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An Integrated HPLC Assay Technique for the Detection of Omeprazole in Mixed Dose Forms with Aspirin, Diclofenac or Domperidone

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Omeprazole (OMP), which is given for treatment in conjunction with a number of medications, is drawing increased attention for its capacity to reduce gastric acid formation. Commonly administered OMP-loaded dose forms include aspirin (ASN), diclofenac (DCF) and domperidone (DMP). As a result, a single liquid chromatographic approach, which can simultaneously determine OMP-ASN, OMP-DCF or OMP-DMP in commercial formulations was developed. On a reverse-phase Agilent Eclipse XBD C18 analytical column (4.6 × 150 mm; 3.5 (m), the requisite separation was accomplished utilizing a gradient flow of the mobile phase made up of a mixture of solvents A and B (50:50 v/v) and diode array detection at 272 nm. Where solvent A is acetonitrile:buffer-A:buffer-B (10:90:50 v/v/v) and solvent B is acetonitrile:buffer-A:buffer-B (90:10:50 v/v/v). The buffer-B is made up of an equal mixture of 100 mM acetic acid and 100 mM triethyl-amine with a final attained pH of 5.2, whereas the buffer-A is made up of 10 mM trifluoroacetic acid (pH adjusted to 2.2). According to ICH Q2(R1) criteria, the method had been validated and verified the senstivity of the proposed method with a linearity range of 0.5-20 mcg/mL for all the analytes. The established technique for concurrently quantifying OMP with DCF, ASN or DMP in commercial formulations can be regularly employed in the quality control laboratory in accordance with the validation conditions.

Keywords: Aspirin, Diclofenac, Domperidone, Gradient flow, HPLC, Omeprazole.

INTRODUCTION

Omeprazole (OMP), a proton pump inhibitor, is used to treat excessive stomach acid [1]. It is usually indicated during the diagnosis of erosive esophagitis, Zollinger-Ellison syndrome, peptic ulcers and gastroesophageal reflux disease (GERD) [2]. In 2003, OMP was approved by The Food and Medicine Administration (FDA) USA for sale as an over-the-counter drug to treat recurrent heartburn [3]. Almost all the medicinal drugs tend to stimulate hydrochloric acid output, which might cause gastroesophageal reflux disease (GERD). As a result, in today's practice, every prescription contains a proton pump inhibitor. When a patient is advised of multiple medication therapies, the coadministration of a proton pump inhibitor becomes critical. Meanwhile, various mixed-dose versions of OMP and other APIs are available in the market. In adult asthmatics with gastroesophageal reflux, combined therapy with OMP and DMP can reduce symptoms and improve pulmonary function [4]. In 2016, the FDA approved a new fixed-dose combination formulations of OMP with non-steroidal anti-inflammatory drugs such as ASN and DCF. The OMP-ASN combination has been shown to prevent cardiovascular and cerebrovascular events in patients at risk of ASN-associated stomach ulcers. The OMP-DCF combination is useful in individuals with osteoarthritis or rheumatoid arthritis, reducing secondary dysmenorrhea problems [5].

Numerous HPLC techniques are available for the analysis of omeprazole [6-8], aspirin [9-11], diclofenac [12,13] and domperidone [14]. The measurement of OMP, coupled with ASN [15-19], DCF [20] and DMP [21] in mixed dose forms, has been done using HPLC. However, there is currently no HPLC method that can analyze all four of the aforementioned medications in their commercial formulations. The primary goal of the suggested technique is to develop a standardized

HPLC method for the quantitative determination of omeprazole (OMP) in bulk, mixed dose forms or physical mixtures with aspirin (ASN), diclofenac (DCF) and domperidone (DMP).

EXPERIMENTAL

Drugs *viz*. omeprazole (OMP), diclofenac (DCF), aspirin (ASN) and domperidone (DMP) of pharmaceutical grade were supplied by the pharmaceutical industries in Visakhapatnam, India. To make dilutions, HPLC-grade water was purchased from Qualigens in Mumbai, India. Acetonitrile of the HPLC grade was bought from Finar, India. All other chemicals and solvents of analytical quality were also procured from the commerical sources.

The study was carried out using a binary gradient HPLC system (Shimadzu, Kyoto, Japan) equipped with two pumps (LC-20AD) and a diode array detector (SPD-M20A) with a manual injector.The four drugs were separated chromatographically using a reverse-phase Agilent Eclipse XBD C18 analytical column (4.6 mm × 150 mm; 3.5 µm). LC-Solution software (Shimadzu, Japan) was used to record chromatographic analysis and data integration on a computer. A reverse-phase Agilent Eclipse XBD C_{18} analytical column (4.6 mm × 150 mm; 3.5 mm internal diameter) was used to chromatographically separate the four medicines. Computerized chromatographic analysis and data integration was recorded using LC-Solution software (Shimadzu, Japan).

Development and optimization of the HPLC method: Water with acetonitrile or methanol as mobile phase was tested in preliminary experiments with varied ratio and flow rates. Due to the poor separation, the pH of the mobile phase had to be adjusted. Gradient elution of the organic phase and acidic pH buffer systems (formic acid, orthophosphoric acid and trifluoro acetic acid) were utilized to enhance the separation. Experiments were run with triethylamine, ammonium acetate, trifluoroacetic acid, formic acid and ammonium bicarbonate buffers alone or in combination to generate a nice peak pattern (shape, narrow and sharp) and considerable separation. Faster and more reliable separation is typically achieved by altering buffer strength and capacity, pH, composition and the addition of ion-pair reagents. Many columns were trailed to test the influence of stationary phases for achieving the desired separation such as Agilent Eclipse XDB-C18 (4.6 × 150 mm; 3.5 µm), Fortis C-18 (4.6 × 250 mm; 5.0 µm), Altima HP C-18 Amide (4.6 × 150 mm; 5.0 µm), XTerra® RP 18 (4.6 × 100 mm, 5.0 µm), Enable C-18G (4.6 × 250 mm; 5.0 µm).

Preparation of buffers

Buffer A [10 mM trifluoroacetic acid (TFA)]: Accurate volume of 0.80 mL of trifluoroacetic acid was taken in 100 mL of HPLC grade water. The solution was adjusted to pH 2.2 followed by vacuum filtration through a 0.45 µm membrane filter.

Buffer B (100 mM acetic acid (AA):100 mM triethyl amine (TEA) (50:50)]: Accurate volume of 0.280 mL of acetic acid was taken in 50 mL of HPLC grade water and pH was measured to be 2.7. Now, 0.695 mL of triethylamine was taken in another 50 mL of HPLC grade water and the pH of the solution was again measured (pH: 10.98). A mixture of the prepared acetic acid and triethylamine buffers was made in a ratio of 50:50 and subjected to pH measurement (final pH: 5.2) followed by filtration through a 0.45 µm filter membrane under vacuum.

Preparation of mobile phase: The gradient flow programme uses a mixture of solvents A and B (50:50 v/v) as the mobile phase (Table-1). Solvent A is a 10:90:50 (v/v/v) combination of acetonitrile, buffer A and buffer B; solvent B is a 90:10:50 (v/v/v) combination of the same ingredients. Immediately following their respective preparation, solvents A and B were filtered through 0.45 µm Millipore membrane filters and sonicated for 1 h. A diluent is also made using a 50/50 acetonitrile/water mixture for use in making our standards and quality control solutions.

TABLE-1
PROGRAM FOR MOBILE PHASE GRADIENT FLOW

Time (min)	0.01	2.50	10.0	15.0
Flow rate (mL/min)	0.2	0.4	0.8	0.4

Standards and quality control solutions: Accurately weighed 25 mg of OMP, DCF, ASN and DMP and placed them separately in different volumetric flasks. Complete drug solubility was achieved by adding a little amount of methanol to each flask. Each analyte was diluted to a concentration of 1000 µg/mL in its own diluent and the volume was then adjusted accordingly. Dilutions of each drug stock solution were used to create working standards (100 µg/mL). Similarly, a new mixture of all the analytes was generated before analysis to generate a set of calibration standards with suitable concentrations. The calibration curves required the preparation of five standard solutions ranging in concentration from 0.5 to 20 µg/mL (five points). Diluted working (all analyte) or calibration standards were used to prepare quality control (QC) samples for accuracy and robustness testing.

Several different combinations of commercial brands were purchased from the local pharmacy shop that all shared OMP as their common drug. Ten tablets of each brand were weighed on a precision scale, and the powder was dissolved in 100 mL of diluents using ultrasonication for around 10 min, resulting in an active ingredient equivalent to 50 mg. To get three separate concentrations of the extracted samples, the supernatant liquid was filtered and appropriately diluted with diluent. The assay result was confirmed to be comparable with the labelled claim.

Chromatographic conditions: For the chromatographic analysis, a diode array detector set at 272 nm and an Agilent Eclipse XBD C18 analytical column (4.6 mm × 150 mm; 3.5 µm were applied. A gradient flow of solvent A and solvent B (50:50 v/v) was used to enhance the separation of the analytes (Table-1). Specifically, solvent A is a mixture of 10% (v/v) acetonitrile, 90% (v/v) acetonitrile and 50% (v/v) buffer A, while solvent B is a mixture of 90% (v/v) acetonitrile, 10% (v/v) buffer A and 50% (v/v) buffer B. With a 20 µL injection volume, the experiment proved successful.

RESULTS AND DISCUSSION

Method development and optimization: Several experiments were conducted using various buffers, but no ideal values were found for peak shape and separation. The chromatogram and chromatographic state of each experiment were recorded. An Agilent Eclipse XBD C_{18} analytical column (4.6 mm × 150 mm; 3.5 µm) equipped with a mobile phase of solvent A (acetonitrile:triethylamine:acetic acid:trifluoroacetic acid (10:50:90) and solvent B (acetonitrile:triethylamine:acetic acid:trifluoroacetic acid (90:50:10) pumped at a gradient flow was used for The eluent was measured at 272 nm using a PDA detector and the injection volume was set to 20 µL. Fig. 1 shows the chromatograms obtained under the optimal condition.

Validation of assay method: The following characteristics were investigated to validate that the suggested analytical method met ICH Q2 (R1) criteria [22].

Linearity, range and sensitivity: Plotting individual peak areas against drug concentrations yielded calibration curves. In the concentration range of 0.5 to 20 µg/mL, the technique displayed good linearity ($r^2 = 0.999$) (Table-2). The method's sensitivity was investigated by setting the limit of detection (LOD) = 3.3 (σ/S) and limit of quantization (LOQ) = 10 (σ/S), where S is the slope of calibration curve and σ is the standard deviation of response. The measured LODs were 0.446792, 0.472355, 0.423956 and 0.406991 µg/mL, respectively and LOQs were 1.353915, 1.43138, 1.284714 and 1.233306 µg/mL for aspirin (ASN), domperidone (DMP), omeprazole (OMP) and diclofenac (DCF), respectively.

Method precision: All the experiments were carried out in triplicates and the results are compiled in Table-3. For QC samples at three different levels of 5, 10 and 20 µg/mL, studies were conducted to determine the precision of the novel RP-HPLC method. The approach was determined to be precise

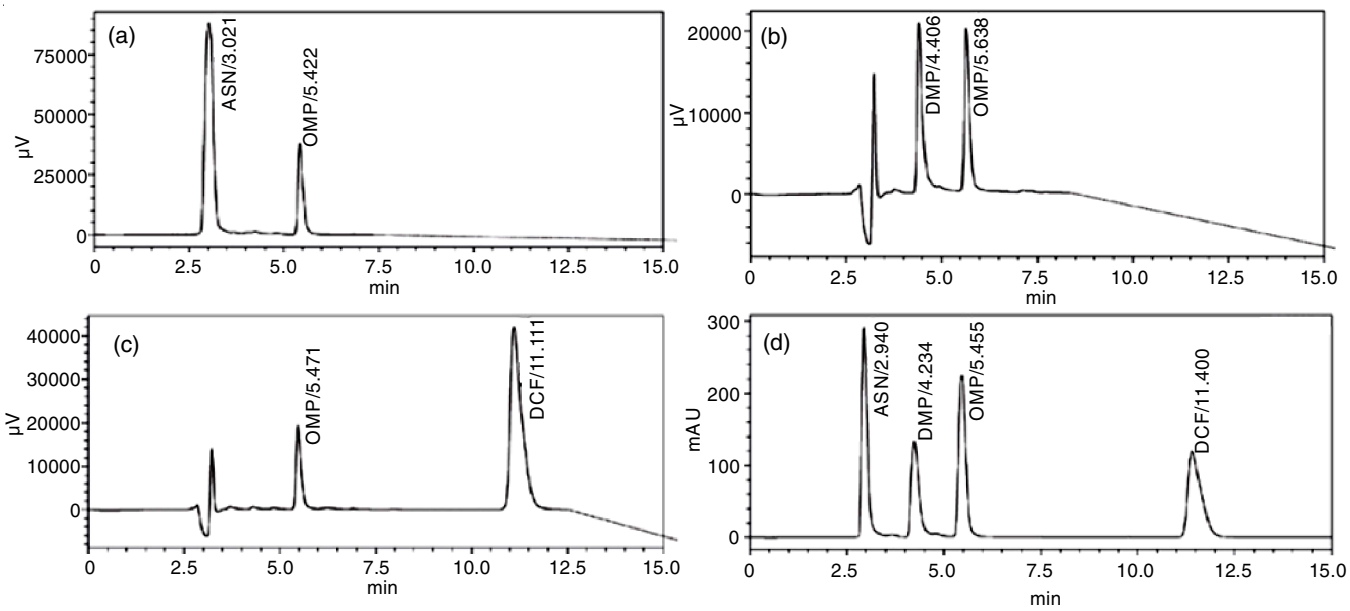

Fig. 1. Typical chromatograms of commercial formulations combining (a) OMP and ASN; (b) OMP and DMP; (c) OMP and DCF; and (d) the synthetic mixture of all four analytes (standard concentration 10 µg/mL)

TABLE-2
DATA FROM THE CALIBRATION CURVE AND SENSITIVITY OF THE ANALYTICAL METHOD

Particulars	Aspirin	Domperidone	Omeprazole	Diclofenac
Range	0.5 to 20 µg/mL	0.5 to 20 µg/mL	0.5 to 20 µg/mL	0.5 to 20 µg/mL
Y = mx + c	40504x + 32720	36539x – 7444.6	48895x – 6444.6	42619X + 3339.2
R^2	0.999	0.998	0.998	0.998
SD	5520.183	12409.604	15082.320	12662.314
LOD (µg/mL)	0.449	1.120	1.017	0.980
LOQ (µg/mL)	1.362	3.396	3.084	2.971

TABLE 3
PRECISION DATA WHEN PERFORMED IN TRIPLICATE (n = 3)

Sample ID	Peak area (mean ± SD; %RSD); n = 3			
	Omeprazole	Aspirin	Domperidone	Diclofenac
QC1 (5 µg/mL)	230991 ± 148.109; 0.06	232631 ± 365.230; 0.16	153514 ± 1094.81; 0.71	195013 ± 149.408; 0.08
QC2 (10 µg/mL)	483249 ± 365.831; 0.08	437327 ± 406.793; 0.09	354060 ± 113.61; 0.03	441344 ± 194.656; 0.04
QC3 (20 µg/mL)	976196 ± 186.875; 0.02	843994 ± 185.599; 0.02	728429 ± 1312.414; 0.18	853978 ± 292.716; 0.03

TABLE-4
RECOVERY RESULTS OF THE METHOD WHEN EXPERIMENTED BY SPIKING STANDARD DRUGS AT THREE LEVELS TO THE FIXED CONCENTRATION OF COMMERCIAL SAMPLES

Drugs	Amount of reference standard spiked (n = 3)		
	50%	100%	150%
Amount added (µg/mL)	2.5	5	7.5
OMP-Amount found (µg/mL), (Recovery ± RSD)%	2.49, (99.6 ± 0.28)	4.91, (98.2 ± 0.16)	7.31, (97.46 ± 0.02)
DMP-Amount found (µg/mL), (Recovery ± RSD)%	2.41, (96.4 ± 0.16)	4.97, (99.4 ± 0.12)	7.42, (98.93 ± 0.13)
DCF-Amount found (µg/mL), (Recovery ± RSD)%	2.46, (98.4 ± 0.27)	5.02, (100.4 ± 0.12)	7.48, (99.73 ± 0.07)
ASN-Amount found (µg/mL), (Recovery ± RSD)%	2.48, (99.27 ± 0.70)	4.90, (98.02 ± 0.44)	7.39, (98.55 ± 0.59)

TABLE-5
ROBUSTNESS RESULTS OF THE METHOD WHEN DELIBERATE VARIATIONS IN THE OPTIMAL CONDITION WAS STUDIED

Condition	Level	Mean ± SD; %RSD (n = 3)			
		OMP		DMP	
		RT (min)	A_s	RT (min)	A_s
Optimal	–	5.42 ± 0.002; 0.040	1.640 ± 0.027; 1.70	4.406 ± 0.0510; 0.54	1.90 ± 0.023; 1.28
Flow rate at start of the gradient (± 0.1 mL/min)	0.1	5.42 ± 0.030; 0.060	1.624 ± 0.015; 0.93	4.044 ± 0.1200; 0.13	1.70 ± 0.024; 1.34
	0.3	5.47 ± 0.007; 0.012	1.670 ± 0.022; 1.33	4.630 ± 0.1160; 1.21	1.87 ± 0.011; 0.51
Buffer B pH (± 0.2)	5.0	5.41 ± 0.011; 0.190	1.610 ± 0.007; 0.48	4.400 ± 0.6100; 1.12	1.97 ± 0.090; 0.49
	5.4	5.47 ± 0.240; 0.454	1.630 ± 0.110; 0.71	4.400 ± 0.0093; 0.98	1.78 ± 0.026; 1.48
Condition	Level	DCF		ASN	
		RT (min)	A_s	RT (min)	A_s
Optimal	–	11.11 ± 0.0026; 0.42	1.83 ± 0.037; 1.95	3.021 ± 0.023; 0.30	1.19 ± 0.0250; 1.64
Flow rate at start of the gradient (± 0.1 mL/min)	0.1	11.06 ± 0.0390; 0.62	1.65 ± 0.015; 0.97	3.042 ± 0.003; 0.04	1.15 ± 0.0150; 0.97
	0.3	11.07 ± 0.0240; 0.38	1.62 ± 0.015; 0.79	3.001 ± 0.005; 0.07	1.183 ± 0.015; 0.83
Buffer B pH (± 0.2)	5.0	11.09 ± 0.1200; 1.85	1.86 ± 0.011; 0.73	3.210 ± 0.004; 0.05	1.178 ± 0.010; 0.56
	5.4	11.11 ± 0.0060; 0.10	1.82 ± 0.020; 1.06	3.021 ± 0.013; 0.17	1.166 ± 0.020; 1.35

and the calculated percent coefficient of variation (CV) was < 0.639.

Accuracy: Accuracy was determined by analyzing a known concentration of standard drug spiked with marketed formulation at 50%, 100% and 150% levels and then determining the recovery percentage. The recovery results from the accuracy experiments of the proposed method are given in Table-4.

Robustness: Variations in separation parameters (optimal condition) *i.e.* the mobile phase flow rate at the start of the gradient program (± 0.1 mL/min) and buffer B pH (± 0.2) have been experimented. Reproducibility and conformity to the necessary standards were validated by the statistical comparison with the recommended approach (Table-5).

Assay of commercial formulations: Omeprazole (OMP) containing combination medication dosage forms sold on the commercial market were collected for this study. Ten tablets of each brand were weighed and broken into a powder; then, the powder was dissolved in acetonitrile in 100 mL volumetric flask using ultrasonication for about 5 min; finally, the solution was diluted to marked volume with acetonitrile. The filtrate was diluted to the appropriate quantities using diluent and then analyzed. Three separate concentrations were performed within the linearity range and the findings were consistent with the percribed values for the extracted sample (Table-6).

TABLE-6
ASSAY OF COMMERCIALLY AVAILABLE COMBINED FORMULATIONS

Formulation	Labelled amount (mg)	Amount found (mg)	%RSD (n = 3)	Assay (% w/w)
OMP + DMP	OMP 20	19.55	0.69	97.75
	DMP 10	9.883	0.918	98.83
OMP + ASN	OMP 40	40.24	1.55	100.6
	ASN 81	80.81	1.281	99.765
OMP + DCF	OMP 20	19.783	0.682	98.915
	DCF 100	100.31	0.424	100.31

Conclusion

A standardized RP-HPLC method has been developed for the analysis of omeprazole (OMP) drug in the dosage forms, with aspirin (ASN), diclofenac (DCF) and domperidone (DMP). Satisfactory performance was measured in terms of linearity, accuracy, precision and robustness of the method. Regular analysis of the four medications in either isolated or mixed dosage forms is found to be feasible using the proposed RP-HPLC method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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