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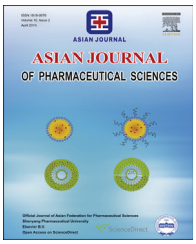

Short Communication

Gradient high performance liquid chromatography method for simultaneous determination of ilaprazole and its related impurities in commercial tablets

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ABSTRACT

A methodology (HPLC) proposed in this paper for simultaneously quantitative determination of ilaprazole and its related impurities in commercial tablets was developed and validated. The chromatographic separation was carried out by gradient elution using an Agilent C_8 column (4.6 mm × 250 mm, 5 μm) which was maintained at 25 °C. The mobile phase composed of solvent A (methanol) and solvent B (solution consisting 0.02 mmol/l monopotassium phosphate and 0.025 mmol/l sodium hydroxide) was at a flow rate of 1.0 ml/min. The samples were detected and quantified at 237 nm using an ultraviolet absorbance detector. Calibration curves of all analytes from 0.5 to 3.5 μg/ml were good linearity ($r \geq 0.9990$) and recovery was greater than 99.5% for each analyte. The lower limit of detection (LLOD) and quantification (LOQ) of this analytical method were 10 ng/ml and 25 ng/ml for all impurities, respectively. The stress studies indicated that the degradation products could not interfere with the detection of ilaprazole and its related impurities and the assay can thus be considered stability-indicating. The method precisions were in the range of 0.41–1.21 while the instrument precisions were in the range of 0.38–0.95 in terms of peak area RSD% for all impurities, respectively. This method is considered stability-indicating and is applicable for accurate and simultaneous measuring of the ilaprazole and its related impurities in commercial enteric-coated tablets.

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1. Introduction

Ilaprazole, [IY-81149, 2 (((4-methoxy-3-methyl)-2-pyridinyl)-methylsulfinyl)-5-(1H-pyrrol-1-lyl) 1H-benzimidazole] (Fig. 1A), is a potent inhibitor of the enzyme gastric proton pump H+/K+-ATPase in inhibiting gastric acid secretion, which includes gastric and duodenal ulcer, reflux oesophagitis and Zollinger-Ellison syndrome [1–3]. Unlike other proton pump

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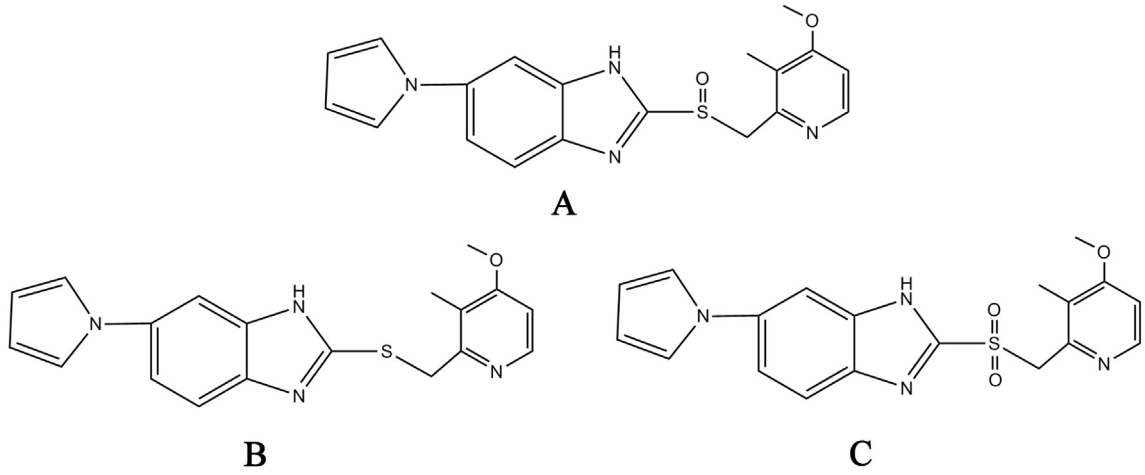

Fig. 1 – Chemical structures of (A) ilaprazole, (B) ilaprazole sulfur ether and (C) ilaprazole sulphone.

inhibitors (PPIs), ilaprazole with a longer plasma half-life (about 3.6 h) [4] produces a greater and prolongs acid-suppressing effect [5], avoiding the occurrence of nocturnal acid break (NAB) during the PPIs therapy [6]. Besides, a greater safety could be assured by at least two factors. One is the significant reduction of dosage and lower toxicity for ilaprazole comparing to omeprazole [7]. And the other one is the low variability of the pharmacokinetics and pharmacodynamics of ilaprazole attributed to its metabolism independent of CYP2C19 [8], which played the predominant role in metabolism of other PPIs [9,10].

Recently, there were a lot of efforts committed to researching metabolism in vivo [11–14]; however, there was only one paper reporting a UPLC method for the simultaneous determination of ilaprazole and its impurities in tablets [15]. A ternary liquid system (acetonitrile, methanol and ammonium acetate buffer) was used to separated five known impurities. In light of the requirements of a more widely available method and better quality control for ilaprazole involved solid preparations, the development of a novel analytical method was demanded and necessary. Therefore, we developed a sensitive, selective and accurate gradient HPLC method, using a simple methanol-water binary system, for the quantification of ilaprazole and seven known impurities including ilaprazole sulfur ether and ilaprazole sulphone (Fig. 1B and C) and impurity A, B, C, D, E (structures not for publication) in commercial tablets.

2. Materials and methods

2.1. Materials

Ilaprazole (5 mg enteric-coated tablet) were purchased from Liyzon Pharmaceutical Group Inc. (Zhuhai, China). Ilaprazole and related impurities (ilaprazole sulphone, ilaprazole sulfur ether and impurity A, B, C, D, and E) (purity > 99.0%, purities were detected by HPLC using area normalization method and structures were validated by nuclear magnetism resonance (NMR) and mass spectrum (MS)) were supplied by Medicinal Chemistry Lab of Shenyang Pharmaceutical University (Shenyang, China). HPLC-grade methanol was from Fisher Scientific Worldwide (Shanghai) Co., Ltd. (Shanghai, China). Deionized-distilled water was used throughout this study. All other reagents were of analytical grade.

2.2. HPLC operating conditions

HPLC separations were performed by an Agilent C_8 column (4.6 mm × 250 mm, 5 μm) (Agilent, USA). Analytical HPLC apparatus was consisted of an L-2130 pump (Hitachi, Japan) and an L-2400 ultraviolet absorbance detector (Hitachi, Japan).

Gradient HPLC method was used for the analysis of ilaprazole and its related impurities at 25 °C by using an ultraviolet absorbance detector at a wavelength of 237 nm. The mobile phase consisted of solvent A (methanol) and solvent B (0.02 mmol/l monopotassium phosphate and 0.025 mmol/l sodium hydroxide) with a flow rate of 1.0 ml/min. The initial mobile phase composition was maintained at 42% solvent A for 30 min, changed linearly to 55% (30–35 min) and held 30 min (35–65 min), then followed by a return to the initial conditions within 5 min (65–70 min) and kept 5 min (70–75 min) for the chromatograph column equilibrium. The mobile phase was filtered through a 0.22 μm PTFE filter (Millipore, USA) and ultrasounded for degasification before use. The injection volume was of 20 μl.

2.3. Preparation of stock and standard solutions

On account of the instability of ilaprazole, its freshly solution was prepared immediately before use and protected from light. Stock solutions (100 μg/ml) of ilaprazole impurities were separately prepared with methanol. The stock solutions of different impurity were kept from light and stored at 4 °C for four weeks with no evidence of decomposition.

Aliquots of the stock solutions of different impurity were transferred into 10 ml volumetric flasks with bulb pipettes and the solutions were made up to volume with methanol to yield final concentrations of 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 μg/ml for each impurity.

Twenty commercial tablets were weighed, crushed and mixed with a mortar and pestle for 15 min. A portion of powder equivalent to 10 mg ilaprazole was precisely weighed to 10 ml volumetric flask and 5 ml methanol was added. The volumetric flask was sonicated for 10 min to completely dissolve ilaprazole and then the solutions were made up to volume with methanol. After centrifugation for 5 min at 13,000 rpm, aliquots of the solutions were filtered through 0.22 μm nylon filters.

2.4. System suitability test

System suitability test was conducted by injections of the system suitability solution ($n = 6$). The acceptance criteria were as follows: relative standard deviation (RSD) for peak areas within 2%, column plates greater than 5000, the USP tailing factor within 1.5, and the resolution greater than 2.0.

2.5. Forced degradation studies of ilaprazole

The conditions for forced degradation studies were screened and finally fixed as follows to obtain 10–20% degradation of ilaprazole [16].

2.5.1. Acid degradation

Solution containing 1 mg/ml of ilaprazole was treated with 1 mol/l HCl for 30 s. The resultant solution was neutralized immediately as needed and analyzed.

2.5.2. Alkali degradation

Solution containing 1 mg/ml of ilaprazole was disposed with 1 mol/l NaOH for 2 h. The resultant solutions were neutralized and analyzed promptly.

2.5.3. Oxidative condition

Solution containing 1 mg/ml ilaprazole was obtained with 30% w/v H_2O_2, and the resultant solutions were analyzed 1 h later.

2.5.4. Thermal degradation study

Tablet powders were subjected to 100 °C in an oven for 1 h, and then 1 mg/ml ilaprazole solution was achieved with methanol for analysis.

2.5.5. Photostability study

Tablet powders were exposed to 4500 lx ± 500 lx light for 20 days to determine the effect of irradiation on the stability of the drug. The sample solution (equivalent to approximately 1 mg/ml of ilaprazole) was prepared for further HPLC analysis.

3. Results and discussion

3.1. HPLC method development and optimization

As we all know, more impurities controlled were beneficial to guarantee quality of commercial drugs and to ensure safety of drug therapy. From this prespective, the development of method with a capability of more known impurities detected than the reported method in the literature [15] was necessary and salutary. At the begin of method development, an isocratic elution (42% solvent A as the initial mobile phase composition described in section "2.2") was used to separate ilaprazole and seven kown impurities, leading to a total runtime over 120 min. Considering the polarity difference of ilaprazole and its related impurities, a gradient HPLC method was developed to achieve a shorter runtime and desired sensitivity. An Agilent C_8 column (4.6 mm × 250 mm, 5 μm) (Agilent, USA) maintained at 25 °C was used for the separation and this method was validated to assure the reliability of determination of ilaprazole and its impurities. The composition, pH and the flow rate of the mobile phase were changed to optimize the separation conditions. A gradient HPLC method which was described in section "2.2" was selected for further studies after several preliminary investigatory chromatographic runs. Under the experimental conditions, all the peaks were well defined and free from tailing. The deliberate changes in the mobile phase composition and flow rate were evaluated to test the method robustness.

3.2. Validation of the method

The developed method was characterized by evaluation of specificity, limit of detection (LLOD), limit of quantization (LOQ), linearity, precision, accuracy, and robustness/ruggedness.

3.2.1. System suitability test

The results of system suitability test indicated that all the parameters obtained were within the acceptable limits. Fig. 2 shows the chromatogram of the system suitability test solution.

3.2.2. Specificity

The specificity of the method was guaranteed by observing potential interferences caused by degradation products under stress conditions. The stress testing studies indicated a preferable specificity of this method for both ilaprazole and its related impurities. The results of stress testing are shown in Fig. 3.

3.2.3. LLOD and LOQ

The LOQs for ilaprazole impurity A, B, C, D, E, ilaprazole sulphone and ilaprazole sulfur ether corresponding to a signal-to-noise ratio of 10 were 25 ng/ml, 200 ng/ml, 75 ng/ml, 125 ng/ml, 750 ng/ml, 200 ng/ml and 200 ng/ml, respectively.

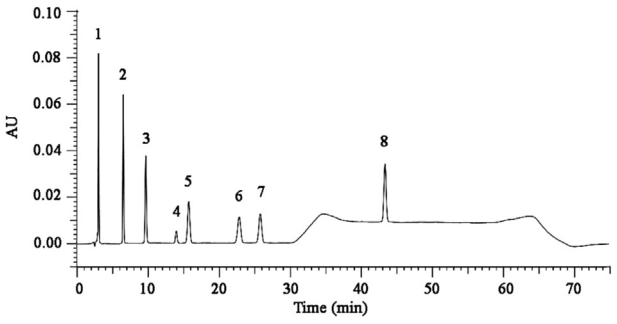

Fig. 2 – Chromatogram of the system suitability test solution (1-Impurity A, 2-Impurity C, 3-Impurity D, 4-Impurity E, 5-Ilaprazole sulphone, 6-Ilaprazole, 7-Impurity B, 8-Ilaprazole sulfur ether).

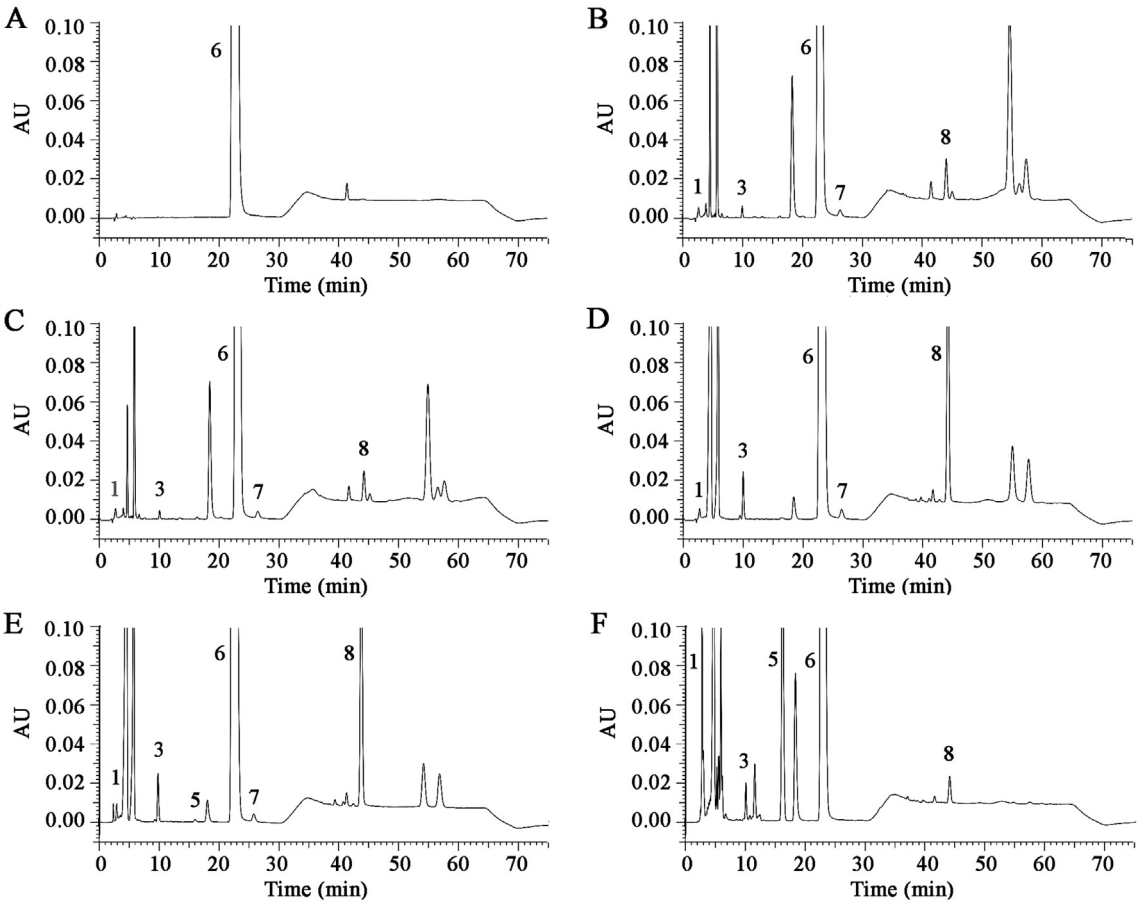

Fig. 3 – Chromatograms of stress test. (A) Untreated tablet powders; (B) acid hydrolysis-degraded tablet powders; (C) base hydrolysis-degraded tablet powders; (D) dry-heated tablet powders; (E) light degraded tablet powders; (F) oxidation degraded tablet powders (the peak number is the same as in Fig. 2).

And the LLODs for ilaprazole related impurities corresponding to a signal-to-noise ratio of 3 were 10 ng/ml, 80 ng/ml, 30 ng/ml, 50 ng/ml, 300 ng/ml, 80 ng/ml and 80 ng/ml. The resultant RSD values (%) for these studies were ≤2.0%.

3.2.4. *Linearity*

The linearity was performed with the standard solutions of ilaprazole impurities ($n = 7$) over the concentration range of 0.5–3.5 μg/ml, respectively. Least squares linear regression analysis of the calibration curve was employed to establish the linearity. Typical sequent regression equations for ilaprazole impurity A, B, C, D, E, ilaprazole sulphone and ilaprazole sulfur ether are listed in Table 1.

Table 1 – Typical sequent regression equations of ilaprazole related impurities.

Compound	Regression equation	Correlation coefficient (r)
Impurity A	$A = 147.39C - 5162.7$	0.9997
Impurity B	$A = 84.09C - 2570.1$	0.9999
Impurity C	$A = 95.128C - 2427.7$	0.9996
Impurity D	$A = 102.61C - 2903.1$	0.9999
Impurity E	$A = 14.371C + 701.86$	0.9994
Ilaprazole sulphone	$A = 70.984C - 2730.1$	0.9992
Ilaprazole sulfur ether	$A = 107.66C - 3309.3$	0.9990

Note: A being the peak area and C the concentration in μg/ml.

3.2.5. *Precision*

The precision of the HPLC method were assessed by analyzing 6 replicate sample solutions in three different concentrations of mixed impurities standard solutions (i.e. 0.8, 1.0, 1.2 μg/ml). The same sample solution (1.0 μg/ml) was injected 6 times to investigate the instrument precision. The RSD (%) of the

Table 2 – Results of determination of precision for ilaprazole related impurities.

Compound	Method precision RSD (%)	Instrument precision RSD (%)
Impurity A	0.41	0.38
Impurity B	0.69	0.44
Impurity C	1.09	0.85
Impurity D	0.85	0.42
Impurity E	1.21	0.95
Ilaprazole sulphone	0.71	0.51
Ilaprazole sulfur ether	0.68	0.78

Table 3 – Accuracy results for the determination of ilaprazole related impurities.

Compound	Added (μg/ml)	Found (μg/ml)	Recovery (%)	RSD (%)	Average recovery (%)	Average RSD (%)
Impurity A	0.80	0.80	99.8	0.54	99.8	0.61
	1.00	1.00	99.9	0.31		
	1.20	1.20	99.7	0.98		
Impurity B	0.80	0.80	100.5	1.08	100.3	0.92
	1.00	1.00	100	0.86		
	1.20	1.21	100.5	0.82		
Impurity C	0.80	0.80	100.2	0.28	100.2	0.34
	1.00	1.00	100.3	0.32		
	1.20	1.20	100.2	0.43		
Impurity D	0.80	0.80	100	0.70	99.8	0.54
	1.00	1.00	99.8	0.49		
	1.20	1.19	99.5	0.43		
Impurity E	0.80	0.81	100.5	1.21	100.2	0.87
	1.00	1.00	99.8	0.45		
	1.20	1.21	100.4	0.96		
Ilaprazole sulphone	0.80	0.81	100.9	0.42	100.7	0.37
	1.00	1.00	100.3	0.16		
	1.20	1.21	100.9	0.51		
Ilaprazole sulfur ether	0.80	0.80	99.7	1.19	99.8	1.01
	1.00	1.00	100	0.94		
	1.20	1.20	99.8	0.88		

proposed method precision and instrument precision were all less than 1.21 and 0.95% for impurities A, B, C, D, E, ilaprazole sulphone and ilaprazole sulfur ether (Table 2).

3.2.6. *Accuracy*

Results of method accuracy are summarized in Table 3. The accuracy for the determination of ilaprazole and its impurities was determined by preparing drug substance sample at 80, 100 and 120% of the target (1 μg/ml). The apparent recovery of ilaprazole and its impurities were found to be from 99.5 to 100.9%. The average percent recovery was 99.8 (RSD% 0.61), 100.3 (RSD% 0.92), 100.2% (RSD% 0.34), 99.8% (RSD% 0.54), 100.2% (RSD% 0.87), 100.7% (RSD% 0.37) and 99.8% (RSD% 1.01) for ilaprazole and its related impurities.

3.2.7. *Stability*

The stability of ilaprazole in methanol was evaluated at ambient temperature and 4 °C in refrigeration for 12 h (Table 4). Compared with ambient temperature, the stability of sample was improved to some extent, however, the change of ilaprazole peak area was approximated 2% in 2 h, so the fresh sample needed to be prepared prior to use. The mixed impurities solution was stable at ambient temperature for 12 h (Table 5). No significant changes (<2%) of the chromatographic responses were observed for the stock solutions of different impurity, relative to freshly prepared standards (Table 6).

Table 4 – Results of stability of ilaprazole solution at ambient temperature and 4 °C.

Conditions	Rate of change (%) of ilaprazole peak area under different conditions					
	2 h	4 h	6 h	8 h	10 h	12 h
Ambient temperature	−2.91	−4.44	−6.79	−8.87	−11.01	−13.40
4 °C	−1.84	−3.13	−5.50	−5.64	−7.78	−10.17

3.2.8. *Robustness*

The robustness of the HPLC method was studied under a variety of conditions including changes of flow rate and the proportion of buffer and organic phase. The small deliberate variations of these parameters were not significantly affected the reproducibility of the proposed method, which proved good robustness of the HPLC method.

4. Conclusions

A sensitive, selective and accurate gradient HPLC method has been developed and validated for the quantization of ilaprazole and its related impurities in commercial tablets. The stress testing results revealed that the method was specific and selective. Validation experiments demonstrated that linear, precise and accurate of method met the acceptance

Table 5 – Results of stability of ilaprazole related impurities solution at ambient temperature.

Compound	Rate of change (%)of different impurity peak area at ambient temperature					
	2 h	4 h	6 h	8 h	10 h	12 h
Impurity A	−0.67	−0.97	−0.38	−0.30	−0.77	−0.45
Impurity B	−0.56	−0.34	−0.59	−0.89	−0.98	−0.37
Impurity C	−0.22	−0.06	−0.77	−0.78	−0.76	−0.66
Impurity D	−0.54	−0.54	0.92	0.79	−0.77	0.59
Impurity E	−0.70	0.11	−0.87	0.08	−0.82	−0.31
Ilaprazole sulphone	0.65	0.35	0.60	−0.21	0.27	0.50
Ilaprazole sulfur ether	−0.57	−0.45	−0.59	−0.24	−0.26	−0.47

Table 6 – Results of stability of the stock solutions of different impurity at 4 °C for four weeks.

Compound	Impurity A	Impurity B	Impurity C	Impurity D	Impurity E	Ilaprazole sulphone	Ilaprazole sulfur ether
Rate of change (%)	0.48	−0.17	−0.41	0.39	−0.64	−0.37	−0.85

criteria. The method proposed here was found to be sufficient for quantitation of ilaprazole and its related impurities in commercial enteric-coated tablets.

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