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

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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, 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

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.

RESULTS

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.

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...
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.

## 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
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.

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REFERENCES

1. Available from: http://www.drugbank.ca/drugs/DB00338.
2. Available from: http://www.rxlist.com/prilosec-drug.htm.
3. Available from: http://www.medicine.nevada.edu/wps/Proceedings/52/18-20_PWPS-52-0111.pdf.
4. Available from: http://www.questhealthlibrary.com/herbs/piperine.
5. Available from: http://www.drmajeed.com/articles/2000TheMedicinalUsesOfPepper.pdf.
6. Snyder LR, Kirkland JJ, Glajch JI. Practical HPLC Method Development. 2nd ed. New York: Wiley; 1997.
7. ICH QIA (R2). Stability Testing of New Drug Substances and Products; 2003.
8. ICH, Q2B. Validation of Analytical Procedure: Methodology. Geneva: International Conference on Harmonization, IFPMA; 2005.
9. ICH. Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology; 1996.
10. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartite Guidelines; 1996.
11. Skoog, DA, Holler DM, Crouch SR. Fundamentals of Analytical Chemistry. 8th ed. Belmont, CA: Thomson-Brooks/Cole; 2004. p. 644-55.
12. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed., Vol. 2. New Delhi: CBS Publishers and Distributors; 1997. p. 157-74.
13. Connors KA. A Textbook of Pharmaceutical Analysis. 3rd ed. New Delhi: Wiley Inter Sciences Inc.; 1997. p. 157-74.
14. Douglas A, Holler SF, Nieman TA. Principles of Instrumental Analysis. Singapore City: Thomson Asia Pte Ltd.; 2005. p. 725-60.

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

Table 8: System suitability parameters for omeprazole and piperine

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.
16. Watson DG. Pharmaceutical Analysis. In: A Text Book for Pharmacy Students and Pharmaceutical Chemists. 2nd ed. Harcourt Publishers Limited.; 2005. p. 221-32, 311.
17. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental Methods of Analysis. 6th ed. New Delhi: CBS Publishers and Distributors; 1986. p. 1-15.
18. Sharma BK. Instrumental Methods of Chemical Analysis. 19th ed. Meerut: Goel Publishing House; 2000.
19. Sethi PD. High-Performance Liquid Chromatography. 1st ed. New Delhi: CBS Publisher; 2001. p. 1-103.
20. Vogel AI, Bassett J. Vogel’s Text Book of Quantitative Inorganic Analysis. London, New York: Longman; 1962. p. 193-208.
21. Ewing GW. Instrumental Methods of Chemical Analysis. New York: McGraw-Hill Book Co., Inc.; 1985. p. 340-5.
22. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd ed. New Delhi: CBS Publisher; 1997. p. 51-64.
23. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. Gurgaon, Mumbai: Himalaya Publishing House Pvt. Ltd.; 2007. p. 2566-638.
24. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development. 2nd ed. New York: Wiley Inter Science; 1997. p. 1-56, 234-89, 685-712.