SUMMARY

Various novel analytical methods were developed for the quality control of drugs and formulations which included analytical methods combined with statistical techniques such as Quality by Design and chemometrics, stability indicating analytical methods, bioanalytical methods and analytical chemometric models to study quality of formulations. The summary of the entire work is as follows:

Method 1A: Development of RP-HPLC and chemometric assisted spectrophotometric methods for the simultaneous determination of five active ingredients in cough and cold tablets and their application to dissolution study

An isocratic RP-HPLC method was developed for the simultaneous determination of phenylephrine hydrochloride (PEP), paracetamol (PCM), guaifenesin (GNF), chlorpheniramine maleate (CPM) and bromhexine hydrochloride (BRM).

For optimisation of the method various trials were carried out with different ratios of mobile phase and at different pH. Finally an isocratic elution method was developed with the mobile phase comprising MeOH, ACN and 10 mM phosphate buffer (pH 3) in the ratio of 27.5:22.5:50 respectively. The detection wavelength was 218 nm.

The retention time was found to be 2.6, 3.3, 4.6, 6.0 and 12.8 min for PEP, PCM, GNF, CPM and BRM resp. The linearity was found in the concentration range of 5-20 µg/mL for PEP, 225-900 µg/mL for PCM, 50-200 µg/mL for GNF, 1-4 µg/mL for CPM and 4-16 µg/mL for BRM. The percentage recoveries were found to be 97.57-99.65% for PEP, 99.6-100.99% for PCM, 99.16-101.32% for GNF, 99.91-101.39% for CPM and 98.74-99.18% for BRM. The method was found to be accurate, specific and less time consuming due to short run time for the five components.

The method was applied to study the dissolution of the five components in tablet formulation. The dissolution media was 0.01 N HCl. The percentage release of all the five components was found to be greater than 85% within 60 minutes.

Method 1B: Development of chemometric assisted spectrophotometric methods for the simultaneous determination of five active ingredients in cough and cold tablets and their application to dissolution study

Two chemometric methods i.e. principal component regression (PCR) method and partial least squares (PLS) method were developed to quantify the five components i.e. PEP, PCM, GNF, CPM and BRM.

The mixture solutions of all the five components were prepared in varied concentration and ratios and two sets: training and test sets were prepared. The UV absorption spectra were analysed in the range of 200-400 nm.

The data matrix for concentration of components and absorbance (at various wavelengths) was constructed. The wavelength range of 210-320 nm was used to construct the PLS and PCR models. 51 wavelengths ranging from 210-320 nm at an interval of 2 nm were selected and the models were successfully developed. The percentage recoveries found by PLS method were 99.65-100.37% for PEP, 99.78-100.37% for PCM, 99.65-100.11% for GNF, 99.77-100.42% for CPM and 99.83-100.37% for BRM. The percentage recoveries found by PLS method were 99.62-99.97% for PEP, 99.85-100.17% for PCM, 99.44-100.38% for GNF, 99.81-100.83% for CPM and 100.08-100.44% for BRM.

The methods were applied to study the dissolution of the five components in tablet formulation and the percentage release of all the five components was found to be greater than 85% within 60 minutes by all both the chemometric methods.

The statistical comparison of RP-HPLC method developed in Method 1A and the PLS and PCR methods developed in Method 1B was performed by the ANOVA test. The three methods were not found to be significantly different from each other.

The chemometric assisted spectrophotometric methods (PLS and PCR) and RP-HPLC method have been proposed and successfully applied for the simultaneous determination of PEP, PCM, GNF, CPM, and BRM in their commercial tablet formulation. The assay and dissolution results obtained by chemometric methods were found to be in a good coincidence with that of RP-HPLC method. The methods thus developed were found to be suitable and can be successfully used for the determination of the PEP, PCM, GNF, CPM and BRM in pharmaceutical tablet formulation as well as for dissolution study.

Method 2: QbD approach for development and optimisation of RP-HPLC method for the simultaneous estimation of four component cream formulation: Application to permeability study

The concept of Design of Experiments (DoE) approach was explored to study the effect of various factors influencing the optimisation of RP-HPLC method for the simultaneous estimation of the four drugs *viz.* Ofloxacin (OFX), Ornidazole (ORN), Terbinafine hydrochloride (TBH) and Clobetasol propionate (CBP) in bulk drug and in cream formulation.

A full factorial design was used to study the factors which significantly affected the chromatographic separation of the four drugs, such as pH of the buffer, initial percentage of organic content (%BI) and gradient time (Tg) and the DoE model was constructed. The optimal conditions obtained after applying the design with good system suitability parameters for all four drugs were found to be at pH 2.6, %BI as 24% of ACN and gradient time of 4 min.

The optimal conditions predicted by the DoE model were found to be in a good agreement with the experimental results. The retention time was found to be 4, 9.2, 15.8 and 19.8 min for OFX, ORN, TBH and CBP resp. The RP-HPLC method was validated using ICH guidelines and was applied for the assay of cream formulation. The linearity was found to be 150-750 µg/mL for OFX, 400-2000 µg/mL for ORN, 200-1000 µg/mL for TBH and 10-50 µg/mL for CBP. The percentage recoveries were found to be 98-99.84% for OFX, 98.56-98.84% for ORN, 98.56-100.13% for TBH and 98.22-100.82% for CBP.

The RP-HPLC method was successfully applied to study the *in vitro* permeability of cream formulation in rat skin using Franzch diffusion cell. The study was carried out upto 24 h and the receptor media used for the study was mixture of PBS (pH 7.4): ethanol 70:30. The cumulative percentage release for all drugs was found to be above 45 % within 7 h.

Thus the QbD approach was successfully applied for the optimisation and development of RP-HPLC method for the simultaneous estimation of the four drugs in cream formulation.

Method 3: Development of RP-HPLC method for the simultaneous estimation of Azelastine hydrochloride and Budesonide in bulk drug and laboratory mixture: QbD based optimisation

QbD based Design of Experiments (DoE) approach was used for the development of RPHPLC method for the simultaneous estimation of the Azelastine hydrochloride (AZ) and Budesonide (BD) in bulk drug and laboratory mixture.

Plackett-Burman design (PBD) was used for the preliminary screening of various factors. Various factors such as: type of buffer (phosphate or ammonium buffer), concentration of buffer, type of column (C8 or C18), pH of buffer, temperature, flow rate and organic concentration were studied and the PBD model was constructed using 12 runs.

The results of PBD gave three prominent factors *i.e.* pH, organic concentration and flow-rate, which affected the chromatographic separation. These three factors were further optimised by Box-Behnken design (BBD). The range of the factors used for BBD was: pH in the range of 3 to 5, organic concentration in the range of 40 to 60% and flow-rate in the range of 0.8 to 1.2 mL/min. 14 runs were designed and the BBD model was constructed. The optimal conditions obtained from the BBD results were pH 3, organic concentration as 60% v/v of ACN and flow rate as 1 mL/min. The optimal conditions were found to be in a good agreement with the experimental results.

The retention time was found to be 4.15 and 7.3 min for AZ and BD resp. The RP-HPLC method was validated using ICH guidelines and was successfully applied for the simultaneous estimation of AZ and BD in laboratory mixture. The linearity was found to be 4.28-342.4 µg/mL for AZ and 1-80 µg/mL for BD. The percentage recoveries were found to be 99.02-99.85% for AZ and 98.78-100.3% for BD.

The method thus developed was found to be LC-MS compatible and hence was further extended for the development of LC-MS bioanalytical method for the estimation of AZ and BD in human plasma.

Method 4: Simultaneous estimation of Azelastine hydrochloride and Budesonide in human plasma by LC-MS/MS method using solid phase extraction technique

As an extension of the RP-HPLC method developed in Method 3, the LC-MS/MS method was developed for the simultaneous estimation of Azelastine hydrochloride (AZ) and Budesonide (BD) in human plasma. The same RP-HPLC method developed in Method 3 was used and the mass parameters were optimised.

The separation was performed via isocratic elution of AZ and BD using 10 mM ammonium acetate buffer (pH 3): ACN (40:60, v/v) as mobile phase at a flow rate of 1.0 mL/min. The extraction of AZ and BD from human plasma was carried out by solid phase extraction using a mixture of MeOH, ACN and ammonium acetate buffer pH 3 in the ratio 40:30:30. After trying various internal standards, Diclofenac (DC) was used as an internal standard with respect to the resolution and elution.

The electron spray ionisation (ESI) mass spectrometric detection was done in positive ion selected multiple reaction mode (MRM) target for [M^+] ions at 382 m/z, 431 m/z and 295 m/z for AZ, BD and DC respectively. A complete analytical run was achieved within 12 min. The method was validated and found to be linear at a concentration range of 21.4-428 ng/mL of AZ and 5-100 ng/mL of BD. The limit of quantification was 21.4 ng/mL and 5 ng/mL for AZ and BD respectively. Intra and inter batch accuracy and precision were within the acceptable limits. The percentage accuracy and precision (±%CV) for within batch were in the range of 98.27-99.85% (0.37-1.99 %CV) for AZ and 98.06-101.57% (0.43-3.68 %CV) for BD and for between batch it was in the range of 98.17-100.66% (0.5-2.5 %CV) for AZ and 97.53-100.96% (0.59-2.4 %CV) for BD. Analytes were stable in a battery of stability studies.

The LC-MS/MS method was found to be fast, simple, selective, sensitive, accurate and reproducible and was sensitive upto nanogram level.

Method 5: Development of stability indicating analytical method for simultaneous estimation of Azelastine hydrochloride and Budesonide in bulk drug and laboratory mixture: Characterisation of major degradation products by LC-MS/MS technique

A specific stability indicating RP-HPLC method for Azelastine Hydrochloride (AZ) and Budesonide (BD) in synthetic laboratory formulation was developed. The degradation of AZ and BD was carried out in various degradation conditions such as acid hydrolysis, base hydrolysis, neutral hydrolysis, peroxide degradation, thermal and photolytic stress.

The degradation was first studied for individual drugs and thereafter the degradation conditions were optimised for the simultaneous study of both. The optimised conditions were: 1M HCl, 30 min, 80 ºC for acid hydrolysis; 1MNaOH, 1h, 80 ºC for alkaline hydrolysis; 6% H_2O_2, 30 min, 80 ºC for oxidative degradation; 5382 LUX and 144UW/cm^2, 21 days for photolytic degradation; 3 h, 80 ºC for neutral hydrolysis and 80 ºC, 14 days for dry heat degradation. Six major degradation products (DPs) were formed wherein one for acid hydrolysis of BD (DP1) Rt=5.6, one for base hydrolysis of BD (DP2) Rt=7.4, two for peroxide degradation of AZ (DP3, DP4) Rt=11.4, 12.3, one for peroxide degradation of BD (DP5) Rt=13.6 and one for neutral hydrolysis of BD (DP6) Rt=7.5.

A specific SIAM was thus developed with six DPs and two APIs. Chromatographic separation was achieved on Oyster C8 column (250 mm length x 4.6 mm i.d., 5µm particle size) with gradient elution using 10 mM ammonium formate buffer (pH 3) as solvent A and ACN as solvent B, at a flow rate of 0.8 mL/min and ambient temperature. AZ and two epimers of BD were eluted at retention time of about 10.5, 14.3 and 14.5 min respectively. The method was specific as both the drugs could be estimated without the interference of degradation impurities.

The DPs were further analysed by LC-MS/MS technique and their structures as well as degradation pathways were proposed on the basis of the mass and fragmentation pattern.

Method 6: Development of stability indicating analytical method for Silodosin in bulk drug and formulation: Degradation kinetics and characterisation of major degradation products by LC-MS/MS technique

A simple, accurate, precise and sensitive stability indicating analytical method was developed for the determination of Silodosin (SDN) in bulk and formulation. The degradation of AZ and BD was carried out in various degradation conditions such as acid hydrolysis, base hydrolysis, neutral hydrolysis, peroxide degradation, thermal and photolytic stress. . The degradation conditions were: 1M HCl, 6 h, 80 ºC for acid hydrolysis; 1M NaOH, 6 h, 80 ºC for alkaline hydrolysis; 3% H_2O_2, 6 h, 80 ºC for oxidative degradation; 5382 LUX and 144UW/cm^2, 21 days for photolytic degradation; 6 h, 80 ºC for neutral hydrolysis and 9 h, 80 ºC, for dry heat degradation. Three major degradation products (DPs) were formed wherein one for acid hydrolysis of SDN (DP1) Rt=3.4 and two for peroxide degradation of SDN (DP2, DP3) Rt=4.1, 5.6.

A specific SIAM was thus developed with three DPs and SDN. Chromatographic separation was achieved on Thermo C18 (150 mm x 4.6 mm x 5 µ) column, using ACN and 10 mM phosphate buffer (pH 6.5) in the ratio of 65:35. The detector was set at 220 nm and the flow rate was kept at 1.0 mL/min.

Acid and peroxide stability of Silodosin, was evaluated specifically. The acid and peroxide degradation products were characterised by using LC-MS/MS technique and the structures of degradation products were proposed using m/z ratio of DPs and their fragmentation pattern.

The degradation kinetics of these two degradations i.e. acid hydrolysis and peroxide degradation was studied which showed that both the degradations followed first order kinetics and the activation energy was obtained as 12.44 and 16.09 kJ/mole resp.

Thus a specific SIAM was developed for SDN and structures and degradation pathways for the degradation products were proposed as well as the kinetics of the two major degradations were specifically studied.

Method 7A: Development of chemometric models to study the quality of amoxicillin in commercial formulations by using NIR, Raman and FTIR techniques

An effort to study the quality of different commercial brands of AMX was carried out. Various brands of Amoxicillin trihydrate tablet and capsules were analysed with three analytical techniques i.e. FTIR, NIR and Raman spectroscopy. Total of 15 commercial capsules and 19 commercial tablets were taken for analysis. 10 placebo samples, 10 counterfeit samples and 1 standard tablet were prepared in the laboratory.

A matrix of 55×1763 for IR data, 55×100 for NIR data, 55×2301 for Raman data was constructed and the Principle component analysis (PCA) models and hierarchical cluster analysis models (HCA) were prepared and cluster analysis was performed.

The model built up using PCA could resolve and separate the various marketed drugs from the lab prepared counterfeit and placebo ones. The PCA showed three major groups viz. one group of marketed formulation including laboratory prepared tablet, second group of counterfeit samples and the third group of placebo samples.

The best results were obtained by NIR technique. In zero order NIR PCA model cluster of formulation samples was found to be closer to clusters of counterfeits, hence this proved the benefit of using SGolay derivatisation. The PCA model and the HCA dendrogram, for first derivative of NIR spectra proved to be the best as the most clear distinction was possible by PCA as well as for cluster analysis.

However FTIR and Raman also proved to be promising for the analysis. The NIR model could clearly differentiate the three groups for formulation, counterfeit and placebo. The Raman and IR-PCA models were found to be somewhat similar in context of the gradient observed in the clusters. The PCA obtained with zero order Raman-PCA was similar to that obtained by SGolay first derivative IR-PCA model. Raman-HCA dendrogram was better than IR-HCA as quite clear classification and correlation could be observed. Both models Raman-PCA and IR-PCA, although, could discriminate the samples.

Method 7B: Development of chemometric models to study the quality of amoxicillin and Potassium clavulanate in commercial formulations by using NIR, Raman and FTIR techniques

An effort to study the quality of different commercial brands of AMX and PC was carried out. Various brands of the combination of AMX and PC tablets were analysed with three analytical techniques i.e. FTIR, NIR and Raman spectroscopy. Total of 15 commercial tablets were taken for analysis. 10 placebo samples, 10 counterfeit samples and 1 standard tablet were prepared in the laboratory.

A matrix of 36×1763 for IR data, 36×100 for NIR data, 36×2301 for Raman data was constructed and the Principle component analysis (PCA) models and hierarchical cluster analysis models (HCA) were prepared and cluster analysis was performed.

The model built up using PCA could resolve and separate the genuine marketed drugs from the counterfeit ones. The PCA showed three major groups viz. one group of marketed formulation including laboratory prepared tablet, second group of counterfeit samples and the third group of placebo samples. The hierarchical cluster analysis could also classify the three sample groups specifically. The best results were obtained by NIR and IR techniques, however Raman also proved promising for the analysis.

The PCA model for zero order NIR spectra and zero order IR spectra proved to be the best as the most clear distinction was possible by PCA as well as for cluster analysis. The Raman PCA plot could not specifically differentiate the FM samples from the CF and PL ones. The HCA classification was quite clear and distinct in all the three models but the best results were obtained for NIR and IR model. The PCA models developed from SGolay derivatisation caused the merging of FM samples with the CF samples hence could not be beneficial in this case.