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FORMATION AND VALIDATION OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS ASSESSMENT OF ASPIRIN AND OMEPRAZOLE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

YOSPRALA is a newly developed tablet that, due to its immediate release of Omeprazole (40 mg) and delayed release of Aspirin (81 mg) or (325 mg) dose power, is effective for cardiovascular as well as gastrointestinal safety. Yosprala was approved for cardiovascular and cerebrovascular diseases by the USFDA in Sept 2016. Aspirin is an antiplatelet agent & Omeprazole is an inhibitor of the proton pump. **Purpose:** To develop a modern, reliable, clear, fast, inexpensive and sensitive RP-HPLC method to quantify aspirin and Omeprazole tablet form. **Method:** On RP-HPLC, the separation of these molecules is accomplished by using the column of PHENOMENEX C8 (150 × 4.6mm, 5µm). This accomplishment is accomplished with the help of a mobile phase containing a 75:25 ratio of phosphate buffer (ph 7.5) and acetonitrile, with a sample induction of 20µl. At 280 nm, the wavelength is chosen with a flow rate of 01 ml/min. **Results:** The retention time of Aspirin was found to be 2.2 and for Omeprazole it was 8.4min. The linearity range is 32-48µg/ml and 8-12µg/ml for Aspirin and omeprazole respectively. The correlation

coefficient was 0.9996 forAspirin and omeprazole. The procedure was validated for accuracy and less than 2.0 percent RSD was found for both aspirin and omeprazole. For standard deviation, relative standard deviation, coefficient of variance and the results were within the range, the methodology was statistically validated. The above method is therefore simple, affordable, cost-effective, economical and durable.

KEYWORDS: Yosprala; Aspirin; Omeprazole; HPLC.

INTRODUCTION

The leading cause of death in the United States is cardiovascular disease (CVD). In fact, CVD accounted for 30, 8% of all deaths in the U.S. in 2013.[1] The efficacy of aspirin is well known in the literature in primary and secondary prevention of CVD (including coronary heart disease, cerebrovascular disease, and peripheral artery disease) and has long been integrated in clinical guidelines.[2–4]

Aspirin [2-(acetyloxy) benzoic acid] functions as a cyclooxygenase inhibitor, leading to inhibition of prostaglandin biosynthesis. It also prevents the accumulation of platelets and is used to avoid arterial and venous thrombosis.

However, aspirin raises the possibility of bleeding, most even from the gastrointestinal (GI) tract, though sometimes intracranially, in spite of its beneficial effects.

In secondary prevention of cardiovascular disease, aspirin has a well-established effect, but increases gastrointestinal bleeding, especially when used in high-risk patients. Unfortunately, gastrointestinal bleeding is widely expected in patients following acute coronary syndrome and is separately correlated with an increased risk of adverse cardiovascular incidents and mortality.[5]

Proton pump inhibitors (PPIs) have revolutionized the treatment of gastric acid-related disorders ever since their introduction in the 1980s. They are the most effective gastric acid secretion inhibitors available, and are thus the key therapy for many gastric problems today, ranging from dyspepsia and gastroesophageal reflux disease to peptic ulcers and bleeding from GI. The protective effect of PPIs has been shown in many studies to prevent aspirin-induced gastric ulcers and bleeding.[6]

In patients needing dual antiplatelet therapy (DAPT).[7] proton-pump inhibitors (PPIs) have been shown to effectively reduce the risk of gastrointestinal bleeding and can increase aspirin adherence due to a reduction in dyspepsia.[8-9] The prophylactic use of PPIs in high-risk patients needing antiplatelet therapy is also recommended by multidisciplinary consensus guidelines. However, there has been increasing concern in potential adverse drug interactions between aspirin and PPIs that affect the efficacy of aspirin, antiplatelet activity. A possible pharmacological association between these commonly prescribed agents has been confirmed by early preclinical evidence, however this theoretical role is substantiated by insufficient clinical data.

Yosprala is a newly developed tablet that, due to its immediate release of Omeprazole (40 mg) and delayed release of Aspirin (81 mg), is effective for cardiovascular as well as gastrointestinal protection. Yosprala was approved for cardiovascular and cerebrovascular diseases by the USFDA in Sept 2016. Aspirin is an antiplatelet agent and Omeprazole is an inhibitor of the proton pump.

Structure

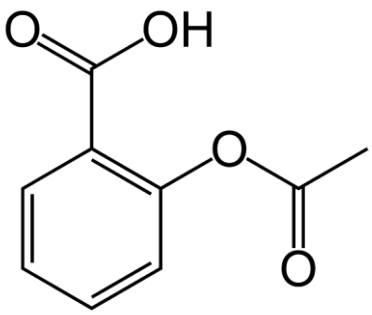

Figure: Aspirin

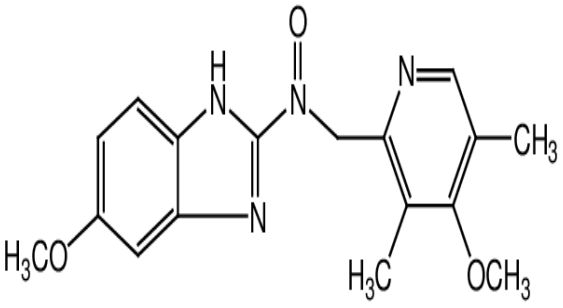

Figure: Omeprazole

MATERIALS AND METHODS

Chemicals and Reagent: The commercially accessible Epclusa formula containing Omeprazole 100mg and Aspirin 400 mg bought from the US market. Global Pharmaceuticals provides the reference work standards for both drugs. E.Merck Ltd, Pakistan acquired potassium dihydrogen phosphate, phosphoric acid and acetonitrile (HPLC grade).

Instrument used: A Hitachi, Model (5110-5410), Adwa pH meter, Model AD 1020.

Solutions preparation

Diluent: Acetonitrile and distilled water were used as a diluent in the ratio of 80:20.

Preparation of solutions

Solution A

Dissolve 0.725gm of monobasic sodium phosphate and 4.472gm anhydrous dibasic sodium phosphate in 1000ml D.I water, adjust pH to 7.6 with phosphoric acid.

Solution B

Dissolve 1.045gm of tribasic sodium phosphate dodecahydrate and 1.958gm dibasic sodium phosphate dihydrate in 100ml D.I water.

Mobile phase

Mobile phase was prepared by mixing Acetonitrile and Solution A (Phosphate buffer having h 7.6) in the ratio of 25:75. Mobile phase was filtered by using 0.4 µm membrane filter paper and sonicate for 5 minute.

Standard stock solution

Accurately weigh working standard of Aspirin 81.2mg and Omeprazole 10mg in 250ml volumetric flask, add 10ml methanol and dissolve. Add 10ml Solution B and makeup volume with D.I water.

Standard preparation

Transferred 5ml in 50ml volumetric flask from Standard stock solution and volume was made up with DI.Water. Final solution having a concentration of Aspirin 120 µg/ml and Omeprazole 30 µg/ml.

Sample stock solution

Weigh and crush 20 tablets and then accurately Weigh equivalent to 81.2mg Aspirin and 10mg Omeprazole in 250ml volumetric flask, add 10ml methanol and dissolve. Add 10ml Solution B and 150ml D.I water, stir for 10-15 minutes and makeup volume to the mark with D.I water.

Sample preparation

Filter Sample stock solution and transfer 5ml of the filtrate solution in 50ml volumetric flask and makeup volume to the mark with D.I water.

Chromatographic condition: Separation and quantification of the two drugs was achieved by using a C8 column of PHENOMENEX (150 x 4.6 mm x 5 µm). A 20 µl solution sample

was injected. Flow rate was set to be1 ml / min and absorbance was measured at 280 nm. Column temperature has been adjustedto 25 ° C.

RESULTS AND DISCUSSIONS

Method development: Drug analysis is the main role of drug production and development. There are no analytical procedures for new drugs in the Pharmacopoeia, so a simple, precise, and specific linear method of analysis needs to be developed.

The choice of mobile phase was based on the separation of two drugs with ideal resolution and spikes of Aspirin and Omeprazole, which were achieved on a PHENOMENEX C8-HPLC column.

Method validation: The advanced technique like system suitability, specificity, accuracy linearity, robustness, LOD and LOQ has been certified according to ICH and USP rules.

System suitability test: According to the USP guideline, suitability tests were performed prior to running samples for verification. The RSD of six sample of both drugs was 0.21% and 0.49%, indicating that the HPLC system has good precision. Table 1 shows the results obtained by suitability.

Table 1(a): System Suitability of Aspirin Table 1(b): System Suitability of Omeprazole.

%RSD (<2.0)	Tailing Factor	Theoretical Plates	%RSD (<2.0)	Tailing Factor	Theoretical Plates
0.32	1.42	4960	0.98	1.42	4759

Specificity: It is the capacity to determine the interference between analyte and other compound. Figure 1 shows no interference between analyte and other compound like mobile phase and placebo, indicating the specificity of analytical technique.

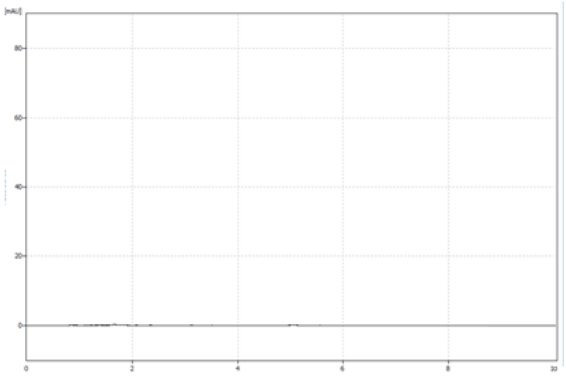

Fig. 1(a): Chromatogram of Placebo.

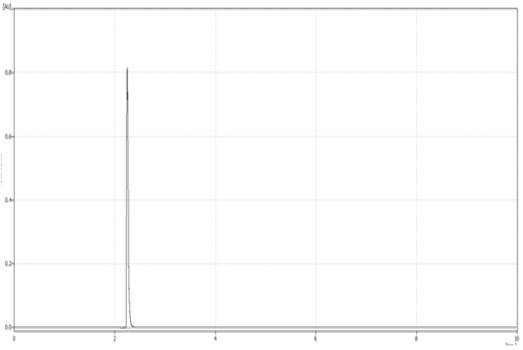

Fig. 1(b): Chromatogram of aspirin.

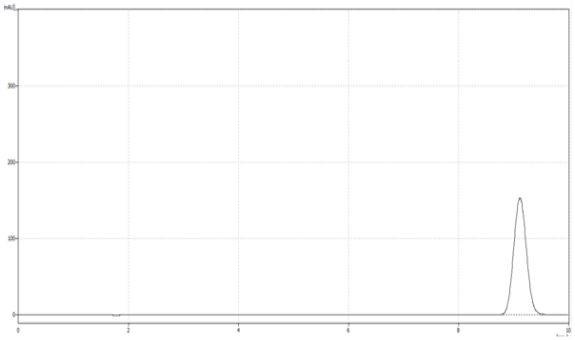

Fig. 1 (c): Chromatogram of omeprazole.

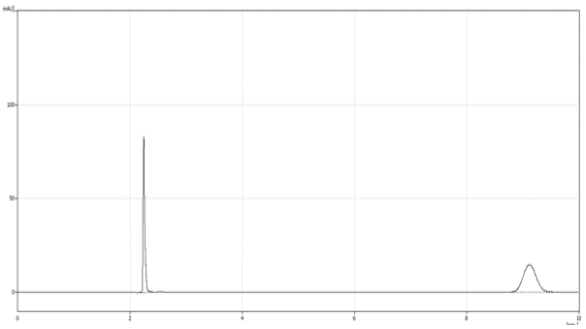

Fig 1(d): Chromatogram of both drugs.

Linearity: Linearity is the relation between absorbance and concentration of drugs. That was shown by plotting the X-axis and Y-axis plots at concentrations from 96 ug / ml to 144 ug / ml Aspirin and 24 ug / ml to 36 ug / ml Omeprazole. The LOD and LOQ of Aspirin are 2.633ug / ml and 7.978ug / ml, respectively, and Omeprazole is 1.116ug / ml and 3.382ug / mlrespectively. Results are shown in Figure 2(a) and 2(b).

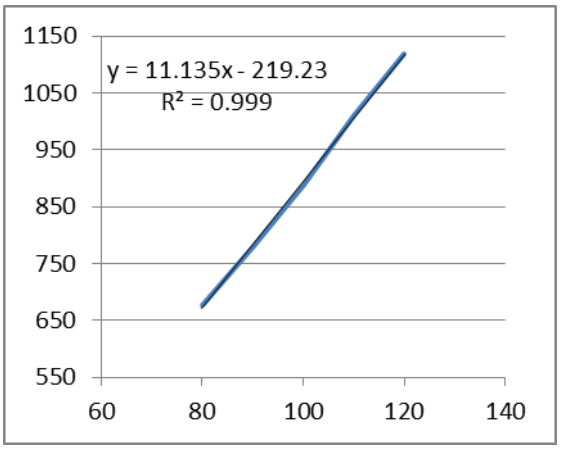

Fig. 2 (a): Aspirin Linearity plot

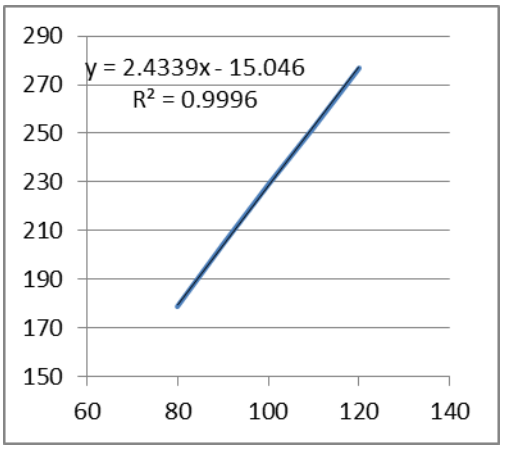

Fig. 2 (b): Omeprazole Linearity plot

Recovery: After spiking 80%, 100% and 120% sample concentration of Omeprazole andAspirin in Placebo, Recovery was achieved by injecting replicate samples.

Table 4 shows spiked and retrieved values.

Table 4: Recovery of Aspirin and Omeprazole.

Recovery Level	Drugs	Amount Spiked (mg/ml)	Amount Recovered (mg/ml)	Recovery (%age)	Standard Deviation	RSD(%)
80%	Aspirin	260 260 260	255.87 255.67 256.03	98.40	0.182	0.07
	Omeprazole	32 32 32	32.92 32.752 32.048	101.79	0.37	1.15
100%	Aspirin	325 325 325	318.98 329.48 328.54	100.20	5.80	1.78
	Omeprazole	40 40 40	40.06 40.88 39.60	100.45	0.52	1.31
120%	Aspirin	390 390 390	391.17 384.96 391.04	99.75	3.54	0.91
	Omeprazole	48 48 48	48.028 48.30 48.384	100.49	0.15	0.31
			Aspirin	Omeprazole		
Overall Mean			99.45%	100.91%		
Overall Standard Deviation			0.936	0.762		
Overall % RSD			0.94	0.755		

Precision: Precision of analytical method shows the degree of scattering between samples. Proposed precision by evaluating the six sample replicates. Assay of each duplicate and calculation of the percentage RSD of the sample. The results obtained are in table 5.

Table 5: Precision results of Aspirin and Omeprazole.

Drugs	Peak Areasof Replicate	Average Peak Areas of each replicate	Assay %	Average	Standard Deviation	%RSD
Aspirin	915.67	952.126	101.49	100.243	5.62	0.61
	988.582					
	906.119	905.2565	99.35			
	904.394					
	904.092	910.127	99.88			
	916.162					
Omeprazole	229.864	232.616	101.71	99.80	2.459	1.077
	235.369					
	222.586	226.741	99.01			
	230.896					
	225.637	225.679	98.68			
	225.721					

Robustness: It is the little variation in different perimeter of analysis, to check sample stability. Variations such as flow rate and column temperature do not affect the performance of the method. Table 6 shows the results obtained.

Table 6 (a): Robustness result of omeprazole.

Level		Peak Areas of Replicate		Theoretic alplates	Tailing factor	Resolution	Standard Deviation	%RSD
100% conc.	Change in flow rate	0.9ml/min	217.111	5597	1.456	21.45	0.43	0.19
			216.251	5735	1.459	21.66		
		1.0ml/min	215.350	5808	1.470	21.66	1.20	0.55
			217.756	5881	1.463	21.77		
		1.1ml/min	215.418	5957	1.470	21.88	0.53	0.24
			214.357	6031	1.470	21.98		
	Change in column Temp.	25°C	219.551	5148	1.437	20.95	0.37	0.16
			218.810	5270	1.441	21.14		
		30 °C	217.517	5332	1.451	21.24	0.48	0.22
			218.483	5397	1.455	21.34		
		35 °C	217.979	5461	1.448	21.34	0.37	0.17
			217.238	5595	1.459	21.45		

Table 6 (b): Robustness result of aspirin.

Level		Peak Areas of Replicate		Theoretical plates	Tailing factor	Resolution	Standard Deviation	%RSD
100% conc.	Change in flow rate	0.9ml/min	909.930	4111	1.50	2.001	1.08	0.11
			907.753	4111	1.50	1.988		
		1.0ml/min	904.938	3951	1.514	1.954	0.42	0.04
			904.092	3951	1.514	1.954		
		1.1ml/min	904.027	3951	1.514	1.949	0.96	0.104
			902.106	3951	1.514	1.941		
	Change in column Temp.	25°C	909.593	4460	1.472	2.086	0.53	0.05
			910.655	4460	1.486	2.063		
		30 °C	903.599	4460	1.486	2.049	3.7	0.40
			911.032	4460	1.486	2.049		
			908.155	4280	1.459	2.043		
		35 °C	909.930	4111	1.50	2.001	0.92	0.10
			910.008	4111	1.500	1.993		

CONCLUSION

In this study, quantitative method of analysis in tablet dosage forms for Aspirin and Omeprazole were developed. Resolution between Aspirin and Omeprazole gives goodresults. USP and ICH guidelines were used to validate the method based on the above experimental results. The validated method can therefore be used in a tablet dosage form for numerical analysis of Aspirin and Omeprazole.

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