ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Special Issue 4, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF REVERSE PHASE-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE FOR THE CONCOMITANT ASSESSMENT OF OMEPRAZOLE AND PIPERINE IN BULK FORM

SUSITHRA E*, PAVANI CH[2]

Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. Email: esusithra@gmail.com

Received: 10 October 2018, Revised and Accepted: 11 December 2018

ABSTRACT

Objective: The immense literature study was carried out and disclosed that here no method arrived for the concomitant assessment of omeprazole and piperine in bulk form by using RP-HPLC. Hence, an effort was assembled to arise a easy, specific, precise, reliable, linear, rapid, and validated reverse phase-high-performance liquid chromatography (RP-HPLC) technique for the simultaneous assessment of omeprazole and piperine in bulk form.

Methods: The chromatographic analysis of omeprazole and piperine was performed using a RP-HPLC (WATERS) provided with autosampler and ultraviolet (UV) detector with the software of EMPOWER Version 2. The chosen conditions were isocratic separation with two mobile phase composed of acetonitrile:buffer (phosphate buffer: pH 6.5 ± 0.1) (55:45). Detection was carried out using UV/visible double-beam spectrophotometer at 320 nm. The method was validated as per the ICH guidelines.

Results: The retention time for omeprazole and piperine by proposed HPLC method was found to be 2.767 and 4.029 min, respectively. The correlation coefficients are 0.999. The developed chromatographic method was found to be accurate with recovery 99.2–99.8% and was found within the acceptance criteria (i.e., 98.0–102.0%) with acceptable % relative standard deviation of not >2% at each level.

Conclusion: Thus, the proposed HPLC procedure for the concomitant assessment of omeprazole and piperine was accurate, precise, linear, robust, simple, and economic.

Keywords: Omeprazole, Piperine, Reverse phase-high-performance liquid chromatography, Validation, Simultaneous evalution.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11s4.31718

INTRODUCTION

Omeprazole is a proton-pump inhibitor by apprehension of gastric H+, K+ -ATPase, and in this way, it controls the gastric acid production and omeprazole containing substituted benzimidazole ring in its structure [1-5].

Piperine is a major alkaloid of black pepper, having several pharmacological activities. Along with that, it also enhances the bioavailability of various drugs. Currently, it has been patented as bioavailability enhancer and as an important ingredient of incapacitating composition [6-10].

The immense literature study was carried out and disclosed that here no method arrived for the concomitant assessment of omeprazole and piperine in bulk form by using RP-HPLC. Hence, an effort was made to originate a easy, specific, close, accurate, linear, rapid, and validated high-performance liquid chromatography (HPLC) method for the simultaneous assessment of omeprazole and piperine.

METHODS

Instrumentation

A HPLC (WATERS) equipped with autosampler and ultraviolet detector with the software of EMPOWER Version 2 was used. Complete weighings are done on single pan weighing balance (Shimadzu).

Reagents and standards

Omeprazole and piperine (Fig. 1) standards were obtained from Piramal, India. Analytical grade methanol and acetonitrile were purchased from Merck Specialties Pvt. Ltd., Mumbai. Double-distilled water was used throughout the experiment.

Standard stock solution preparation procedure

To the 10 ml volumetric flask, add 0.1 g of omeprazole and 0.1 g of piperine and 7 ml of mobile phase. The above mixture was sonicated up to become a solution and finally build the solution 10 ml with mobile phase. From the prepared above stock solution (omeprazole and piperine), pipette out 0.3 ml into a 10 ml volumetric flask and thinned out up to 10 ml with similar mobile phase to acquire 30 µg/ml concentration, respectively. The above-prepared stock solutions were filtered across 0.45 µm membrane filter paper using vacuum filter.

RESULTS

Method development

About 20 mL of the standard solution injected into the injector port and calculated the areas (omeprazole and piperine peaks) (Fig. 2).

Optimized chromatographic conditions

Optimized chromatographic conditions were obtained after using mobile phase, acetonitrile:buffer (Phosphate buffer: pH 6.5±0.1) (55:45), by selecting 1 ml/min flow rate and 240 nm detection wavelength. The separation achieved on XTerra RP8 column (4.6 mm×150 mm and 3.5 µm).

Validation of analytical method

Precision

The method precision was performed by intraday precision studies. In the intraday precision studies, five replicated standard solutions were prepared and injected into port, and % relative standard deviation (RSD) and response factor were determined and are reported in Table 1. In the same manner for the interday precision, five replicated

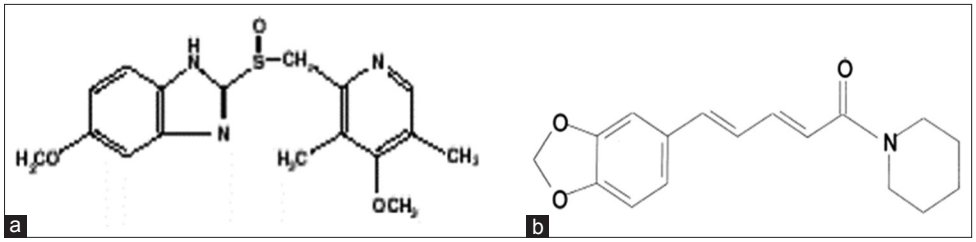

Fig 1: Chemical structures of (a) Omeprazole and (b) Piperine [2]

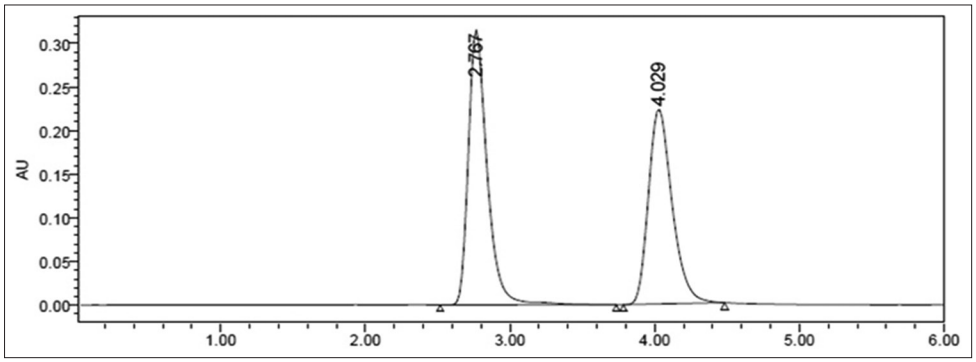

Fig 2: Chromatogram of standard solution (omeprazole 30 µg/ml AND piperine 30 µg/ml)

standard solutions were prepared and injected into port, and % RSD and response factor were determined and are reported in Table 2.

Linearity

The linearity study was made from a series of standard solutions of omeprazole and piperine. For omeprazole and piperine, suitable volumes of stock solution of 1000 µg/ml were diluted to obtain a series of solutions having concentrations of 10–50 µg/ml of omeprazole and piperine. Each solution was injected, and chromatograms were recorded. The peak areas were represented against concentration to get calibration curves for omeprazole and piperine. The calibration curves were linear in the range of 10–50 µg/ml of omeprazole and piperine and are reported in Table 3.

Accuracy

The accuracy of the method was checked at three different levels of 80, 100, and 120% solutions made from standard solutions of omeprazole and piperine and calculated the individual recovery and mean recovery values. The percentage average recoveries were obtained in between 99.7 and 99.9 and are reported in Tables 4 and 5.

Detection limit (LOD) and quantification limit (LOQ)

LOD and LOQ were found out from the signal-to-noise ratio. Resolution of the signal-to-noise ratio is accomplished by differentiating calculated signals from samples with familiar small concentrations of analyte with those of blank samples and determining the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 and 2:1 is acceptable for estimating the LOD and for LOQ signal-to-noise ratio 10:1 is generally acceptable [11-15].

LOD and LOQ values of omeprazole and piperine are reported in Table 6 and 7.

Robustness

It is defined as a degree of its potential to endure unchanged by negligible predetermined variations in optimized method specifications such as variation of flow rate (+1 ml/min), mobile phase composition, and temperature [16-20]. Here, no consequence effect on peak area and holding time was constructed.

System suitability parameters

These are the parameters to assure that the method can originate the results of defensible accuracy and precision. System suitability parameters were assessed by injecting mixed standard preparation in replicate. Parameters such as tailing factor, theoretical plates, and resolution were determined [21-24]. The system suitability parameters for the method are listed in Table 8.

Table 1: Precision results for omeprazole and piperine

Injection	Area of omeprazole	Area of piperine
Injection-1	2,849,314	2,534,539
Injection-2	2,860,134	2,539,247
Injection-3	2,861,298	2,544,661
Injection-4	2,863,959	2,548,839
Injection-5	2,874,416	2,558,822
Average	2,861,824	2,545,221
Standard deviation	8983.0	9330.0
%RSD	0.31	0.37

RSD: Relative standard deviation

Table 2: ID precision results for omeprazole and piperine

Injection	Area of omeprazole	Area of piperine
Injection-1	2,837,703	2,540,424
Injection-2	2,837,396	2,545,953
Injection-3	2,800,105	2,552,894
Injection-4	2,864,566	2,514,155
Injection-5	2,859,837	2,558,072
Average	2,839,922	2,542,300
Standard deviation	25498.3	17102.9
%RSD	0.90	0.67

RSD: Relative standard deviation

RESULTS AND DISCUSSION

The current research was run out to arise a simple, diplomatic, accurate, and precise reverse phase (RP)-HPLC technique for the study of omeprazole and piperine in bulk form. The holding times for omeprazole and piperine were found to be 2.767 and 4.029 min, respectively. Each standard was injected 5 times and for each standard get the consistent peak areas. A fine correlation coefficient (r=0.999) was noticed between the deliberations and area under the curves. Precision was found out, and the reports were showed the % RSD value which is below 1.00 and reveals that the recommended HPLC

Table 3: Linearity results for omeprazole and piperine

S. No.	Linearity level	Omeprazole		Piperine	
		Concentration (microgram/mL)	Area under the curve	Concentration (microgram/mL)	Area under the curve
1	I	10	894,043	10	920,032
2	II	20	1,913,389	20	1,752,782
3	III	30	2,906,620	30	2,521,426
4	IV	40	3,800,672	40	3,326,009
5	V	50	4738193	50	4,217,393
Correlation coefficient		0.999		0.999	

Table 4: Accuracy results for omeprazole (analyte recovery)

Percentage concentration at specification level (%)	Area under the curve	Weight added (mg)	Weight found (mg)	Percentage recovery (%)	Average recovery (%)
80	4,306,922	7.58	7.43	98.0	99.7
100	5,784,168	10.0	9.98	99.8	
120	7,162,858	12.2	12.3	101.3	

Table 5: Accuracy results for piperine (analyte recovery)

Percentage concentration at specification level (%)	Area under the curve	Weight added (mg)	Weight found (mg)	Percentage recovery (%)	Average recovery (%)
80	3,769,304	7.63	7.48	98.1	99.9
100	5,023,657	10.0	9.98	99.8	
120	6,311,333	12.3	12.5	101.9	

Table 6: LOD results for omeprazole and piperine

Parameters	Omeprazole	Piperine
Concentration (µg/mL)	0.012	0.016
Retention time (Rt)	2.772	4.043
Height (µv)	128	132
Area	1147	1512

LOD: Detection limit

Table 7: LOQ results for omeprazole and piperine

Parameters	Omeprazole	Piperine
Concentration (µg/mL)	0.042	0.053
Retention time (R_t)	2.771	4.044
Height (µv)	429	427
Area	3844	4892

LOQ: Quantification limit

Table 8: System suitability parameters for omeprazole and piperine

S. No.	Parameters	Omeprazole	Piperine
1	Area	2,849,708	2,534,375
2	Retention time (Rt)	2.776	4.042
3	Resolution (R_s)	-	4.7
4	Tailing factor (T)	1.4	1.3
5	Number of theoretical plates (N)	2313	2979

method was accurate and particular. The quantity of drug retrieved was presented in Table 4 and 5. The proposed technique was sturdy as noticed from unsubstantial changes in the consequences of study by small changes in mobile phase composition, flow rate, and temperature. Thus, the proposed RP-HPLC technique was built to be easy, specific, definite, correct, and less time-consuming.

CONCLUSION

Thus, the proposed RP-HPLC technique for the concomitant assessment of omeprazole and piperine in bulk form was accurate, precise, linear, robust, simple, and economic.

ACKNOWLEDGEMENTS

The writers are thankful to the Piramal, India, for contributing omeprazole and piperine drugs.

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