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QUANTITATIVE SIMULTANEOUS ESTIMATION OF ASPIRIN AND OMEPRAZOLE BY RP-HPLC METHOD IMPLEMENTING AQbD APPROACH IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

Aspirin, Omeprazole, RP-HPLC, Analytical Quality by Design, method development, Validation

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ABSTRACT: A simple, accurate, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Aspirin (ASP) and Omeprazole (OMP). By using box benchen design method have been developed and optimized. Effective chromatographic separation achieved using C18 column (250 × 4.6 mm, 5 µm) as a stationary phase and mobile phase consisted of methanol: Disodium hydrogen phosphate buffer (68: 32 v/v), pH adjusted to 4.5 with phosphoric acid at a flow rate 1.15 mL/min at a detection wavelength of 280 nm. Analytical Quality by Design approach was applied to evaluate the effect of three factors are the volume of the organic phase in the mobile phase, pH of mobile phase and flow rate on chromatographic responses like retention time and tailing factor. The retention time of ASP and OMP were found to be 2.94 and 5.87 min respectively. Calibration curves were found to be linear over the concentration range of 10-60 µg/mL for ASP and 5-30 µg/mL for OMP. The % recovery of drugs by developed method was found in the range of 98 - 102%. The proposed method was found to be precise and robust. The method was successfully applied for the quantitative determination of ASP and OMP in the tablet dosage form.

INTRODUCTION: [1-16] MI commonly known as a heart attack occurs when blood flow stops to a part of the heart causing damage to the heart muscle. Yosprala, fixed-dose combination is available containing the antiplatelet agent aspirin and the proton pump inhibitor omeprazole [1-5]. According to ICH Q8 (Quality by Design), QbD is defined as A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [6-10].

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AQbD (Analytical QbD) is a science and risk-based paradigm for analytical method development, endeavoring for understanding the predefined objectives to control the Critical Method Variables (CMVs) affecting the Critical Method Attributes (CMAs) to achieve enhanced method performance, high robustness, ruggedness and flexibility for continuous improvement [11-14].

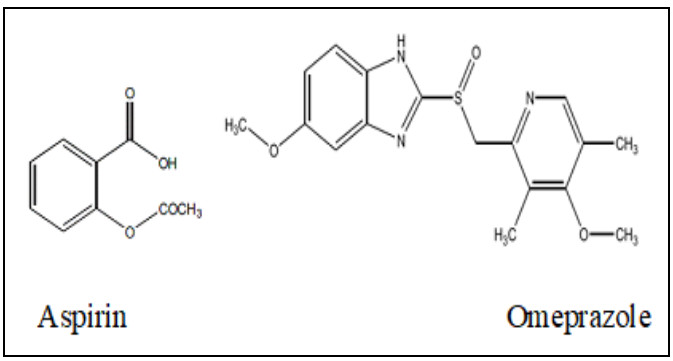

FIG. 1: CHEMICAL STRUCTURE OF ASPIRIN AND OMEPRAZOLE

Literature survey reveals only one analytical method reported for quantitative estimation of the aspirin and omeprazole are UV [15] and only one method was reported for reverse phase HPLC [16] in combination. To the best of our knowledge, no analytical method has been reported for the Quantitative estimation of the aspirin and omeprazole by utilizing experimental designs. So, to improve quality and to reduce cost and for an accurate result, the aim of the present study was to develop, optimize and validate RP-HPLC method for simultaneous determination of aspirin and omeprazole by AQBD approach.

According to ICH Q8 (Quality by Design), QbD is defined as A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [6-10]. AQbD (Analytical QbD) is a science and risk-based paradigm for analytical method development, endeavoring for understanding the predefined objectives to control the Critical Method Variables (CMVs) affecting the Critical Method Attributes (CMAs) to achieve enhanced method performance, high robustness, ruggedness and flexibility for continuous improvement [11-14].

Literature survey reveals only one analytical method reported for quantitative estimation of the aspirin and omeprazole are UV [15], and only one method was reported for reverse phase HPLC [16] in combination. To the best of our knowledge, no analytical method has been reported for the Quantitative estimation of the aspirin and omeprazole by utilizing experimental designs. So, to improve quality and to reduce cost and for an accurate result, the aim of the present study was to develop, optimize and validate RP-HPLC method for simultaneous determination of aspirin and omeprazole by AQBD approach.

MATERIALS AND METHODS:

Chemicals and Reagents: Standard sample of Aspirin was given as a gift sample from Sidmak, Valsad, India, and omeprazole were given as a gift sample from Mangalam drugs, Vapi. Methanol HPLC-grade, water HPLC-grade, and Disodium hydrogen phosphate were purchased from Rankem, RFCL Limited, New Delhi, India

Instruments:

HPLC: A LC-2010 AHT HPLC of Shimadzu corporation equipped with LC P-100 pump, a PDA detector, a high-pressure gradient mixer of 1500 µl, a loop injector of 20 µl capacity and Class-VP software was used for the analysis.

Sonicator: A digital ultrasonic cleaner (Equitron) was used for mixing.

Selection of Wavelength: Standard solutions were prepared for both drugs in methanol individually and were scanned in the wavelength range of 200-400 nm, and the overlain spectrum was obtained. From the overlain spectrum isoabsorptive point was found to be 280 nm **Fig. 2**.

Thus, 280 nm was selected as a detection wavelength for simultaneous determination of aspirin and omeprazole.

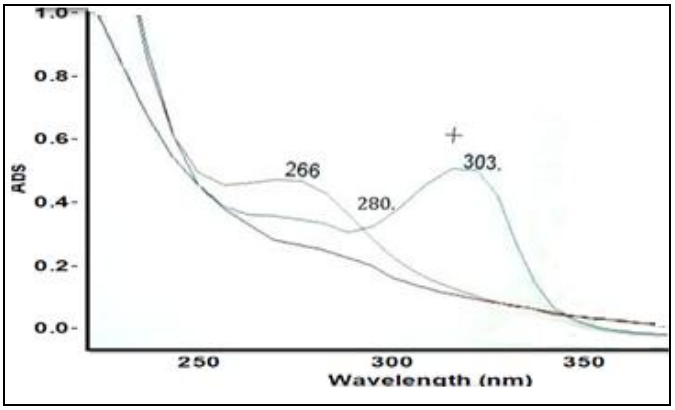

FIG. 2: OVERLAIN UV SPECTRA FOR ASP AND OMP

TABLE 1: VARIABLES SELECTED FOR QBD DESIGN

Factors dependent variables	Level used		
	Low (-1)	Medium (1)	High (+1)
X1 = % organic modifier	40	60	80
X2 = pH	3.0	4.5	6.0
X3 = flow rate (mL/min)	0.8	1.15	1.5
Dependant Variable	Y1 (retention time)	≤ 10	
	Y2 (tailing factor)	≤ 1.5	

TABLE 2: SUMMARY OF RESULTS FOR METHOD OPTIMIZATION BY QBD DESIGN

Run	Level	Factors			Responses			
		X1: Organic modifier (v/v)	X2: pH of the mobile phase	X3: Flow rate (ml/min)	Y1: RT of D1 (ASP) (min)	Y2: RT of D2 (OME) (min)	Y3: TF of D1 (ASP)	Y4: TF of D2 (OME)
1	-1, -1, 0	40	3.00	1.15	10.53	37.24	0.99	0.99
2	0, -1, -1	60	3.00	0.80	5.93	11.65	1.24	1.00
3	0,-1,+1	60	3.00	1.50	6.48	7.22	0.93	1.17
4	+1,+1,0	80	6.00	1.15	3.72	5.30	0.93	1.17
5	-1, 0, -1	40	4.50	0.80	3.71	7.29	1.21	0.99
6	0,+1, +1	60	6.00	1.50	2.05	6.58	0.73	0.89
7	-1, 0,+1	40	4.50	1.50	3.42	3.80	1.01	1.01
8	+1, 0,+1	80	4.50	1.50	1.98	2.84	0.72	0.94
9	+1,0,-1	80	4.50	0.80	3.72	5.30	0.71	0.95
10	0,+1,-1	60	6.00	0.80	3.85	12.20	0.73	0.89
11	-1,+1,0	40	6.00	1.15	3.51	4.52	0.71	0.95
12	+1,-1,0	80	3.00	1.15	2.95	3.80	0.88	0.99
13	0,0,0	60	4.50	1.15	2.93	8.57	1.11	0.95

RESULTS AND DISCUSSION: By using box benchen design 13 runs were performed for aspirin and omeprazole. The dependent and independent variables of all runs are shown in **Table 1**. The proposed regression equations for various chromatographic responses of both regression equations for various chromatographic responses of both the drugs are given in **Table 3**. It was observed that the best-fitted model for both drugs was quadratic and linear model. The optimization, while a positive value represents an effect that favors optimization while a negative value indicates the inverse relationship between factors and response. In case of ASP, it is clear from the equations that the factor a (volume of organic modifier), factor B (pH of mobile phase) and factor C (flow rate) had negative effects on retention time. For tailing factor A and B shows negative and factor C shows a positive effect. In the case of OMP factor, A had a negative effect on both responses Y2 and Y4 and factor B and C had a negative effect on Y2 and had a positive effect on Y4.

The ANOVA results indicate all model terms were found to be significant for both drugs **Table 2**. 3D response surface plots presented For ASP OMP which are used for determination of the relationship between the response and factors. In the case of ASP, plot 3 indicates that all three factors had a negative effect on retention time. With the decrease in volume of organic phase, pH and Flow rate, the retention time increases individually. But, When two factors are changing, it produces a different effect on responses like for all three factors had positive effects, when mobile phase ratio and pH, mobile phase ratio and flow rate and pH and flow rate and mobile phase ratio increases, retention time increases. Plot 4 indicates that all two factors had a negative effect and one factor had a positive effect on tailing factor. With the decrease in the volume of the organic phase and pH increases the tailing factor. But tailing factor increases with increasing Flow rate.

In the case of OMP, plot 5 indicates that all three factors had a negative effect on retention time. With the decrease in volume of organic phase, pH and flow rate, the retention time increases individually. But when two factors are changing, it produces a different effect on responses like when mobile phase ratio and pH, mobile phase ratio and flow rate had on negative effects on retention time, and pH and flow rate had a positive effect. The plot 6 indicates that all one factor had a negative effect and two factors had a positive effect on tailing factor. Tailing factor decreases with increasing organic phase modifier but tailing factor increases with decreasing pH and Flow rate.

Software Aided Method Optimization: The final optimized conditions were determined by evaluating the effect of three factors X1 (mobile phase ratio), X2 (pH of mobile phase) and X3 (Flow rate). The desirability plot for aspirin and omeprazole was generated by the Design Expert software. The desirability factor of X1, X2 and X3 were found to be less than 1 **Fig. 5**. Based on retention time and tailing factor, the optimized conditions selected was mobile phase were methanol: Buffer (68: 32) at pH 4.5 and the flow rate of 1.15 ml/min **Fig. 6**.

TABLE 3: REGRESSION EQUATIONS FOR ASPIRIN AND OMEPRAZOLE

Drug	Regression equation
ASP	$Y_1 = 4.21 - 1.45(*A) - 1.25 (* B) - 1.10 (* C) +1.95 (*A*B) + 0.33 (*A*C) + 0.10 (*B*C)$
	$Y_3 = 1.44714 -3.31250E-003 * Mobile phase -0.071667* pH +3.57143E-003 * Flow rate$
OMP	$Y_2 = 8.90 – 4.51 (*A) – 4.00 (* B) - 1.98 (* C) + 8.42 (*A*B) + 0.24 (*A*C) – 0.31 (*B*C)$
	$Y_4 = 1.00830 - 7.50000E-004 * Mobile phase + 0.00012* pH+0.028571 * Flow rate$

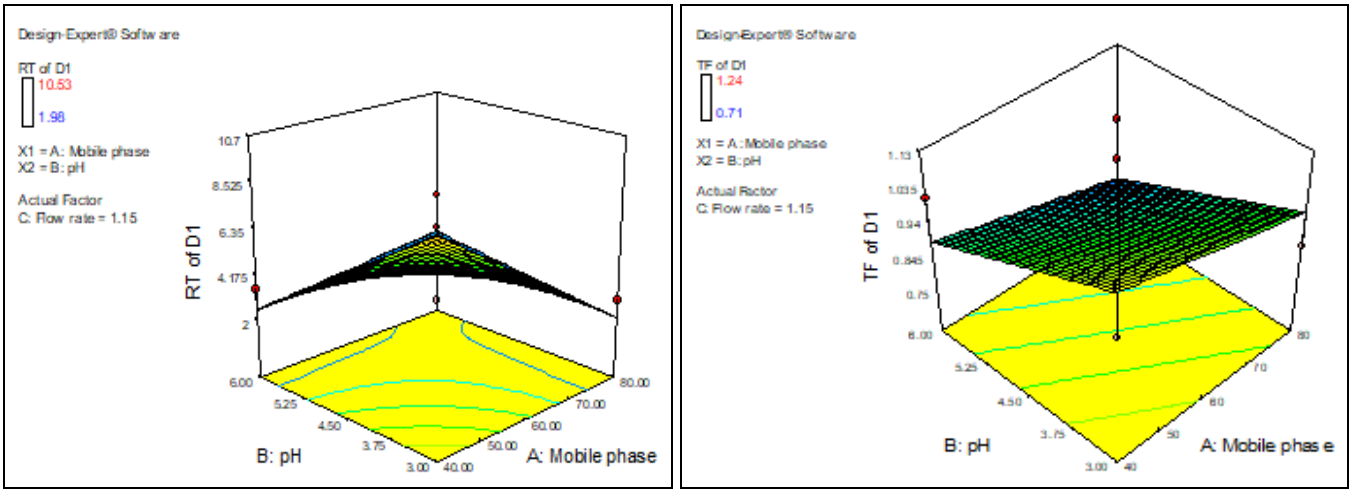

FIG. 3: 3D SURFACE PLOT OF EFFECT OF INTERACTION OF X1, X2 AND X3 ON RETENTION TIME AND TAILING FACTOR OF ASPIRIN

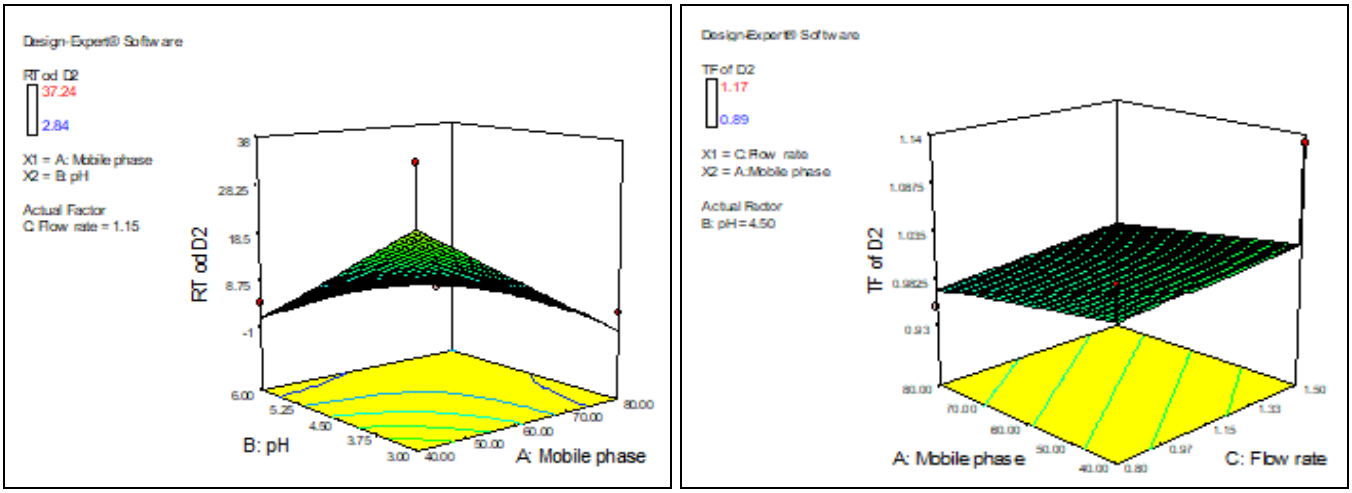

FIG. 4: 3D SURFACE PLOT OF EFFECT OF INTERACTION OF X1, X2 AND X3 ON RETENTION TIME AND TAILING FACTOR OF OMEPRAZOLE

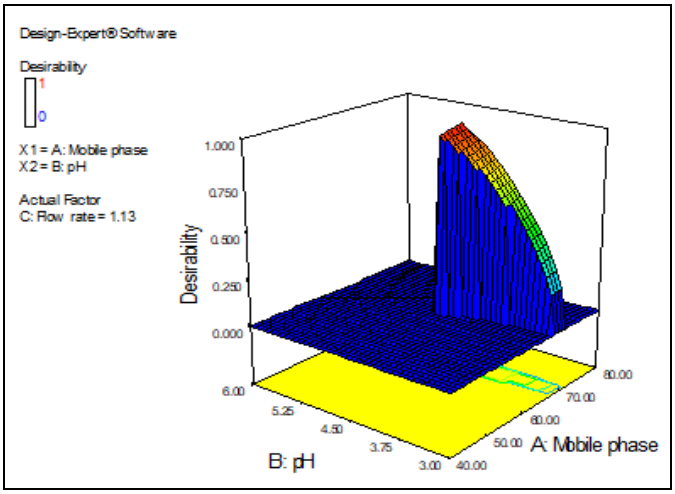

FIG. 5: 3D SURFACE PLOT OF DESIRABILITY

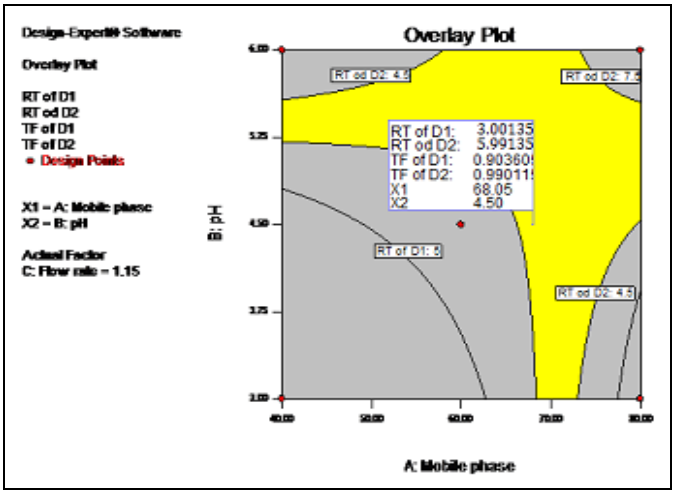

FIG. 6: OVERLAY PLOT

Preparation of Solutions for Initial Trials:

Preparation of Standard Stock Solution: Standard stock solution containing aspirin and omeprazole were prepared by dissolving 10 mg of each drug separately in separate volumetric flasks using methanol as a solvents up to 50 ml of methanol, it was then sonicated for 10 min, and the final volume of the solution was made up to 100 ml with methanol to get stock solution containing 100 µg/ml of aspirin and omeprazole respectively.

Preparation of Working Standard Solutions: Working standard solution of ASP (40 µg/ml) was prepared by withdrawing 4 ml from standard stock solution of aspirin into 10 ml volumetric flask and the volume was made up to the mark by using methanol as a solvent to get solution containing 40 µg/ml of aspirin and working standard of OME (20 µg/ml) was prepared by withdrawing 2 ml from standard stock solution of omeprazole into 10 ml volumetric flask and the volume was made up to the mark by using methanol as a solvent to get solution containing 20 µg/ml of omeprazole.

Preparation of Sample Solution: Twenty tablets of yosprala (each tablet containing 81 mg of aspirin and 40 mg of omeprazole) were weighed; average weight was calculated and triturated. Accurately weighed tablet powder equivalent to 10 mg of yosprala was transferred to a 100 ml of volumetric flask. About 50 ml of methanol was added to the flask and sonicated for 15 min. The volume of the solution was made up to the mark to get a solution containing 100 µg/ml of test solution. The resulting solution was then filtered through a Whatman filter paper followed by a syringe filter.

Optimized Conditions: The final optimized conditions were determined by evaluating the effect of three factors X1 (mobile phase ratio) and X2 (pH) and X3 (flow rate). The desirability plot for omeprazole was generated by the Design Expert software. The desirability factor of X1 was found to be less than 1 for all three factors. Based on retention time and tailing factor, the optimized conditions selected was mobile phase were Methanol: Disodium hydrogen phosphate buffer (68: 32 v/v), pH 4.5 and flow rate 1.15 ml/min.

Method Validation: The method developed by applying the concept of QbD was validated for various parameters.

Specificity: It was found that there was no interference from the blank (mobile phase) or excipient present in a tablet which states that the developed method was specific **Fig. 7, 8**.

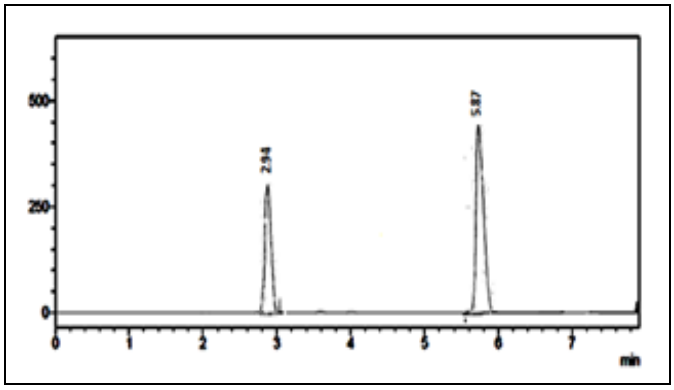

FIG. 7: CHROMATOGRAM OF STANDARD SOLUTION OF ASP AND OMP

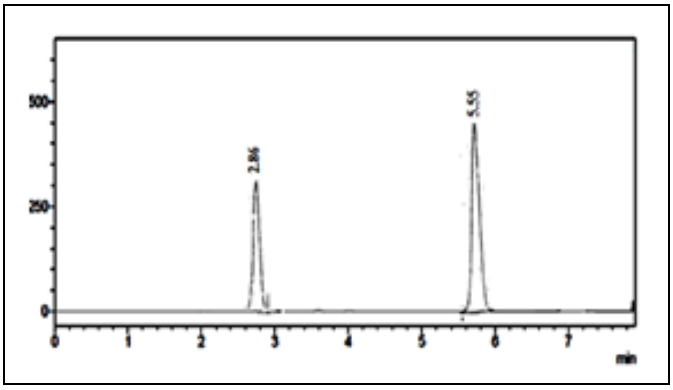

FIG. 8: CHROMATOGRAM OF TEST SOLUTION OF ASPIRIN AND OMEPRAZOLE

System Suitability Test: Numbers of theoretical plates (N) was found to be 2607.30 and 3710.71 for aspirin and omeprazole respectively which are greater than 2000. The tailing factor obtained was 1.04 for both the drug which was less than 2.0 **Table 4**.

TABLE 4: RESULTS OF SYSTEM SUITABILITY PARAMETERS

Parameters	Mean ± SD of Aspirin	%RSD	Mean ± SD of Omeprazole	%RSD
Area	127165 ± 2.6079	0.0002	529360 ± 2.6076	0.0004
No. of theoretical plates	2608.30 ± 37.20	1.4062	3710.71 ± 20.29	0.0054
Tailing factor	1.0416 ± 0.0075	0.7200	1.0416 ± 0.0054	0.5184

*n=6 (six measurements)

As per the results of system suitability tests, the proposed method was found to be suitable for carrying out analysis.

LOD and LOQ: LOD and LOQ for aspirin and omeprazole were found to be 0.014 and 0.044 for aspirin, and 0.020 and 0.062 respectively **Table 5**. As the proposed method could detect the drug under study up to microgram level, the method was found to be sensitive.

TABLE 5: REGRESSION ANALYSIS DATA FOR PROPOSED METHOD

Parameters	Aspirin	Omeprazole
Linearity (µg/ml)	10-70 µg/ml	5-35 µg/ml
Regression coefficient (R^2)	0.998	0.998
Y – intercept ± SD	19.143 ± 1.21	48.48 ± 1.61
Slope ± SD	269.15 ± 0.577	256.69 ± 0.577
LOD	0.014	0.020
LOQ	0.044	0.062

LOD = Limit of detection, LOQ= Limit of quantification

Linearity and Range: Linearity was determined by evaluating different concentrations of standard solutions of Aspirin in the range of 10-60 µg/ml and standard solutions of omeprazole in the range of 5-30 µg/ml.

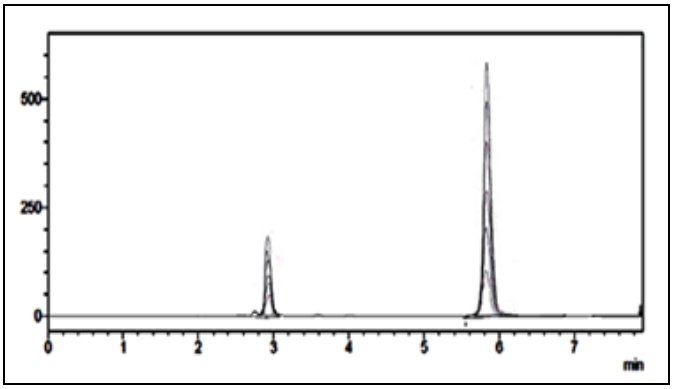

FIG. 9: OVERLAY PLOT OF ASPIRIN AND OMEPRAZOLE FOR LINEARITY STUDY

Accuracy: The % recovery was calculated by analyzing solutions prepared by adding a known amount of standard aspirin and omeprazole solution to pre-analyzed test solution. Values of % RSD at each level were less than 2.0.

The % recovery for each drug was 99.99-100.15% and 99.99-100% for aspirin and omeprazole respectively, which was found within the limit (98-102%) at each level, so the proposed method was found to be accurate **Table 6**.

TABLE 6: RESULT OF ACCURACY STUDIES OF ASPIRIN AND OMEPRAZOLE

% Level	Amount present (µg/ml)		Amount recovered		% Recovery	
	ASP	OME	ASP	OME	ASP	OME
50	30	15	30.04	14.99	100.15	99.99
100	50	25	49.99	25	99.99	100
150	70	35	70.10	34.99	100.15	99.99

Precision: The precision of the method was demonstrated by intraday and interday precision studies at three concentration levels 20 µg/ml, 40 µg/ml and 60 µg/ml for aspirin and 10 µg/ml, 20 µg/ml and 30 µg/ml for omeprazole respectively. The values of % RSD obtained at each level of both intraday and the interday precision study was less than 2. So, the proposed method was found to be precise **Table 7**.

TABLE 7: RESULT OF PRECISION STUDIES OF ASPIRIN AND OMEPRAZOLE

Drug	Concentration	Intraday (%RSD)	Intraday (%RSD)	Interday (%RSD)	Interday (%RSD)
Aspirin	20	0.0027	0.0039	0.0047	0.0039
	40	0.0019	0.0019	0.0019	0.0027
	60	0.0019	0.0017	0.0019	0.0011
Omeprazole	10	0.0013	0.0009	0.0009	0.0009
	20	0.0008	0.0008	0.0006	0.0006
	30	0.0003	0.0003	0.0003	0.0003

Robustness: In Robustness study % RSD was found to be less than 2%. In the case of the area of standard solution and % content was found to be in the range 98-102% **Table 8**. Hence, the developed method was robust.

TABLE 8: EVOLUTION OF ROBUSTNESS FOR DETERMINATION OF ASP AND OMP

Parameter	Average					
	Area of aspirin	Area of Omeprazole	The retention time of aspirin	The retention time of Omeprazole	% content of aspirin	% content of Omeprazole
pH						
4.49	127177	529379	2.91	5.90	101.1	99.96
4.50	127154	529364	2.94	5.87	99.98	99.99
4.51	127128	529383	2.98	5.83	98.35	100.2
Average	127153	529379	2.94	5.87	99.81	100.05
%RSD	0.019	0.001	0.026	0.028	0.138	0.129
Flow rate (ml/min)						
1.13	127137	529355	2.92	5.84	99.93	99.95
1.15	127154	529364	2.94	5.87	100.2	100.3
1.17	127173	529375	2.96	5.91	101.3	99.98
Average	127154	529364	2.94	5.87	100.47	100.07
%RSD	0.014	0.001	0.680	0.598	0.722	0.139
Wavelength						
278 nm	127167	529355	2.97	5.89	98.99	99.49
280 nm	127155	529364	2.94	5.87	99.23	98.99
282 nm	127145	529374	2.96	5.85	99.89	99.52
Average	127155	529364	2.95	5.87	99.37	99.34
%RSD	0.008	0.001	0.517	0.340	0.519	0.299

Assay: The content of aspirin and omeprazole in tablet were found to be 99.58% ± 0.00 and 100.04% ± 0.00 respectively **Table 9**. The value of assay obtained for yosprala was within limits (98.00% to 102.00%).

TABLE 9: RESULT OF ANALYSIS OF ASPIRIN AND OMEPRAZOLE IN TABLET FORMULATION

Amount took (µg/mL)		Amount obtained (µg/mL)		Assay (%w/w)	
Aspirin	Omeprazole	Aspirin	Omeprazole	Aspirin	Omeprazole
40 ppm	20 ppm	39.93	20.02	99.58	100.04

CONCLUSION: The method was developed and optimized by applying AQbD approach for simultaneous determination of ASP and OMP. The run time is 8 min for proposed method so rapid determination of analytes is carried out within which the two drugs are well resolved. The AQbD method applied to reduce trails so less time consuming and accurate method was optimized and validation parameters were performed.

ACKNOWLEDGEMENT: I would acknowledge to sidmak and mangalam drugs for providing the API of aspirin and omeprazole respectively as a gift sample.

CONFLICT OF INTEREST: Nil

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