Article

Development and Validation of a Novel Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Related Substances of Ketoprofen and Omeprazole in Combined Capsule Dosage Form

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Abstract

A novel, simple, sensitive, selective and reproducible stability-indicating high performance liquid chromatographic method was developed for the quantitative determination of degradation products and process-related impurities of ketoprofen (KET) and omeprazole (OMZ) in combined oral solid dosage form. Chromatographic separation was achieved on a Phenomenex Luna C18 (2) column (150 × 4.6 mm, 5 µm) under gradient elution by using a binary mixture of potassium dihydrogen phosphate buffer and acetonitrile at a flow rate of 0.8 mL/min. Chromatogram was monitored at 233 nm for KET impurities and at 305 nm for OMZ impurities using a dual wavelength UV detector. Resolution for KET and OMZ and 14 impurities was found to be >1.5 for any pair of components. Typical retention behaviors of impurities at various pH values were depicted graphically. To prove the stability-indicating power of the method, the drug product was subjected to hydrolytic, oxidative, photolytic, humidity and thermal stress conditions as per ICH. The developed method was validated according to the current ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

Introduction

Ketoprofen (KET) is chemically known as (2*RS*)-2-(3-benzoylphenyl) propanoic acid (Figure 1A). Its empirical formula is C₁₆H₁₄O₃ and the molecular weight is 254.28. KET is a nonsteroidal anti-inflammatory drug (NSAID), which is used in reducing pain caused by inflammation (1). Omeprazole (OMZ) is chemically known as (i*RS*)-5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl)-1*H*-benzo[d]imidazole (Figure 1B). Its empirical formula is C₁₇H₁₉N₃O₃S and the molecular weight is 345.4. OMZ is a proton pump inhibitor, which decreases the amount of acid produced in the stomach. OMZ is used to treat symptoms of gastroesophageal reflux disease (GERD)

and other conditions caused by excess stomach acid (2). Recently, KET has been marketed in combination with OMZ in modified release capsules formulation (Axorid). In combination, these are available in 100/20, 150/20 and 200/20 mg of KET and OMZ, respectively (3). A literature survey revealed that individually both KET and OMZ are officially approved in USP and EP (4, 5). But a pharmaceutical combination of KET and OMZ is not officially mentioned in any pharmacopeia. An extensive literature survey found that a number of analytical methods have been reported in the literature for the individual determination of KET or OMZ. These methods include HPLC estimation (6–20), UV spectrophotometry (21–23) and LC–MS

Fig.	Name	Chemical Structure	Chemical name
1A	KET		(2 <i>RS</i>)-2-(3-benzoylphenyl) propanoic acid
1B	KET Imp-A		3-[(1 <i>RS</i>)-1-carboxyethyl] benzoic acid
1C	KET Imp-B		(3-benzoylphenyl) acetic acid
1D	KET Imp-C		(2 <i>RS</i>)-2-[3-(4-methylbenzoyl) phenyl] propanoic acid
1E	KET Imp-D		(2 <i>RS</i>)-2-(3-benzoylphenyl) propanamide
1F	KET Imp-E		1-(3-benzoylphenyl) ethanone
1G	KET Imp-F		(2 <i>RS</i>)-2-(3-benzoylphenyl) propanenitrile
1H	OMZ		(<i>RS</i>)-5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methylsulfinyl)-1 <i>H</i> -benzo[<i>d</i>]imidazole
1I	OMZ Imp-A		1,4-dihydro-1-(5-methoxy-1 <i>H</i> -benzo[<i>d</i>]imidazol-2-yl)-3,5-dimethyl-4-oxopyridine-2-carboxylic acid

Figure 1. Chemical structure and name of KET, OMZ and its impurities. (A–G) KET Imp-A to KET Imp-F. (H–P) OMZ Imp-A to OMZ Imp-H.

Figure 1. Continued

estimation (24–26). The simultaneous assay determination of KET and OMZ in capsules was also reported (27). However, to the best of our knowledge, no LC method for the simultaneous determination of related substances of KET and OMZ in combined dosage forms has been reported so far. Hence it is felt essential to develop a single liquid

chromatographic method which will serve as a reliable, accurate, sensitive and stability-indicating HPLC method for the simultaneous determination of related substances of KET and OMZ in combined dosage forms. The developed method was successfully validated according to the ICH guidelines (28).

Experimental

Materials and reagents

KET and OMZ combined capsules (KOC), KET and OMZ active pharmaceutical ingredients (API) and standards of impurities were supplied by Dr Reddy's Laboratories Limited, IPDO, Hyderabad, India. The HPLC grade acetonitrile (ACN), analytical grade potassium dihydrogen phosphate (KH_2PO_4), triethyl amine (TEA), ortho phosphoric acid (H_3PO_4), hydrochloric acid (HCl), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were purchased from Merck, Darmstadt, Germany. Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA).

Instrumentation

LC analysis was performed on a Waters Acquity HPLC system equipped with a 2995 photo diode array detector. The output signal was monitored and processed using Empower 2 software (Waters Corporation, Milford, MA, USA).

Chromatographic conditions

Separation was accomplished on a Phenomenex Luna C18 (2) column (150 mm, 4.6 mm, 5 μm). Mobile phase A consisted of 0.01 M KH_2PO_4 and 3 mL of TEA (pH 7.0 adjusted with H_3PO_4). Mobile phase B contained a mixture of MilliQ water and ACN in the ratio of 10:90 (v/v). The mobile phases were filtered through a nylon 0.45- μm membrane filter. The flow rate of mobile phase was 0.8 mL/min. The HPLC gradient program (time in min)/%B was set as 0.01/12, 3/12, 25/35, 35/40, 50/60, 55/70, 60/70, 60.1/12 and 65/12. The column temperature was maintained at 25°C. The detection was monitored at a wavelength of 233 nm for KET impurities and of 305 nm for OMZ impurities. The injection volume was set as 10.0 μL . A mixture of 0.1 M NaOH and ACN in the proportion of 50 : 50 (v/v) was used as a diluent.

Preparation of stock and standard solutions

The individual stock solutions (100 $\mu\text{g/mL}$) of KET Imp-A, KET Imp-B, KET Imp-C, KET Imp-D, KET Imp-E, KET Imp-F and KET were prepared in methanol. OMZ Imp-A, OMZ Imp-B, OMZ Imp-C, OMZ Imp-D, OMZ Imp-E, OMZ Imp-F, OMZ Imp-G, OMZ Imp-H and OMZ individual stock solutions (80 $\mu\text{g/mL}$) were prepared in diluent. These solutions were prepared freshly and diluted (with same diluent) further quantitatively to study the validation attributes. The specification limits considered for validation studies were 0.5% for OMZ impurities and 0.2% for KET impurities. Diluted standard solution of 4 $\mu\text{g/mL}$ was prepared from the KET stock solution for the determination of the KET-related compounds and 0.8 $\mu\text{g/mL}$ was prepared from the OMZ stock solution for the determination of the OMZ-related compounds.

Preparation of sample solution

The entire pellets content of KET and OMZ capsule's (100 mg of KET/20 mg of OMZ) ($n = 10$) were transferred into a 500-mL volumetric flask. Then, 350 mL of diluent was added and the flask is kept on a rotator shaker for 15 min followed by 15 min sonication. The solution was diluted to volume with the diluent, and a portion of the sample was centrifuged at 4,000 rpm for 10 min. This solution contained 2,000 $\mu\text{g/mL}$ of KET and 400 $\mu\text{g/mL}$ of OMZ.

Specificity and mass balance study

Stress degradation studies was performed according to the ICH guidelines Q1A (R2) (29) to demonstrate the stability-indicating nature and specificity of the proposed method. The entire pellets content of KET and OMZ capsule's (100 mg of KET/20 mg of OMZ) ($n = 10$) were transferred into a 500-mL volumetric flask and were subjected to forced degradation study under acid (0.5 N HCl at 25°C for 30 min), base (0.5 N NaOH at 60°C temperature for 4 h), neutral (water at 60°C for 4 h) and oxidation conditions (3.0% v/v H_2O_2 at room temperature for 30 min). The stressed samples of acid and base degradation were neutralized with 0.5 N NaOH and 0.5 N HCl, respectively, and made up to volume with the diluent. The drug product was placed in a thermally controlled oven at 90°C up to 24 h for the thermal stress study. Photolytic degradation was performed by exposing the drug to visible light and UV with minimum exposure of 1.2 million lux-hours and 200 W-h/m². The drug product was placed into a humidity chamber up to 7 days for humidity degradation. The degradation samples were analyzed by the HPLC method as described in the chromatographic condition. A peak purity test was carried out for KET and OMZ peaks by using a Photodiode Array (PDA) detector in all stressed samples. The assay of stressed samples was performed (at 200 $\mu\text{g/mL}$ for KET and 40 $\mu\text{g/mL}$ for OMZ) by comparison with the qualified reference standard and the mass balance (% assay + % impurities + % degradation products) was calculated.

Validation parameters

The proposed method was validated according to the ICH guidelines for its limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, solution stability and robustness.

Results

Forced degradation studies

Forced degradation samples under various conditions were analyzed at an initial concentration of 2,000 $\mu\text{g/mL}$ of KET and 400 $\mu\text{g/mL}$ of OMZ by the HPLC method (chromatographic condition) using a PDA detector to ensure homogeneity of KET and OMZ peaks. When the drug was subjected to acidic hydrolysis (0.5 N HCl at 25°C for 30 min), OMZ immediately degraded into OMZ Imp-A and OMZ Imp-F and low level KET Imp-E was observed. Under base hydrolysis (0.5 N NaOH at 60°C temperature for 4 h), five known degradation products such as OMZ Imp-A, OMZ Imp-B, OMZ Imp-F, OMZ Imp-H, KET Imp-E and one low level OMZ unknown degradation product were formed. In water hydrolysis (water at 60°C for 4 h), five known degradation products such as OMZ Imp-A, OMZ Imp-F, OMZ Imp-H and low level KET Imp-B and KET Imp-E were formed. When the drug was subjected to peroxide degradation (10% H_2O_2 at room temperature 24 h), OMZ completely degraded into OMZ Imp-F. Hence gradually the concentration of peroxide and holding time were reduced to 3.0% v/v H_2O_2 at room temperature for 30 min, where five known degradation products such as OMZ Imp-A, OMZ Imp-F (major), OMZ Imp-H and KET Imp-E and two OMZ unknown degradation products were formed. In a humidity degradation study, four known degradation products such as OMZ Imp-A, OMZ Imp-B, OMZ Imp-C, OMZ Imp-H and one OMZ unknown degradation product were formed. In a thermal and photolytic stress study, no considerable degradation was noticed. The above results confirmed that the drug product was very sensitive toward acid hydrolysis and peroxide degradation (Figure 2).

Figure 2. Typical chromatograms of KET and OMZ under stress conditions. (A1) OMZ acid hydrolysis and (A2) KET acid hydrolysis. (B1) OMZ base hydrolysis and (B2) KET base hydrolysis. (C1) OMZ peroxide degradation and (C2) KET peroxide degradation. (D1) OMZ water hydrolysis and (D2) KET water hydrolysis. (E1) OMZ humidity degradation and (E2) KET humidity degradation. (F1) OMZ photo degradation and (F2) KET photo degradation. (G1) OMZ thermal degradation and (G2) KET thermal degradation. This figure is available in black and white in print and in color at JCS online.

Figure 2 Continued

All the forced degradation samples were analyzed using a PDA detector to ensure the homogeneity and purity of the KET and OMZ peaks. The results from the peak purity assessment revealed that the purity angle was less than the purity threshold in all of the stressed samples. The mass balance (% assay + % sum of all impurities) results were calculated and found to be more than 99.0% (Table I). The purity of KET and OMZ were unaffected by the presence of its

impurities, degradation products and other excipients (placebo) and thus confirms the stability-indicating power of the method.

Results of method validation

Sensitivity

The LOD and LOQ values for all impurities were determined by injecting a series of diluted solutions with known concentration to obtain

Table I. Summary of Forced Degradation Results

Degradation condition	% Assay		RS by HPLC % degradation		Mass balance (% assay + % deg. products)		Remarks/observation
	OMZ	KET	OMZ	KET	OMZ	KET	
Acid hydrolysis (0.5 N HCl, 25°C, 30 min)	96.2	99.2	3.7	0.17	99.9	99.4	OMZ Imp-A, OMZ Imp-F, OMZ Imp-H and KET Imp-B and KET Imp-E degradation products were formed
Base hydrolysis (0.5 N NaOH, 60°C, 4 h)	97.5	99.5	2.6	0.13	100.1	99.6	OMZ Imp-A, OMZ Imp-B, OMZ Imp-F, OMZ Imp-H and KET Imp-E degradation products were formed
Water hydrolysis (60°C, 4 h)	94.1	99.8	5.4	0.15	99.5	100.0	OMZ Imp-A, OMZ Imp-F, OMZ Imp-H and KET Imp-B and KET Imp-E degradation products were formed
Oxidation (3.0% H ₂ O ₂ , 25°C, 30 min)	82.1	98.9	17.2	0.25	99.3	99.2	OMZ Imp-A, OMZ Imp-F, OMZ UK-1, OMZ UK-2 and KET Imp-E degradation products were formed
Photo degradation: exposed to 200 W h/m ² and 1.2 million lux hour in photo stability chamber for 16 h	99.6	99.3	0.4	0.22	100.0	99.5	Low level OMZ Imp-F and KET Imp-E degradation products were formed
Thermal degradation (90°C, 24 h)	99.4	99.7	0.7	0.1	100.1	99.8	No significant degradation observed
Humidity at 90% RH (25°C, 7 days)	90.2	99.1	10.5	0.0	100.7	99.1	OMZ Imp-A, OMZ Imp-B, OMZ Imp-C, OMZ Imp-H, and OMZ UK-1 degradation products were formed

UK, unknown impurity.

S/N ratio values of 3 and 10, respectively. For LOQ, the signal-to-noise ratio's for all the impurities were ranging from 9.7 to 10.3. A precision study was performed at the LOQ level by injecting six individual preparations of KET, OMZ and its impurities and calculated the % relative standard deviation (RSD) for the areas of each peak. The RSDs were found to be between 2.52 and 5.81%. Accuracy at the LOQ level was verified by injecting three individual preparations of KET and OMZ spiked with impurities at the LOQ level. The percent recoveries were calculated for each impurity and those are ranging from 97.1 to 104.1%. The results were in the range of 0.14–0.58 µg/mL for LOQ and 0.05–0.17 µg/mL for LOD (Table II).

Precision

The repeatability and ruggedness of the method was performed by six individual determinations of KET (2,000 µg/mL) and OMZ (400 µg/mL) by spiking with impurities at the specification level. The ruggedness was determined by repeating the same experiment on two different days by different analysts using a different equipment. The % RSD was calculated for each impurity (Table II). These results confirmed the high precision.

Linearity

Linearity test solutions were prepared from impurity stock solution at seven different concentration levels ranging from the LOQ to 150% of the specification level (i.e., LOQ, 1, 2, 3, 4, 5, 6 µg/mL for KET Imp-A to KET Imp-F; LOQ, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µg/mL for OMZ Imp-A to OMZ Imp-H). The calibration curve was drawn by the plotting impurity area versus the concentration. The correlation coefficients, slopes and y-intercepts of the calibration plots are reported. Calibration plots for the seven related substances were linear over the ranges tested. The correlation coefficients were >0.999 for all the components (Table II). These results show that there was an excellent

correlation between the peak area and the concentration for the 14 impurities.

Accuracy

Accuracy of the method was evaluated by spiking with known amounts of impurities to the KET (2,000 µg/mL) and OMZ (400 µg/mL) test sample at the level of 50, 100 and 150% of specification in triplicate. The percent recoveries were calculated for related substances, and those are ranging from 99.1 to 104.1% (Table II).

Robustness

To determine the robustness of the method, experimental conditions were deliberately altered. The factors chosen for this study, which were the critical sources of variability in the operating procedures, such as flow rate (0.8 ± 0.2 mL/min), mobile phase pH (7.0 ± 0.2), mobile phase composition (mobile phase B; $\pm 5\%$ ACN) and column oven temperature ($25 \pm 5^\circ\text{C}$) were identified. The sample spiked with all known impurities at specification level was injected, and the resolution among the impurities was monitored. The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions except the change in pH of the buffer. KET, KET Imp-A, KET Imp-C and OMZ Imp-F move toward right with decreasing pH while toward left with increasing pH. Hence it was concluded that the method is sensitive to mobile phase buffer pH.

Solution stability and mobile phase stability

No significant changes in the amounts of the 14 impurities were observed during solution stability and mobile phase stability experiments when performed using the related substances method. The results from solution stability and mobile phase stability experiments confirmed that the standard solutions and sample were stable for up to 24 h

Table II. Summary of method validation for KET, OMZ and its impurities

Validation parameter	KET Imp-A	KET Imp-B	KET Imp-C	KET Imp-D	KET Imp-E	KET Imp-F	KET	OMZ Imp-A	OMZ Imp-B	OMZ Imp-C	OMZ Imp-D	OMZ Imp-E	OMZ Imp-F	OMZ Imp-G	OMZ Imp-H	OMZ
Specifications (%)	0.2	0.2	0.2	0.2	0.2	0.2	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	–
LOD (µg/mL)	0.15	0.13	0.13	0.15	0.17	0.17	0.15	0.04	0.05	0.05	0.05	0.04	0.04	0.05	0.05	0.06
LOQ (µg/mL)	0.55	0.44	0.43	0.51	0.58	0.56	0.48	0.14	0.161	0.17	0.166	0.14	0.14	0.16	0.165	0.17
LOQ accuracy	101.2	102.1	97.1	97.3	99.1	100.1	102.1	101.2	102.2	99.9	101.1	100.2	103.5	103.5	104.1	103.1
% RSD (<i>n</i> = 6) ^a	4.21	3.81	3.95	4.25	4.51	4.24	2.52	4.15	3.25	5.8	5.81	5.42	5.73	4.65	5.35	4.35
% RSD (<i>n</i> = 6) ^b	0.72	0.63	0.85	0.62	0.54	0.65	–	0.91	0.88	0.73	0.81	0.95	0.74	0.94	0.81	–
% RSD (<i>n</i> = 6) ^c	0.56	0.74	0.63	0.75	0.92	0.74	–	0.75	0.68	0.91	0.89	0.92	0.86	0.91	0.87	–
Accuracy at 50%	98.7	101.1	100.9	102.3	104.1	106.1	–	99.8	100.2	99.5	102.3	97.8	100.2	99.8	99.2	–
Accuracy at 100%	98.2	100.1	101.5	103.2	103.8	105.1	–	98.7	101.1	98.8	101.5	98.1	101.2	99.7	98.5	–
Accuracy at 150%	99.1	100.5	102.1	102.1	102.5	104.1	–	99.1	100.5	99.8	100.5	99.5	101.4	100.2	99.5	–
Regression equation (<i>y</i>)																
Correlation	0.9992	0.9996	0.9995	0.9999	0.9998	0.9999	0.999	0.9998	0.9996	0.9998	0.9997	0.9998	0.9997	0.9999	0.9995	0.9997
% Y-intercept	1.6	1.8	1.9	0.6	0.8	1.2	1.4	1.6	0.7	0.9	1.2	1.7	0.6	0.7	0.9	1.2

Linearity range is LOQ–150% with respect to 0.2% specification level for KET and its impurities; 0.5% specification level for OMZ and its impurities.

^aLOQ precision.

^bRepeatability.

^cIntermediate precision.

during determination of related substances. The mobile phase was stable up to 2 days.

Discussion

The main objective of method development was to achieve simple and efficient separation between KET and OMZ and its related compounds. The main difficulty was to obtain sufficient selectivity and resolution between structurally similar impurities and degradants. Initially, experiments were carried out based on the available OMZ and KET USP/EP monograph HPLC methods. KET monograph methods have the mobile phase pH (3.5) on acidic side but OMZ highly sensitive toward acidic side so that this method is incapable of estimating OMZ impurities in KET and OMZ combined dosage form.

As per OMZ USP monograph HPLC method, three compounds namely, KET Imp-B, OMZ Imp-C and OMZ Imp-D were coeluted with KET; KET Imp-C and OMZ Imp-E were merged together and also KET Imp-E and KET Imp-F were strongly retained. Considering the demerits of the existing methods, we felt that it is necessary to develop a new stability-indicating method for the related substance of KET and OMZ in combined dosage forms. To achieve final chromatographic conditions, the following chromatographic parameters have been optimized.

Selection of wavelength and stationary phase

A detection wavelength of 233 nm for KET-related compounds and 305 nm for OMZ-related compounds were selected based on the full-range UV spectral data due to its high sensitivity for all related substances and a minimal difference in response factors. Due to the OMZ molecule stable at basic pH, a basic buffer pH (pH 7.0) was selected as mobile phase A. Because the KET impurities have a wide range of polarities, the need for a gradient run was assessed using mobile phase A: phosphate buffer (pH 7.0; 0.01 M) and mobile phase B: ACN–water (80 : 20, v/v) on phenyl, cyano, C8 and C18 columns. The chromatographic separation has been verified on different C18 stationary phases with slightly different selectivities and

hydrophobicities. Finally, a Phenomenex Luna C18(2) column provided the largest number of peaks. Thus, further experiments were carried out in the Phenomenex Luna C18 (2) column (150 × 4.6 mm, 5 µm).

Evaluation of mobile phase and gradient program

The initial gradient run (time in min)/(%B); (0/0 and 80/100) provided an estimate of the percentage of organic ratio and approximate retention times for the impurities. The retention times of the first and last impurity peaks were 64 and 69 min, respectively. To minimize the run time with better separations, many attempts were made with different organic solvent compositions in mobile phases A and B, along with different gradient elutions. It was found that 100% phosphate buffer (pH 7.0) as mobile phase A; water and ACN in the ratio of 10 : 90 (v/v) as mobile phase B; with gradient elution (time(min)/%B) 0.01/12, 3/12, 25/35, 35/40, 50/60, 55/70, 60/70, 60.1/12 and 65/12 enabled separation for all components with good peak shape, resolution and retention.

Evaluation of buffer pH and modifier

The effect of the buffer pH on the retention times of KET and OMZ and its related compounds was studied in the pH range 5.0–9.0 while keeping other chromatographic parameters unchanged. The retention behavior was illustrated in Figure 3, which plotted the retentions of the 14 impurities and KET and OMZ as a function of mobile phase buffer pH. At pH 6.0–9.0, the early elution of KET Imp-A was observed. This is due to the presence of two hydrophilic ionizable functional groups (–COOH). A distinctive retention behavior was observed for KET Imp-B and OMZ Imp-C, OMZ Imp-D and KET, KET Imp-C and OMZ Imp-E and OMZ Imp-F and OMZ Imp-G. No changes were found for OMZ Imp-A, OMZ Imp-B, OMZ Imp-H, KET Imp-E and KET Imp-F in terms of ionization and retention in the studied range. At pH 7.0, the best resolutions were obtained for all impurities except for OMZ Imp-D and KET.

TEA was added to the mobile phase to separate OMZ Imp-D and KET peaks. To increase the ionic interaction between TEA and KET, the TEA concentration was optimized to 0.3% in the mobile phase while maintaining the buffer at pH 7.0. The addition of TEA not

Figure 3. Effect of mobile phase buffer pH on retention of impurities. This figure is available in black and white in print and in color at JCS online.

Figure 4. Typical chromatogram of the test sample spiked with all impurities in the final chromatographic conditions. This figure is available in black and white in print and in color at JCS online.

Table III. System suitability data

S. no.	Impurity name	RT (in min)	USP resolution	USP tailing factor	USP plate count	% RSD ^a (n = 6)
1	KET Imp-A	3.94		0.9	5,723.9	–
2	OMZ Imp-A	15.31	53.1	1.2	54,553.9	–
3	OMZ Imp-B	18.12	10.2	1.1	64,800.3	–
4	KET Imp-B	23.66	21.2	1.1	168,294	–
5	OMZ Imp-C	24.19	2.1	1.1	136,571.9	–
6	OMZ Imp-D	25.00	3.2	1.1	170,109.6	–
7	KET	25.73	2.0	1.2	15,081.9	0.5
8	KET Imp-C	30.61	8.7	1.2	195,030.5	–
9	OMZ Imp-E	31.62	3.1	1.1	127,900.1	–
10	OMZ	32.79	3.3	1.0	141,925.7	0.7
11	OMZ Imp-F	35.36	6.7	1.1	117,048.3	–
12	OMZ Imp-G	36.85	3.2	0.9	92,847.4	–
13	KET Imp-D	39.29	4.8	1.0	115,185.2	–
14	OMZ Imp-H	44.86	11.5	1.1	147,117.7	–
15	KET Imp-E	50.16	10.6	1.0	219,159.1	–
16	KET Imp-F	55.15	11.1	1.1	402,901.8	–

n, Number of determinations; RSD, relative standard deviation.

^aKET diluted standard (4 µg/mL) and OMZ diluted standard (0.8 µg/mL).

only increase the resolution between OMZ Imp-D and KET peaks ($R_s \sim 1.8$) but also enhanced all impurities' peak shapes and KET Imp-A retention increased to 3.9 min. Therefore, the mobile phase pH was set at 7.0 and the TEA concentration was set at 0.3% for the separation of all impurities (Figure 4).

Evaluation of diluent and placebo interference

In this study, 0.1 M NaOH was chosen as diluent for dispersion of OMZ enteric-coated pellets and OMZ was found to be stable in 0.1 M NaOH. KET is also found to be more soluble in 0.1 M NaOH due to the presence of a carboxylic acid moiety. ACN is required to enhance the dispersion of the KET pellets. Hence, 0.1 M NaOH and ACN in the ratio of 1:1 was taken as the diluent.

This diluent was found to be more suitable for determination of related substances of KET and OMZ in combined dosage forms. The blank and placebo interference were also verified and found that no interference was observed at the retention time of any of the impurities and KET and OMZ.

Efficient separation and selectivity were achieved on the Phenomenex Luna C18(2) column (150 mm, 4.6 mm, 5 µm) using mobile

phase A (KH_2PO_4 buffer and 3 mL TEA, pH adjusted to 7.0 with H_3PO_4) and mobile phase B (ACN–water, 90 : 10 v/v). The chromatogram was monitored at 233 nm for related compounds of KET and 305 nm for related compounds for OMZ using a gradient program (time in min)/%B): 0.01/12, 3/12, 25/35, 35/40, 50/60, 55/70, 60/70, 60.1/12 and 65/12 with a mobile phase flow rate of 0.8 mL/min. The system suitability parameters were evaluated for KET and OMZ and its impurities (Figure 4). The USP tailing factor for all impurities and KET and OMZ were found to be <1.2. The USP resolution (R_s) between all components is >1.5 (Table III).

Conclusion

A simple and selective stability-indicating gradient RP-HPLC method has been developed for the quantitative determination of related substances of KET and OMZ in combined oral solid dosage form. The developed RP-HPLC method has several advantages, which are follows: simple mobile phase, simple test sample preparation, single chromatographic method, efficient separation within a reasonable analysis time and low cost analysis.

The developed method was validated as per the ICH guidelines and found to be specific, precise, accurate and linear. Thus, the method can be used for routine analysis, quality control and stability studies of APIs and their related substances in combined oral dosage forms.

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