HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF KETOPROFEN AND OMEPRAZOLE

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Abstract: A simple, specific, precise and accurate reversed phase liquid chromatographic method has been proposed for the simultaneous determination of ketoprofen and omeprazole in laboratorymixture.

The chromatographic separation was performed on a LiChrosorb C_{18}, 125 mm x 4.6 mm, 5 μmcolumn at a detector wavelength of 230 nm and a flow rate of 1.5 ml/min. The mobile phase was composed of phosphate buffer (pH adjudted to 4.6 with ortho-phosphoric acid) and methanol (25:75*v/v*)

The retention times of ketoprofen and omeprazole were found to be 3.05 and 6.96 min, respectively. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantitation and limit of detection. The calibration curves were linear in the concentration range of 25.00-200.0 μg/ml for ketoprofen and 5.00-40.00 μg/ml for omeprazole. The estimated relative standard deviation values of % recovery at accuracy studies were found to be not greater than 3 %.

The presented method for the simultaneous determination of ketoprofen and omeprazole in synthetic mixture is specific, rapid and simple with good sensitivity.

Key words: Ketoprofen, omeprazole, HPLC, validation, laboratory mixture, quality control

Introduction

Omeprazole (OME), chemically is (RS)-5-methoxy-2-[4 –methoxy-3, 5 dimethyl pyridin-2-yl) methyl] sulphinyl]-1Hbenzimidazole. It functions as proton pump inhibitor and used in the treatment of gastro-esophageal reflux disease, duodenal, gastric, esophageal ulceration and Zollinger-Ellison syndrome [1]. Literature survey revealed that OME can be analyzed alone and in combination with other drugs in various dosage forms and biological fluids. The methods include stability indicating high-performance thin layer chromatography [4], high-performance liquid chromatography [3-5], TLC densitometry [6, 9], UV spectrophotometry [8, 9], capillary electrophoresis [10].

Ketoprofen (KET), chemically is (RS)-2-[3-(benzoyl) phenyl] propanoic acid which is non-steroidal antiinflammatory drug with analgesic, antipyretic effects used in

arthritis, severe toothaches, post-herpetic neuralgia, pain for radiculopathy, joint disorders, dysmenorrheal, menstrual cramps and gout [1]. Literature survey reveals that for KET can be analyzed alone and in combination with other drugs in various dosage forms and biological fluids. It includes separation techniques [11-14], UV spectrophotometry [15, 16] and flowinjection analysis [17].

Combination of NSAID with a H_2-receptor antagonists is beneficial as it suppress gastric acid secretion[18, 19].

The aim of the present study was to develop and validate a HPLC method for the simultaneous determination of ketoprofen and omeprazole in laboratory mixture contained 100 mg KET and 20 mg OME. The method described complied with validation requirements of ICH [20] and could be used for routine quality control of pharmaceutical formulations in ordinary laboratories.

Experimental section

Materials and methods

Chemicals and Reagents

Working standards of ketoprofen RS (purity 100.2 %) and omeprazole RS (purity 99.91 %) were provided by (Sigma-Aldrich). LC-grade methanol and ortho-phosphoric acid were procured from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C_{18}, 125 mm x 4.6 mm, 5 µm column eluted with a mixture of phosphate buffer (pH adjusted to 4.6 with ortho-phosphoric acid) and methanol (25:75*v/v*) as the mobile phase at flow rate of 1.5 ml/min. Detection was carried out by absorbance at 230 nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

Preparation of standard solutions

For ketoprofen: 50 mg of ketoprofen was accurately weighed and transferred to a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution A-1000 µ g/ml).

For omeprazole: 20 mg of omeprazole was accurately weighed and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol (Stock solution B-200 µ g/ml).

For mixed standard: From the stock solutions A and B dilutions of different concentration were made as mentioned in the Table 1.

Table 1. Preparation of mixed standards

Stock solution → Volume taken (ml)		Total volume	Concentration in µg/ml	
A	B	(ml)	KET	OME
5	5	200	25	5
5	5	100	50	10
5	5	50	100	20
15	15	100	150	30
10	10	50	200	40

Preparation of synthetic mixture

A bulk mixture of both drugs wasprepared using 100 mg of KET and 20 mg of OME.Common excipients which are used in tabletformulation were added in this laboratory mixture,triturated well and weighed. A powder equivalent to 50 mg of KET and 10 mg of OMEwas weighedaccurately and transferred to 100 ml of volumetricflask, dissolved in sufficient quantity of methanol andvolume was adjusted up to the mark with methanol.The stock solution was filtered through a 0.45 µm Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 25.0 ml volumetric flask to give a test solution containing 100 µg/ml KET and 20.00 µg/ml OME.

Results and discussion

The proposed method was validated with respect to selectivity,linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirementsto show it could be used for simultaneous determination of ketoprofenand omeprazole in laboratory mixture.

Selectivity

From the chromatogram shown in Fig. 1, it is evident, that under the chosen chromatographic conditions ketoprofen (Tr=3.05 min) and omeprazole (Tr=6.96 min) were completely separated. The specificity of the proposed method was confirmed by injecting blank sample. The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there were not another peaks on the retention times of ketoprofen and omeprazole.

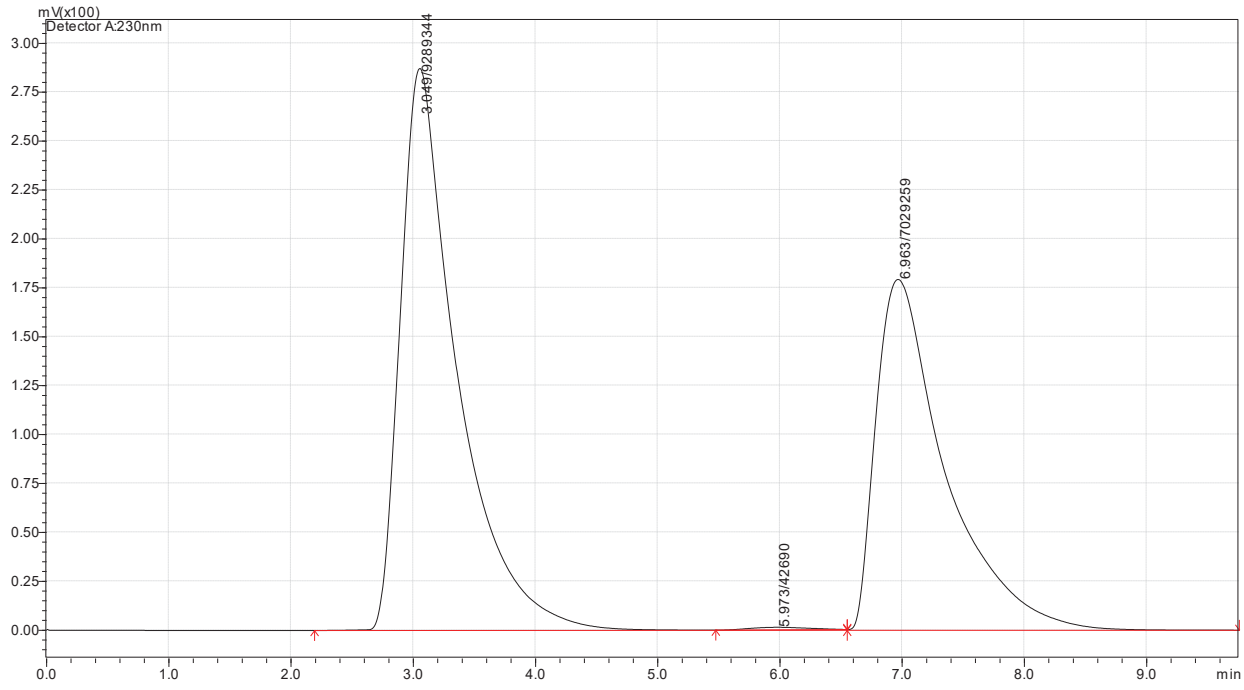

Figure1. Chromatogram from analysis of sample

Linearity

Calibration curves were constructed in the range of 25.00-200.0 µg/ml for KET and 5.00-40.0 µg/ml for OME to encompass the expected concentration in measured samples. An excellent correlation existed between the peak areas and the concentrations of both compounds as can be seen from correlation coefficients. The

limit of quantitation and limit of detection were calculated from the standard deviations and slopes of the responses using a signal-to-noise ratio as per ICH guidelines. Data concerning linearity and sensitivity of the method were shown in Table 2.

Table 2. Linearity data

Parameter	ketoprofen	omeprazole
Linearity range	25.00-200.0 μg/ml	5.00-40.0 μg/ml
Slope	13654	21452
Intercept	-1098	1017
Regression coefficient	0.9999	0.9998
Limit of quantitation, ng	8	12
Limit of detection, ng	4	5

Accuracy

The accuracy of the method was determined by calculating the recoveries of KET and OME by the standard addition method. Known amounts of standard solutions of both KET and OME (50, 100, and 150%) were added to prequantified sample solutions of drug formulation.The method was found to be accurate with recoveries of 98.33%–99.85% and an acceptable RSD of not more than 3% at each level. The recoveries obtained by the proposed method for KET and OME were shown in Table 3.

Table 3. Results from study of accuracy

Amount of sample (μg/ml)		Sets	Amount drug of spiked (μg/ml)		Average amount recovered (μg/ml)		Mean recovery (%) ± SD		% RSD	
KET	OME		KET	OME	KET	OME	KET	OME	KET	OME
50	10	1	0	0						
50	10	2	0	0	49.85	9.81	99.48±1.15	98.77±1.80	1.15	1.82
50	10	3	0	0						
50	10	1	25	5						
50	10	2	25	5	74.82	14.87	99.81±0.57	99.55±2.54	0.57	2.55
50	10	3	25	5						
50	10	1	50	10						
50	10	2	50	10	99.74	19.57	99.75±0.90	98.28±2.14	0.87	2.18
50	10	3	50	10						

50	10	1	75	15						
50	10	2	75	15	125.1	24.80	99.85±0.68	99.64±1.40	0.68	1.40
50	10	3	75	15						

Precision

Precision is determined by studying the repeatability and intermediate precision. Repeatability (interday precision) result indicates the precisionunder the same operating conditions over a short interval of time. Intermediate precision study expresses within laboratory variation in different days. In both inter- and intraday precision study the values of % RSD were not more than 2.0% indicates good repeatability and intermediate precision (Table 4).

Table 4. Precision of the method

Drug	Interday precision		Intraday precision	
	% Amount found± SD*	% RSD	% Amount found± SD*	% RSD
KET	99.52±0.56	0.56	100.4±0.47	0.47
OME	98.93±0.69	0.70	99.21±0.39	0.39

**Average of six determinations*

Conclusion

The newly developed RP-LC method for simultaneous determination of ketoprofen and omeprazole in laboratory mixture is specific, precise, accurate and rapid. Hence the proposed method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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