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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF INDOMETHACIN AND OMEPRAZOLE IN RABBIT PLASMA BY USING HPLC

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ABSTRACT

A novel approach was used to develop and validate a bioanalytical RP-HPLC method for the simultaneous estimation of Indomethacin and Omeprazole in rabbit plasma using Rabeprazole as internal standard. Evaluation of the content of drugs were done by employing a mixture of phosphate buffer (pH 4.6) and acetonitrile (65:35, v/v) as the mobile phase and measure the absorbance at 260 nm for Indomethacin and Omeprazole. Retention time was established to be 2.014 min for Rabeprazole, 3.310 min for Indomethacin and 5.479 min for Omeprazole. The results have shown that the analytical technique furnished here establishes acceptable accuracy and precision, shorter and easy sample preparation, reduced the complications for equipment on satisfactory analysis time.

Key words: Indomethacin, Omeprazole, Bioanalytical, Estimation, Plasma.

INTRODUCTION

Indomethacin (IDM) (Fig. 1) is chemically 2-(1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl) acetic acid and it was a non selective, non steroidal anti-inflammatory drug[1]. Indomethacin was used for reducing fever, pain, swelling, hemicranias continua, hypnic headache and migraine. Indomethacin half-life was 0.7 hrs. Indomethacin acts by inhibiting the prostaglandin biosynthesis[2].

Omeprazole (OMP) (Fig. 2) is chemically 6-methoxy-2-[(4-methoxy-3,5-dimethyl-

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pyridine-2-yl)methanesulfinyl]-1*H*-1,3-benzodiazole and it is a selective irreversible proton pump inhibitor[3]. Omeprazole was used for the treatment of dyspepsia, peptic ulcers, gastroesophageal reflux disease, laryngopharyngeal reflux and zollinger ellison syndrome. Omeprazole acts by inhibiting acid production[4]. Indomethacin and Omeprazole recommended as a fixed dose combination capsule containing 75 mg of Indomethacin and 20 mg of Omeprazole daily dosing used to acute gout, enclosing spondylitis, dysmenorrhoea, musculoskeletal disorders, osteoarthritis, patency of ductus arteriosus and rheumatoid arthritis[5,6].

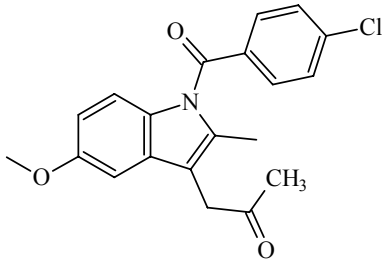

Fig. 1: Structure of Indomethacin

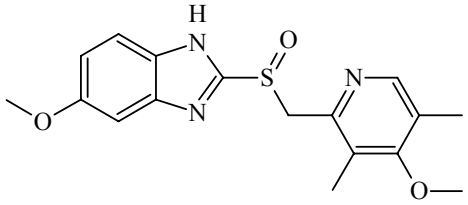

Fig. 2: Structure of Omeprazole

Rabeprazole (RBP) (Fig. 3) is chemically (RS)-2-([4-(3-methoxypropoxy)-3-methylpyridine-2-yl)-1*H*-benzo(d)imidazole and it is a selective gastric proton pump inhibitor[7]. Rabeprzole was used for the treatment for gastroesophageal reflux disease and it acts by preventing the gastric acid secrection[8,9].

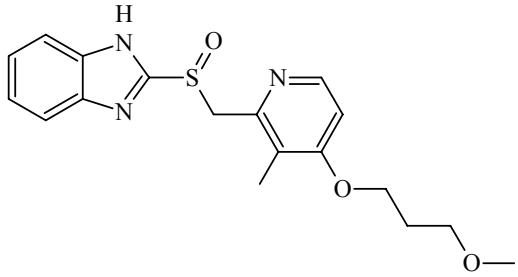

Fig. 3: Structure of Rabeprazole

Literature survy revaled that few analytical methods was reported such as spectrophotometric[10,11], HPLC[12-23] and LC-MS[24,25] for deterimination by Indomethacin and Omeprazole with individual and in combination with other drugs. Although no method was reported for the simultaneous estimation of Indomethacin and Omeprazole in combined pharmaceutical formulations.

The present study was aimed to develop a simple, sensitive, rapid, precise and accurate bionalytical method and validated[26] for simultaneous estimation of Indomethacin and Omeprazole according to ICH guidelines[27,28] by using high performance liquid chromatography in rabbit plasma.

EXPERIMENTAL

Chemicals, reagents, standards and samples

Blank rabbit plasma, pure samples of drugs like Indomethacin, Omeprazole and Rabeprazole was obtained from Spectrum Pharma Research Solutions, Hyderabad, India. Analytical grade of orthophosporic acid was obtained from SD Fine Chemicals Ltd., Mumbai, India. HPLC grade of acetonitrile and water was obtained from Qualigens Fine Chemicals, Mumbai, India.

Instrumentation

The analysis was performed by using a chromatographic system Waters 2695 series HPLC comprised of vacuum degasser, auto injector, dual gradient pump with photo diode array detector. The HPLC system was equipped with Empower 2 software.

Chromatographic conditions

Indomethacin and Omeprazole was analysed in Kromosil C18 column (250 mm x 4.6 mm, 5 μm) for the chromatographic separation. The mobile phase was composed of phosphate buffer (pH 4.6) and acetonitrile (65:35, v/v) and was used diluent. Filtered through 0.45 μm nylon membrane filter under vacuum filtration and pumped at a temperature of 30^{o}C, at a flow rate of 1 mL/min with UV detection wavelength at 260 nm and injection volume was 10 μL. The run time was 8 min and the retention time of Indomethacin and Omeprazole was found to be 3.310 min and 5.479 min, respectively.

Preparation of stock solution

Primary stock solutions of Indomethacin and Omeprazole were prepared individually by dissolving 10 mg of each in 10 mL volumetric flasks in diluent. Series of working

solutions of Indomethacin and Omeprazole were prepared by the suitable dilution of the stock solutions with same diluent to achieve the concentration of 20-200 µg/mL for Indomethacin and 10-100 µg/mL for Omeprazole.

Procedure

The standard solutions were prepared by dilution of the standard stock solution with diluent to obtain a concentration range 20-200 µg/mL for Indomethacin and 10-100 µg/mL for Omeprazole. Triplicate 10 µL injections were prepared for each concentration and chromatographed under the conditions reported above. The peak area of each concentration was plotted against the corresponding concentration to get the calibration curve and regression equation was computed.

Preparation of spiked plasma sample

250 µL of rabbit plasma, 50 µL of internal standard, 10 µL of Indomethcin and 10 µL of Omeprazole were pipetted into 10 mL centrifuge tube and to it 2 mL of acetonitrile was added. The mixture was mixed shortly, standing for 5 min at room temperature then vortex for 2 min, finally the mixture was centrifuged at 3200 rpm for 10 min. After the centrifugation, 10 µL of the supernatant layer was collected and injected into HPLC.

Method validation

Linearity: Calibration curve showed the relation between the concentration of drugs versus peak area were established. Results manifest linear relationship in the range of 20-200 µg/mL and 10-100 µg/mL for Indomethacin and Omeprazole respectively. In two drugs run from linear regression equation was measured by following ICH guidelines shown in Fig. 4 and 5.

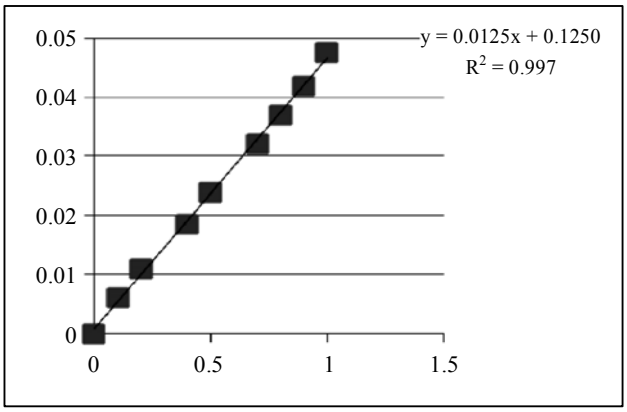

Fig. 4: Calibration curve of Indomethacin

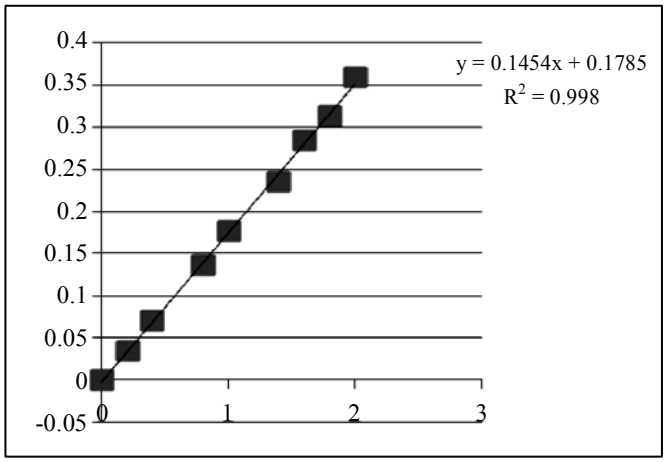

Fig. 5: Calibration curve of Omeprazole

Precision and accuracy

Results from the validation data of rabbit plasma were within acceptable limits. Within batch precision method was measured range from 6.50 to 9.49% for Indomethacin and 5.31 to 7.73% for Omeprazole. The accuracy of the method was measured in the range of 97.33 to 105.28 % for Indomethacin and 94.07 to 108.17% for Omeprazole and the results are shown in Table 1.

Table 1: Accuracy and precision

	LLOQQC		LQC		MQC		HQC	
	IDM	**OMP**	**IDM**	**OMP**	**IDM**	**OMP**	**IDM**	**OMP**
Mean	105.28	108.17	97.33	103.92	97.96	96.33	100.02	94.07
S.D. (+/-)	0.02	0.006	0.05	0.01	0.13	0.03	0.24	0.05
C.V. (%)	6.50	6.14	9.49	5.31	9.02	7.73	8.92	6.46

Recovery

The analyte recovery for the Indomethacin and Omeprazole were measured to be 50.97% and 51.43%, respectively. The internal standard for Rabeprazole was found to 51.60% and the results are furnished in Table 2.

Table 2: Recovery studies

	Indomethacin	Omeprazole	Rabeprazole
Mean	50.97	51.43	51.60
S.D. (+/-)	3.06	1.35	0.43
C.V. (%)	6.02	2.64	0.45

Stability studies

Long term stability

The concentration of sample of stability was ranged between 100.63% to 101.63% for Indomethacin and 100.94% to 101.24% for Omeprazole and the results are furnished in Table 3.

Table 3: Long term stability studies

	LQC		HQC	
	Indomethacin	Omeprazole	Indomethacin	Omeprazole
Mean	100.63	101.24	101.63	100.94
S.D. (+/-)	0.059	0.011	0.29	0.097
C.V. (%)	9.72	5.69	10.46	10.27

Freeze thaw stability

The concentration of sample of stability was measured between 88.71% to 104.33% for Indomethacin and 94.40% to 104.08% for Omeprazole and the results are shown in Table 4.

Table 4: Freeze thaw stability studies

	LQC		HQC	
	Indomethacin	Omeprazole	Indomethacin	Omeprazole
Mean	104.33	104.08	88.71	94.40
S.D. (+/-)	0.035	0.013	0.273	0.06
C.V. (%)	5.84	6.75	10.69	6.80

Bench top stability

The drug was developed to be stable for at least 3 hours on bench top at room temperature. The freshly spiked calibration standards against back calculated concentration was within a range of 89.16% to 105.01% for Indomethacin and 99.30% to 106.33% for Omeprazole and the results are shown in Table 5.

Table 5: Bench top stability studies

	LQC		HQC	
	Indomethacin	**Omeprazole**	**Indomethacin**	**Omeprazole**
Mean	105.01	106.33	89.16	99.30
S.D. (+/-)	0.046	0.014	0.262	0.065
C.V. (%)	7.66	6.87	10.21	6.88

Processed sample stability

The concentration of sample of stability was measured range of 98.37% to 101.38% at 2 to 8^{o}C and 99.46% to 99.94% at room temperature for Indomethacin and Omeprazole, respectively and the results are shown in Table 6.

Table 6: Processed sample stability studies

	LQC		HQC	
	Indomethacin	**Omeprazole**	**Indomethacin**	**Omeprazole**
Mean	98.37	99.46	101.38	99.94
S.D. (+/-)	0.018	0.018	0.95	0.94
N	3	3	3	3

RESULTS AND DISCUSSION

Different stationary phases were tried with various columns, poor and bend peaks were observed with different columns while Kromosil C18 column gave satisfactory resolution and free from tailing. Several mobile phases were used like methanol with acetonitrile were found to separate the compounds doubtful and potassium dihydrogen phosphate buffer with acetonitrile lead to good separation. The present study objective was acquired using mobile phase composed of phosphate buffer with acetonitrile (65:35, v/v)

with the pH adjusted to 4.6 ± 0.2 with orthophosphoric acid. The mobile phase composition is optimized under described conditions, free from tailing, peaks are well defined, resolved, the tailing factors were < 2 for all peaks. The retention times for Indomethacin was 3.310 min and for Omeprazole was 5.479 min, respectively, at a flow rate of 1 mL/min. The optimum wavelength for detection was 260 nm at where much better detector responses for the two drugs were obtained. The chromatograms of rabbit plasma blank and drugs Rabeprazole, Indomethacin and Omeprazole were shown in Fig. 6 and 7, respectively.

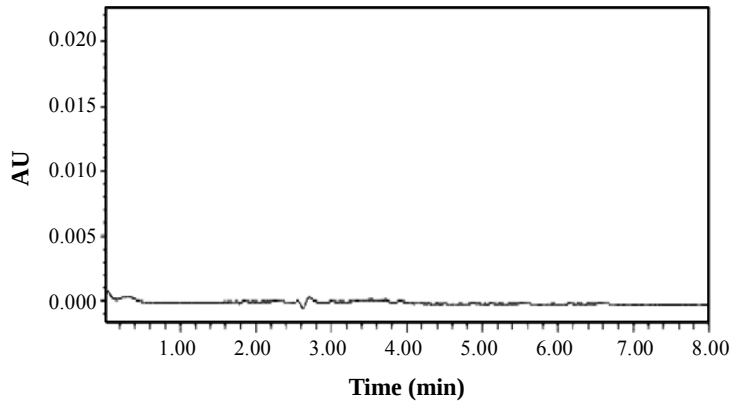

Fig. 6: Chromatogram of rabbit plasma blank

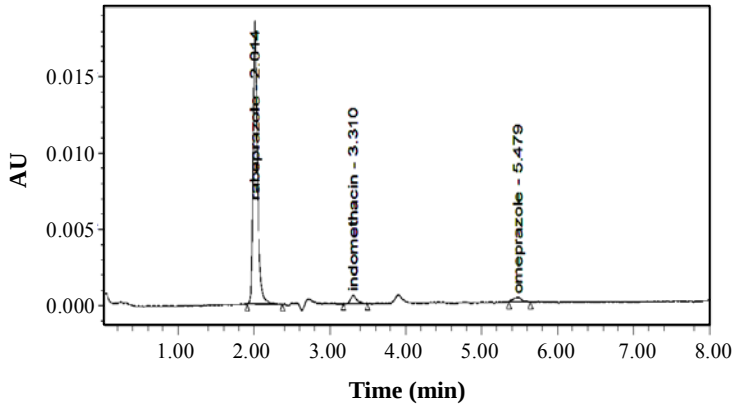

Fig. 7: Chromatogram of Rabeprazole, Indomethacin and omeprazole in spiked rabbit plasma

CONCLUSION

The proposed HPLC method was simple, rapid, precise and accurate, highly sensitive and cost effective for simultaneous estimation for the Indomethacin and Omeprazole in rabbit

plasma extracted by liquid-liquid extraction method. The percentage relative standard deviation of all parameters was found with in limits, which indicates this method will be validated. Hence, this method was suitable for pharmacokinetic studies.

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