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Research Article

RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ASPIRIN AND OMEPRAZOLE IN BULK AND TABLET DOSAGEFORM

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A R T I C L E I N F O

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A B S T R A C T

A validated RP-HPLC method was developed for simultaneous estimation of aspirin and omeprazole for bulk and pharmaceutical dosage form. The method was developed under optimized chromatographic conditions using a reversed phase C_{18} column and a mobile phase composition of acetonitrile-methanol (20:80 v/v). The flow rate used was 0.6 ml/min and UV detection was carried out at 233 nm. The retention time of aspirin and omeprazole was found to be 1.720 and 2.667 min respectively. The method was found to be linear over a concentration range of 10-50 µg/ml and 5-25 µg/ml for aspirin and omeprazole, respectively. The proposed method could be applied for the routine quality control analysis of this new combination in pure powder and pharmaceutical formulation.

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Introduction

Aspirin (ASP, Figure 1) is a non-steroidal anti-inflammatory drug used to treat inflammation, pain, fever. It also possessantithrombotic action. Aspirin is given shortly after a heart attack and decreases the risk of heart failure and also can be used in longterm to help prevention

of heart attacks, strokes and blood clots (1). Omeprazole (OME, Figure 2), is a proton pump inhibitor used in the treatment of gastro esophageal reflux disease and peptic ulcer disease (2). Yosprala is a formulation approved by USFDA in 2016 for patients who require aspirin for secondary prevention of cardiovascular and cerebrovascular events and who are at risk of developing aspirin associated gastric ulcers (3). UV spectroscopy and HPTLC methods for estimation of Aspirin in combination with omeprazole have been reported in the literature (4-6). Recently few HPLC methods for simultaneous estimation of aspirin and omeprazole in bulk and pharmaceutical dosage forms have been developed (7-12). Majority of the methods employed complex mobile phases containing buffer. Hence an attempt was made to develop a new, simple, sensitive, accurate and cost effective method for simultaneous estimation of aspirin and omeprazole.

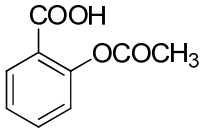

Figure 1: Structure of Aspirin

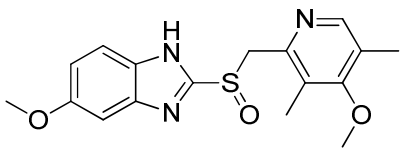

Figure 2: Structure of Omeprazole

Material and Methods

Pure ASP (99 %) and OME (99 %) kindly supplied by Lee pharma Ltd as gift sample. Methanol, Acetonitrile, (HPLC grade, Fischer Scientific, India). Methanol was of an analytical grade, (SD-Fine chemicals, India).

Instruments:

RP- HPLC- Agilent 1220 Infinity LC system provided with a G4 288c series 2-Channel, Dual plunger pump, Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µm particle size),Variable wavelength Detector (VWD) with Deuterium lamp and Holmium oxide filter and detects ranging from 190-600 nm, Agilent Cary 60 UV-Visible double beam Spectrophotometer, manual injector. The chromatographic analysis was carried out using (Ez Chrome-versionA.04.05) data analysis program.

Standard solutions

Accurately weighed 10 mg of ASP and OME pure powders were transferred to a 100 ml volumetric flask. Then the volume was made up to the mark with the mobile phase and mixed well. Standard working solutions with concentration (100 µg/mL) of ASP and OPZ were obtained.

Chromatographic conditions

A mobile phase system of acetonitrile-methanol (20:80 v/v) was used at a flow rate of 0.6 mL/min. The injection volume was

20µL. The column used was Agilent C18 column (150 mm x 4.6 mm, 5 µm particle size). The eluents were monitored at 233 nm.

Calibration curves for ASP and OME

Accurate volumes of ASP and OME standard solutions were transferred into two separate series of 10 ml volumetric flasks and diluted to volume with the mobile phase to obtain final concentrations of 10-50 and 5-25 µg/mL of ASP and OMErespectively. 20 µL aliquots were injected and eluted with the mobile phase under the optimum chromatographic conditions. The average peak areas of ASP and OME were plotted versus the corresponding drug concentrations (µg/mL) to get the calibration graphs. Then, the corresponding regression equations were derived.

Analysis of pharmaceutical formulation

Twenty tablets (81 mg of ASP/40 mg of OME per tablet) were weighed and then finely powdered. Appropriate weight of powder equivalent to one tablet was accurately weighed, transferred to 100 mL volumetric flask and the volume was made up to 50 mL with mobile phase. The solution was shaken vigorously for 20 min then sonicated for 30 min and filtered. The volume was adjusted up to 100 mL with the mobile phase to produce a stock solution labeled to contain 810 and 400 µg/mL of ASP and OME respectively. Necessary dilutions of the stock solution were made with the mobile phase to obtain different concentrations of ASP and OME covering the concentration range. 20 µL aliquots were injected and eluted with the mobile phase under the optimum chromatographic conditions. Tablet contents of both drugs were calculated using the corresponding regression equation.

Results and Discussion

Determination of wavelength maxima

Both the standard solution was scanned between 400nm to 200nm.The overlain spectrum of both drugs was recorded. From the overlain spectrum, 299.4nm (λmax of omeprazole) and 229.2nm (λmax of Aspirin) the isobestic point was found to be at 233.8nm and it is shown in Figure 3 .This wavelength was utilized for HPLC method development.

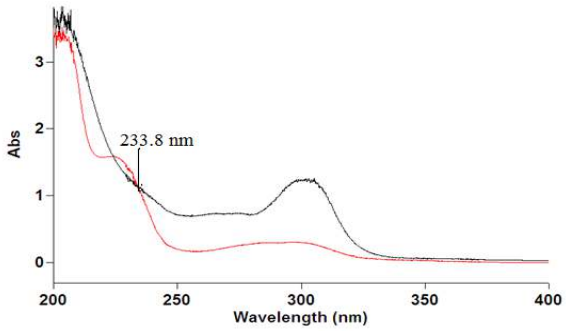

Figure 3: Isobestic point for Aspirin and Omeprazole

Method development

Different conditions affecting the chromatographic separation were optimized regarding the resolution between the two studied drugs. Several mobile phases were tried in order to improve the separation process. Acetonitrile and methanol in different ratios were applied and good separation was carried out on Agilent C_{18} column (150 mm x 4.6 mm, 5 µm particle size) using an optimized mobilephase consists of acetonitrile-methanol (20:80) v/v at flow rate 0.6 ml/ min and UV detection at 233.8nm. HPLC chromatogram (Figure 4) revealed that ASP and OME were clearly separated from each other with good resolution at retention times of 1.717 and 2.667 min. for ASP and OME respectively.

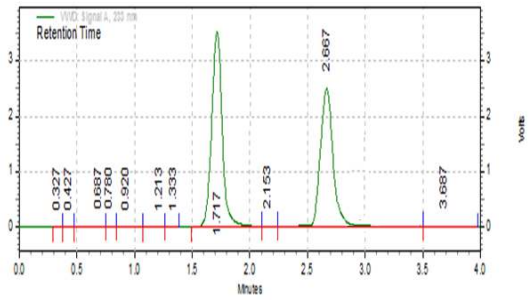

Figure 4: HPLC chromatogram of mixture of Aspirin and Omeprazole

Method validation

The proposed method was validating according to ICH guidelines. The validation parameters were checked regarding linearity, accuracy, precision, specificity, limit of quantification, limit of detection and robustness (13, 14).

Linearity

Under the optimized experimental conditions, the calibration graphs for the method were constructed by plotting the average peak areas versus drug concentrations in µg/mL for both ASP and OME. Linearity ranges and corresponding peak areas are presented in table 1. Regression equations, intercepts, slopes and coefficient of determination for the calibration data were presented in Figure 5 and 6.

Table 1: Linearity results for Aspirin and Omeprazole

ASP		OME	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
10	259972	5	229709
20	479944	10	429418
30	694916	15	619127
40	939889	20	838837
50	1184861	25	1018546

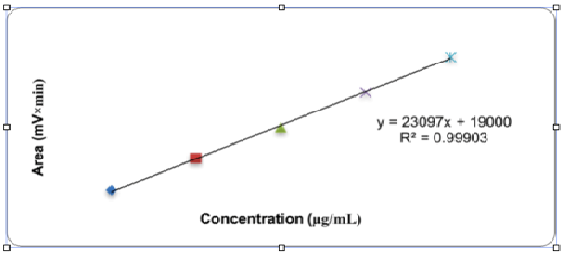

Figure 5: Calibration curve of Aspirin (Linearity)

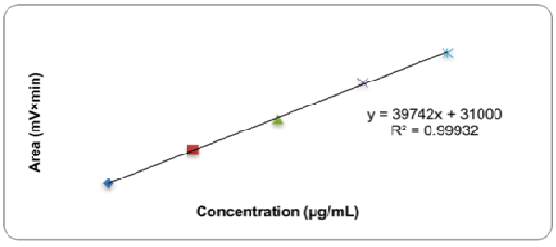

Figure 6: Calibration curve of Omeprazole (Linearity)

Accuracy (recovery study)

The accuracy of the method was determined by applying the general procedure for determination over 40 µg/mLconcentrations for ASP and 20 µg/mL concentrations for OMP. Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and the percentage recovery was calculated by using the corresponding regression equation. The mean %Recovery was found to be 99.93% and 99.84% for Aspirin and Omeprazole respectively. The results were represented in Table 2

Table 2: Accuracy results for Aspirin and Omeprazole

Analyte	Accuracy level	Amount added	Amount found	Recovery (%)	Mean recovery ± SD (%)
ASP	80%	32	31.90	99.68	99.76±0.08
		32	31.92	99.75	
		32	31.95	99.84	
	100%	40	39.95	99.87	99.99±0.11
		40	40.01	100.02	
		40	40.04	100.10	
	120%	48	47.98	99.95	100.04±0.07
		48	48.03	100.06	
		48	48.05	100.10	
OME	80%	16	15.92	99.50	99.62±0.16
		16	15.93	99.56	
		16	15.97	99.81	
	100%	20	19.95	99.75	99.90±0.15
		20	19.98	99.90	
		20	20.01	100.05	
	120%	24	23.97	99.87	100.01±0.12
		24	24.01	100.04	
		24	24.03	100.12	

Precision

The precision of analytical procedure express degree of the agreement among individual test when the procedure is applied repeatedly to multiple sampling of homogenous samples. Intra-day precission was determined by applying six injections on the same day following the procedure of repeatability. Mean area, standard deviation and % RSD were calculated for the two drugs. The data were given in Table 3.

Table 3: Precision results of Aspirin and Omeprazole

Inj.	Peak Area	
	ASP	OME
1	938929	834315
2	939231	834292
3	939889	834837
4	941284	831293
5	939721	832041
6	938727	833889
Mean	939630.2	833444.5
SD	924.88	1429.08
%RSD	0.09	0.17

Specificity

Specificity can be described as accurate measure the response of the analyzed compounds with no interferences from sample matrix. The specificity test was performed for Omeprazole and Aspirin. It was found that there was no interference of sample matrix in retention time of analytical peak. The chromatograms are represented in Figure 7, 8 and 9.

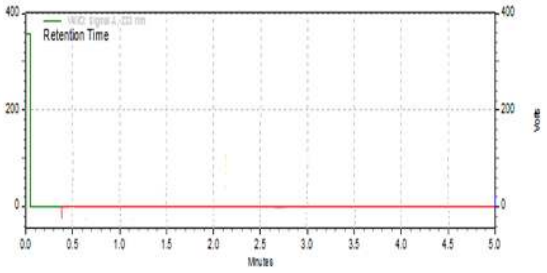

Figure 7: Chromatogram of blank (mobile phase)

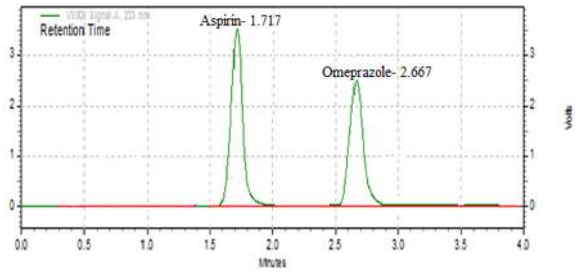

Figure 8: Chromatogram of standard injection

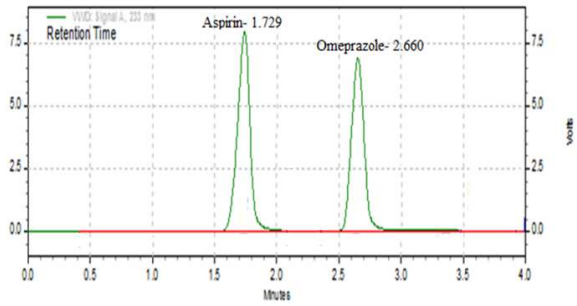

Figure 9: Chromatogram of sample injection

System suitability

Parameters of the HPLC method were assessed by six replicate injections of a solution of each drug (40 µg/mL in mobile phase) to the HPLC system. Various parameters such as the number of theoretical plates (N), resolution factor (R) and tailing factor (T) were calculated. The data were given in Table 4.

Table 4: System suitability parameters for Aspirin and Omeprazole

	ASP			OME			
Inj.	RT (min)	USP Plate count	Tailing	RT (min)	USP Plate count	Tailing	Resolution
1	1.710	2369	1.09	2.663	3457	1.07	3.9
2	1.717	2418	1.04	2.665	3498	1.09	3.6
3	1.720	2459	0.94	2.667	3482	1.02	3.4
4	1.729	2512	0.96	2.660	3518	0.98	3.6
5	1.729	2541	1.09	2.667	3504	1.11	4.1

Limit of detection & Limit of quantification

Limit of detection (LOD) was lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of quantification (LOQ) the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. The standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD is calculated according to formula LOD = 3.3 (SD/M) and LOQ = 10(SD/M). The results were given in Table 5.

Table: 5 LOD & LOQ table of Aspirin and Omeprazole

Analyte	LOD	LOQ
ASP	1.85	5.26
OME	1.09	3.31

Robustness

The robustness study was established by introducing small changes in various parameters like flow rate and mobile phase

composition. Minor changes in parameters did not affect the separation revealed the sensitivity of the method. The results were given in Table 6.

Table 6: Robustness results of Aspirin and Omeprazole:

Parameter		Retention Time (min)		Peak area (mV× min)		Tailing factor	
		OME	ASP	OME	ASP	OME	ASP
Flow rate (mL/min)	Standard	2.660	1.729	838837	939889	1.14	1.04
	0.5	2.665	1.721	838874	939834	1.09	1.11
	0.7	2.653	1.697	838812	939881	0.94	0.94
Mobile phase (Acetonitri:Methanol)	22:78	2.594	1.709	838754	939856	1.35	1.24
	18:82	2.696	1.740	838923	939984	1.29	1.27

Application of the validated method to pharmaceutical formulation

The simultaneous determination of ASP and OME in tablet dosage form was determined by the proposed method. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives as shown in Table7.

Table 7: Determination of aspirin and omeprazole combination in tablet dosage forms by the proposed method.

Drug	ASP	OME
Lebeled amount (mg)	81	40
Found (µg/mL)	80.65	39.83
	80.72	39.59
	80.66	39.75
Mean±SD	80.67±0.03	39.72±0.12
Recovery (%)	99.59	99.30

Conclusions

This study described a simple, selective and reliable HPLC method for the estimation of ASPand OME. The method was validated according to the ICH recommendation and can be used for the routine analysis of pharmaceutical preparations contaiining the mentioned drugs.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest

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