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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND LANSOPRAZOLE IN BULK AND LABORATORY SAMPLE BY DIFFERENT UV SPECTROPHOTOMETRIC TECHNIQUES

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Keywords:

Aspirin, Lansoprazole, First Derivative Spectrophotometry, Multicomponent Quantitation mode, Validation

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ABSTRACT: Four simple, rapid, inexpensive, precise and accurate UV spectrophotometric methods have been developed for simultaneous estimation of Aspirin (ASP) and Lansoprazole (LANSO). Method A was Simultaneous equation method (Vierodt's method) which applies measurement of absorptivities at two wavelengths, 276.00 nm, (λmax of Aspirin) and 284.00 nm, (λmax of Lansoprazole) in zero order spectra. The concentrations were calculated from the derived equations. Method B was based on zero crossing first Derivative (D^1) spectrophotometry where Aspirin showed zero crossing point at 303nm and Lansoprazole showed zero crossing point at 244.5nm.Method C was Dual wavelength technique, in which absorbance difference between two points on the mixture was measured where difference for one drug is zero and amplitude of other drug was directly proportional to the concentration. Analytical wavelengths for Aspirin were 262nm and 295.7nm; while for Lansoprazole 270nm and 282.5nm were selected. Method D was based on Multicomponent mode technique, in which sampling wavelengths selected were 276 and 284 nm. Linearity for Aspirin was between 26-130 µg/mL and Lansoprazole was 4-20 µg/mL. Accuracy of all above methods was determined by recovery studies and % recovery was estimated between 97 to 103%. 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 Aspirin and Lansoprazole in laboratory sample. Statistical Analysis was done to compare all the four developed spectrophotometric methods.

INTRODUCTION: Chemically, Aspirin is 2-(acetyloxy) benzoic acid (Figure 1) which is one of the widely used Non-steroidal anti-inflammatory category drug. Aspirin is official in Indian Pharmacopeia, British Pharmacopoeia and United States Pharmacopoeia which describe acid-base titration for aspirin [1, 2, 3].

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Lansoprazole as shown in Figure 2 is chemically 2-({[3-methyl-4-(2, 2, 2-trifluoroethoxy) pyridin-2-yl] methane} sulfinyl)-1H-1, 3-benzodiazole. It is a potent Proton Pump Inhibitor used in acidity, ulcers, Gastro-esophageal Reflux Disease, etc. BP 2009 includes potentiometric estimation of Lansoprazole while USP 2007 and IP 2014 include a Liquid chromatographic method for assay of Lansoprazole [4].

Aspirin in low dose acts as a platelet-aggregation inhibitor. The number of patients taking low-dose aspirin for prevention of the recurrence of cerebral infarction or myocardial infarction is increasing.

But administration of low-dose aspirin may cause gastric or duodenal ulcers, thus preventing the onset of ulcers in that patient population is important. Takeda Pharmaceuticals launched Takelda® combination tablets, a fixed-dose combination ("FDC") of low-dose aspirin (ASP) with Lansoprazole (LANSO), a proton pump inhibitor[5]. Such a combination is useful for risk reduction of thrombosis and embolism in patients with a history of gastric ulcer or duodenal ulcer, which have had angina, myocardial infarction, coronary artery bypass grafting or percutaneous transluminal coronary angioplasty.

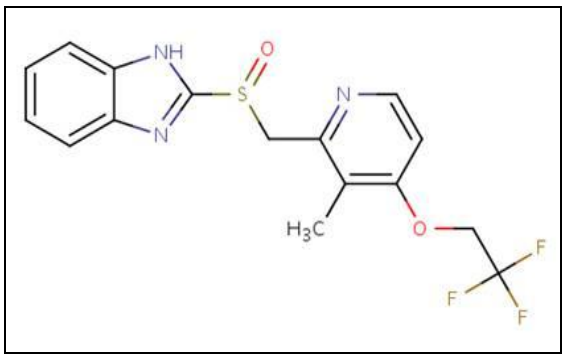

FIG. 1: STRUCTURE OF LANSOPRAZOLE

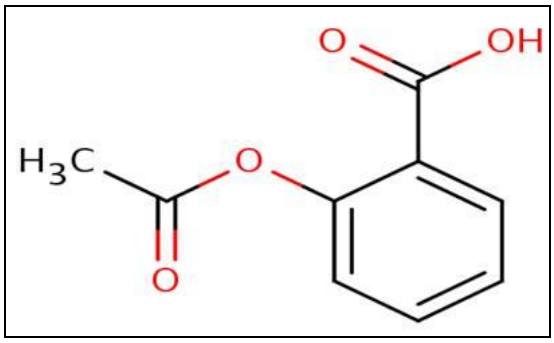

FIG. 2: STRUCTURE OF ASPIRIN

Survey of literature revealed that number of methods has been reported for the individual analysis of Aspirin and Lansoprazole by UV spectrophotometric and RP-HPLC method. Sunil Singh et al have reported UV spectrophotometric method for simultaneous estimation of Aspirin, Clopidogrel and Atorvastatin[6]. Shahabuddin N. Alvi et al have reported Derivative spectroscopic methods for combination of Aspirin with Prasugrel[7]. Chaudhary N. et al. reported a UV spectrophotometric method for simultaneous estimation of Lansoprazole and Naproxen[8].

Several UV spectrophotometric, RP-HPLC, HPTLC, UPHPLC, GC and Spectrofluorimetric methods have been reported for Aspirin and Lansoprazole individually or in combination with other drugs [9-16].

However, to best of our knowledge, there is no reported UV-spectrophotometric method available for simultaneous estimation of Aspirin (ASP) and Lansoprazole (LANSO). The aim of the present work was to develop easy, economic, accurate, specific and precise spectrophotometric methods for simultaneous estimation of Aspirin and Lansoprazole in bulk and synthetic mixture, and validation of the newly developed analytical methods.

MATERIALS AND METHODS:

Apparatus 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 were carried out in 1cm 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 2010 and Graphpad Prism v 5.0.0 software.

Materials:

Gift samples of standards- Aspirin and Lansoprazole were provided by Sun Pharmaceuticals advance Research center, Vadodara and Zydus Cadila, Ahmedabad respectively.

Reagents and Chemicals:

Methanol analytical reagent grade (Spectrochem Pvt. Ltd, Mumbai, India) was used as the solvent and diluent.

Year and site of Experimentation:

The experiment was performed at Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, Pharmacy Department, G. H. Patel Building, Donor's Plaza, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara – 390002, Gujarat, India in the year 2014.

Preparation of Stock Solution:

25mg of ASP and LANSO were separately weighed accurately and transferred into two 25 mL

volumetric flasks. Methanol was added into the volumetric flasks to dissolve the standards and finally volume was made upto the mark with Methanol to obtain standard solutions of ASP (1000µg/mL) and LANSO (1000µg/mL) respectively.

Preparation of Working Standard Solutions: From the stock above solution of Lansoprazole, working standard solution of LANSO (100 µg/mL) was prepared by transferring 5 mL aliquot to 50 mL volumetric flask and making up the volume with methanol.

Preparation of Calibration Curve of Standard ASP and LANSO:

From standard stock solution of ASP (1000µg/mL), aliquots of 0.26mL, 0.52mL, 0.78mL, 1.04mL and 1.3mL were withdrawn and transferred to 10mL volumetric flasks. Volume was made upto the mark with Methanol to produce 26µg/mL, 52µg/mL, 78µg/mL, 104µg/mL and 130µg/mL of ASP respectively. From the working standard solution of LANSO (100µg/mL), aliquots of 0.4mL, 0.8mL, 1.2mL, 1.6mL and 2mL were transferred to 10mL volumetric flasks and volume was made upto the mark with Methanol to produce 4µg/mL, 8µg/mL, 12µg/mL, 16µg/mL and 20µg/mL of LANSO respectively. Mixed standard solutions of ASP and LANSO were prepared in ratio of 6.5:1 as present in the marketed formulation.

Preparation of Laboratory Sample Solution: The Combined Dosage Formulation of ASP and LANSO is TAKELDA® combination tablets launched by Takeda Pharmaceuticals, which is not yet available in Indian market, so a laboratory sample was prepared using the excipients mentioned in the literature[17, 18]. The ingredients used to prepare laboratory sample are shown in Table 1. 100mg of prepared synthetic mixture was accurately weighed and transferred to a 100mL volumetric flask. 50mL methanol was added and sample was sonicated for 5 minutes. Finally volume was made upto the mark with methanol and filtered through Whatman Filter Paper 41. Suitable aliquots were withdrawn to obtain the final solutions in the concentration range from 26 to 130 µg/mL of ASP and 4 to 20 µg/mL of LANSO for Recovery studies and assay of synthetic mixture.

TABLE 1: FORMULA FOR THE LABORATORY SAMPLE

Sr. No.	Ingredient	Quantity (mg)
1	Lansoprazole	15
2	Aspirin	100
3	Sucrose	30
4	Starch	70
5	MCC (Microcrystalline cellulose)	50
6	PEG(polyethylene glycol)	23
7	Talc	5
8	Magnesium stearate	7
	TOTAL	300

Stability of Solutions:

Stock Solutions of ASP (1000 µg/mL) and LANSO (1000 µg/mL) were prepared in methanol and stored at room temperature for 24 hours. Absorbances of solutions were noted at 0hr, 2hr, 3hr, 4hr, 6hr, 12hr and 24hr time intervals. Solution of LANSO was found to be stable for 24 hours while fresh solution of ASP was prepared every four hours.

METHOD A: SIMULTANEOUS EQUATION METHOD (VIERODT'S METHOD):

If a sample containing two absorbing drugs (X and Y) each of which absorbs at λmax of other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) provided that certain criteria apply[19]. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2, the absorbance of the mixture is the sum of the individual absorbance of X and Y. As mentioned earlier, dilutions for ASP and LANSO were prepared in concentration range of 26-130 µg/mL and 4-20 µg/mL respectively were prepared and scanned between 200 to 400 nm.

The zero order overlain spectra of ASP and LANSO are shown in Figure 3. Calibration curve of absorbance versus concentration were prepared. The calibration curves were found to be linear in the concentration range under study as depicted in Figure 4 and Figure 5. The analytical wavelength for ASP and LANSO were 276nm and 284nm respectively.

Absorptivity of ASP and LANSO were calculated at both the wavelengths. The concentrations of ASP

and LANSO can be calculated from following equations:

Cx(ASP)=(A2 ay1 – A1 ay2) / (ax2 ay1 – ax1 ay2)

Cy (LANSO)=(A1 ax2–A2 ax1)/(ax2 ay1–ax1 ay2)

Where; Cx & Cy are concentrations of ASP and LANSO respectively in gm/100 ml in the sample solution. A1 & A2 are the absorbances of the mixture at 276.00 nm & 284.00 nm respectively; aX1 and aX2 = Absorptivity of ASP at 276.00 nm and 284.00 nm; aY1 and aY2 = Absorptivity of LANSO at 276.00nm and 284.00 nm respectively.

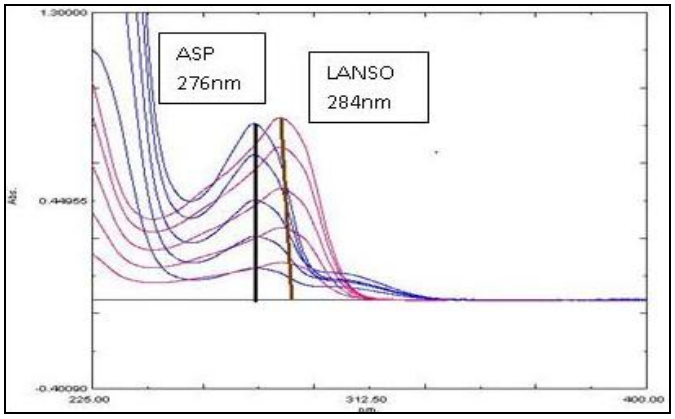

FIG. 3: ZERO ORDER OVERLAIN SPECTRA (Absorbance vs. Wavelength) OF ASP (26-10 µg/ml, blue) AND LANSO (4-20 µg/ml, pink).

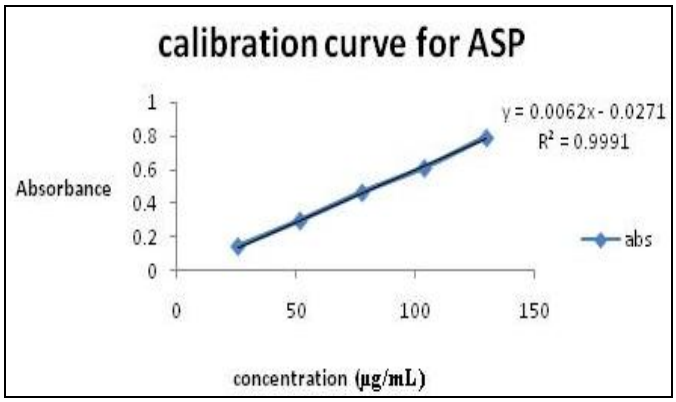

FIG.4: CALIBRATION CURVE OF ASP AT 276nm

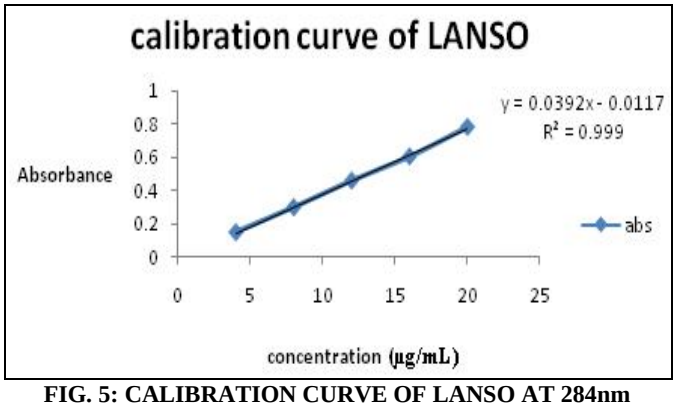

FIG. 5: CALIBRATION CURVE OF LANSO AT 284nm

METHOD B: FIRST DERIVATIVE ZERO CROSSING POINT METHOD (ZCP): Derivative spectroscopy on the basis of zero-crossing measurements involves measurement 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[19]. The absorption spectra of the solutions of ASP and LANSO were recorded in the range of 200 nm to 400 nm and were stored in the memory of the instrument and transformed to first derivative with $\Delta\lambda$ = 8nm and scaling factor = 1. **Fig. 6** shows that at 303 nm, ASP shows zero crossing point and hence LANSO can be determined while at 244.5 nm, LANSO shows zero crossing point and hence ASP can be determined.

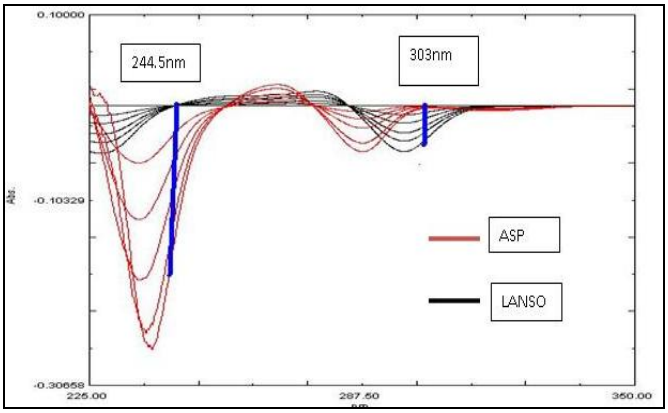

FIG.6: OVERLAIN FIRST DERIVATIVE SPECTRA OF ASP (red) AND LANSO (black) WITH THEIR ZERO CROSSING POINTS.

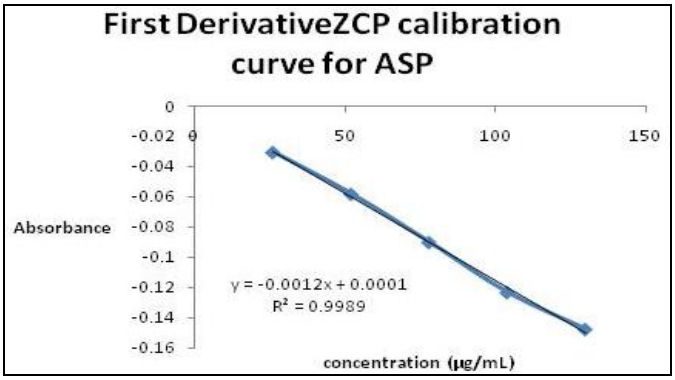

FIG.7: CALIBRATION GRAPH OF FIRST DERIVATIVE ASP at 244.5nm

Calibration curves were constructed with five different concentrations in the range between 26-130 µg/mL and 4-20 µg/mL for ASP and LANSO respectively. Each concentration was analyzed thrice. The concentration of the drug present in the

Laboratory Sample solution was determined against the calibration curve.**Fig.7** and **Fig. 8** show calibration graphs of ASP and LANSO at 244.5nm and 303nm respectively.

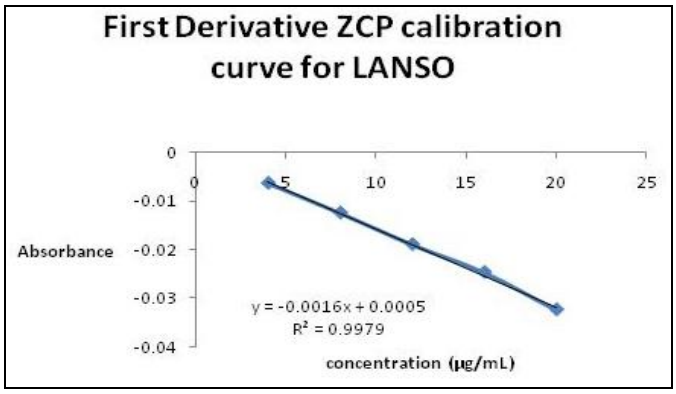

FIG. 8: CALIBRATION GRAPH OF FIRST DERIVATIVE LANSO AT 303nm

METHOD C: DUAL WAVELENGTH METHOD:

The principle for dual wavelength method is that the absorbance difference between two points on the mixture where difference for one drug is zero and spectra is directly proportional to the concentration of the component of interest [20]. Solutions with concentrations 26 to 130 µg/mL (for ASP) and 4-20 µg/mL (for LANSO) were prepared and scanned between 200 to 400 nm. ASP showed identical absorbance at 270nm and 282.5nm, so difference of absorbances of ASP at 270nm and 282.5nm were zero at which LANSO exhibited linearity **(Fig.9).**

Similarly, LANSO showed identical absorbance at 262nm and 295.7nm, hence difference of absorbances of LANSO at 262nm and 295.7nm were found to be zero; at which ASP was found to be linear. Results of these studies are explained in **Table 2**. The analytical wavelengths selected in zero order overlain spectra for ASP and LANSO for Dual Wavelength method are shown in **Fig. 9**. **Fig.10** and **11** show the calibration graphs of ASP and LANSO respectively at the mentioned wavelengths.

TABLE 2: DETERMINATION OF ASPIRIN AND LANSOPRAZOLE USING DUAL WAVELENGTH METHOD

AT 262nm- 295.7nm				AT 270nm- 282.5nm			
Conc. Of Aspirin (µg/mL)	**Absorbance of ASP**	**Conc. Of LANSO (µg/mL)**	**Absorbance of LANSO**	**Conc. Of ASP(µg/mL)**	**Absorbance of ASP**	**Conc. Of LANSO (µg/mL)**	**Absorbance of LANSO**
26	0.0597	4	0.000	26	0.001	4	0.0382
52	0.1364	8	0.001	52	0.000	8	0.0794
78	0.2287	12	0.000	78	0.001	12	0.1227
104	0.3261	16	0.000	104	0.000	16	0.1671
130	0.4291	20	0.000	130	0.000	20	0.2149

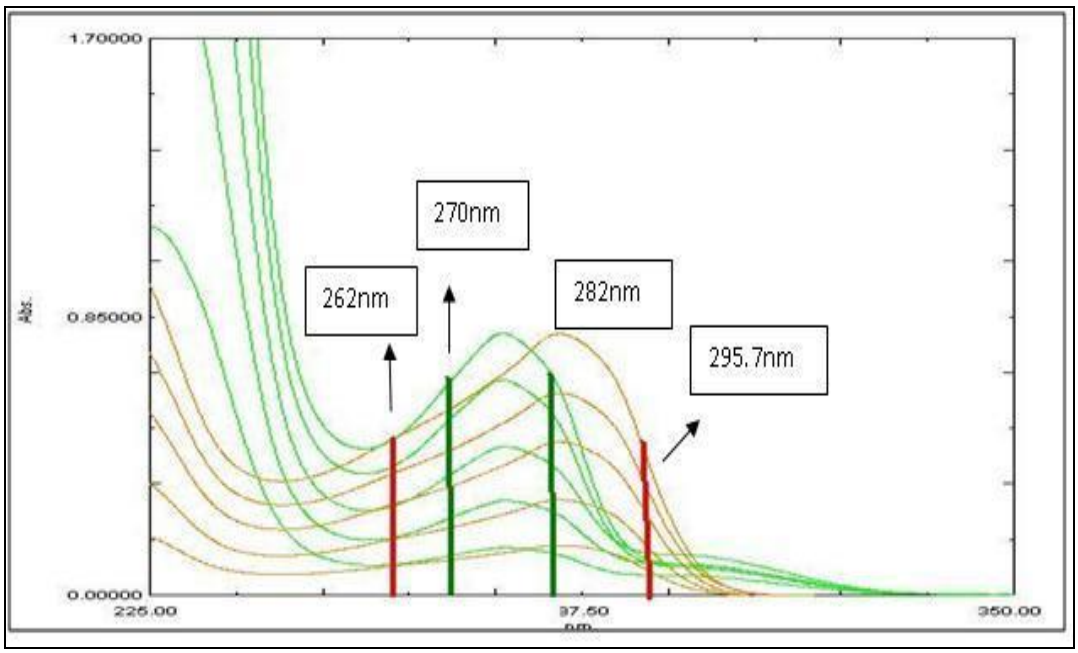

FIG.9: ANALYTICAL WAVELENGTH FOR ASP IS 262 AND 295.7 nm AND FOR LANSO IS 270 AND 282.5 nm.

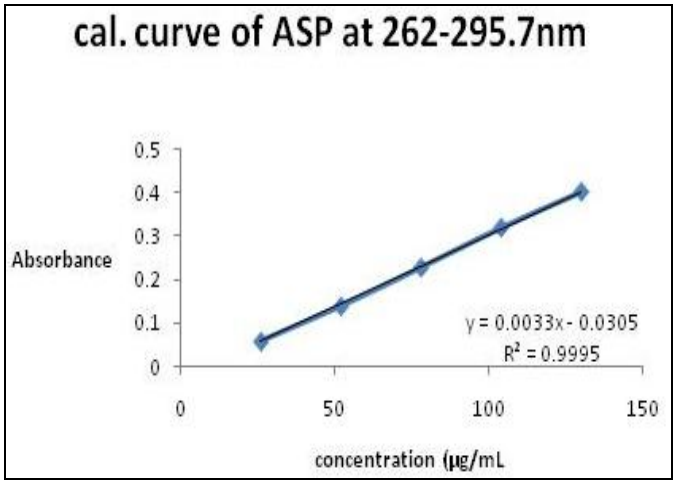

FIG.10: CALIBRATION GRAPH OF ASP AT 262nm-295.7nm

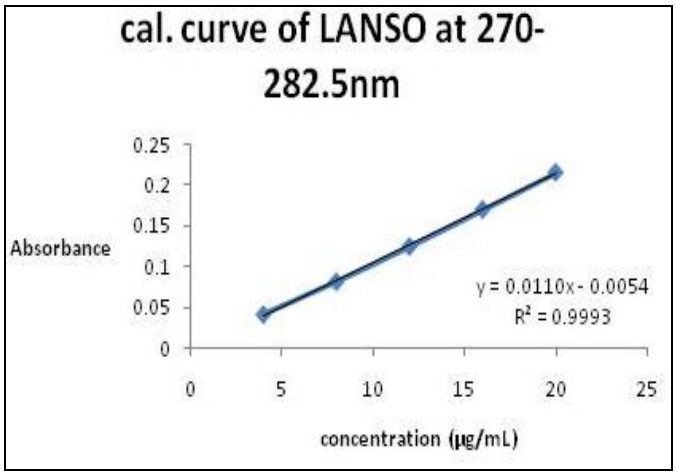

FIG.11: CALIBRATION GRAPH OF LANSO AT 270nm-282.5nm

METHOD D: MULTICOMPONENT QUANTITATION MODE ANALYSIS:

For this method 276.0 nm (λmax of ASP) and 284.0 nm (λmax of LANSO) were selected as two sampling wavelengths for ASP and LANSO and Multicomponent mode of spectrophotometer was used. The data from these scans was used to determine concentrations of two drugs in the prepared synthetic mixture sample solutions **(Fig. 12)**.

The overlain spectra of five standard binary mixtures (26:4, 52:8, 78:12, 104:16, and 130:20) were employed to determine the concentration of drug in sample solution by analysis of spectral data of sample solutions with reference to mixture standards[21]. **Fig.13** and **14** display the calibration graphs of ASP and LANSO by Multicomponent Quantitation Mode Analysis.

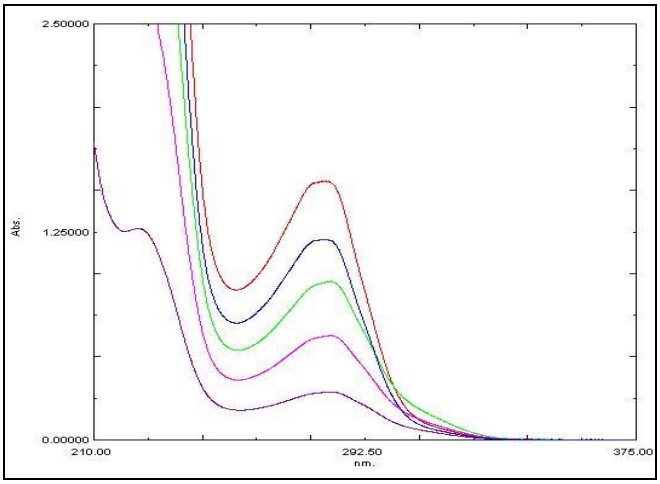

FIG.12: MULTICOMPONENT MODE SPECTRA FOR ASP AND LANSO

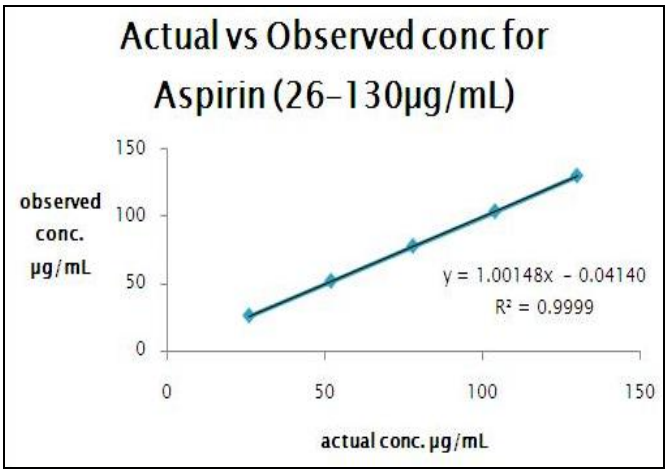

FIG. 13: CALIBRATION GRAPH OF ASP BY MULTICOMPONENT MODE METHOD

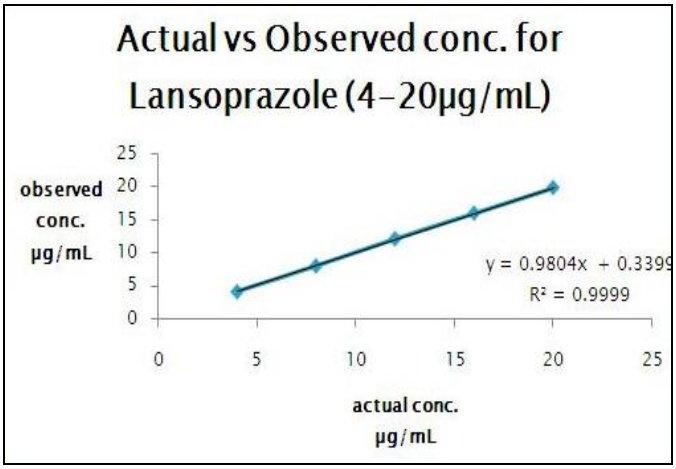

FIG. 14: CALIBRATION GRAPH OF LANSO BY MULTICOMPONENT MODE METHOD

Application of the Proposed Methods for the Determination of Aspirin and Lansoprazole in Laboratory Sample:

100mg of prepared synthetic mixture was accurately weighed and transferred to a 100mL volumetric flask. 50mL methanol was added and sample was sonicated for 5 minutes. Finally volume was made upto the mark with methanol and filtered through Whatman Filter Paper 41. Suitable aliquots were withdrawn and analyzed by Method A, Method B, Method C and Method D as explained above. Results for the assay of laboratory sample are discussed in **Table 3.** Analysis was performed by taking six replicate samples for each (n=6).

TABLE 3: RESULTS OF SIMULTANEOUS ESTIMATION OF ASP AND LANSO IN SYNTHETIC MIXTURE BY METHODS-A, B, C AND D. (SD= Standard Deviation)

Synthetic Mixture Labelled Claim : LANSO: ASP= 15mg:100mg			
Sr No.	**Method**	**% Assay**	
		ASP ± SD	**LANSO ± SD**
A	Simultaneous eqn. (Vierodt's method)	100.365 ± 0.548	100.105 ± 0.364
B	First derivative ZCP method	98.815 ± 0.703	99.863 ± 0.324
C	Dual wavelength method	101.27 ± 0.743	99.56 ± 0.479
D	Multi-component Quantitation Mode	100.783 ± 0.967	100.3195 ± 0.505

VALIDATION OF THE DEVELOPED METHODS [22]:
Developed spectrophotometric methods for the simultaneous estimation of ASP and LANSO were validated according to ICH Q2 (R1) guidelines and data complying with the standards were obtained.

Linearity and Sensitivity: The linearity of method was evaluated thrice by analyzing five concentration 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 4** reveals the Summary of Validation parameters of ASP and LANSO by the four developed methods.

TABLE 4: SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHODS

Parameter	**Simultaneous eqn method (A)**		**First derivative ZCP method (B)**		**Dual wavelength method (C)**		**Multi-component Quant. Mode (D)**	
	ASP	**LANSO**	**ASP**	**LANSO**	**ASP**	**LANSO**	**ASP**	**LANSO**
Analytical wavelength (nm)	276nm	284nm	244.5 nm	303nm	262nm, 295.7 nm	270nm, 282.5nm	276nm, 284 nm	
Beer's range (µg/ml)	26- 130 µg/mL	4-20 µg/mL	26- 130 µg/mL	4-20 µg/mL	26- 130 µg/mL	4-20 µg/mL	26- 130 µg/mL	4-20 µg/mL
Slope	0.0062	0.0392	-0.0012	-0.0016	0.0110	0.0033	1.0015	0.9804
Intercept	-0.0271	-0.0117	0.0001	0.005	-0.0054	-0.0305	-0.0414	0.3399
Correlation coefficient	0.9991	0.999	0.9989	0.9979	0.9993	0.9995	0.9999	0.9999
Standard Deviation of Intercepts	0.0023	0.0017	0.0002	0.00309	0.00113	0.00967	0.0955	0.1199
Limit Of Detection (LOD)	1.2602	0.1478	0.4626	0.62364	8.03441	0.34114	0.3135	0.3987
Limit Of Quantitation (LOQ)	3.8188	0.4479	1.4018	1.88982	21.3770	1.0337	0.9501	1.2083

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). Results are expressed in terms of standard deviation and %Relative standard Deviation (%RSD) as shown in **Table 5.** It can be observed that the %RSD was less than 2 for all the four proposed methods.

TABLE 5: RESULTS OF INTRADAY AND INTERDAY PRECISION FOR ASP AND LANSO BY THE PROPOSED FOUR METHODS

Parameter	Simultaneous eqn method (A)		First derivative ZCP method (B)		Dual wavelength method (C)		Multi-component Quant. Mode (D)	
	ASP	LANSO	ASP	LANSO	ASP	LANSO	ASP	LANSO
Intraday precision (SD)	0.000563	0.00069	0.000104	0.0000652	0.00059	0.000914	0.2559	0.0259
Intraday precision (%RSD)	0.17%	0.1613 %	0.12002 %	0.4011 %	0.40125 %	0.89177 %	0.2876 %	0.1929 %
Interday precision (SD)	0.003809	0.00548	0.000268	0.000246	0.00292	0.00139	0.5038	0.08354
Interday precision (%RSD)	0.739 %	1.101 %	0. 3693 %	1.1401 %	1.3445%	1.334 %	0.7954 %	0.857 %

Accuracy[22]: To check the Accuracy of different methods, Recovery studies were carried out from pre-analyzed sample at three deferent level of standard addition 80%, 100% and 120%. Results of Recovery studies are shown in **Table 6**. For each of the method explained above, %Recovery was the average of three determinations at each standard addition level. %Recovery for different methods was found to be between 97%-103% which prove that all the methods were accurate.

TABLE 6: RESULTS OF ACCURACY (% recovery) FOR ASP AND LANSO BY THE PROPOSED FOUR METHODS (SD=Standard Deviation)

Method	% spiking	Conc. ACTUAL µg/ml		Conc. ADDED µg/ml		Conc. RECOVERED µg/ml		% RECOVERY ± SD	
		ASP	LANSO	ASP	LANSO	ASP	LANSO	ASP	LANSO
Simultaneous	80	56.15	8.5	44.9	6.8	43.638	6.72	97.189 ±1.126	98.824 ±0.320
equation	100	56.15	8.5	56.15	8.5	56.726	8.443	101.026 ±0.863	99.329 ±0.186
method	120	56.15	8.5	67.4	10.2	68.43	10.402	101.528 ±0..953	101.98 ±0.846
First	80	56.15	8.5	44.9	6.8	44.991	6.6433	100.157 ±0.443	97.699 ±1.173
Derivative	100	56.15	8.5	56.15	8.5	56.213	8.5812	100.112 ±0.167	100.955 ±0.819
ZCP method	120	56.15	8.5	67.4	10.2	67.443	10.423	100.094 ±0.153	102.183 ±0.776
Dual	80	56.15	8.5	44.9	6.8	44.368	6.8067	98.77 ±0.4355	100.09 ±1.155
wavelength	100	56.15	8.5	56.15	8.5	57.139	8.607	101.759±0.3214	101.3451 ±1.19
method	120	56.15	8.5	67.4	10.2	66.941	10.132	99.3543 ±0.4596	99.32 ±1.453
Multi-	80	56.15	8.5	44.9	6.8	44.65	6.8057	99.36 ±0.217	100.576 ±0.931
component	100	56.15	8.5	56.15	8.5	56.48	8.616	100.586 ±0.0108	101.38 ±0.1705
Quant. Mode	120	56.15	8.5	67.4	10.2	67.347	10.209	99.95 ± 0.0136	100.092 ± 0.3075

Statistical Analysis:

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 larger) 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 four developed methods were subjected to ANOVA. The analysis was done 6 times by each method (count = 6).

Data analysis was done using Microsoft Excel 2010 and Graphpad Prism v 5.0.0 software. Results of ANOVA for ASP and LANSO are shown in **Table 7** and **Table 8** respectively. Results of Post-hoc analysis using Tukey's multiple comparison test for ASP are depicted in **Table 9**.

TABLE 7: ANOVA FOR COMPARISON OF DIFFERENT METHODS FOR ASPIRIN

ANOVA: Single Factor						
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Simultaneous equation method	6	602.19	100.365	0.30067		
First derivative ZCP	6	592.89	98.815	0.49395		
Dual wavelength	6	607.62	101.27	0.55216		
Multicomponent Quantitation Mode Analysis	6	603.696	100.616	0.940351		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	19.4743	3	6.491434	11.35297	0.000145	3.098391
Within Groups	11.43565	20	0.571783			
Total	30.90996	23				

TABLE 8: ANOVA FOR COMPARISON OF DIFFERENT METHODS FOR LANSOPRAZOLE

ANOVA: Single Factor						
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Simultaneous equation method	6	600.63	100.105	0.13235		
First derivative ZCP	6	599.18	99.86333	0.104867		
Dual wavelength	6	597.36	99.56	0.22976		
Multicomponent Quantitation Mode Analysis	6	600.737	100.1228	0.133486		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	1.247818	3	0.415939	2.770793	0.068274	3.098391
Within Groups	3.002312	20	0.150116			
Total	4.25013	23				

TABLE 9: RESULTS OF POST-HOC ANALYSIS USING TUKEY'S MULTIPLE COMPARISON TEST FOR ASPIRIN

ANOVA Table	**SS**	**df**	**MS**	
Treatment (between columns)	19.47	3	6.491	
Residual (within columns)	11.44	20	0.5718	
Total	30.91	23		
Tukey's Multiple Comparison Test	**Mean Diff.**	**95% CI of Mean diff**	**q value**	**Significant? P < 0.05?**
Method A vs. Method B	1.55	0.3282 to 2.772	5.021	Yes
Method A vs. Method C	-0.905	-2.127 to 0.3168	2.932	No
Method A vs. Method D	-0.251	-1.473 to 0.9708	0.8131	No
Method B vs. Method C	-2.455	-3.677 to -1.233	7.953	Yes
Method B vs. Method D	-1.801	-3.023 to -0.5792	5.834	Yes
Method C vs. Method D	0.654	-0.5678 to 1.876	2.119	No

RESULTS AND DISCUSSION: All the four developed methods were found to be linear with acceptable correlation coefficients as discussed above. Linearity range was exhibited from 26-130µg/mL for ASP and 4-20 µg/mL for LANSO at their respective selected wavelengths for the proposed methods. Analysis of prepared laboratory samples showed that % assay was in range of 98-102 %. All methods showed to have %RSD value less than 2 for intraday and interday precision. Intraday precision indicated the precision under same operating conditions at a short interval of time. The validity and reliability of all methods were assessed by recovery studies by spiking standards at different levels. The mean percentage recovery between 97-103 % reflected that the methods were sufficiently accurate.

The means of percentage recovery (%RSD) were found to be low values (less than 2) for all four

methods. This reveals that any small change in the concentration of drug solution could be accurately determined by the proposed methods. Statistical analysis of all the four methods was done. It can be seen that P-value for LANSO was greater than α (0.05) and observed F value was lower than $F_{critical}$ values, hence there was no significant difference between all four methods for LANSO. But, for ASP there was a statistically significant difference as P-value for ASP 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 ASP. This revealed that Method B (First Derivative ZCP) was significantly different from other methods.

CONCLUSION: The proposed validated Four Spectrophotometric methods were simple, rapid, accurate, precise and Inexpensive. The sample recovery for all four methods was in good agreement with their respective label claims, which suggested non-interference of formulation additives in its estimation. Hence, the developed methods could be successfully applied for simultaneous estimation of Aspirin and Lansoprazole in Fixed Dose combinations.

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