Development and Validation of an HPTLC Method for Simultaneous Estimation of Omeprazole and Ketoprofen in a Developed Tablet Formulation

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Abstract: Nonsteroidal anti-inflammatory drugs (NSAIDs) are often used for their anti-inflammatory, analgesic, antipyretic effect and also used to alleviate pain and inflammation in rheumatic diseases. There are some side effects of NSAIDs like ulcers, diarrhoea, dyspepsia, gastric ulceration/bleeding which are life threatening. Attempts are made to reduce the risk of GI with the use of proton pump inhibitor, for the patients on long term treatment with NSAIDs. Combination of NSAID with a proton pump inhibitor is beneficial as it suppress gastric acid secretion. It is recently available in UK market.

An attempt was made to develop simple, precise and accurate high performance thin layer chromatographic (HPTLC) method for the simultaneous estimation of Omeprazole (OME) and Ketoprofen (KET) in bulk and in developed formulation on aluminum backed TLC plates, coated with silica gel $60F_{254}$ as stationary phase. The separation was carried out in chloroform: methanol 9:1 (v/v) as mobile phase. The detection was carried out densitometrically using a UV detector at 283 nm. The two drugs were satisfactorily resolved with Rf 0.45± 0.02 and 0.32± 0.02 for OME and KET respectively. Linearity was found in the range of 30-120 ng/ band (r^2 = 0.999) for OME and 150-600 ng/ band (r^2 = 0.999) for KET. The assay results were found to be 99.44% w/w and 99.303 % w/w for OME and KET respectively. The % Recovery for both analytes was in the range of 98.9-100.8%. The proposed method can be used for routine simultaneous analysis of OME and KET. The suitability of this HPTLC method for estimation of these compounds is proved by validation in accordance with the requirements of ICH Guidelines.

Keywords: Omeprazole, Ketoprofen, HPTLC, Validation, ICH Guideline.

1 INTRODUCTION:

Omeprazole, chemically is (RS)-5-methoxy-2-[4 –methoxy-3, 5 dimethyl pyridin-2-yl) methyl] sulphinyl]-1H-benzimidazole (Figure 1). 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]. OME is official in IP and BP [2,3]. Literature survey revealed that OME can be analyzed alone and in combination with other drugs in

various dosage forms and biological fluids. These methods includes stability indicating high-performance thin layer chromatography (HPTLC)[4], high-performance liquid chromatography (HPLC)[5-7], TLC densitometry[8,9], simultaneous UV spectrophotometry[10,11], Capillary electrophoresis[12],HPLC employing electrochemical and coulometric detection[13].

Ketoprofen, chemically is (RS)-2-[3-(benzoyl) phenyl] propanoic acid (Figure 2) which is non-steroidal anti-inflammatory drug with analgesic, antipyretic effects used in arthritis, severe toothaches, nerve pain like sciatica, post-herpetic neuralgia, pain for radiculopathy, joint disorders, dysmenorrheal, menstrual cramps and gout[1]. KET is official in IP [14]. 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 high-performance liquid chromatography (HPLC)[15-17], TLC and HPTLC[18], simultaneous UV spectrophotometric methods[19,20] and flow-injection analysis[21]. This present study reports for the first time the simultaneous quantitation of OME and KET in bulk drug and developed tablet formulation by HPTLC.
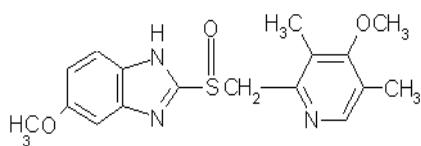

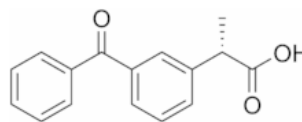**Figure 1 Structure of Omeprazole**   **Figure 2 Structure of Ketoprofen**

## 2 EXPERIMENTAL:

2.1 Chemicals, Reagents and Methods:

Pure drugs of OME and KET were kindly provided by Cipla Ltd as a gift sample. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2 Instrumentation and Chromatographic Conditions:

Chromatography was performed on aluminum-backed TLC plates (20 cm × 10 cm), coated with 250 μm layer of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany supplied by Anchrom Technologists, Mumbai). Samples were applied to the plates as 8 mm bands, by means of 25 microlitre Linomat V applicator (Camag, Muttenz Switzerland) equipped with a Hamilton syringe (Bonaduz., Switzerland). The slit dimensions were 6.00 mm × 0.30 mm and the scanning speed was 20mm s^{-1}. A constant application rate of 1 μL/s was used and the space between two bands was 5 mm. The monochromator bandwidth was set at 20 nm, each track was scanned thrice and the baseline correction was used. Ascending development of the plates was performed with chloroform: methanol 9:1 (v/v) as mobile phase, in a Camag 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 10 min at room temperature. The length of chromatogram run was 8 cm. The average development time was 20 min. After development, plates were dried. Densitometric scanning was performed on Camag TLC Scanner 3 in the reflectance absorbance mode at 283 nm for all measurements and operated by Wincats software version 3.15 supplied by Anchrom technologists, (Mumbai). A unidirectional air flow was maintained in the laboratory. The source of radiation utilized was deuterium lamp, which emits UV spectrum between 190 nm to 400 nm.

2.3 Preparation of standard solutions:

Twenty five milligram of each drug OME and KET were weighed separately and dissolved in 10 ml of HPLC grade methanol separately. The volume was made upto 25 ml so as to get the concentration 1 mg mL^{-1}. Standard stock solutions were further diluted with methanol to concentration of 100 μg/mL for each drug separately. Both drugs have optimum absorbance at 283 nm as indicated in Fig 3.

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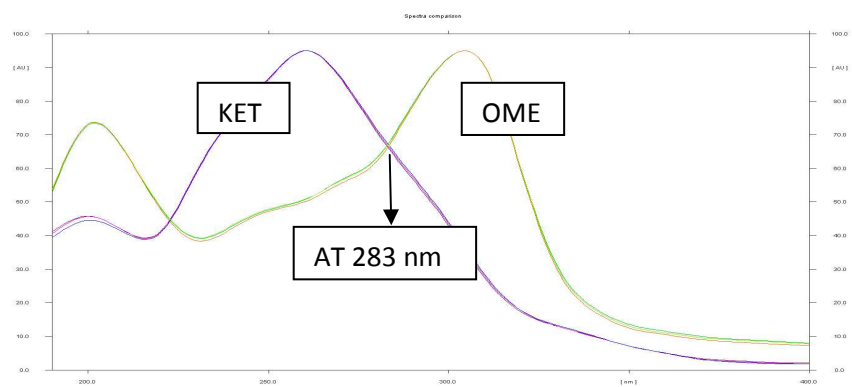
Figure 3: Typical overlay spectra of KET and OME (100 µg/ml)

2.4 Optimization of Mobile Phase

Optimization of mobile phase was initially started with the use of neat solvents like methanol, chloroform, and toluene. Various solvent mixtures like Dichloromethane: Methanol (9:1 v/v), Ethyl acetate: Methanol (9:1 v/v), Chloroform: Toluene: Methanol (7:2:1 v/v/v), Chloroform: Methanol (9:1 v/v) were tried to achieve optimum resolution between OME and KET. Mixture of Chloroform: Methanol (9:1 v/v) was chosen as the mobile phase for analysis.

At 283 nm, concentration range of 30-120 ng/ band and 150-600 ng/ band for OME and KET respectively was found to be linear.

2.5 Analysis of formulation:

Axorid capsules containing OME (20mg) and KET (100mg) in combination is marketed by Meda Pharmaceuticals, Scotland as modified-release capsules[22].

From the developed formulation powder equivalent to 10 mg of OME and 50 mg of KET was accurately weighed in 50 ml volumetric flask and dissolved in 35 ml methanol, ultrasonicated for 10 min and was filtered through Whatman filter paper no. 41.

The matrix and filter paper was further washed with methanol and washings were added to volumetric flask to the volume. The above stock solution was diluted with methanol to give a concentration of 100 µg/ml of OME and 500 µg/ml of KET. The amount of OME and KET present in tablet formulation was calculated by comparing peak area of sample with that of standard.

2.4 Method Validation:

Validation of the TLC method was carried out as per ICH guidelines [23] with the following parameters:

2.4.1 Linearity and range

Eight independent dilutions of stock solution in mobile phase containing OME and KET in the range of 30-120 ng/ band and 150-600 ng/ band respectively were applied to the plate thrice. The plate was developed as described above. Peak areas measured at 283 nm were plotted against corresponding concentrations to furnish the calibration plot. Residuals for both the drugs were plotted by calculating the distance of response from the regression line and plotting it against the concentration of analyte.

2.4.2 Precision

The precision of the method was carried by repeatability, Intraday and Interday precision studies. Repeatability studies were performed with six repeated measurements of 40 ng/ band and 200 ng/ band concentration of OME and KET respectively for standard and sample solutions. Each dilution was made by independent weighing. Intraday variation studies were performed with six repeated measurements at 100% of the test concentration

(40ng/ band for OME and 200 ng/ band for KET) on the same day. The Interday precision of the method was checked by repeating a study on three different days and % RSD was calculated.

2.4.3 Accuracy

Accuracy of the method was carried out in terms of percent recovery. The method was applied to drug samples (40 ng/ band and 200 ng/ band for OME and KET respectively) to which known amount of standard OME and KET corresponding to 80, 100 and 120% of label claim were added. Each mixture was analyzed six times, area of peak was measured and % Relative standard deviation of percentage drug content was calculated.

2.4.4 Limit of detection and limit of quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = $3.3 \times \delta /S$ and LOQ = $10 \times \delta/S$, where δ is the standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and S is the slope of the corresponding calibration plot.

2.4.4 Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for OME and KET in the samples were confirmed by comparing the R_f with that of a standard. The mobile phase resolved both the drugs very efficiently, as shown in Figure 4. The peak purity of OME and KET was determined by comparing the spectrum at three different regions of the spot i.e. peak start, peak apex and peak end.
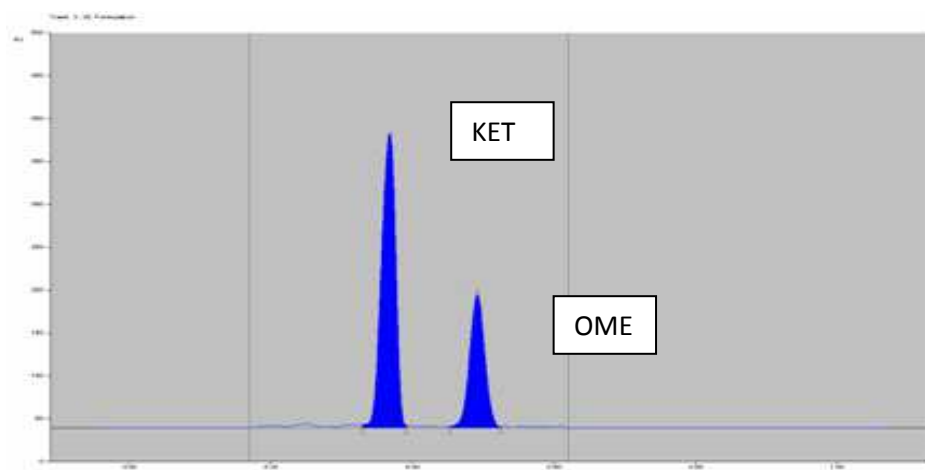
Figure 4: Chromatogram of OME and KET at 283nm with Rf value of 0.45 ± 0.02 and 0.32 ± 0.02 respectively.

2.4.5. Robustness

Small deliberate changes in the experimental parameters were done. These include time from spotting to chromatography and from chromatography to scanning were varied by $\pm$ 5 mins, Ultrasonication time of the mixture was varied by $\pm$ 3 min. Plates from different lot number were used.

3 RESULTS AND DISCUSSION:

The developed HPTLC method for simultaneous estimation of OME and KET in tablet formulation was found to be simple and convenient for the routine analysis of two drugs.

3.1 Optimization of mobile phase: Use of Dichloromethane: Methanol (9:1) as a mobile phase has given Rf values closer to 1. In Ethyl acetate: Methanol (9:1) no proper resolution was obtained. Tailing was observed in Chloroform: Toluene: Methanol (7:2:1). Well resolved spots and symmetrical peak shapes were obtained in Chloroform: Methanol (9:1) as a mobile phase.

3.2 Optimization of other chromatographic conditions: This validated method uses silica gel 60 F_{254} as a stationary phase, 8 mm band application, the scanning speed of 20mm s^{-1}, an application rate of 1 μL/s, 5 mm space between two bands, chamber saturation time of 10 min at room temperature, 8 cm chromatogram run and the average development time of 20 min.

3.3 Linearity: Linearity of the method was studied by spotting eight concentrations of the drugs in the range of 30-120 ng/band and 150-600 ng/ band for OME and KET respectively. Residuals are randomly distributed around the regression function as shown in Fig 5 and do not show any tendency proving the linearity of the method. The regression equations for calibration curve were found to be y= 71063x+ 341.6 and y= 7120x+347 for OME and KET respectively. High slope value indicates strong UV absorption by OME than KET. The correlation coefficient of these drugs was found to be close to 1.00 indicates good linearity.

(OME)   **(KET)**
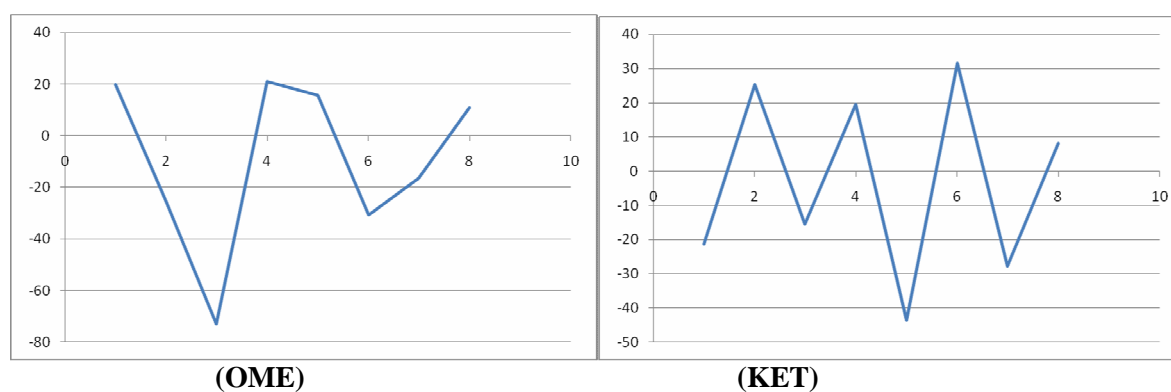
Figure 5: Residuals of OME and KET obtained from regression data at 283nm by plotting distance of response from regression line.

3.4 Analysis of Tablet formulation: Three replicate determinations were performed and assay results obtained are shown in Table 1.

3.5 Precision: The method is found to be precise as evidenced from % RSD value less than 2 (Table 2). The results of Intraday and Interday studies prove precision of the method.

3.6 Accuracy: Percent recovery of the proposed method when applied for drug- excipient blend after spiking with standard was found to be 98.9-100.8 % for both drugs (Table 3).

3.7 Sensitivity: Sensitivity of the method was proved by low limit of detections 4.68/29.9 ng/band (OME/KET) and limit of quantitation 14/90.9 ng/band (OME/KET).

3.8 Specificity and robustness: Values of r as shown in Table 1 are greater than 0.99 indicates peak purity of both analytes. The low values of % RSD in peak area after doing deliberate minor changes in the method parameters indicates robustness of the method (Table 4).

This method utilizes simple binary mobile phase. As compared to other reported chromatographic methods proposed method is economic. Hence it can be used for routine analysis of two drugs in combined dosage forms.

Table 1: Linearity Data, Assay Values of OME and KET

Parameters	OME	KET
Range as per Beers law	30-150ng/spot	150-600ng/spot
Regression equation (y= mx + c)	y= 71063x+ 341.6	y= 7120x+347
Correlation Coefficient (r^2)	0.999	0.999
Residuals	Random	Random
Assay $\pm$ S.D. (% RSD)	99.44 $\pm$ 0.2854 (0.3544)	99.303 $\pm$0.2386 (0.596)
Sample peak purity r (S, M) r (M,E)	0.9994 0.9995	0.9996 0.9997
LOD (ng/band)	4.68	29.9
LOQ(ng/band)	14	90.9

Table 2: It Shows Statistical Analysis for Precision of the Proposed Method.

Drug	Concentration (ng/spot)	Repeatability	Intra day	Inter-day
			%Assay ± RSD	
OME	40	99.67 ± 0.8956	99.41 ± 0.7685	99.12 ± 1.2354
KET	200	99.87 ± 0.5678	99.30 ± 0.4534	99.21 ± 1.1452

n= 6

Table 3: It Shows Statistical Analysis for Recovery of the Proposed Method.

Drug (ng/spot)	Amount spiked (% level)	Amount spiked (ng/spot)	Mean concentrationfound (ng/spot)	% Mean Recovery	% RSD
OME (40)	80	20	19.882	99.41	0.3401
	100	40	39.784	99.46	0.2973
	120	60	59.688	99.48	0.3941
KET (200)	80	100	99.26	99.26	0.841
	100	200	199.4	99.70	0.452
	120	300	298.59	99.53	0.639

n= 6

Table 4 Robustness Study of OME and KET

Parameter	SD of peak area (% RSD) OME	SD of peak area (% RSD)KET
Time from spotting to chromatography	15.78 (1.08)	9.34 (0.67)
Time from chromatography to scanning	13.87 (1.12)	9.67 (0.71)
Ultrasonication time	9.56 (0.54)	7.08 (0.38)
late from different lot numbers	4.68 (0.39)	5.68 (0.43)

4 CONCLUSION:

The developed TLC technique for simultaneous determination of OME and KET is precise, specific, robust and accurate. Statistical analysis proves that the method is suitable for the analysis of OME and KET from the bulk drug and solid dosage form without any interference from the excipients. This is the first reported HPTLC method for simultaneous estimation of OME and KET to the best of our knowledge.

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