Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2025, 17(5): 757-773
(http://www.derpharmachemica.com/archive.html)

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Aspirin and Omeprazole in Bulk and Tablet Dosage Form

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Received: 15-Jan-2026, Manuscript no: DPC-26-186595 **Editor assigned:** 19-Jan-2026, Pre QC No: DPC-6-186595 (PQ), **Reviewed:** 02-Feb-2025, QC No: DPC-26-186595, **Revised:** 01-Jun-2026, Manuscript No: DPC-26-186595 (R), **Published:** 17-Jun-2026, DOI: 10.4172/0975-413X.17.3.757-773

ABSTRACT

Aim: *The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of aspirin and omeprazole in bulk and tablet dosage form.*

Objectives: *Following are the objectives of the present work:*

- *To develop a new RP-HPLC method for simultaneous estimation of "aspirin and omeprazole" and validate the method according to ICH guidelines.*
- *To apply the validated method for the simultaneous estimation of aspirin and omeprazole in tablet dosage form.*

Plan of Work: *This was carried out in two steps*

- *RP-HPLC method development*
- *Method validation.*

Keywords: Quality assurance, Safety, RP-HPLC, Efficacy, Aspirin, Omeprazole

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data. Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry and fluorimetry methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as Nuclear Magnetic Resonance (NMR) and Para Magnetic Resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing [1].

Analytical method development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeias) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure, comparative laboratory data including merits/demerits should be made available [2].

Steps involved in method development

Documentation starts at the very beginning of the development process. A system for full documentation of development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

Analyte standard characterization

- All known information about the analyte and its structure is collected *i.e.*, physical and chemical properties.
- The standard analyte (100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desiccators and freezer).
- When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.
- Only those methods (spectroscopic, MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

Method requirements

The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.

Literature search and prior methodology

The literature for all types of information related to the analyte is surveyed. solubility profile (solubility of Drug in different solvents and at different pH conditions), analytical profile (Physico-chemical properties, Eg: pKa, melting point, degradation pathways, etc) and stability profile (sensitivity of the drug towards light, heat, moisture etc) and relevant analytical methods, books, periodicals, chemical manufacturers and regulatory agency compendia such as USP/NF, are reviewed [3].

Aspirin

Drug profile

Aspirin is a Non-Steroidal Anti-Inflamatory Drug (NSAID) and antiplatelet agent. Aspirin is also known as acetylsalicylic acid (ASA), that is used to treat pain, fever and inflammation. Aspirin is also used long-term to help prevent heart attacks, strokes
and blood clots in people at high cardiac risk (Figure 1).

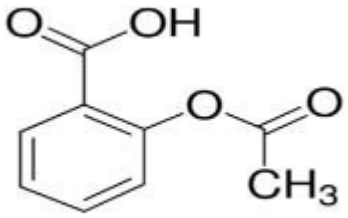

Figure 1: Chemical structure of Aspirin.

Nomenclature: 2-(ethyloxy)benzenecarboxylic acid
CAS Number: 50-78-2
Molecular Weight: 180.157 g/mol Molecular Formula: $C_9H_8O_4$
Physical State: Solid
Solubility: Acetylsalicylic acid is slightly soluble in water, with a limit of
solubility reported as approximately 3 mg/ml at 25°C. It is also soluble in ethanol at 50 mg/ml and will dissolve in solutions of alkali hydroxides and carbonates with decomposition.
Storage: Store it at room temperature

Melting Point: 135°C
Indication: Aspirin is similarly efficient as paracetamol on trivial acute pain (e.g. headaches, dental pain, or colds). However, it is also used for chronic states of pain, e.g. for cancer patients and (in high doses) for rheumatic fever.
Mechanism of action: Aspirin's ability to suppress the production of prostaglandins and
thromboxanes is due to its irreversible inactivation of the Cyclooxygenase (COX) enzyme. Cyclooxygenase is required for prostaglandin and thromboxane synthesis
Pharmacodynamics: The chemical name for aspirin is 2-ethanoyloxybenzoic acid (old name: Acetylsalicylic acid). Aspirin is a weak organic acid. It is absorbed mainly in its unionised form in the stomach and upper small intestine.
Protein binding: About 50%-80% of salicylate in the blood is bound to albumin protein, while the rest remains in the active, ionized state. The volume of distribution is 0.1-0.2 L/kg.
Metabolism: Hepatic salicylate, in turn, is mainly metabolized by the liver. This metabolism occurs primarily by hepatic conjugation with glycin or glucuronic acid, each involving different metabolic pathways.
Elimination: Elimination follows zero-order kinetics. Renal elimination of unchanged drug depends on urine pH.
Half-life: 15 to 20 min.
Toxicity: Nausea, vomiting, diaphoresis, and tinnitus are the earliest signs and ofsalicylate toxicity. Other early symptoms and signs symptoms are vertigo, hyperventilation, tachycardia and hyperactivity. As toxicity progresses, agitation, delirium, hallucinations, convulsions, lethargy, and stupor may occur.
Affected organisms: Humans, microorganisms and other mammals.

Omeprazole

Omeprazole is a proton pump inhibitor. It is used in the treatment of gastroesophageal reflux disease, peptic ulcer disease and Zollinger-Ellison syndrome. It is also used to prevent upper gastrointestinal bleeding in people who are at high risk (Figure 2) [4].

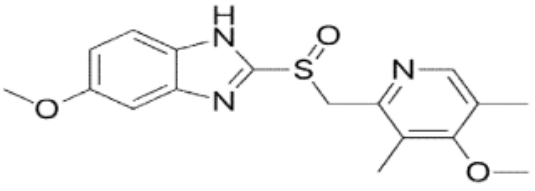

Figure 2: Chemical structure of Omeprazole.

Nomenclature: 5-Methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl) methanesulfinyl]-1H-benzimidazole
CAS number: 73590-58-6
Molecular weight: 345.42 g/mol
Molecular formula: $C_{17}H_{19}N_3O_3S$
Physical state: Solid
Solubility: The solubility of omeprazole in ethanol is approximately 5 mg/ml and approximately 30 mg/m in DMSO and DMF.

MATERIALS AND METHODS

The various materials and instruments's used for the development of RP-HPLC method. The present study is summarized as follows (Tables 1-5):

Chemicals/reagents and solvents

Table 1: List of various materials.

S. No	Materials	Materials	Company
1	Methanol	HPLC	Fischer scientific
2	Acetonitrile	HPLC	Fischer scientific
3	Methanol	AR	Sd-fine chemicals

Table 2: Working standard/reference standard/ API.

S. No	Working standard	Potency	Company
1	Aspirin	99.9	Lee pharma
2	Omeprazole	99.9	Lee pharma

Table 3: Test sample.

S .No	Test sample	Composition
1	Bilayer tablet of Aspirin and Omeprazole	Aspirin - 81mg Omeprazole - 40mg

Table 4: Excipients used in bilayer tablet.

S. No	Excipients	Company
1	Hydroxy propyl methyl cellulose	Yarrow chemproducts (Mumbai)
2	Starch	Yarrow chem products (Mumbai)
3	Microcrystalline cellulose reagents	Otto chemical bio chemical
4	Magnesium stearate	Otto chemical bio chemical reagents
5	Cross povidone	Yarrow chem products (Mumbai)

Table 5: List of various equipment's.

S. No	Name of equipment	Software	Model	Company
1	HPLC	Ez Chrome (version A.04.05)	1220 Infinity LC	Agilent
2	UV–spectrophotometer	Cary winUV (version 5.0.0.999)	Cary 60	Agilent
3	pH meter	N/A	LI 120	ELICO
4	Weighing balance	N/A	BL 220H	SHIMADZU
5	Sonicator	N/A	SS304	SISCA

Experimental work

Selection of detection wave length

Instrument and materials: A Cary 60-UV-Visible double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of AR grade from SD fine chem limited.
Solvent Selection: In order to select suitable solvent for simultaneous estimation of aspirin and omeprazole in various solvents were selected for the solubility studies and it was found that they were freely soluble in methanol, ethanol and acetonitrile. Hence in the present work, drugs were dissolved in methanol for all the dilutions [5-9].
Preparation of stock solution aspirin: Accurately weighed and transferred 25 mg of aspirin working standard into a 25 mL clean dry volumetric flask and it was dissolved by using methanol and the volume was made up to the mark with methanol (1000 µg/mL).
Preparation of working standard solution aspirin: 2.5 mL of the above standard stock solution was pipetted into 25 mL volumetric flask and diluted up to the mark with methanol to get concentration of 100 µg/mL. Futher dilution was carried out to prepare solution strength of 30 µg/mL.
Preparation of stock solution omeprazole: Accurately weighed and transferred 25 mg of omeprazole working standard into a 25 mL clean dry volumetric flask and it was dissolved by using methanol and the volume was made up to the mark with methanol (1000 µg/mL).
Preparation of working standard solution omeprazole: 2.5 mL of the above standard stock solution was pipetted into 25 mL volumetric flask and diluted up to the mark with methanol to get concentration of 100 µg/mL. Further dilution was carried out to prepare solution strength of 30 µg/mL.
Detection of isobestic point: Absorption maximum of aspirin and omeprazole was determined in UV Spectrophotometer and was found to be 229.2 and 299.4 nm. The isobestic point of two drugs shows at 233.8 nm. Hence this λmax was utilized for HPLC method development (Figure 4) [10].

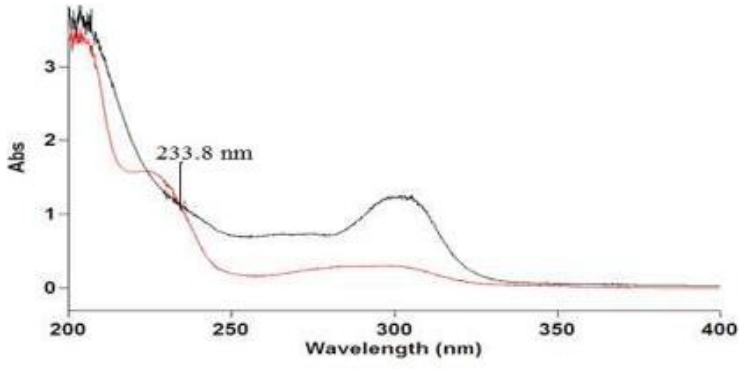

Figure 4: Isobestic point for aspirin and omeprazole

HPLC method development

Instrument and reagents: An Agilent model-1220 Infinity LC–HPLC system with Agilent openLAB CDS (EZ Chrome) software "version A.04.05" equipped with a variable wavelength detector (VWD) and a manual injector was used. It was manufactured by Agilent Technologies, USA. An Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 µm particle size) was used for the analytical separation and quantification of the mixtures.
Methanol: HPLC grade
Acetonitrile: HPLC grade

Trials
The present study describes the development and validation of RP-HPLC method for simultaneous estimation of aspirin and omeprazole in bulk and tablet dosage form. During development of the analytical method methanol: Acetonitrile (50:50), methanol: Acetonitrile (70:30), methanol: Acetonitrile (90:10) mobile phase ra were tried at a flow rate of 1 mL/min. Good resolved peaks were observed at 80:20 ratio of methanol: acetonitrile. Trials were further performed by changing the chromatographic parameter like flow rate (1-0.6 mL/min). Good separation with adequate resolution was observed at a flow rate of 0.6 mL/min in methanol: acetonitrile (80:20) [11].

Chromatographic conditions
Mobile phase: methanol: acetonitrile
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 µmparticlesize)
Detector wavelength: 233.8 nm
Injection volume: 20 µl
Run time: 10 min

Preparation of analytical solutions

Preparation of mobile phase: Based up on the solubility of the drugs methanol and acetonitrile was used a mobile phase in various ratio.

Aspirin standard solution
Stock solution: 25 gm of aspirin was accurately weighed and transferred to a 25 mL volumetric flask. The solution was diluted with 3/4th of diluent and sonicated for 10 minutes. Flask were made upto 25 ml with mobile phase to get
a1000 µg/mL solution.
Working standard solution: 1 mL of stock solution was diluted to 25mL with the mobile phase to obtain a 40 µg/mL solution [12-15].

Omeprazole standard solution
Stock solution: 25 mg of omeprazole was accurately weighed and transferred to 25 mL volumetric flask. The solution was diluted with 3/4th of diluent and sonicated for 10 minutes. Flask were made upto 25 mL with mobile phase to get a1000 µg/mL solution.
Working standard solution: 0.5 mL of stock solution was diluted to 25 mL.
with the mobile phase to obtain a 20 µg/mL solution.

Preparation of mixed standard stock solution
Stock solution: 100 mg of aspirin and 50 mg of omeprazole were weighed accurately into a 100 mL volumetric flask and dissolved in sufficient quantity of mobile phase and finally made upto 100 mL with the mobile phase.
Working standard solution: 4 mL of standard stock solution was taken into a 100 mL volumetric flask and diluted to 100 mL with mobile phase to get a concentration of 40 µg/mL of aspirin and 20 µg/mL of omeprazole [16].

Conditions for optimized method

Observation: Better resolution and symmetric Peaks.
Mobile phase: Methanol: Acetonitrile (80:20% v/v)
Flow rate: 0.6 mL/min
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm,5 µ particle size)
Detector wavelength: 233.8 nm
Injection volume: 20 µl
Run time: 5 min
Retention time: 1.729 min (Aspirin), 2.660 min (Omeprazole)

Validation

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of aspirin and omeprazole, and the solutions were injected five times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of five standard injections results should not be more than 2%.
Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific [17-20].
Linearity: 10% Standard solution: 0.1mL each from two standard stock solutions was pipetted out and made up to 10 ml. (10µg/mL of Aspirin and 5µg/mL of Omeprazole).
20% Standard solution: 0.2mL each from two standard stock solutions was pipetted out and made up to 10ml. (20 µg/mL of Aspirin and 10µg/mL of Omeprazole).
30% Standard solution: 0.3mL each from two standard stock solutions was pipetted out and made up to 10ml. (30 µg/mL of Aspirin and 15 µg/mL of Omeprazole).

40% Standard solution: 0.4 mL each from two standard stock solutions was pipetted out and made up to 10ml. (40 µg/mL of Aspirin and 20 µg/mL of Omeprazole)
50% Standard solution: 0.5 mL each from two standard stock solutions was pipetted out and made up to 10ml. (50 µg/mL of Aspirin and 25µg/mL of Omeprazole).
Precision:

- Preparation of Standard stock solutions: Accurately weighed 10 mg of aspirin, 5mg of omeprazole and transferred to 10 mL flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000 µg/mL of Aspirin and 500 µg/mL Omeprazole).
- Preparation of Standard working solutions (40% solution): 0.4 mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent. (40µg/mL of Aspirin and 20 µg/mL of Omeprazole).

Accuracy:

- Preparation of Standard stock solutions: Accurately weighed 10 mg of aspirin, 5 mg of omeprazole and transferred to 10 mL flasks and $3/4^{th}$ of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (1000 µg/mL of Aspirin and 500 µg/mL Omeprazole)
- Preparation of 80% Spiked Solution: 0.32 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 0.4 mL from each standard stock solution was pipetted out, and made up to the mark with diluent.
- Preparation of 100% Spiked Solution: 0.4 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 0.4 mL from each standard stock solution was pipetted out, and made up to the mark with diluent.
- Preparation of 120% Spiked Solution: 0.48 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 0.4 mL from each standard stock solution was pipetted out and made up to the mark with diluent.

Acceptance criteria: The % Recovery for each level should be between 98.0 to 102 %
Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.5mL/min), Flow plus (0.7mL/min), mobile phase minus, mobile phase plus, temperature minus (28°C) and temperature plus (32°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. % RSD was within the limit [21-25].
Limit of detection and limit of quantitation: The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined by considering stand deviation of the response and the slope. The slope was estimated from the calibration curve of the analyte. Stand deviation (σ) was calculated in excel using the function STEYX.

Tablet preparation:

Compression of bilayer tablet: Based on physiochemical characterization, solvent evaporation mixture containing PVP K-30 in the ratio of 1:3 (equivalent to 10 mg of drug) was selected to formulate into immediate release tablets. All the ingredients of immediate release
layer and sustained release layer were weighed separately and passed through sieve #.
Ingredients of immediate release layer and sustained release layer taken in a mortar separately and mixed for a period of 20 min using pestle. The prepared powder blends of the two layers (immediate and sustained) were evaluated for precompression parameters separately. These powder blends were used for the preparation of immediate and sustained release tablets by direct compression method. Based on the dissolution profiles of immediate release tablets and sustained release tablets the formulation IF (immediate release) and SF (sustained release) were selected for the preparation of bilayer tablets with varying concentration of Aspirin in the sustained release layer (Tables 6 and 7) [27-30].

Formulation of bilayer tablets:

Table 6: Ingredients of sustained release layer.

Ingredients	Quantity for a single tablet (mg)
Omeprazole	40 mg
Dry starch	10 mg
Cross povidone	10 mg
Microcrystalline cellulose	38 mg
Magnesium stearate	2 mg

Table 7: Ingredients of sustained release layer.

Ingredients	Quantity for a single tablet (mg)
Aspirin	80 mg
Hydroxy propyl methyl cellulose	20 mg
Starch	40 mg
Microcrystalline cellulose	56 mg
Magnesium stearate	4 mg

Manufacturing of bilayer tablets

The steps involved in manufacturing of bilayer tablets are shown in below.

Step 1: Ingredients of immediate release and sustained release layer with varying concentration of aspirin in sustained release layer were accurately weighed.

Step 2: Passed through 44 # sieve

Step 3: The powder was blend for 20 min

Step 4: Accurately weigh 200mg powder blend and fed into die cavity of tablet punching machine and compressed at 3 kg/cm^2 using 8 mm flat punches.

Step 5: Similarly, immediate release layer equivalent to 100 mg powder blend was fed to die cavity containing to sustained release layer so that the final hardness obtained for a bilayer floating tablet should be between 5.5-6.6 kg/cm^2 (Figure 5) [31-33].

Figure 5: Bilayer tablets.

Estimation of aspirin and omeprazole in bilayer tablet:

Preparation of sample stock solutions: 20 tablets were weighed, then the quantity of powder equivalent to 80 mg of aspirin was transferred into a 100 mL volumetric flask and 50 mL of diluents was added and sonicated for 25 min, then filtered by HPLC filters and the volume was made up with mobile phase. (800 µg/mL of Aspirin and 400 µg/mL of Omeprazole).

Preparation of sample working solutions: 5 mL of filtered sample stock solution was transferred to 100 mL volumetric flask and made up with mobile phase. (40 µg/mL of Aspirin and 20 µg/mL of Omeprazole).

This solution was injected into the column, and peak areas and retention times were recorded. Three different batches of bilayer tablet were analyzed using the validated method. For the analysis three replicates of each batch were assayed. Mean peak area of the drug was calculated and the drug contents in the tablet was quantified. The results shown were found to be in good agreement with labelled amounts, which confirms the suitability of the method for the analysis of drug in tablet dosage form.

RESULTS AND DISCUSSION

Optimized wavelength selected was 233 nm.

Method development: Method development was done by changing mobile phase ratios and flow rate.

Trail 1: Chromatographic conditions

Mobile phase: Methanol: Acetonitrile
Flow rate: 1 ml/min
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 µm particlesize)
Detector wave length: 233 nm
Column temperature: 30°C
Injection volume: 20 µl
Run time: 4 min
Diluent: Methanol and Acetonitrile in the ratio 50:50
Results: Unknown peaks with principal peaks Were observed, further trial is carried out (Figure 6) [34,35].

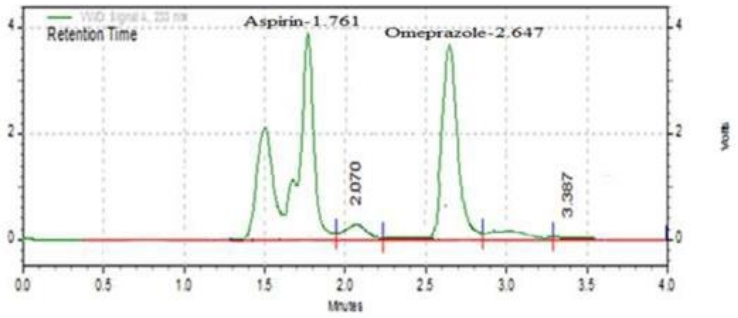

Figure 6: Trial chromatogram 1.

Trial 2:

Chromatographic conditions:

Mobile phase: Methanol: Acetonitrile
Flow rate: 1 ml/min
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm, 5 µmparticlesize)
Detector wave length: 233 nm
Column temperature: 30°C
Injection volume: 20 µl
Run time: 5 min
Diluent: Methanol and Acetonitrile in the ratio 70:30
Results: Broad peak of aspirin was obseved, Further trial is carried out (Figure 7).

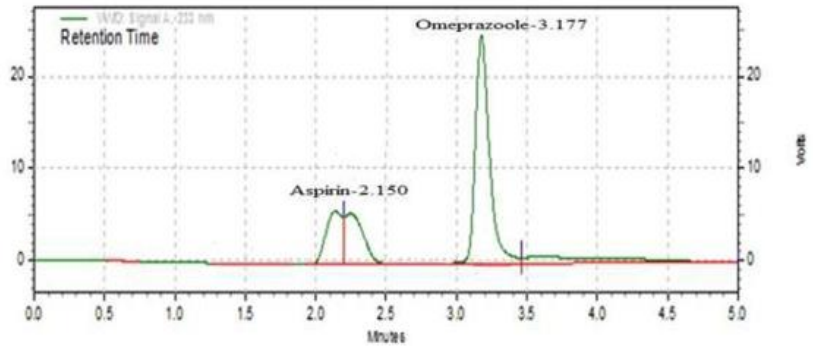

Figure 7: Trial chromatogram 2.

Trial 3:

Chromatographic conditions
Mobile phase: Methanol: Acetonitrile
Flow rate: 1 ml/min
Column: Eclipse XDB plus C18 Column (4.6 × 150mm, 5µmparticlesize)
Detector wave length: 233 nm
Column temperature: 30°C
Injection volume: 20 µl
Run time: 5 min
Diluent: Methanol and Acetonitrile in the ratio 80:20
Results: Elution of peaks was good, but retention time is less, further trial is carried out (Figure 8) [36-38].

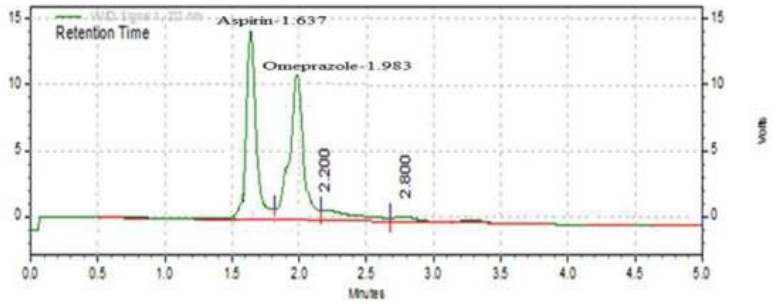

Figure 8: Trial chromatogram 3.

Trial 4:
Chromatographic conditions:
Mobile phase: Methanol: Acetonitrile
Flow rate: 0.8 ml/min
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µmparticlesize)
Detector wave length: 233 nm
Column temperature: 30°C
Injection volume: 20 µl
Run time: 5 min
Diluent: Methanol and Acetonitrile in the ratio 80:20
Results: Elution of peaks were good, but retention time is less, further trial is carried out (Figure 9) [39].

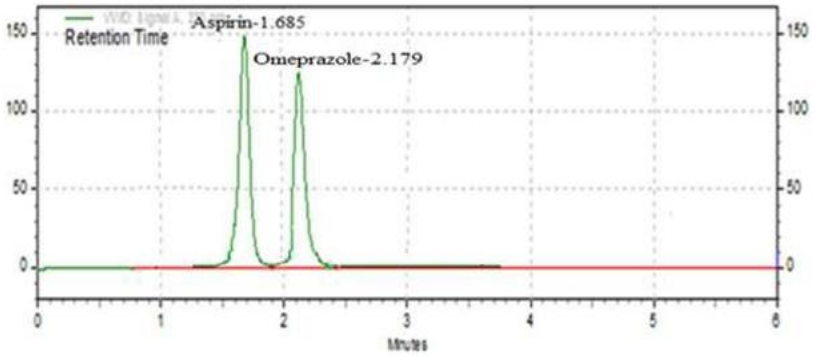

Figure 9: Trial chromatogram 4.

Optimized method

Chromatographic conditions:

Mobile phase: Methanol: Acetonitrile
Flow rate: 0.6 ml/min
Column: Eclipse XDB plus C18 Column (4.6 × 150 mm, 5µmparticlesize)
Detector wave length: 233 nm
Column temperature: 30°C
Injection volume: 20 µl
Run time: 5 min
Diluent: Methanol and Acetonitrile in the ratio 80:20
Results: Both peaks have good resolution, tailing Factor, theoretical plate count and resolution (Figure 10).

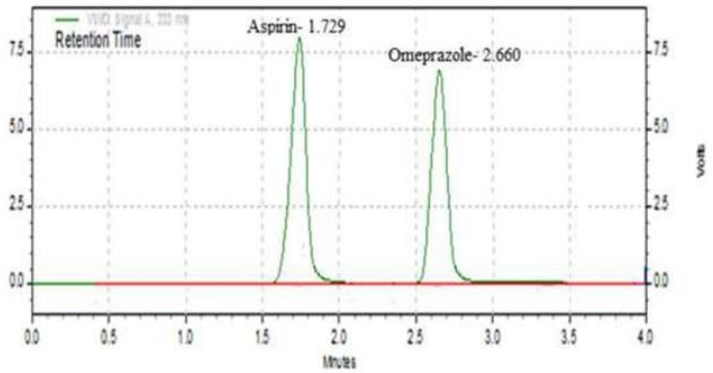

Figure 10: Optimized chromatogram.

Observation: Aspirin and Omeprazole were eluted at 1.729 min and 2.660 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.
System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines (Table 8) [40-42].

Table 8: System suitability parameters for Aspirin and Omeprazole.

S. no	Aspirin			Omeprazole			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	1.71	2369	1.09	2.663	3457	1.07	3.9
2	1.717	2418	1.04	2.665	3498	1.09	3.6
3	1.72	2459	0.94	2.667	3482	1.02	3.4
4	1.729	2512	0.96	2.66	3518	0.98	3.6
5	1.729	2541	1.09	2.667	3504	1.11	4.1

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation

Validation can be defined as a documented evidence which provide high degree of assurance that a process of a product consistently meets its pre-defined quality characteristics and attributes [43-45].

Specificity

Specificity is the quality of being exact, particular, and detailed rather than general or vague (Figures 11-13).

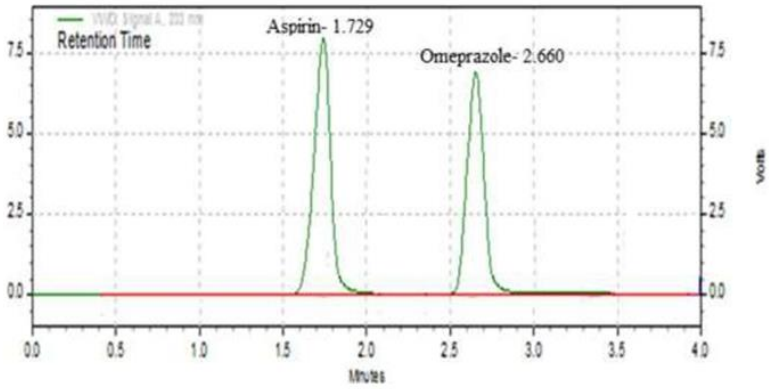

Figure 11: Chromatogram of placebo.

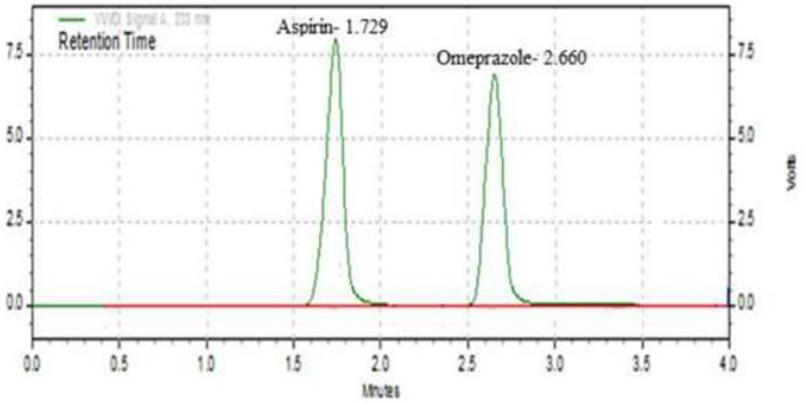

Figure 12: Chromatogram of standard.

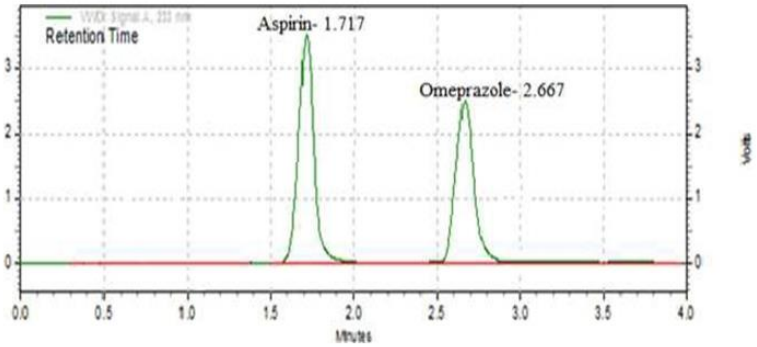

Figure 13: Chromatogram of standard with placebo.

Discussion: Retention times of Aspirin and Omeprazole were 1.717 min and 2.667 min respectively. We did not found and interfering peaks in placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Linearity is the property of a mathematical relationship, system or process where the output is directly proportional to the input (Table 9) (Figures 14 and 15).

Table 9: Linearity table for Aspirin and Omeprazole.

Aspirin		**Omeprazole**	
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

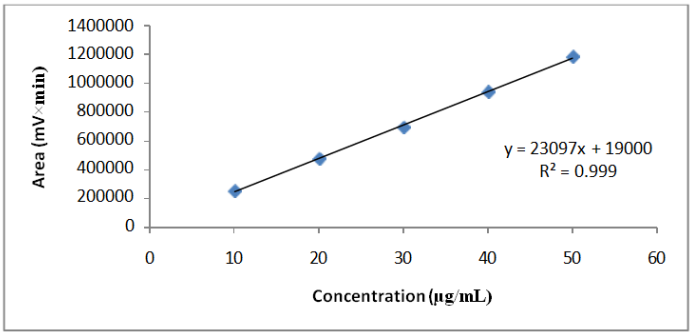

Figure 14: Calibration curve of Aspirin (Linearity).

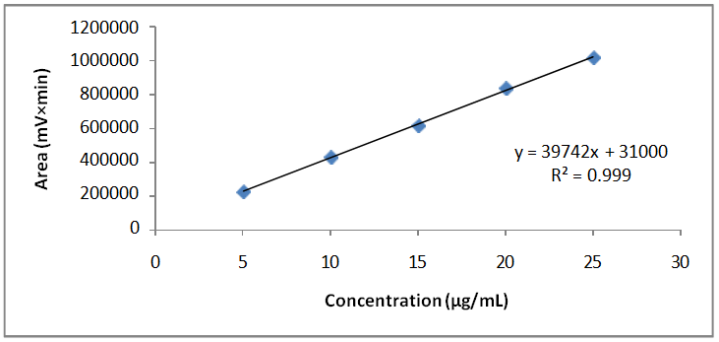

Figure 15: Calibration curve of Omeprazole (Linearity).

Discussion: Five linear concentrations of aspirin (10-50 µg/ml) and omeprazole (5-25 µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for aspirin was y=23097x + 19000 and of omeprazole was y=39742x + 31000 correlation coefficient obtained was 0.998 for the two drugs [46].

Precision

Precision is the degree to which repeated measurements, calculations, or estimates under unchanged conditions show the same results.

Repeatability

System precision: It is the degree of closeness (agreement) between individual test results when an analytical method is applied repeatedly to multiple samplings of a homogeneous sample under identical, unchanged condition (Table 10).

Table 10: System precision table of Aspirin and Omeprazole.

S. No	Area of Aspirin	Area of Omeprazole
1	938929	834315
2	939231	834292
3	939889	834837
4	941284	831293
5	939721	832041
6	938727	833889
Mean	939630.2	833444.5
S.D	924.88	1429.08
%RSD	0.09	0.17

Intermediate precision (Day - Day Precision)

It measures the variation in analytical results obtained within a single laboratory over an extended period, accounting for random events like different days, analysts, equipment, or reagent batches (Table 11) [47].

Table 11: Intermediate precision table of Aspirin and Omeprazol.

Days	Area of Aspirin	Area of Omeprazole
Day - 1	939285	835217
	938171	834101
Day - 2	939273	835184
	938115	834075
Day - 3	939242	835245
	938093	834139
Mean	938696.5	834660.2
S.D	625.26	608.8
%RSD	0.06	0.07

Discussion: Multiple sampling from a sample stock solution was done and three working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.06% and 0.07% respectively for Aspirin and Omeprazole. As the limit of Precision was less than "2" the system precision was passed in this method [48].

Accuracy

Accuracy is the degree to which a measurement, calculation, or piece of information aligns with its true, actual, or accepted value (Table 12).

Table 12: Evaluation data of accuracy study.

Analyte	Accuracy level	Amount added	Amount found	Recovery (%)	Mean recovery (%)
Aspirin	80%	32	31.9	99.68	99.76
		32	31.92	99.75	
		32	31.95	99.84	
	100%	40	39.95	99.87	99.99
		40	40.01	100.02	
		40	40.04	100.1	
Omeprazole	120%	48	47.98	99.95	100.04
		48	48.03	100.06	
		48	48.05	100.1	
	80%	16	15.92	99.5	99.62
		16	15.93	99.56	
		16	15.97	99.81	
	100%	20	19.95	99.75	99.9
		20	19.98	99.9	
		20	20.01	100.05	
	120%	24	23.97	99.87	100.01
		24	24.01	100.04	
		24	24.03	100.12	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.93% and 99.84% for Aspirin and Omeprazole respectively [49].

Limit of detection and limit of quantitation

It is the lowest concentration of an analyte that can be reliably detected but not necessarily quantified (Table 13) (Figures 16 and 17).

Table 13: LOD & LOQ table of Aspirin and Omeprazole.

Molecule	LOD	LOQ
Aspirin	1.85	5.62
Omeprazole	1.09	3.31

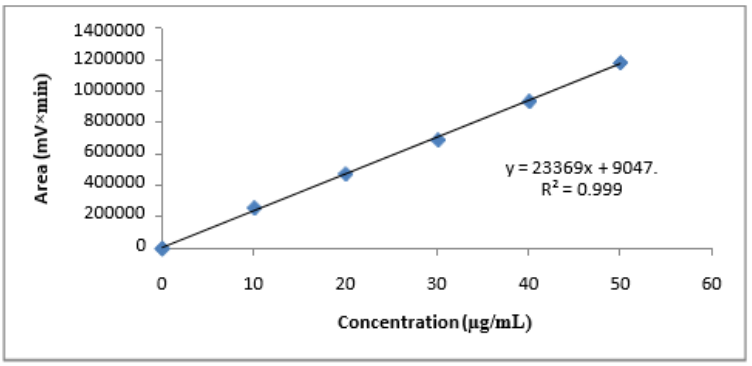

Figure 16: LOD calibration curve of Aspirin.

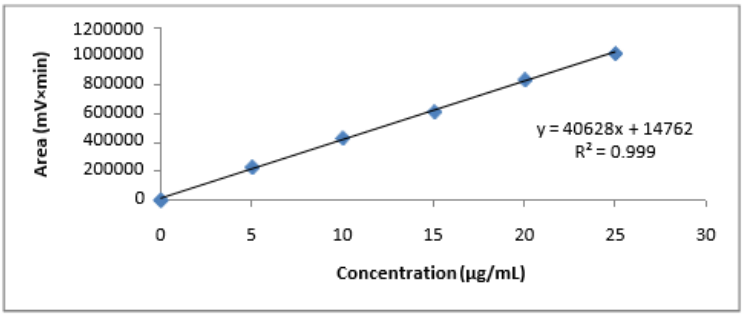

Figure 17: LOQ calibration curve of Omeprazole.

Robustness

Robustness is the ability of a system, process, or concept to withstand stress, variations, or adverse conditions without failing or experiencing significant degradation (Tables 14 and 15).

Table 14: Robustness data for Aspirin.

S.No	Parameter	Retention time(min)	Peak area (mV × min)	Tailing factor
1	Standard	1.729	939889	1.04
2	Flow rate (-) 0.5ml/min	1.721	939834	1.11
3	Flow rate (+) 0.7ml/min	1.697	939881	0.94
4	Mobile phase (-) 78B:22A	1.709	939856	1.24
5	Mobile phase (+) 82B:18A	1.74	939984	1.27
6	Temperature (-) 28°C	1.72	939874	1.01
7	Temperature (+) 32°C	1.745	939814	1.15

Table 15: Robustness data for Omeprazole.

S.NO	Parameter	Retention time(min)	Peak area (mV × min)	Tailing factor
1	Standard	2.66	838837	1.14
2	Flow rate (-) 0.5ml/min	2.665	838874	1.09
3	Flow rate (+) 0.7ml/min	2.653	838812	0.94
4	Mobile phase (-) 78B:22A	2.594	838754	1.35
5	Mobile phase (+) 82B:18A	2.696	838923	1.29
6	Temperature (-) 28°C	2.654	838805	1.16
7	Temperature (+) 32°C	2.669	838893	1.21

Discussion: (0.7ml/min), Robustness conditions like Flow minus (0.5ml/min), Flow plus mobile phase minus (78B:22A), mobile phase plus (82B:18A), temperature minus (28°C) and temperature plus(32°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table 16) (Figure 18).

Estimation of aspirin and omeprazole in bilayer tablet

Table 16: Estimation data for bilayer tablet.

Sample tablet		Batch	Lable claim (mg)	Amount found	% Amount found	% RSD
Bilayer tablet	Aspirin	1	80	79.68	99.6	0.13
		2	80	79.72	99.65	
		3	80	79.52	99.4	
	Omeprazole	1	40	39.57	98.42	0.14
		2	40	39.68	99.2	
		3	40	39.59	98.97	

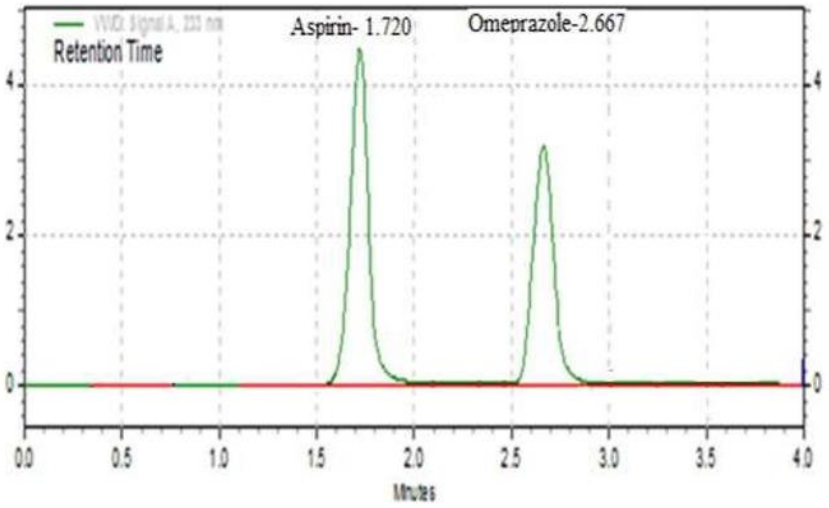

Figure 18: Chromatogram of working sample solution.

Discussion: The developed method was used to analyse the bilayer tablets manufactured in our laboratories. A clear resolution of the drugs was achieved with no interference from excipients. Total amount of drugs actually present in the bilayer tablet were estimated to be >98% for both drugs.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Aspirin and Omeprazole in Tablet dosage form. Retention time of Aspirin and Omeprazole were found to be 1.729 min and 2.660. The method was validated as per International Conference on Harmonization (ICH) guidelines. % RSD of the Aspirin and Omeprazole were and found to be 0.09 and 0.17 respectively. % Recovery was obtained as 99.93% and 99.84% for Aspirin and Omeprazole respectively. LOD, LOQ values obtained from regression equations of Aspirin and Omeprazole were 1.85, 1.09 and 5.62, 3.31 respectively. Regression equation of Aspirin was y=23097x+19000, and of Omeprazole was y = 39742x + 31000. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

The statistical parameters and recovery data reveals the good accuracy and

precision of the method. Finally, since no pharmacopoeial method for determination of ASP & OMP in bulk and pharmaceutical formulations have been reported yet. The method could be useful and suitable for the estimation of the ASP & OMP in bulk and pharmaceutical formulations.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

ETHICAL APPROVAL

Not applicable.

CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

Nill.

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