation \( y = 53027x + 34065 \) and correlation coefficient 0.999. LOD and LOQ were 1.069 µg/ml and 3.56 µg/ml with accuracy 97.31%. Intraday-interday precision was 0.80%, 0.76% respectively. Theoretical plates were
3347 (more than 2000) with asymmetry 0.90151 (≤2). All the validation parameters were within the acceptance range indicating the developed method was simple, sensitive, accurate and precise; hence the developed method was proper for routine analysis of withaferin A.

The method for estimation of glycyrrhetic acid developed at 252nm in the range 20-120µg/ml had showed regression equation $y=447.3 \times + 2810$ and correlation coefficient 0.997. LOD and LOQ of developed method were 8.37µg/ml and 27.92µg/ml respectively with accuracy 95.99%. Intraday precision was 1.34% and interday was 1.21%. Theoretical plates were 3808 (more than 2000) with asymmetry 1.1132 (≤2). Validated parameters were within the acceptance range indicating the method was simple, sensitive, accurate and precise; hence the method was suitable for routine analysis of glycyrrhetic acid.

The developed method for simultaneous estimation of berberine and glycyrrhetic acid at 230nm in the range 10-60µg/ml showed regression equation $Y = 15254x + 53712$ for berberine and $Y = 2374x + 2085$ for glycyrrhetic acid with correlation coefficient 0.999. LOD and LOQ were 0.24µg/ml, 0.72µg/ml for berberine and 0.87µg/ml, 2.63µg/ml for glycyrrhetic acid. Accuracy was 95.92% for berberine and 95.98% for glycyrrhetic acid. Intraday-interday precision of berberine was 1.24%, 0.97% and glycyrrhetic acid was 1.08%, 1.12% Theoretical plates were 3760 and 6034 for berberine and glycyrrhetic acid (more than 2000) respectively. Asymmetry was 1.02 for berberine and 0.82 for glycyrrhetic acid (≤2). Resolution was 7.95min. The validation parameters were within the acceptance range from the prescribed standard indicating the developed method was simple, sensitive, accurate and precise for routine simultaneous analysis in herbs and herbal formulation.

The developed method for simultaneous estimation of berberine and curcumin at 425nm in the range 10-60µg/ml had showed regression equation $Y=13090x+8598$ for berberine and $Y=62854x+10400$ for curcumin with correlation coefficient 0.997. LOD and LOQ were 0.38µg/ml, 1.16µg/ml respectively for berberine and 0.61µg/ml, 2.03µg/ml respectively for curcumin. Accuracy was 99.08% for berberine and 96.96% for curcumin. Intraday-interday precision of berberine was 0.65%, 1.10% and curcumin was 0.98%, 0.92%. Theoretical plates were 2460 and 4680 for berberine and curcumin respectively (more than 2000). Asymmetry was 2.00
and 0.89280 for berberine and curcumin respectively (≤2). Resolution was 22.21382 min. The validation parameters were within the acceptance range indicating method was simple, sensitive, accurate and precise, hence was suitable for routine simultaneous analysis in herbs.

The developed method for simultaneous estimation of embelin and piperine at 292 nm in the range 10-60 µg/ml had shown regression equation \( y=11145x-15324 \) for embelin and \( y=13435x-1453 \) for piperine with correlation coefficient 0.997. LOD and LOQ were 0.31 µg/ml, 1.04 µg/ml respectively for embelin and 0.27 µg/ml, 0.92 µg/ml respectively for piperine. Accuracy was 95.23% for embelin and 96.44% for piperine. Intraday-interday precision of embelin was 0.84%, 1.01% respectively and for piperine was 1.36% and 0.84 respectively. Theoretical plates were 2353 for piperine and 2898 for embelin (more than 2000). Asymmetry was 1.32420 and 1.42123 for embelin and piperine respectively (≤2). Resolution was 4.24005 min. The validation parameters were within the acceptance range indicating the method was simple, sensitive, accurate and precise and hence was suitable for routine simultaneous analysis of embelin and piperine in herbs and herbal formulation.

All the developed method for analysis of individual biomarker and simultaneous analysis of biomarkers utilized cheaper solvents like water, methanol, acetonitrile, triethylamine, orthophosphoric acid and glacial acetic acid. Column used was C\(_{18}\) which is easily available and most commonly used. Separation in all mobile phase is achieved before 10 min except in mixture of berberine and curcumin, demonstrated developed methods were economic and protective for column, ultimately most suitable for routine analysis of biomarkers individually and combination.