A REVIEW ON CHEMOMETRIC ASSISTED UV SPECTROSCOPIC AND RP-HPLC METHODS FOR MULTICOMPONENT ANALYSIS

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ABSTRACT

In the present study, chemometric assisted analytical methods for various multicomponent formulations were reviewed. The intricacy of analysing the multicomponent samples with the conventional spectroscopic methods presents a vital challenge in the modern pharmaceutical analysis. Use of chemometric models in UV spectral data analysis has become popular, efficient and makes multicomponent formulation analysis an easier one and various articles which reported the usage of chemometric models to UV spectroscopic methods were presented. Ordinary chromatographic methods were usually based on one variable at a time approach (OVAT) which appears to be easier, but this approach is not reliable due to non-consideration of interaction effects between variables, requirement of a greater number of trial runs, more time consumption and complicated optimization involving several factors. These problems can be overcome by the application of chemometric tools to the chromatographic methods.

Key words: chemometrics, multicomponent samples. one variable at a time approach

Introduction:

Multicomponent formulations have acquired importance in the field of pharmaceuticals by exhibiting beneficial effects with respect to the efficacy and potency (Gostner JM et al., 2012) [1]. The intricacy of analyzing these samples with various components presents a vital challenge in the modern pharmaceutical analysis. Analysts have enlarged their interest in the area of complex formulations analysis by using different analytical methods especially chromatographic and spectroscopic methods (Kumar et al., 2014)[2].

Application of chemometric tools in UV-VIS spectroscopy

UV-VIS spectroscopic methods have the advantageous of being simple, easy to operate, relatively lesser expensive when compared to the other popular quantitative methods and have been the extensively used techniques in quantitative analysis. But, it is difficult to analyze the multicomponent formulations which show grievous overlapping in their absorption spectra by using direct and conventional UV spectrophotometric methods without any prior separation.

Moreover, ordinary spectrophotometric methods require more time and the obtained results were deplorable and contrived by low resolution (Luca M et al., 2016 and Attimarad M et al., 2020)[3-4]. Chemometrics, an emerging trend in the field of analytical chemistry, extricates large amount of data by the application of statistical and mathematical methods is used to assist the UV spectroscopic methods to overcome the drawbacks and difficulties (Tawakkol et al., 2017)[5]. Implementation of chemometric models in UV spectral data analysis has become more popular, efficient and powerful in the determination of components in multicomponent formulations.

Chemometric assisted UV spectroscopic methods were found to enhance the spectral data quality, selectivity, sensitivity and have the ability to resolve the complex spectra with greater degree of accuracy and precision (Hopke et al., 2003)[6]. Spectral analysis of multicomponent formulations is mainly intended to contrive a calibration model correlating the outputs of the spectrophotometer to the quantities of analytical samples. Chemometric tools used in UV spectrophotometry are based on multivariate data analysis which extracts relevant information from the whole spectra to utilize concurrently an elevated number of signals (Sahin et al., 2016 and Darwish et al., 2013)[7-8].

PCR and PLS chemometric models:

Multivariate calibration methods can markedly enhance the precision and accuracy of spectrophotometric studies because of the inclusion of numerous spectral intensities (Darwish et al., 2016 and Shaalan et al., 2009) [9-10]. Among the various existed chemometric multivariate data methods, regression and factor based methods like principal component regression (PCR) and partial least squares regression (PLS) have been widely reported in the literature for the UV spectroscopic data analysis of multicomponent formulations (Patel et al., 2014 and Katsarov et al., 2018) [11-12]. These two powerful and efficient chemometric models possess the ability to overcome the difficulties caused by conventional methods and are being successfully adapted in

the simultaneous determination of drugs (Hemmateenaj et al., 2006 and Abersturi et al., 2002)[13-14].

PCR and PLS chemometric approaches in UV spectrophotometric method development comprises of the following steps which include

- Selection of the solvent based on solubilities of drugs
- Construction of calibration curves as per Beer-Lambert's law
- Preparation of mixture solutions of working standards in the calibration curve ranges
- Scanning of the standard mixture solutions in the instrument and importing the spectral data into the software
- Selection of optimal wavelengths for analysis
- Development and optimization of calibration models based on factors
- Validation of the optimized models and applied for quantification

Chemometric assisted UV spectrophotometric methods for simultaneous analysis of multicomponent formulations:

Chemometric assisted UV spectrophotometric methods like PCR & PLS methods were reported for the simultaneous estimation of Ambroxol and Doxycycline in capsule dosage form. The PCR and PLS models were developed using 25 calibration mixtures in the wavelength range of 220-320 nm at 1 nm data interval. Multicomponent analysis quality is based on the wavelength range selected. Considering this, non-informative spectral wavelengths which degrade the models performance were not chosen. Appropriate choice of factors and principal components is essential for PLS and PCR calibration. The factors number should not result in over fitting of the data. Cross validation method is used to choose the optimal number of factors (Hadad et al., 2008) [15].

Determination of ternary mixture in antidiabetic pill was done by using PLS and ANN models. In PLS model, both the absorbance and concentration data matrix was used in data analysis where as in PCR model, only absorbance matrix is used. Construction of calibration model is the primary step in PLS which is done by using nineteen mixtures and to test this, additional seven mixtures were used in the validation set. Selection of more LVs results in addition of more amount of noise and less LVs results in inadequate usage of calibration data. Hence, selection of optimal number of factors is a critical parameter in PLS model and was chosen based on the RMSECV values (Belal et al., 2020) [16].

Simultaneous analysis of Sofusbuvir and Ledipasvir in their combined dosage form was executed by PLS, derivative and wavelet transform spectroscopic techniques. Two sets of standard solutions, training and validation sets were prepared. K fold cross validation method was used to choose optimal factors in the PLS model and the model was applied in the wavelength range of 200-400 nm with a data interval of 1 nm (Khalili et al., 2018) [17]. PLS & PCR models were used in spectrophotometric estimation of pyridine bases and among the developed models, PLS was found to be more efficient than PCR. The principal components and factors number that resulted

in the minimum PRESS values were chosen for calibration of the models (Khajeh Sharif et al., 2017) [18].

Comparative study of PCR and PLS methods was performed for quantitative analysis of Zidovudine and Lamivudine in tablets which revealed that the results obtained were similar (Ustundag et al., 2015) [19]. Simultaneous estimation of Etodolac and Thiocolchicoside was done by PCR & PLS models and the proposed methods can be used as alternative to HPLC method in quantification. The algorithms used in PCR and PLS models were almost same. PCR combines the techniques of PCA and ILS (Albayrak et al., 2019) [20]. Comparison of chemometric PLS and RP-HPLC methods was done for Carbamazepine and Phenytoin in serum samples. PLS model was constructed by framing 12 calibration and 6 validation samples. Factors selection was based on obtained press values. The results obtained by both the methods were found to be almost similar (Rezaei et al., 2005) [21]. Chemometric assisted UV spectroscopic calibration models like PCR and PLS were developed for the simultaneous determination of lansoprazole and domperidone in their combined dosage from. The models were developed in the wavelength range of 260-310 nm at 1 nm data interval in the concentration ranges of 2-18 μg/mL and 4-36 μg/mL for lansoprazole and domperidone respectively (Sami et al., 2017) [22].

Simultaneous determination of Phenobarbitone, Diprophylline and Papaverine was done by using chemometric assisted spectrophotometric and HPLC methods. The chemometric models employed were PLS and PCR in the range of 215-245 nm at 0.2 nm data interval. Cross validation was performed on the training set and further applied to external validation set and the dosage form. Good agreement was found with all the developed methods (Gindy, 2005) [23].

Chemometric models like PCR, PLS and CLS were applied for the determination of Ciprofloxacin and Phenazopyridine in their combined dosage form. All the models were executed in the wavelength range of 205-515 nm at an interval of 5 nm. Two sets of standard mixture solutions, calibration set and validation set were prepared. The models were optimized and successfully applied for the analysis in their dosage form. Simultaneous analysis of Famotidine and Ibuprofen in the presence of their related substances was developed by using PLS and PCR chemometric models. Fourteen mixtures were used as calibration set and the errors were predicted by RMSEP values (Elzanfaly et al., 2018) [24].

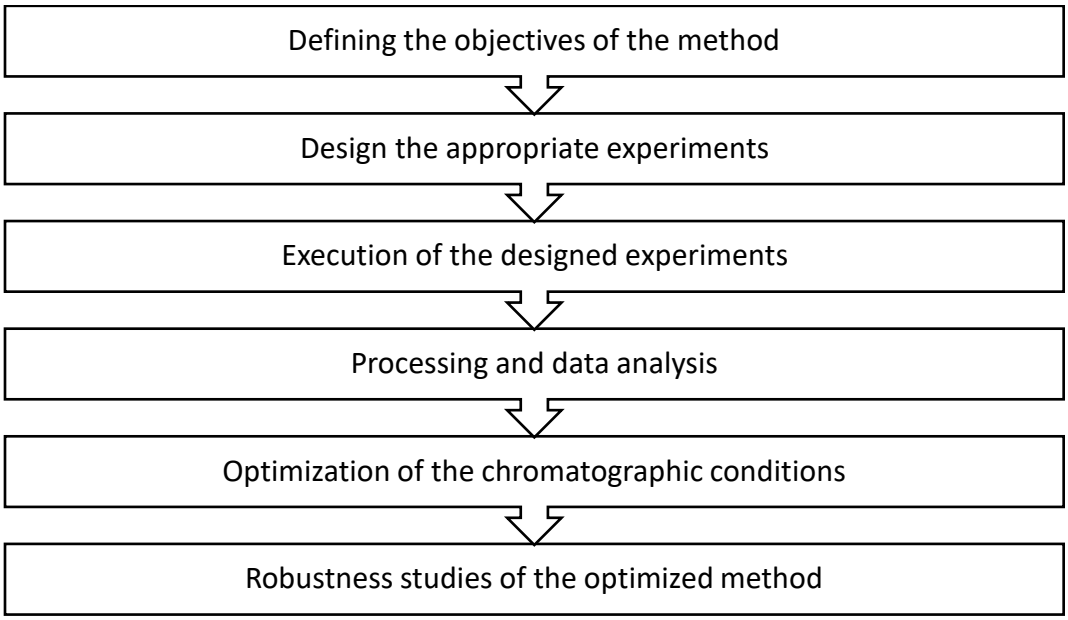
Application of chemometric tools in RP-HPLC

Reversed phase high performance liquid chromatography is one of the most important analytical methods and often used chromatographic technique because of its wider application range, sensitivity, selectivity and accuracy. RP-HPLC has become a greater choice for the analysts to quantify the drugs present in dosage forms (Meenakshi B et al., 2017) [25]. For many years, chromatographic studies were based on univariate approach which uses only one factor or a single variable at a time called as OVAT or OFAT in one experimental series, while all the other variables are kept at constant levels. However, OVAT experimental set up appears to be easier but it should be avoided due to the inconsideration of interaction effects between variables, requirement of more

number of trials and runs, more time consumption and complicated optimization involving several factors (Sree Janardan et al., 2012 and Kowski et al., 2006) [26-27].

The problems with the OVAT approach can be overcome by the application of chemometric tools to the RP-HPLC method. Application of chemometrics in chromatography enables the method development, optimization and also helps in the transfer of procedures and mainly provides the essential and powerful tools for all crucial stages in the method development for separation of drugs (Duarte et al., 2006 and Komsta et al., 2018) [28-29].

Steps involved in chemometric assisted RP-HPLC method development:



Design of Experiments (DOE):

The most popular chemometric tool used in chromatography is Design of experiments which is very effective in sequential and easy optimization and also provides various plots to study the interaction effects between variables. The use of DOE enables multivariate approach which requires minimal experiments, less time and has the ability to predict and find the best optimal responses for the quantification of drugs (Vanbel PF et al., 1993) [30].

Experimental designs enable the analysts to identify the critical factors which have a significant effect on the responses of the experiment and also in optimization of the responses by finding the correct factor values. The main objective is to reduce the experiments number on higher number of factors (Hibbert DB et al., 2012) [31].

The methodology of DOE in RP-HPLC method development involves the following steps which include:

- Selection of the chromatographic instrumental factors which widely influences the separation process
- Defining the levels of variation for the selected factors

- Choosing an appropriate experimental design for the set of factors which provides the main and interaction effects
- Execution of the runs in random order as given by the chosen experimental design.
- Application of statistical regression analysis to compute the mathematical model coefficients of the response surface
- Finding the optimal response in the design space by using the mathematical model
- Validation of the model by verifying the experimental values with predicted values (Cela et al., 2012) [32].

Central composite design- response surface methodology (CCD-RSM):

Central composite design, a subset of response surface methodology is one of the primary and most employed chemometric tool used in the chromatographic optimization in order to find out the best experimental conditions (Ferreira et al., 2007 and Conde FJ et al., 2006) [33-34]. Determination of the significant factors that are affecting the experimental results is the main aim of CCD.

Response surface methodology involves the compilation of statistical and mathematical techniques, which are helpful in analyzing problems in the case where chromatographic responses or dependent variables are influenced by numerous independent variables (Petkovaska et al., 2008 and Elkhoudary et al., 2016) [35-36]. Independent variables are also known as factors which are variables in the experiments that can be changed individually. Typical factors include mobile phase pH, reagents concentration, temperature, flow rate, mobile phase composition among others (Arslan et al., 2016 and Andal et al., 2016) [37-38]. The response surface is a correlation of different responses which contains a function suited to the experimental data, plotted as two-dimensional (contour plots) or three dimensional (rsm plots) curvatures or graphs (Singh et al., 2013) [39].

Optimization of numerous responses at a time becomes easier with another chemometric tool called multicriteria decision making (MCDM). Derringer's desirability function (DDF), an eminent approach of MCDM is widely used in the optimization of multiple responses. This function combines all the discrete desirability which is a geometric mean and possesses the advantage of user flexibility (Derringer et al., 1980 and Deming et al., 1991) [40-41].

Chemometric assisted RP-HPLC methods for simultaneous analysis of multicomponent formulations:

Chemometric tools like experimental design and MCDM approach were applied in the optimization of HPLC conditions for the separation of Pantoprazole and Domperidone. Mobile phase composition, flow rate and buffer molarity were selected as the factors by applying CCD and were used in designing mathematical models. By backward elimination process, insignificant terms ($P > 0.05$) were removed. Optimization of the responses was done by using DDF (Sivakumar et al., 2007) [42]. RSM-CCD was used in optimization of HPLC method for determination of

Captopril and by using this design, maximum information was drawn within short time using small number of experiments. Simultaneous determination of Levetiracetam and Pyridoxine hydrochloride in tablet dosage forms was done by RP-HPLC method using chemometric tool, CCD. The effects of factors were studied by perturbation plots in which, the factors with higher steep slopes are said to be more significant (Hashem et al., 2018) [43].

Simultaneous analysis of Ofloxacin and Nimorazole in dosage form was done by using chemometric assisted RP-HPLC method. Combining chemometric tools to RP-HPLC makes the method more powerful in estimation of drugs. Response surface plots and perturbation plots which represent the effects of factors on a specific response were offered in the models in order to define a better understanding of the proposed method (Giriraj et al., 2014) [44]. Selection of optimal HPLC conditions for Moxifloxacin Hydrochloride & Ketorolac in dosage form was done by applying RSM-CCD. Application of designs enables better sensitivity and interaction effects and to combine the objective responses. Selection of factors was based on preliminary trials and literature reported. Mapping of chromatographic responses by CCD was done to locate the selected factors and RSM enables the description of quadratic models (Kalariya et al., 2014) [45].

Quantitative analysis of seven beta blockers was done by using chemometric assisted UV and RP-HPLC methods. In the developed gradient chromatographic method, CCD was applied to the aqueous phase pH, acetonitrile content and column temperature and optimization was done by DDF. By the application of one way ANOVA, it was found that, there were no differences between the results of developed methods and all the methods can be successfully applied to the commercially available formulations (Abdel Hameed et al., 2011) [46].

RP-HPLC method was developed for Zileuton by applying DOE for robustness study. Applying DOE provides many advantages than the compendial method (Ganorkar et al., 2017) [47]. CCD-RSM was applied for HPLC optimization of triglyceride isomers and the design was found to be efficient, robust and suitable in numerous areas of research applications (Arslan 2017) [48]. RP-HPLC optimization of Emtricitabine loaded formulation was done by using CCD. Response surface mapping is the powerful and efficient way in finding the optimal conditions and to determine this, CCD was used to study the interaction effects between the factors (Gurindersingh et al., 2014) [49].Chemometric RSM was applied for the LC-MS separation of benzopyrene-quinone isomers. In the proposed method, optimization of the multiple responses was done by using DDF and can be successfully applied in the research area of isomers (Gonzalez et al., 2007) [50].

CONCLUSION

The use of chemometric tools like PCR & PLS for UV quantification has surmounted the difficulties caused by the conventional UV methods and showed profound results. Application of chemometric tools like Response surface methodology and Derringer’s desirability for RP-HPLC quantification facilitated the prudent identification of influential factors and also provided the most appropriate conditions for chromatographic analysis of drugs and demonstrated the benefits like user flexibility, reducing the analysis time in the various multicomponent formulations.

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