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Der Pharmacia Lettre, 2018, 10 [4]: 57-67
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ISSN 0975-5071
USA CODEN: DPLEB4

A Validated HPLC-DAD Method for Combined Aspirin and Omeprazole Synthetic Mixture

Vidya V Gawande[1*] and Anil Chandewar V[2]

Pataldhamal Wadhwani College of Pharmacy, Dhamangaon road, Yavatmal (MS) India

[*]***Corresponding author:*** *Vidya VG, Pataldhamal Wadhwani College of Pharmacy, Dhamangaon road, Yavatmal (MS) India. E-mail: viddya.gawande@rediffmail.com*

ABSTRACT

A validated HPLC-DAD method had been developed for combined synthetic mixture of Aspirin (AS) and Omeprazole (OME). The analytes were separated using C18 Water Xterra (50 × 4.6 mm id, particle size 3.5µm) as stationary phase with mobile phase composition water (containing 0.1% Triethylamine pH adjusted to 2.47 ± 0.03 with Orthophosphoric acid): Methanol (50:50% v/v). The Flow rate was 1.2 ml/min opting isocratic mode with runtime of 7 min. and the analytes were detected at 244 nm. The average retention time for both the analytes was found to be 0.89 ± 0.37 min and 4.28 ± 1.91 min for AS and OME respectively. As the Indian market still lacked the selected finished dosage form (YOSPRALA); the laboratory synthetic mixture was prepared and directly compressed into tablets and then analysed successfully using proposed method. The% of label claim for both the drugs was obtained nearly 100 ± % RSD <2. ICH guidelines were followed to validate the method. The method assured linearity for 50-150% of labelled claim for both the analytes. The method assured high degree of precision and accuracy. The method was proved to be robust by assessing robustness parameters.

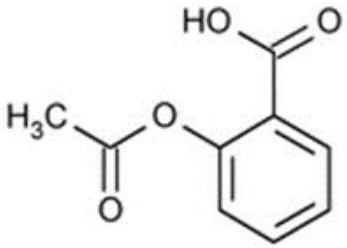
Keywords: Aspirin, Omeprazole, Validation, Linearity, Accuracy.

INTRODUCTION

Aspirin (AS) is chemically an orally administered analgesic, antipyretic and an anti-inflammatory drug. It is pure salicylic acid derivative. It is proved to be effective to treat acute rheumatic fever, rheumatoid arthritis, osteoarthritis, post-myocardial

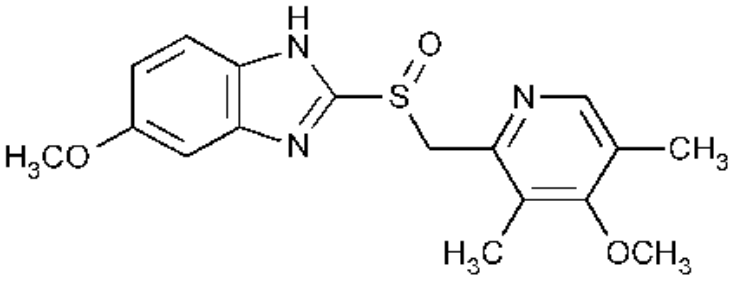
infarction and poststroke patients. Aspirin is irreversible cyclooxygenase-1 inhibitor; acts by acetylating and blocking thromboxane synthesis. It reduces the incidence of myocardial infarction by 30%, stroke by 20%, and all-cause mortality by 18% when used for secondary prevention of thromboembolic events. Omeprazole (OME) is a proton pump inhibitor. It is used in treatment of peptic ulcer, Gastroesophageal reflux disease (GERD), Zollinger Ellison Syndrome, Aspiration pneumonia [1-10].

YOSPRALA is a fixed-dose combination of AS, an anti-platelet agent and OME, a proton pump inhibitor (PPI) consisting of delayed-release AS and immediate-release OME nowadays preferred for secondary prevention of cardiovascular and cerebrovascular events in patients who are at risk of developing aspirin-associated gastric ulcers. *YOSPRALA* manufactured by Aralez pharmaceuticals is not available in Indian market yet. The fixed dose combination containing AS and OME in the ratio 2:1 (Figure 1).



Aspirin

(AS)



Omeprazole

(OME)

Figure 1: Chemical structures of drugs Aspirin (AS) and Omeprazole (OME)

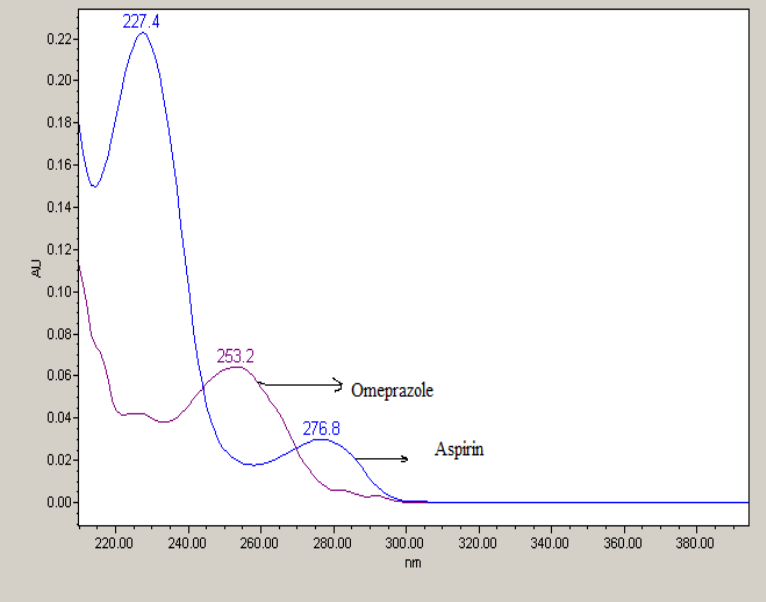


Figure 2: Overlain spectra of Aspirin (AS) and Omeprazole (OME)

MATERIALS AND METHODS

Instrumentation

The assembled analytical instruments used for analysis comprised of WATER liquid chromatography equipped with a model 600 solvent pump, a 996 photodiode array detector, and 515 autosampler. Empower version.2 software (Water Spa, Milford, USA) was used for data acquisition and recording chromatograms. The additional instruments used were Digital Weighing Balance Citizen Model CY104, Ultrasonic Bath Sonicator, Digital pH meter Model EQ 610 [11-15].

Chemicals and reagents

Working standards of AS (99.73%) and OME (99.85%) were obtained as gift samples from Cipla Labs, Mumbai and Triveni chemicals, Vapi. As the marketed formulation *YOSPRALA* is not available in indian market; synthetic mixture was prepared in laboratory and compressed into tablet. All the chemicals Methanol, Water, Orthophosphoric acid, Triethylamine used for HPLC-DAD analysis were of HPLC grade purchased from E. Merck, Mumbai. The excipients used for preparation of synthetic mixture were directly compressible lactose, starch, talc, magnesium stearate.

Chromatographic conditions

The resolution of compounds was obtained using Waters Xterra C18 column with dimensions of (50 ×4.6 mm ID, particle size 3.5 µm). The mobile phase optimization was aimed for simple mobile phase opting isocratic elution mode. So, the developed mobile phase consisting of Water (containing 0.1% Triethylamine pH adjusted to 2.47 ± 0.03 with Orthophosphoric acid): Methanol (50:50% v/v). The mobile phase was filtered through 0.45 µ membrane filter and degassed before use. The flow rate

was 1.2 ml/min and runtime kept was 7 min. The eluents were monitored at 244 nm as the wavelength selected by taking overlain spectra as shown in Figure 2. The sample size for injection was 20 µl with analysis was carried out at ambient temperature [16-19].

Preparation of standard stock solution and sample solution

Preparation of standard stock solution

The ratio of label claim of both the drugs is 2:1. Accordingly AS (81 mg) and OME (40 mg) were weighed out and dissolved in methanol in 100 ml volumetric flasks separately to give standard stock solutions of 810 µg/ml for AS and 400 µg/ml for OME respectively.

Preparation of mixed standard solution

Aliquot portions of stock solutions of both the drugs were taken and transferred to 10 ml volumetric flask and diluted to mark with methanol to get final concentration of 81 µg/ml for AS and 40 µg/ml for OME respectively.

Preparation of laboratory synthetic mixture

As the marketed formulation *YOSPRALA* is not available in Indian market; laboratory synthetic mixture containing active pharmaceutical ingredients with tablet excipients required for direct compression was prepared for 100 tablets. The mixture was compressed into tablets. The formula was developed by taking the required percentage of excipients along with active pharmaceutical ingredients. The weighed ingredients were mixed and blended together. The mixture was then compressed into tablets. The compressed tablets were subjected to quality control tests. Hardness, Friability, weight variation tests, disintegration time of tablets was determined. The results obtained were within the acceptable range. The formula for batch manufacturing with calculated amounts of each ingredient is given in Table 1.

Table 1: Quantities of ingredients for batch manufacturing.

S. No	Name of ingredient	Quantity for one tablet (mg) Each tablet contains	Quantity for 100 tablets
1	Aspirin	81 mg	8.1 g
2	Omeprazole	40 mg	4 g
3	Directly compressible lactose	110 mg	11 g
4	Starch (6.225%)	14.38 mg	1.438 g
5	Talc (1%)	2.31 mg	0.231 g
6	Magnesium stearate (1%)	2.31 mg	0.231 g

	Total	250 mg	25 g

Preparation of sample solution

About 20 pre-formulated and compressed tablets were weighed individually and average weight was determined. The tablets were powdered and an accurate equivalent weight of tablet powder for AS (81 mg) and OME (40 mg) was transferred to 100ml volumetric flask and dissolved in methanol. The content was sonicated for 15 min and required volume was made. Then it was filtered through 0.45 µ membrane filter. An appropriate aliquot was diluted with methanol to get final concentration of 81 µg/ml for AS and 40 µg/ml for OME respectively. The mixed standard solutions and sample solution were analysed using optimized chromatographic conditions (Figure 3).

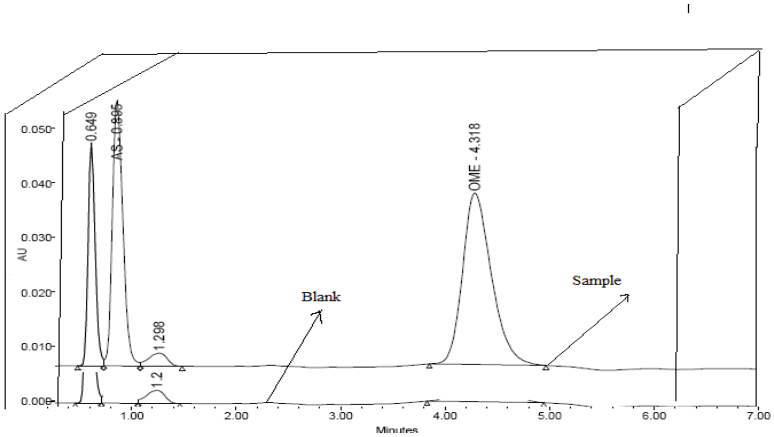


Figure 3: Overlain chromatogram of Aspirin (AS) and Omeprazole (OME) with blank.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

As the marketed formulation *YOSPRALA* was not available in Indian market; laboratory synthetic mixture containing active pharmaceutical ingredients with directly compressible excipients was prepared and tablets were directly compressed. For chromatographic analysis was to be carried out, use of different stationary phases were tried with different column packings; finally, C_{18} Waters Xterra column (50 × 4.6 mm ID, particle size 3.5 µm) was selected as it showed the good resolution and sharp peaks of both the analytes within relatively shorter run time. Several solvent systems comprising binary or ternary mixtures of organic solvents such as methanol, acetonitrile with aqueous solvents like water with varying pH were tried. Finally, isocratic mobile phase comprised of Water (containing 0.1% Triethylamine pH adjusted to 2.47 ± 0.03 with Orthophosphoric acid): Methanol (50:50% v/v) showed early retention with good resolution and sharp peaks of both the analytes therefore was selected. The optimized mobile phase was run opting isocratic mode with flow rate and runtime of 1.2 ml/min and 7 min

respectively. The analytes were detected at 244nm. The mean retention time of AS and OME were found to be 0.89 ± 0.37 min and 4.28 ± 1.91 min respectively. The resolution of eluted peaks was >1.5 indicating higher scale for it. System suitability parameters were recorded for optimized chromatographic conditions. The results for system suitability parameters are summarized in Table 2.

Table 2: System suitability parameters.

S. No.	Mean ± % RSD (n=5)*	Eluted peaks	
		AS (1)	OME (2)
1	t_r (min) ± % RSD	0.89 ± 0.37	4.28 ± 1.91
2	k´ ± % RSD	1.0286 ± 0.73	8.7602 ± 1.76
3	N ± % RSD	3158 ± 1.74	4221 ± 1.35
4	Peak area ± % RSD	263366.2 ± 1.025	760710.6 ± 0.17
5	Asymmetry	1.2536 ± 1.78	1.075 ± 1.48
Eluted Peak Pair 1 And 2			
6	Rs ± % RSD	7.34 ± 1.97	
7	α ± % RSD	3.51 ± 0.56	
Note: *Mean of five determinations, tr – Retention time, k´– Capacity factor, N – Plate number, Tf – Peak asymmetry factor, Rs – Resolution, α – Selectivity (Separation factor)			

Assay of laboratory mixture by proposed method

The mixed standard solutions and sample solution were prepared as given in 2.4.2 and 2.4.4. For six individual weighing of sample, analysis was carried out n=6 for each sample injection. The average peak areas for each sample were considered for calculations. The data is summarized in Table 3.

Table 3: Results of assay of laboratory synthetic mixture.

S. No.	Name of drug	Label claim (mg)	Amount found *(mg) ± % RSD	% of label claim* ± % RSD
1	AS	81	80.47 ± 0.85	99.35 ± 0.84
2	OME	40	39.81 ± 1.22	99.53 ± 1.22

Note: *Mean of six estimations

METHOD VALIDATION

Specificity studies

Specificity studies concluded that Placebo (diluents i.e. mobile phase) and excipients present in compressed formulation didn't found to interfere with analytical results of drugs.

Linearity and Range

The mixed standard solution was prepared as given in 2.4.1 and 2.4.2 and diluted with methanol to get final concentrations ranging from 50-150% of labelled claim for both drugs. The solution of each concentration level was injected five times and response obtained was plotted graphically as mean peak area vs. concentration of analyte in µg/ml. The linear regression equations for AS and OME were found to be Y=3146x+4846 and Y=19387x-11442 respectively. The values for correlation coefficient for both the drugs were found to be 0.999 indicating the acceptable degree of linearity. The method assured linearity in concentration range of 40.5 µg/ml-121.5 µg/ml and 20 µg/ml -60 µg/ml for AS and OME respectively.

Accuracy studies

Accuracy was assessed by Multilevel Recovery Studies with Standard addition method. In this, the standard analytes were spiked to pre-analysed tablet powder sample at three different levels 80%, 100%, 120% of labelled claim and injected in developed chromatographic conditions six times. The mean% recovery values of AS and OME were found to be excellent for all three levels of recovery studies. The results are summarized in Table 4.

Table 4: Recovery data for accuracy studies.

S. No	% Recovery Level	Standard added	Amount added (µg)	Mean Recovery µg ± % RSD at each level (n=6)	Mean% Recovery ± % RSD at each level	Mean% Recovery ± % RSD
1	80	AS	64.8	64.95 ± 0.96	100.23 ± 0.96	99.71 ± 0.46
		OME	32	32.01 ± 1.01	100.03 ± 1.00	--
2	100	AS	81	80.6 ± 1.19	99.5 ± 1.19	--
		OME	40	39.95 ± 1.25	99.83 ± 1.31	--
3	120	AS	97.2	96.6 ± 0.99	99.39 ± 0.99	--
		OME	48	47.88 ± 0.82	99.74 ± 0.82	99.87 ± 0.15

Precision

Precision was assured by comparing determined% (RSD) values at three concentration levels with acceptable range ± <2. Intraday precision included analyzing the standard samples on same day with variations in time interval of analysis using the optimized chromatographic conditions. Interday precision was carried out by analyzing standard samples on three consecutive days. The % RSD values for intraday and interday precision were found to be within acceptable limit (RSD ± <2). The data of precision studies is summarized in Table 5.

Table 5: Data for intraday and inter-day precision (n=6).

S. No	%		Measured Mean Concentration ± % RSD		% Amount ± % RSD	% Amount ± % RSD
	Conc level	Conc (µg/ml)	Intra-day precision (n=6)	Inter-day precision (n=6)	Intra-day Precision (n=6)	Inter-day precision (n=6)
			AS		AS	
1	50	40.5	40.27 ± 0.13	40.33 ± 0.11	99.44 ± 0.13	99.58 ± 0.11
2	80	64.8	65 ± 0.43	64.62 ± 1.37	100.3 ± 0.43	99.72 ± 1.37
3	150	121.5	121.83 ± 0.58	121.09 ± 1.49	100.27 ± 0.57	99.66 ± 1.49
			OME		OME	
1	50	20	19.93 ± 1.83	20.1 ± 0.78	99.64 ± 1.83	100.5 ± 0.78
2	80	32	31.83 ± 0.89	31.87 ± 0.49	99.47 ± 0.89	99.58 ± 0.49
3	150	60	59.91 ± 0.64	59.76 ± 0.58	99.85 ± 0.64	99.61 ± 0.58

Limit of Detection and Limit of Quantitation

Limit of detection refers to the lowest detectable quantity of analytes in a sample.

It can be calculated as,

LOD = 3.3 σ / S

Where, σ = The standard deviation of the response.

S = The slope of the calibration curve.

Limit of quantitation refers to the lowest quantity of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

It can be expressed as:

LOQ = 10 σ / S

Where, σ = The standard deviation of the response.

S = The slope of the calibration curve.

It was determined by injecting the series of known concentration of analytes. The Limit of detection of AS and OME were found to be 0.04517 µg/ml and 0.003620 µg/ml respectively.

The Limit of quantitation of AS and OME were found to be 0.1369 µg/ml and 0.01097 µg/ml respectively.

Robustness studies

Robustness parameter assured the ability of method to remain unaffected by small, deliberate variations in chromatographic parameters. The variations in chromatographic parameters include pH of mobile phase, organic phase composition of mobile phase, flow rate, detection wavelength. Only one parameter was changed deliberately at once and remaining parameters were kept unchanged; in order to observe the accurate results. Firstly, pH was varied ± 0.1 unit and standard analyte solutions were run; the obtained results were unaffected by pH change. Secondly, change in organic phase composition (Methanol) ± 10% was carried out; there was no remarkable change in resolution and retention time of both the analytes. Thirdly, flow rate was altered to ± 10%. (i.e., 1.1 ml/min and 1.3 ml/min). Flow rate of 1.1 ml/min showed increased runtime by 0.5 (from 7 min to 7.5 min). Flow rate of 1.3ml/min revealed decreased runtime by 2 min (from 7 min to 5.0 min). Fourthly, the detection wavelength was varied ± 5nm and system suitability parameters were recorded for change in each parameter. All robustness studies were carried out using mixed standard analyte solutions.

CONCLUSION

So far no HPLC, HPTLC method had been attempted for simultaneous estimation of this combination yet; the proposed research work suggested a validated simple, rapid, adventageous HPLC-DAD method for separation of drugs. The mean% recovery results depicted that there was no interference of excipients in the formulation. The RSD% showed the high degree of precision. The proposed method was found to be robust with respect to flow rate, pH, mobile phase composition, detecting wavelength. Therefore, the developed method was found to be simple, accurate, precise, robust, rapid, advantageous, reliable, economic,

reproducible. Hence, the method can be used for routine analysis of both the drugs individually, and in bulk powder, tablet formulation in routine analysis of pharmaceutical industries as well as quality control laboratories.

ACKNOWLEDGEMENTS

The authors are thankful to management of P. Wadhwani College of Pharmacy, Yavatmal for providing necessary facilities for analytical work. The authors are also thankful to Cipla Labs, Mumbai and Triveni chemicals, Vapi for providing gift samples of drugs.

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