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Stability Indicating RP-HPLC method for simultaneous determination of Aspirin and Omeprazole in the Bulk drug and Synthetic mixture

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Abstract

In the present research work, a successful attempt was made for "Validated HPLC method development for the estimation of some drugs in marketed formulation" which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters o fsampling. The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. Proposed method was found to be linear in the range of 5-25 µg/ml aspirin and omeprazole with the correlation coefficient near to one respictively. The validation and the reliability of preposed method were assessed by recovery study. The recovery of added standards (80%, 100%, 120%) was ranging from 100.042±0.260 to 100.033±0.208, 99.944±0.428 and 100.08±0.641, 100.26±0.321 to 98.54±0.553 for aspirin and omeprazole respectively.

Key Words: Estimation, HPLC, Bulk drug

Introduction

Every year many new drugs and newer drug combinations enter the pharmaceutical area. bioanalytical methods for these new and first timer drugs are mostly confined only to the manufacturing company. However, availability of multiple analytical methods for the same drug/drug combinations in their formulations is always advantageous. Moreover, development of such methods helps in training the analysts for skillfully handling the sophisticated analytical instruments and the way for research approach. Reference literature and general survey reveals that similar work of development of bioanalytical methods for new drugs and their combinations introduced in the market is continuously underway in many academic institutions. The present work is also planned on similarlines.

There are numerous methods for the estimation of Aspirin in fix dosage form. (Gopalakrishnan et al., 2012) worked on analytical method development and validation of HPLC method for the determination of omeprazole in capsule dosage form. Hussein et al., 2016 worked on reversed phase HPLC assay for the determination of omeprazole in human plasma, Reddy et al., (2013) worked on simultaneous determination of aspirin and esomeprozole magnesium in combined tablets by validated ultra performance liquid chromatographic method.

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The non-availability of analytical methods till now for the concurrent analysis of single and multi-component formulations made it worthwhile to pursue the present research work. It was also planned to validate the developed methods as per ICH guidelines.

Hence they offer wide area for research activity with relatively minimum chances of exactly repetitive work. The pharmaceutical dosage forms are widely present with multiple active components i.e. in combined dosage forms. This has opened new task for analyst for simultaneous estimation of aspirin and ticagrelor in combined dosage forms. Therefore, in proposed project, to attempt to develop simple, accurate and precise method for analysis of drugs in the blood plasma and validatethem.

Experimental

Identification and Characterization of drugs

Solubility

Solubility of all three drugs was observed by dissolving them in different solvents.

Melting point- M.P. of the drug for aspirin and omeprazole 133-135 °C and 156-158 °C respectively found through Melting pointapparatus.

Determination of λ max ofDrugs

Standard solution (10g/ml) of pure, Aspirin and Omeprazole was prepared. The pure drug solution was scanned on UV spectrophotometer, and λmax determined.

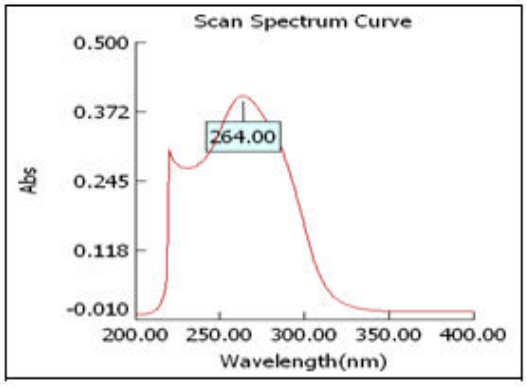

Figure 1: Determination of λmax of Aspirin

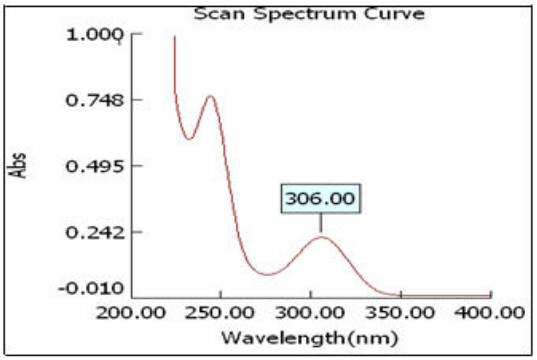

Figure 2: Determination of λmax of Omeprazole

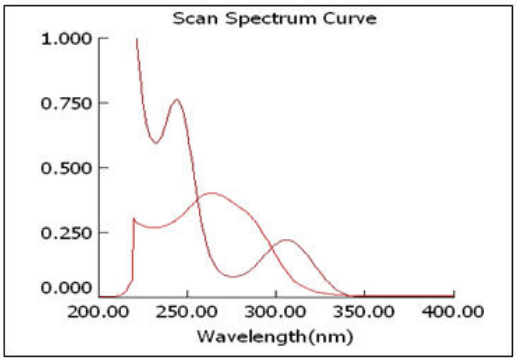

Figure 3: Overlain spectra of both the drugs

Preparation of mobile phase

1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH – 6 with OPA. Filtered through 0.45 filter paper.

Selection of diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials Acetonitrile was used as diluents.

Preparation of standard stock solution Accurately weighed 10 mg of aspirin and omeprazole was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of acetonitrile, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of aspirin and omeprazole was 200 µg/ml. (stock-A)

Preparation of Sub Stock Solution 5 ml of solution was taken from stock-A of aspirin transferred into 10 ml volumetric flask separately

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and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml(Stock-B).

Preparation of DifferentSolution

0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml of omeprazole alsoprepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 g/ml was prepared. All the solution were filtered through 0.2m membrane filter and injected, chromatograms were recorded at 275 nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Table 1: Linearity of aspirin

Std. Conc.	5	10	15	20	25
Rep-1	565.589	1110.256	1565.589	2154.589	2650.145
Rep-2	572.256	1125.565	1560.254	2150.478	2645.589
Rep-3	583.235	1116.658	1547.265	2165.589	2665.458
Mean	573.693	1117.493	1557.703	2156.885	2653.731
S.D.	8.910	7.689	9.425	7.813	10.409
% RSD	1.553	0.688	0.605	0.362	0.392

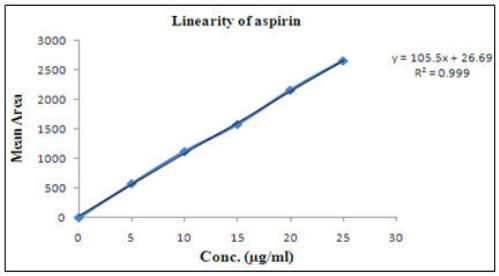

Figure 4: Calibration Curve of aspirin

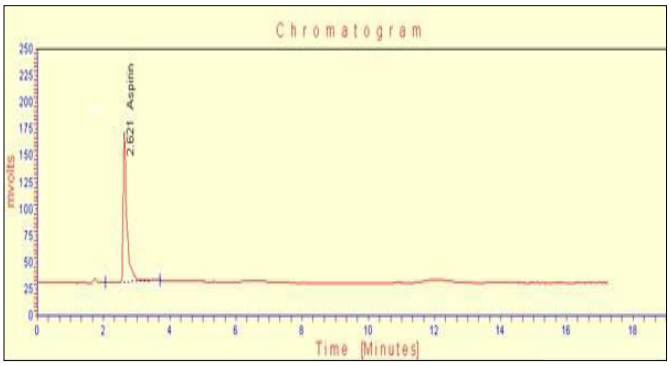

Figure 5: Chromatoram of aspirin

Table 2: Linearity of Omeprazole

Std. Conc.	5	10	15	20	25
Rep-1	825.565	1610.254	2425.658	3250.215	4025.658
Rep-2	830.254	1621.154	2430.145	3245.897	4030.145
Rep-3	832.145	1632.254	2436.658	3247.589	4032.215
Mean	829.321	1621.221	2430.820	3247.900	4029.339
S.D.	3.388	11.000	5.531	2.176	3.352
% RSD	0.408	0.679	0.228	0.067	0.083

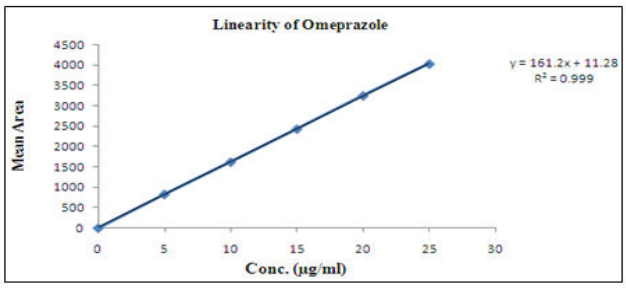

Figure 6: Calibration Curve of Omeprazole

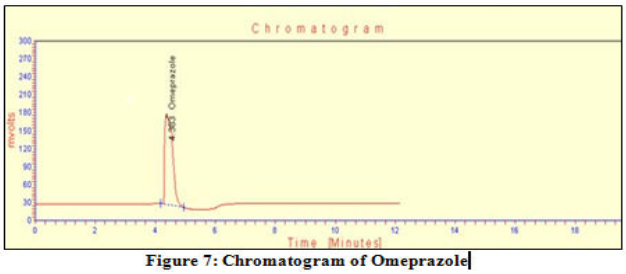

Figure 7: Chromatogram of Omeprazole

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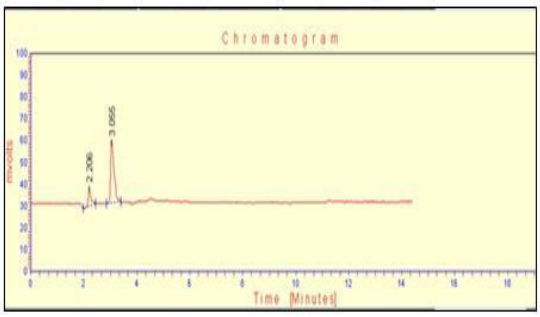

Figure 8: Chromatogram of aspirin and omeprazole

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Aspirin 10 µg/ml was injected separately. Peak report and column performance report were recorded for allchromatogram.

Table 3: System suitability parameters of aspirin

System suitability Parameter	AUC	No. of theoretical plates	Tailing factor
Rep-1	1250.256	3245	1.10
Rep-2	1252.256	3265	1.11
Rep-3	1245.874	3214	1.10
Rep-4	1242.654	3215	1.10
Rep-5	1242.145	3245	1.12
Rep-6	1244.456	3213	1.10
Mean	1246.274	3232.833	1.11
S.D.	3.769589	21.894	0.159

Table 4: System suitability parameters of Omeprazole

System suitability Parameter	AUC	No. of theoretical plates	Tailing factor
Rep-1	1610.254	3050	1.32
Rep-2	1621.154	3045	1.35
Rep-3	1632.254	3045	1.21
Rep-4	1640.578	3047	1.45
Rep-5	1638.987	3050	1.65
Rep-6	1650.547	3056	1.54
Mean	1632.296	3048.833	1.420
S.D.	14.536	4.167	0.159

Laboratory Sample Analysis

The In-house tablet formulation of aspirin is available in the strength of 81mg and omeprazole 40mg. Based on this different standard solutions were prepared for quantitative analysis, which gives satisfactory results. Stock solution was prepared in the same manner. Further dilutions were made to prepare the mixed standard of desiredconcentration.

Table 5: Laboratory sample analyses

	Label claim	
	Aspirin (mg)	**Omeprazole (mg)**
Amount present	81	40
Amount found	80.98±0.45	39.95±0.25
% Assay	99.98±0.56	99.88±0.12

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Table 6.: Response ration data for linearity of Aspirin

Replicates	Concentration (g/ml)	Mean AUC	Response Ratio
Rep-1	5	573.693	114.739
Rep-2	10	1117.493	111.749
Rep-3	15	1557.703	103.847
Rep-4	20	2156.885	107.844
Rep-5	25	2653.731	106.149
SD			4.371
%RSD			4.015

Table 7: Response ration data for linearity of Omeprazole

Replicates	Concentration (g/ml)	Mean AUC	Response Ratio
Rep-1	5	829.321	165.864
Rep-2	10	1621.221	162.122
Rep-3	15	2430.820	162.055
Rep-4	20	3247.900	162.395
Rep-5	25	4029.339	161.174
SD			1.815
%RSD			1.080

Table 6.8: Recovery study of Aspirin

Level of Recovery (%)	80	100	120
Amount present (mg)	10 10 10	10 10 10	10 10 10
Amount of Std. added (mg)	8 8 8	10 10 10	12 12 12
Amount recovered (mg)	7.98 8.01 8.02	9.98 10.02 10.01	11.98 11.95 12.05

% Recovery	99.75 100.12 100.25	99.80 100.20 100.10	99.833 99.583 100.417
Mean % Recovery	100.042±0.260	100.033±0.208	99.944±0.428

Table 9: Recovery study of Omeprazole

Level of Recovery (%)	**80**	**100**	**120**
Amount present (mg)	10 10 10	10 10 10	10 10 10
Amount of Std. added (mg)	8 8 8	10 10 10	12 12 12
Amount recovered (mg)	7.98 8.03 7.95	9.98 9.95 9.98	11.85 12.01 11.98
% Recovery	99.75 100.38 99.38	99.80 99.50 99.80	98.75 100.08 99.83
Mean % Recovery	99.833±0.505	99.700±0.173	99.556±0.708

Table 10: Results of analysis Data of tablet Formulation

Drug	**Label claim (mg)**	**Amount Found (%)**	**Label claim (%)**	**S.D.**
Aspirin	81mg	80.98	99.97	0.154
Omeprazole	40mg	39.95	99.87	0.165

Table 6.11: Intermediate Precision of Aspirin

Intra-day Precision		**Inter-day Precision**	
	% Label Claim		**% Label Claim**
After 1hr	99.98	First day	98.98
After 2hr	99.81	Second day	98.12
After 3hr	99.55	Third day	98.00
After 4hr	99.45		
After 5hr	99.32		

After 6hr	99.05		
Mean	99.527	**Mean**	98.367
SD	0.335	**SD**	0.535
% RSD	0.337	**% RSD**	0.543

Table 6.12: Intermediate Precision of Omeprazole

Intra-day Precision		**Inter-day Precision**	
	% Label Claim		**% Label Claim**
After 1hr	99.12	First day	98.00
After 2hr	99.05	Second day	97.98
After 3hr	99.01	Third day	97.50
After 4hr	98.75		
After 5hr	98.21		
After 6hr	98.05		
Mean	98.698	**Mean**	97.827
SD	0.460	**SD**	0.283
% RSD	0.467	**% RSD**	0.289

Table 12: LOD and LOQ

Name	LOD (g/ml)	LOQ (g/ml)
Aspirin	0.89	2.41
Omeprazole	0.45	1.25

Conclusion

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. Proposed method was found to be linear in the range of 5-25 µg/ml aspirin and omeprazole with the correlation coefficient near to one respictively. The validation and the reliability of preposed method were assessed by recovery study. The recovery of added standards (80%,100%120%) was ranging from 100.042±0.260 to 100.033±0.208, 99.944±0.428 and 100.08±0.641, 100.26±0.321 to 98.54±0.553 for aspirin and omeprazole respectively.

Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted of 1.75 gm KH_2PO_4 in 1000 ml of water add 1 ml of TEA and adjust the pH – 6 with OPA in the ratio of 30:70 v/v at a flow rate of 1.0 ml min^{-1}. A thermo C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 275.0 nm was selected as the detection wavelength for UV-vis. detector.

The proposed methods were found to be linear in the range of 5-25 µg/ml with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form.

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