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Novel UV spectrophotometric & chemometrics assisted spectrophotometric methods for simultaneous estimation of Brexpiprazole and Sertraline: A statistical analysis

Parth Patel and Rajashree Mashru

Abstract
Three simple, rapid, inexpensive, precise and accurate UV spectrophotometric methods have been developed for simultaneous estimation of Brexpiprazole and Sertraline. Method A was simultaneous equation method (vireo's method) which applies measurement of absorptivity at two wavelength 325 nm, (λmax of Brexpiprazole) and 274 nm, (λmax of Sertraline) in zero order spectra. The concentration calculated from the derived equations. Method B was Absorption Ratio Method (Q-ratio) which applies measurement of Absorptivity at two wavelength 275.88 nm (Iso-absorptive point) of both drugs and 325 nm (λmax of Brexpiprazole) in zero order spectra. Method C was based on zero crossing Second derivative (D^2) spectrophotometry where Brexpiprazole showed zero crossing point at 268 nm and Sertraline showed zero crossing point at 280 nm. Linearity for Brexpiprazole was between 1-7 mcg/ml and Sertraline was between 20-140 mcg/ml. Accuracy of all the above methods was determined by recovery studies and % recovery was estimated between 99.01 to 101.80%. Intraday and Inter day precision was checked for all methods and mean % RSD was found to be less than 2. These methods were successfully applied for estimation of Brexpiprazole and Sertraline in Laboratory sample. Four chemometrics methods were also applied to simultaneous determination of Brexpiprazole and Sertraline in simulated mixture. Classical least- square(CLS), inverse least-square(ILS), principal component regression(PCR) and partial least-square(PLS) methods do not need any priori graphical treatment of the overlapping spectra of two drugs in a mixture. For all chemometrics calibration a concentration set of the random mixture consisting of the two drugs in methanol was prepared. The absorbance data in the UV spectra were measured for the 41 wavelength points (from 200 to 400 nm) in the spectral region 200-400 nm considering the intervals of Δλ=5 nm. It is claimed that these new chemometrics-assisted spectrophotometric methods are inexpensive, rapid, and simple and can be trustfully carried out in quality control laboratories. Statistical analysis was done to compare all the three developed spectroscopic methods. Brexpiprazole there was a statistically significant difference as P-value for Brexpiprazole was less than α=0.05 and observed F value was higher than $F_{critical}$ values. Therefore, Post-hoc analysis using multiple comparisons by Tukey's test was performed for Brexpiprazole. This revealed that Method A (Simultaneous equation method [vierdot's method]) was significantly different from other methods.

Keywords: Vierodt's Method, Q-Ratio, ZCP, Post-hoc analysis, Brexpiprazole, Chemometrics calibration methods, Sertraline

Introduction
UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. In multi component sample analysis technique frequently there was a three method used. [1]

I. Simultaneous equation method
II. Absorbance ratio method
III. Derivative zero crossing spectrophotometry

Chemometrics methods are one type of multivariate analysis i.e. considering more than one variable at a time. When applied to UV spectrophotometry, many wavelengths are taken as variable and absorbance at each wavelength is considered. Least square approach involves mathematical modelling by which the square of residual (difference between actual and predicted concentration) is minimized to lowest level. [2] Four different chemometrics methods are used which are

1. Classical Least Square
2. Inverse Least Square
3. Principal component Regression
4. Partial Least Squares or Projection to Latent Structures

Statistics may be defined as the collection, Presentation, analysis and interpretation of numerical data. Analysis of Variance is a technique of separating the total variability in a set of data into components parts, represented by a statistical model. If more than two assay methods are to be compared, the correct statistical procedure to compare the means is the one way analysis of variance (ANOVA). P value in ANOVA is the probability of that random sampling would lead to a difference between sample means as large or longer than you observed. P value threshold is fixed to the value same as alpha probability level. i.e. 0.05 Results of% Assay obtained by all the three developed methods were. Subjected to ANOVA. [3]
Brexpiprazole, an atypical antipsychotic, is available as REXULTI® (Brexpiprazole) tablets. Chemically Brexpiprazole was 7-{4-[4-(1-Benzothiophen-4-yl) piperazin-1-yl]butoxy}quinolin- 2(1*H*)-one. The empirical formula is C25H27N3O2S and its molecular weight is 433.57 g/mol. It is used in the treatment of schizophrenia and as an adjunctive therapy to antidepressants for the treatment of Major Depressive Disorder (MDD). The mechanism of action of brexpiprazole in the treatment of major depressive disorder or schizophrenia is unknown. However, the efficacy of brexpiprazole may be mediated through a combination of partial agonist activity at serotonin 5-HT1A and dopamine D2 receptors, and antagonist activity at serotonin 5-HT2A receptors [4]. ZOLOFT contains sertraline hydrochloride, an SSRI. Sertraline hydrochloride has a molecular weight of 342.7 and has the following chemical name: (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1
naphthalenamine hydrochloride. The empirical formula is C17H17NCl2•HCl. Sertraline hydrochloride is a white crystalline powder that is slightly soluble in water and isopropyl alcohol, and sparingly soluble in ethanol. Sertraline potentiates serotonergic activity in the central nervous system through inhibition of neuronal reuptake of serotonin (5-HT). [5]

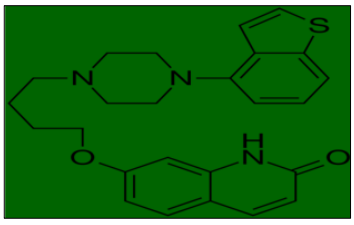


Fig 1: Chemical structure of Brexpiprazole

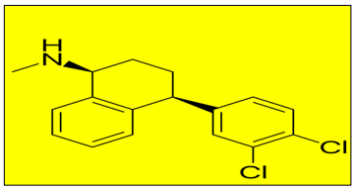


Fig 2: Chemical structure of Sertraline

Why this combination? [6]

- Brexpiprazole used as combination therapy with Sertraline in the treatment of Post-Traumatic Stress Disorder (PTSD). So this combination Provide the synergistic effect. The combination of Brexpiprazole and Sertraline demonstrated a safe and adequate tolerability profile.
- Several UV methods reported for Brexpiprazole. HPLC method reported for estimation of Brexpiprazole in bulk and tablet dosage form. Several UV methods also reported for Sertraline. HPLC method reported for estimation of Sertraline in bulk and also injectable formulations.
- There is no single method is reported for simultaneous estimation of Brexpiprazole and Sertraline in their combination.
- This Combination is in clinical Trial Phase-3 Study. NIH CLINICAL TRIAL ID =NCT04174170

Materials and Methods

Instrumentation and software

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with shimadzu UV probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions was carried out in 1 cm quartz cells over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A*120, shimadzu). Statistical analysis of data was accomplished using Microsoft Excel for Mac version 16.34 and Minitab for mac version 19.2020.1.0 Software.
Matlab 2012b used for CLS and ILS & Unscrambler X used for PCR and PLS. Data extraction from UV probe 2.10 software to Unscrambler X was done using Microsoft Excel for Mac Version 16.34. Design of Calibration and Validation set was done using Design-Expert 11.

Reagents and Chemicals

Methanol analytical reagent grade (Research Lab fine chem industries, Mumbai, India) was used as the solvent and diluent.

Preparation of standard stock solution

10 mg of Brexpiprazole and Sertraline were weighed accurately and transferred into 10 ml volumetric flask Separately. Methanol was added into the volumetric flask to dissolve the standards and finally volume was made up to the mark with Methanol to obtain standard solutions of Brexpiprazole and Sertraline (1000 mcg/ml).

Preparation of working standard solution

From the stock above solution of Brexpiprazole, Working standard solution of Brexpiprazole (100 mcg/ml) was prepared by transferring 1 ml aliquot to 10 ml volumetric flask separately and making up the volume with methanol. Sertraline working standard solution is same as standard stock solution. (1000 mcg/ml)

Preparation of calibration curve of standard of brexpiprazole & sertraline

From the working standard solution of Brexpiprazole(100 mcg/ml), aliquots 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml were withdrawn and transferred to 10 ml volumetric flasks. Volume was made upto the mark with Methanol to produce 1 mcg/ml, 2 mcg/ml, 3 mcg/ml, 4 mcg/ml, 5 mcg/ml, 6 mcg/ml and 7 mcg/ml of Brexpiprazole respectively. From the working standard solution of Sertraline (1000 mcg/ml), aliquots 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml and 1.4

ml were withdrawn and transferred to 10 ml volumetric flasks. Volume was made upto the mark with Methanol to produce 20 mcg/ml, 40 mcg/ml, 60 mcg/ml, 80 mcg/ml, 100 mcg/ml, 120 mcg/ml and 140 mcg/ml of Sertraline respectively. Mixed standard solution of Brexpiprazole and Sertraline were prepared in ratio of 1:20 as approximated in the literature.

Preparation of binary mixtures (Brexpiprazole + Sertraline) for calibration set and validation set
Calibration set [7]
A set of 40 mixtures was prepared in methanol, applying a multilevel multifactor design in which two levels of concentrations of Brexpiprazole and Sertraline within the stated range were introduced as shown in Table-1.

Validation set
A set of 9 mixtures was prepared in methanol, applying a multilevel multifactor design in which two levels of concentrations of Brexpiprazole and Sertraline within the stated range were introduced as shown in Table 2.

Table 1: Calibration set

Sr.no	Brexpiprazole (mcg/ml)	Sertraline. (mcg/ml)
1	1	80
2	1	120
3	7	80
4	4	60
5	1	100
6	3	140
7	2	120
8	6	80
9	1	40
10	3	60
11	3	100
12	2	60
13	6	120
14	1	80
15	3	120
16	3	60
17	2	80
18	6	120
19	5	20
20	3	40
21	5	140
22	3	140
23	5	100
24	2	40
25	7	40
26	6	40
27	5	100
28	6	60
29	6	140
30	4	100
31	2	100
32	2	120
33	1	40
34	1	80
35	4	140
36	7	140
37	4	100
38	7	120
39	6	20
40	1	20

Table 2: Validation set

Sr.no	Brexpiprazole (mcg/ml)	Sertraline (mcg/ml)
1	3	40
2	7	60
3	2	140
4	6	60
5	4	20
6	2	80
7	3	20
8	7	20
9	5	20

1. Simultaneous equation method (Vierodt' Method) (Methoda) [8]
As mentioned earlier dilutions for Brexpiprazole and Sertraline were prepared in concentration range of 1-7 mcg/ml and 20-140 mcg/ml respectively were prepared and scanned between 200 to 400 nm. The zero order overlain spectra of Brexpiprazole and Sertraline are shown in Figure 3 and 4. Calibration curve were found to be linear in the concentrations range under study as depicted in figure 5 and figure 6. The analytical wavelength for Brexpiprazole and Sertraline were 325 nm and 274 nm respectively.
Absorptivity of Brexpiprazole and Sertraline were calculated

at both the wavelengths. The Concentrations of Brexpiprazole and Sertraline can be calculated from the following equations:
C_x (Brexpiprazole) = (A2ay1-A1ay2)/(ax2ay1-ax1ay2) C_y (Sertraline) = (A1ax2-A2ax1)/(ax2ay1-ax1ay2)
Where, C_x and C_y are concentrations of Brexpiprazole and Sertraline respectively in g/100 ml in the sample solution. A1 and A2 are the absorbance of the mixture at 325 nm and 274 nm respectively; ax1 and ax2 =absorptivity of Brexpiprazole at 325 nm and 274 nm; ay1 and ay2 =absorptivity of Sertraline at 325 nm and 274 nm respectively.

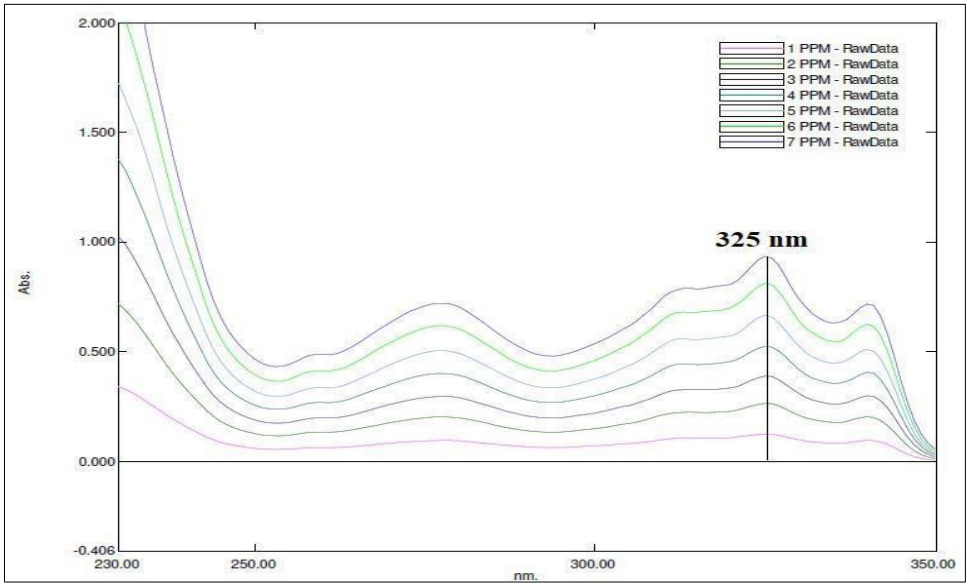


Fig 3: Zero order overlain spectra (Absorbance vs. Concentration) of Brexpiprazole (1-7 mcg/ml)

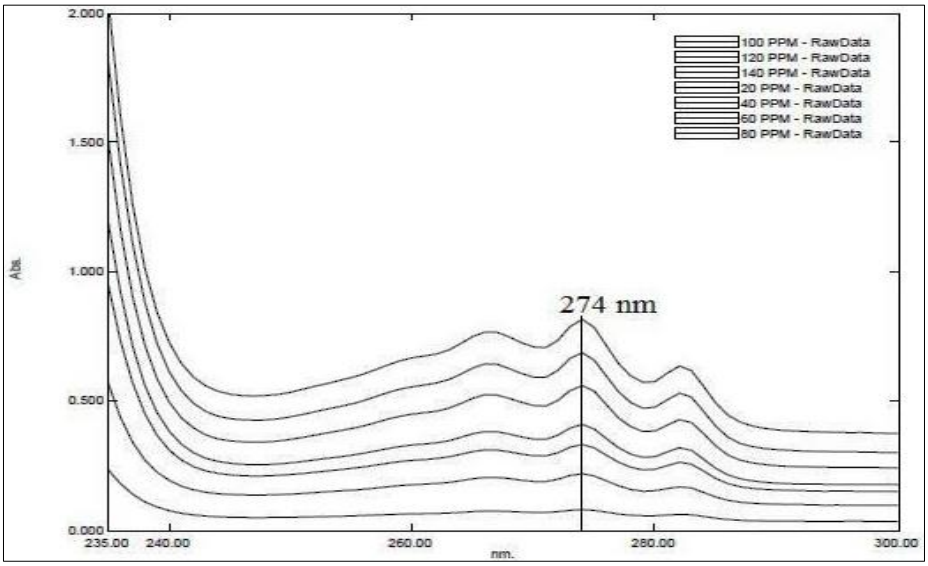


Fig 4: Zero order overlain spectra (Absorbance vs. Concentration) of Sertraline (20-140 mcg/ml)

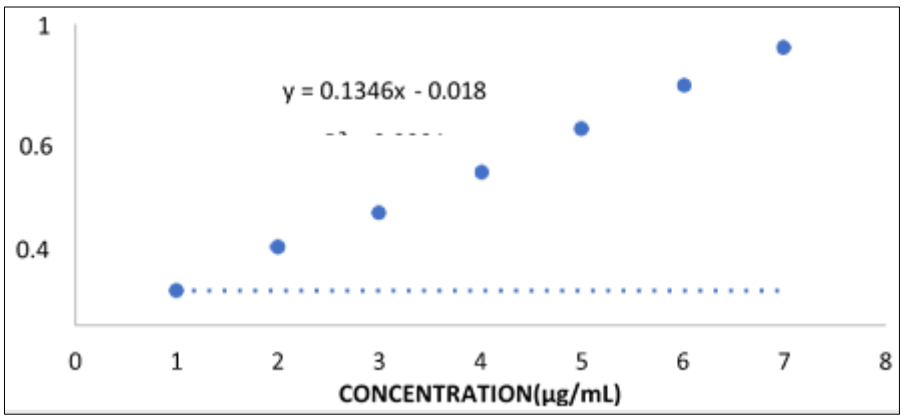


Fig 5: Calibration curve of Brexpiparzole at 325 nm

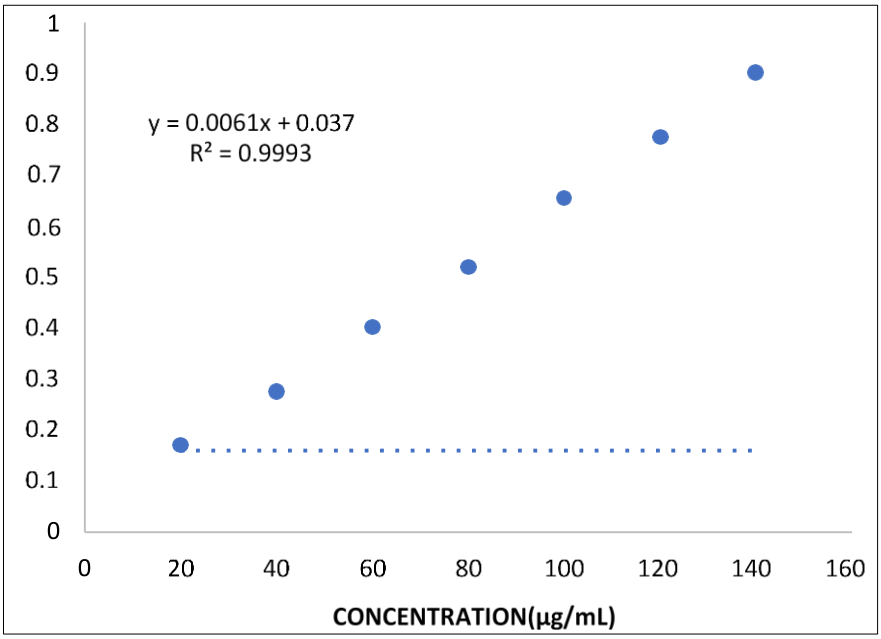


Fig 6: Calibration curve of Sertraline at 274 nm

2. Absorption Ratio Method(Q-Ratio) (Method B)[9]

Absorbance ratio method uses the ratio of absorbance's at two selected wavelengths, one which is an iso absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that Brexpiprazole and Sertraline show an iso absorptive point at 275.88 nm. The second wavelength used is 325 nm, which is the λ-max of Brexpiprazole. As mentioned earlier dilutions for Brexpiprazole and Sertraline were prepared in concentration range of 1-7 mcg/ml and 20-140 mcg/ml respectively were prepared and scanned between 200 to 400 nm. and the absorbance's at 275.88 nm (iso absorptive point) and 325 nm (λ-max of Brexpiprazole) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

CX (Brexpiprazole) = [(QM – QY) / (QX -QY)] × A1/ax1

CY (Sertraline) = [(QM – QX) / (QY -QX)] × A1/ay1

Where, A1 and A2 are absorbance's of mixture at 275.88 nm and 325 nm; ax1 and ay1 are absorptivity's of Brexpiprazole and Sertraline at 275.88 nm; ax2 and ay2 are absorptivity's of Brexpiprazole and Sertraline respectively at 325 nm; QM=A2 /A1, QX=ax2 /ax1 and QY= ay2 / ay1.

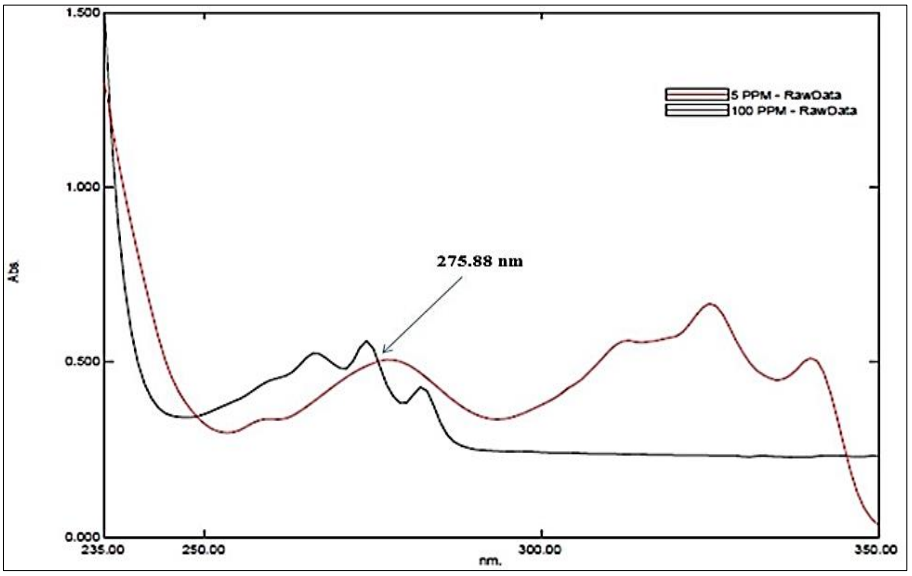


Fig 7: Zero order overlain spectra of Brexpiprazole and Sertraline at Iso- absorptive point=275.88 nm

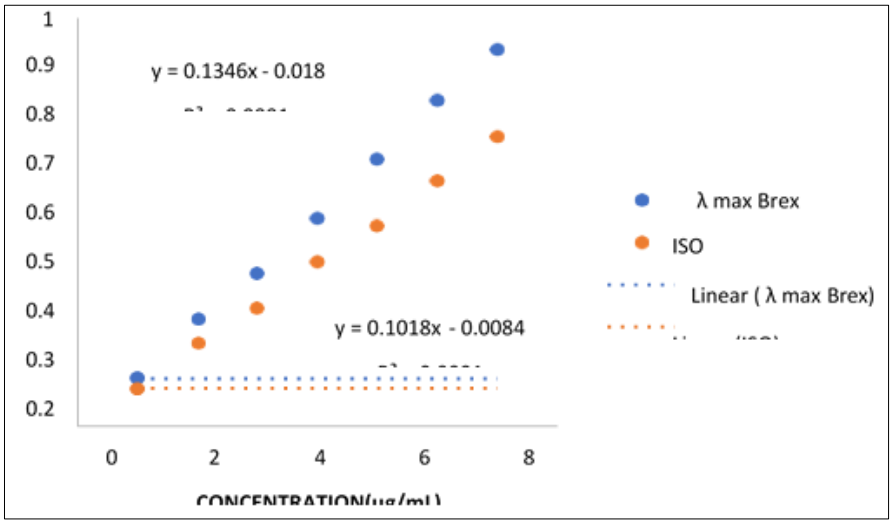


Fig 8: Calibration Curve for Brexpiprazole at 325 nm (λmax of BREX) Calibration Curve for Brexpiprazole at 275.88 nm (Iso-Absorptive Point)

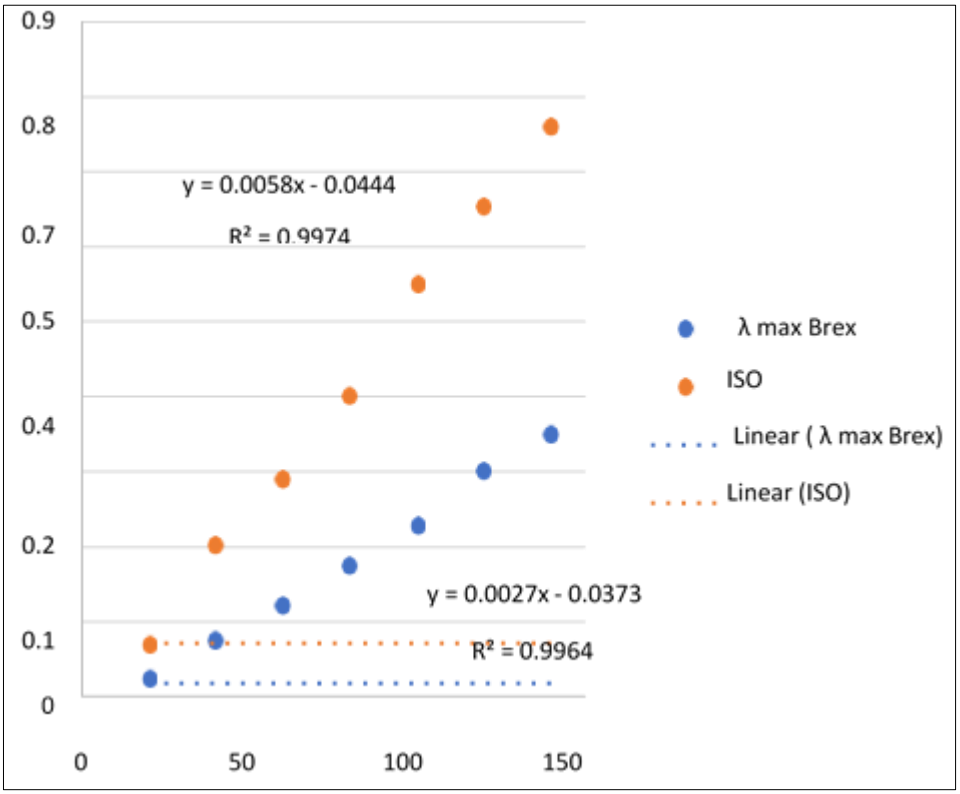


Fig 9: Calibration Curve for Sertraline at 325 nm (λmax of BREX) Calibration Curve for Sertraline at 275.88 nm (Iso-Absorptive Point)

3. Second Derivative Zero Crossing Point Method (Zcp) (Method C) [10]

Derivative spectroscopy on the basis of zero-crossing measurement involves measurements of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other component. The absorption spectra of the solutions of Brexpiprazole and Sertraline were recorded in the range of 200-400 nm and were stored in the memory of the instrument and transformed to second derivative with **Δλ**=10 nm and scaling factor=1. Figure 8.8 shows that at 268 nm Brexpiprazole shows zero crossing point and hence Sertraline can be determined while at 280 nm Sertraline shows zero crossing point and hence Brexpiprazole can be determined. Calibration curves were constructed with seven different concentrations in the range between 1-7 mcg/ml and 20-140 mcg/ml for Brexpiprazole and Sertraline respectively. Each concentrations was analysed thrice. The concentration of the drug present in the laboratory sample solution was determined against the calibration curve. Figure 11 and Figure 12 show calibration graphs of Brexpiprazole and Sertraline 280 nm and 268 nm respectively.

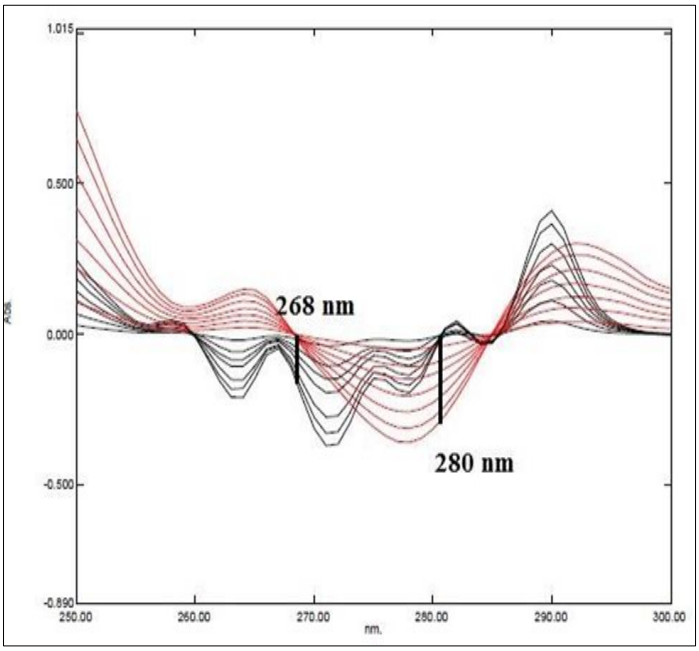


Fig 10: Overlain Second Derivative Spectra Of BREX (RED) & SER(BLACK)With Their Zero Crossing Point ZCP Of BREX=268 nm & ZCP Of SER=280 nm

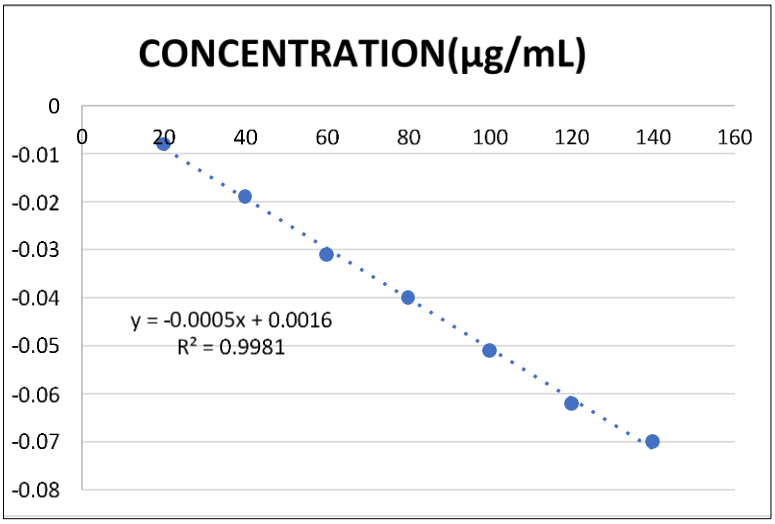


Fig 11: Calibration Graph of Second derivative Brexpiprazole at 268 nm

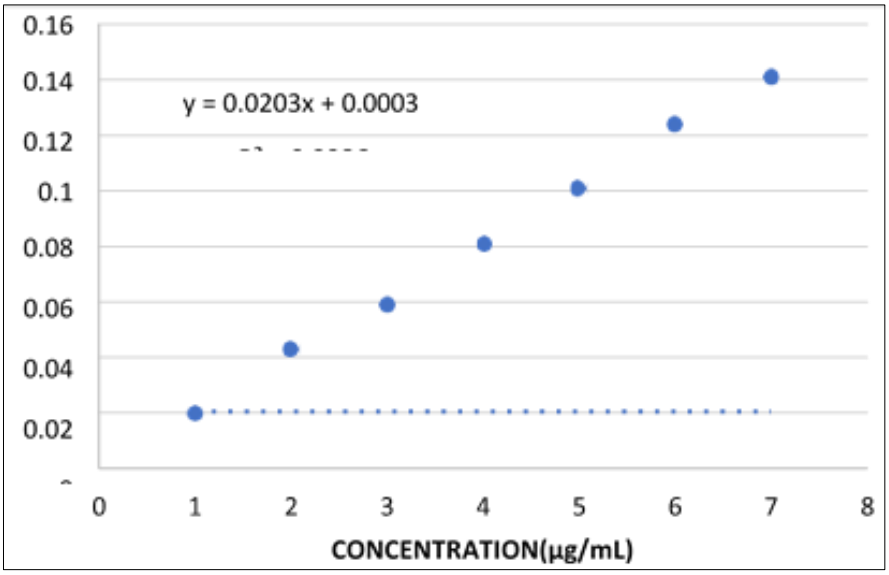


Fig 12: Calibration Graph of Second derivative Sertraline at 280 nm

1. Classical least squares (CLS) method [11]

The mathematical model for this method can be represented by A=CK where A is m×n matrix of calibration spectra, C is m×l matrix of component spectra and K is l×n matrix of absorptivity at unit concentration and unit pathlengh. (m=40,l=2,n=41).The Calibration coefficient matrix(K) was calculated as K=pinv (C)*A and using the K value of calibration coefficient unknown was computed using formula C=A*pinv(K).

Computing the K (calibration coefficient) Matrix

The value of calibration coefficient can be calculated by using following equation K=pinv(C)*

Table 3: K-Matrix

S. No	Wavelength	Brexpiprazole	Sertraline
1	200	0.243	-0.0018
2	205	0.1616	0.0126
3	210	0.2216	0.0163
4	215	0.2178	0.017
5	220	0.1702	0.0195
6	225	0.1387	0.0203
7	230	0.1015	0.0189
8	235	0.0683	0.009
9	240	0.0419	0.0023
10	245	0.025	0.0013
11	250	0.0185	0.0013
12	255	0.0176	0.0015
13	260	0.0192	0.0019
14	265	0.0209	0.0023
15	270	0.0239	0.0021
16	275	0.0264	0.0025
17	280	0.0256	0.0015
18	285	0.0219	0.0019
19	290	0.0184	0.0023
20	295	0.0177	0.0021
21	300	0.0194	0.0005
22	305	0.0218	0.0361
23	310	0.026	0.0246
24	315	0.027	0.0063
25	320	0.0277	0.0136
26	325	0.0317	0.0049
27	330	0.0255	0.0159
28	335	0.0218	0.1072
29	340	0.0248	0.0871
30	345	0.0134	0.049
31	350	0.0032	0.0591
32	355	0.0017	0.0152
33	360	0.0014	0.0181
34	365	0.0172	0.1039
35	370	0.0219	0.0375
36	375	0.0121	0.0089
37	380	0.0381	0.0017
38	385	0.0271	0.0043
39	390	0.0721	0.1017
40	395	0.0612	0.0219
41	400	0.1039	0.0031

Predicting the unknown concentration

Spectra of solution containing unknown concentration of Brexpiprazole and Sertraline were recorded in the range of 200-400 nm and absorbance matrix A was generated. Using the calibration coefficient matrix K, the concentration was computed using following equation C=A*pinv (K)

2. Inverse least squares (ILS) method [12]

The mathematical model for this method can be represented by C=AP where P is l×n matrix of unknown calibration, C and A are same as defined for CLS method mentioned above at unit concentration and unit path length. (m=40,l=2,n=41) The calibration coefficient matrix (P) was calculated as P=pinv (A)*C and using the P value of calibration coefficient unknown was computed using formula C=A*P.

Computing the P (calibration coefficient) Matrix

The value of calibration coefficient can be calculated by using following equation P=pinv (A)*C

Table 4: P-Matrix

S. No	Wavelength	Brexpiprazole	Sertraline
1	200	0.006	0.0005
2	205	0.0011	0.0022
3	210	0.009	0.0061
4	215	0.0028	0.0003
5	220	0.0017	-0.0029
6	225	0.0173	0.0041
7	230	0.0015	0.029
8	235	0.0084	0.1994
9	240	0.0306	0.639
10	245	0.1188	0.675
11	250	0.1618	1.7829
12	255	0.0662	0.0209
13	260	0.0919	0.3801
14	265	0.014	0.153
15	270	0.0015	0.7363
16	275	0.0208	0.9024
17	280	0.0014	0.1468
18	285	0.0983	0.8433
19	290	0.1605	0.0802
20	295	0.0466	0.1597
21	300	0.005	.4732
22	305	0.0023	0.1411
23	310	0.0248	0.7155
24	315	0.0736	0.3916
25	320	0.0257	0.2664
26	325	0.0503	0.275
27	330	0.0386	0.1771
28	335	0.0521	0.3172
29	340	0.095	0.6374
30	345	0.0214	0.6213
31	350	0.0038	0.3449
32	355	0.0672	0.0983
33	360	0.0425	0.0614
34	365	0.0191	0.0449
35	370	0.0081	0.2048
36	375	0.0622	0.0829
37	380	0.2202	0.0562
38	385	0.0803	0.0782
39	390	0.2127	0.0243
40	395	0.0665	0.0173
41	400	0.0157	0.0215

Predicting the unknown concentration

Spectra of solution containing unknown concentration of Brexpiprazole and SERTRALINE were recorded in the range of 200-400 nm and absorbance matrix A was generated. Using the calibration coefficient matrix P, the concentration was computed using following equation C=A*P.

3. Principal Component Regression (PCR) method [13]

PCR is the method which works on the principal of reducing the dimensionality of the original data. Absorbance matrix and concentration matrix as shown above were generated and data was fed to software. The absorbance matrix (X) used for calibration contains total 41 variables i.e. wavelengths at which absorbance values are measured. PCR will compute a few PCs and will perform regression of these PCs with concentration (Y). The algorithm used for PCR was NIPALS i.e. nonlinear iterative partial least squares. Validation was set as full cross validation. The data of absorbance values at 41 wavelengths were used as X space (predictors) and the data containing concentration of BREX and SER in 40 calibration standards were used as Y space (responses).

Determining Optimum Number of Principal Components for PCR

Three major parameters are considered for determination of number of PCs to be taken into account.

1. Total explained Y variance
2. Total residual Y variance and
3. Root Mean Square of Prediction values for validation

(Concentrations are Y space – the responses; and absorbance values at different wavelengths are considered as X space – the predictors. Mixtures prepared as calibration standards may be referred to as samples.)

The model should have as low residual variance as possible. This means that the model should explain most of the variance in the data i.e. explained variance should approach 100%. For this, number of PCs should be optimized. Normally, first 2-3 PCs will explain nearly (not exactly) 100% of variance in data. Moreover, the model should have as low RMSEP values as possible.

The software can validate the model by full cross validation method, where one sample from the calibration set is left out each time and model are calibrated using remaining samples.

Then the prediction is made for left out sample and its residual are calculated. The same process is repeated until each sample is left out once. So, there were total 9 segments for validation, because there were 40 calibration standards or samples. Finally, one can view the plot of residual variance or explained variance (for calibration and validation both) or RMSEP vs. number of PCs. This can help in determining the optimum number of PCs.

Once the model is calibrated with optimum number of PCs, the model can predict the unknown concentration from its absorbance data. Maximum number of PCs was fixed to 7.

4. Partial Least Squares or Projection to Latent Structures (PLS) method [14]

PLS computes factors for X and Y both and then correlates them. It models both the X- and Y-matrices simultaneously to find the latent variables in X that will best predict the latent variables in Y. Full cross validation method is used for determining the optimum number of factors. The algorithm used for PLS was NIPALS i.e. nonlinear iterative partial least squares.

Determining Optimum Number of Principal Components for PLS

The number of factors to be taken into account was determined by full cross validation method and following parameters were considered

1. Total explained Y variance
2. Total residual Y variance
3. RMSEP values for validation

Results and Discussion

UV-Spectrophotometric method validation [15]

Developed spectrophotometric methods for the simultaneous estimation of Brexpiprazole and Sertraline were validated according to ICH Q2 (R1) guidelines and data complying with the standards were obtained.

1. Linearity and sensitivity

The linearity of method was evaluated thrice by analysing six concentrations of each drug. Linear regression equation was obtained over the concentration range (y=mx+c). Limit of detection (LOD) and Limit of quantification(LOQ) were calculated from standard deviation of response and slope of calibration curve. Table 5 reveals the summary of validation parameters of Brexpiprazole and Sertraline by the three developed methods.

Table 5: Summary of validation parameters by developed methods

Parameter	Method A		Method B				Method C	
	BREX	SER	BREX		SER		BREX	SER
Analytical wavelength	325 nm	274 nm	325nm	275.88nm	325nm	275.88 nm	280 nm	268 nm
Beer's Range(mcg/ml)	1-7 mcg/ml	20-140 mcg/ml	1-7 mcg/ml	1-7 mcg/ml	20-140 mcg/ml	20-140 mcg/ml	1-7 mcg/ml	20-140 mcg/ml
Slope	0.1346	0.0061	0.1346	0.1018	0.0058	0.0027	0.0203	0.00015
Intercept	0.018	0.037	0.018	0.0084	0.0444	0.0373	0.0003	0.0016
Correlation coefficient	0.9991	0.9993	0.9991	0.9991	0.9974	0.9964	0.9986	0.9981

2. Precision

Reproducibility of methods was checked by performing intra day precision (three times a day) and inter day precision (repeated triplicates for three consecutive days). Intraday and Interday precision was measured in terms of% RSD. The average% RSD was found to be less than 2 for the three developed methods.

Table 6: Results of intraday and interday precision for Brex and Ser by the proposed three methods

Parameter	Method A		Method B		Method C	
	BREX	SER	BREX	SER	BREX	SER
Intraday Precision (SD)	0.0006	0.0059	0.0062	0.00071	$3.01*10^{-5}$	0.0089
Intraday Precision(%RSD)	1.30	1.19	1.342	1.20	0.89	1.39
Interday Precision (SD)	0.00366	0.0076	0.00087	0.0056	$4.45*10^{-4}$	0.0031
Interday Precision(%RSD)	0.72	0.93	1.01	1.29	1.11	1.41

3. Accuracy

To check the accuracy of different methods, recovery studies were carried out from pre-analyzed sample at three different level of standard addition 80%, 100% and 120%. Results of recovery studies are shown in table 7. For each of the method explained above, % recovery was the average of three determinations at each standard addition level. % recovery study for different method was found to be between 99.01 to 101.80% which prove that all the methods were accurate.

Table 7: Results of Accuracy (% Recovery) for Brex and Ser by the proposed three methods (SD=Stanadrad deviation)

Method	% Spiking	Conc. Actual (mcg/ml)		Conc. Added (mcg/ml)		Conc. Recovered (mcg/ml)		% Recovery±SD	
		BREX	SER	BREX	SER	BREX	SER	BREX	SER
Method A	80	5	100	4	80	4.06	80.81	101.50±0.0054	101.01±0.00051
	100	5	100	5	100	5.01	100.98	100.20±0.0316	100.98±0.00231
	120	5	100	6	120	5.98	119.87	99.66±0.0351	99.89±0.00078
Method B	80	5	100	4	80	3.98	79.87	99.50±0.0031	99.83±0.00020
	100	5	100	5	100	5.09	100.95	101.80±0.0109	100.95±0.00055
	120	5	100	6	120	6.1	119.60	101.66±0.0091	99.66±0.00060
Method C	80	5	100	4	80	3.96	78.66	99.01±0.0816	98.32±0.00512
	100	5	100	5	100	5.05	99.51	101.09±0.0071	99.51±0.00916
	120	5	100	6	120	6.02	121.09	100.33±0.0098	100.90±0.00349

4. LOD & LOQ

Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD=3.3* S.D./ slope of calibration curve

LOQ=10* S.D./ slope of calibration curve

Where, S.D.=Standard deviation of intercepts

Table 8: LOD and LOQ Values of Brex and Ser

Parameter	Method A		Method B				Method C	
	BREX	SER	BREX		SER		BREX	SER
LOD (mcg/ml)	0.19	3.44	0.19	0.20	3.73	3.70	0.24	5.96
LOQ (mcg/ml)	0.59	10.43	0.59	0.61	11.33	11.50	0.73	18.07

5. Robustness

Robustness of UV spectrophotometric method was performed by checking the effect of variation in solvent characteristics on the absorbance of the method. Absorbance of solutions with different analysts were recorded.

Table 9: Robustness value of Brex and Ser

Parameter	Method A							
	BREX		SER		BREX		SER	
Two Different Analysts	A	B	A	B	A	B	A	B
% RSD	0.871	0.641	1.541	1.876	1.561	1.431	0.961	0.653

Validation Of CLS, ILS, PCR and PLS Methods [16]

The validation set Prepared as described was subjected to analysis by developed models of all the four methods. Though The PCR and PLS models are validated using full cross validation, these methods are also applied to validation set.

Root Mean Square Error of Prediction (RMSEP)

The predictive ability of the model can be defined as RMSEP.RMSEP summarizes both precision and accuracy. It is used for examining the errors in the predicted concentrations. The results of future predictions can then be presented as "predicted values ± 2*RMSEP".

Table 10: Statistical values for the simultaneous analysis of Brexpiprazole and Sertraline multivariate calibration methods (CLS, ILS, PCR &PLS)

S. No	Parameter	Brexpiprazole				Sertraline			
		CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS
1	Concentration range	1-7 mcg/ml				20-140 mcg/ml			
2	Spectral region(nm)	200-400 nm				200-400 nm			
Cross-validation result									
3	Optimum number of factors	-	-	7	7	-	-	7	7
4	slope	0.999	0.985	1.020	0.994	1.004	1.003	0.993	1.002
5	intercept	0.058	0.128	0.066	0.008	0.366	0.938	0.673	0.609
6	R2	0.999	0.999	0.999	0.998	0.999	0.999	0.999	0.999
7	RMSE-CV(mcg/ml)	-	-	0.876	0.251	-	-	3.127	5.762
8	RMSEC	0.376	0.187	0.763	0.925	2.542	1.652	1.982	1.092
9	RMSEP	0.983	0.328	0.0342	0.0021	0.734	0.629	0.0035	0.0087
10	SEC	0.276	0.659	-	-	0.176	0.239	-	-
Validation Result									
11	Slope	1.982	2.103	1.527	0.926	2.763	3.872	1.538	1.637
12	C.I. of slope	1.587 2.438	1.782 2.467	1.183 1.827	0.375 1.149	2.273 3.161	3.528 4.192	1.328 1.826	1.283 1.926
13	intercept	0.926	0.621	0.187	0.021	0.523	0.183	0.127	0.062

14	C.I. of intercept	0.815 1.527	0.492 0.927	0.092 0.218	0.002 0.097	0.396 0.819	0.078 0.428	0.098 0.391	0.009 0.106
15	Standard error	0.326	0.217	0.023	0.017	0.229	0.128	0.029	0.018
16	R2	0.997	0.998	0.999	0.999	0.996	0.997	0.999	0.999
17	RMSEC	2.882	1.328	0.872	0.934	3.215	1.762	0.762	0.918

Here,
RMSECV= Root Mean Square Error of Cross-Validation
RMSEC= Root Mean Square Error of Calibration
RMSEP= Root Mean Square Error of Prediction
SEC= Standard Error of Calibration
C.I.= Confidence Interval

Applicability of Proposed Uv Methods

Preparation of Laboratory sample (synthetic mixture)

Laboratory sample was prepared using the excipient mentioned in the literature [17]. The ingredient used to prepare laboratory sample are shown in Table 11

250 mg of prepared synthetic mixture was accurately weighed and transferred to a 100 ml volumetric flask. 50 ml methanol was added and sample was sonicated for 5 min. Finally volume was made upto the mark with methanol and filtered through whatman filter paper 41. Suitable aliquots were withdrawn and analyzed by all three different spectrophotometric methods as explained above. Results for the assay of laboratory sample are discussed in Table 12. Analysis was performed by taking six replicate samples for each (n=6).

Table 11: Formula for the Laboratory sample

Sr.no.	Ingredient	Quantity(mg)
1	Brexpiprazole	1
2	Sertraline	20
3	Sucrose	50
4	Starch	80
5	MCC	60
6	PEG	24
7	Talc	7
8	Magnesium stearate	8
Total		250 mg

Table 12: Results of simultaneous estimation of Brex and Ser in synthetic mixture by three methods (SD=Standard deviation)

S. No	Method	% Assay	
		BREX±SD	SER±SD
1	A	98.02±0.00231	101.01±0.0431
2	B	100.45±0.00041	99.02±0.00076
3	C	101.98±0.0681	98.95±0.00312

Applicability of the chemometrics methods [18]

All four methods were successfully applied for the estimation of Brexpiprazole and Sertraline in simulated mixture. Results are tabulated below:-Table-13

Table 13: Analysis of synthetic mixture

S.No	Method	Brexpiprazole*	Sertraline*
1	CLS	101.527 ±0.0342	99.73 ± 0.0329
2	ILS	98.21 ± 0.8293	100.72 ± 1.428
3	PCR	100.25 ± 0.0039	99.36 ± 0.0538
4	PLS	101.73 ± 0.0273	101.49 ± 0.1737

*Average ± SD (n=3) of three Experiment

Stastical analysis of developed uv methods [19-20]

The analysis done six times by each method (Count-6). Data analysis was done using Microsoft Excel for Mac Version16.34. MINITAB for Mac version 19.2020.1.0 was used for applying various statistical tests. Results of ANOVA for Brexpiprazole and Sertraline are shown in table 14 and table 15 respectively. Results of post-hoc analysis using Tukey‘s multiple comparison test for Brexpiprazole are depicted in Table 16 and Figure 13.

Table 14: ANOVA for comparison of different methods for Brexpiprazole

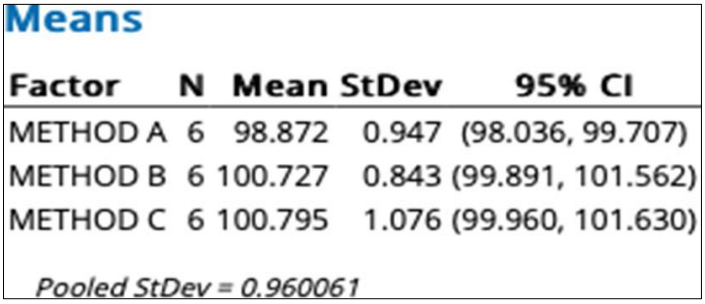
Summary				
Groups	***Count***	***Sum***	***Average***	***Variance***
Column 1	6	593.23	98.8716667	0.89593667
Column 2	6	604.36	100.726667	0.71074667
Column 3	6	604.77	100.795	1.15847
ANOVA				
Source of Variation	*SS*	*df*	*MS*	*F*
Between Groups	14.2898111	2	7.14490556	7.75172805
Within Groups	13.8257667	15	0.92171778	P
Total	28.1155778	17		0.004876

Table 15: ANOVA for comparison of different methods for Sertraline

Summary				
Groups	***Count***	***Sum***	***Average***	***Variance***
Column 1	6	607.34	101.223333	0.24462667
Column 2	6	597.84	99.64	0.63472
Column 3	6	593.67	98.945	1.59743
ANOVA				
Source of Variation	*SS*	*df*	*MS*	*F*
Between Groups	16.3615444	2	8.18077222	3.23897443
Within Groups	12.3838833	15	0.82559222	P
Total	28.7454278	17		0.089196

Table 16: Tukey Simultaneous Tests for Differences of Means for Brexpiprazole

Difference of Levels	**Difference of Means**	**SE of Difference**	**95% CI**	**T-Value**	**Adjusted P-Value**
METHOD B - METHOD A	1.855	0.554	(0.417, 3.293)	3.35	0.012
METHOD C - METHOD A	1.923	0.554	(0.485, 3.362)	3.47	0.009
METHOD C - METHOD B	0.068	0.554	(-1.370, 1.507)	0.12	0.992



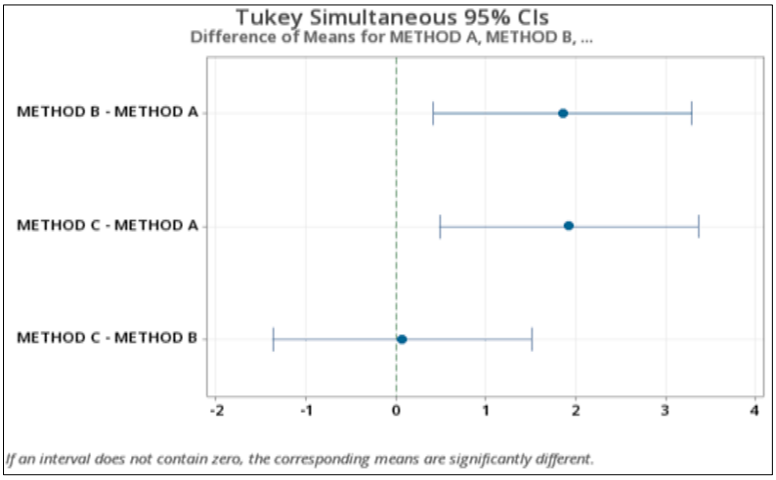


Fig 13: 95% CI of Tukey's method for Brexpiprazole

Conclusion

The proposed validated three spectrophotometric methods were simple, rapid, accurate, precise and Inexpensive. The sample recovery for all three methods was in good agreement with their respective label claims. Which also suggest non-interference of formulation additives in its estimation. Hence, the developed methods could be successfully applied for simultaneous estimation of Brexpiprazole and Sertraline in their fixed dose combination.

The main goal of the proposed work is to develop and

validate the novel chemometrics-assisted algorithm for the simultaneous determination of Brexpiprazole and Sertraline in pharmaceuticals via chemometrics-assisted spectrophotometry method. According to the obtained data, four different chemometrics algorithm exhibited good accuracy and their correlation matrixes showed that there is no difference between each algorithm. Thus, each of them could be confidently used in the simultaneous determination of those pharmaceuticals. These proposed method presents a good alternative to chromatographic separations in routine quality control samples without using mobile phase or any other separation apparatus. Generally, chemometrics methods are very convenient techniques for the simultaneous analysis of multiple compounds in which the overlap of the spectra of the active compounds creates an interference that makes it impossible to determine the concentrations of each compound via classical linear regression equations. Correlation matrix confirmed that each method has a very small difference and the prediction power of PLS and PCR is relatively better. Another advantage of the proposed method is that all analysis was performed neither derivatization nor ratio spectra modes which are expensive and time-consuming steps. Besides, the simplicity of the chemometric calibration methods comes from the ability to evaluate a huge amount of samples in a short time as accurately and precisely in comparison with chromatographic methods. The obtained results demonstrated that the proposed spectrophotometric method can be applicable as a possible alternative method for the simultaneous determination of Brexpiprazole and Sertraline in the routine quality control analysis of pharmaceutical industries.

Statistical analysis of all the three methods were done. It can be seen that P-value for Sertraline was greater than α=0.05 and observed F value was lower than $F_{critical}$ values, hence there was no significant difference between all three methods for Sertraline, But for Brexpiprazole there was a statistically significant difference as P-value for Brexpiprazole was less than α=0.05 and observed F value was higher than $F_{critical}$ values. Therefore, Post-hoc analysis using multiple comparisons by Tukey‘s test was performed for Brexpiprazole. This revealed that Method A (Simultaneous equation method [vierdot‘s method]) was significantly different from other method.

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