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Simultaneous Estimation of Itopride and Gliclazide Potassium by HPLC In API Matrix

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ABSTRACT

An accurate & precise liquid chromatographic method was developed for the simultaneous estimation of Itopride (ITP) and Gliclazide (GCZ) in API matrix. The chromatographic analysis was performed on GRACE C_{18} [250 x 4.6 mm i.d, 5 µ particle size) with mobile phase consisting of 0.05M Potassium Dihydrogen phosphate and 0.5% Tri Ethyl Amine (TEA) buffer having pH 2.7 and Methanol in the ratio of 60:40 v/v at a flow rate of 0.8 mL/ min and UV detection was carried out at 238 nm. The calibration curves of peak area versus concentration, which was linear from 10 - 100 µg/mL for ITP and 5 – 50 µg/mL for GCZ respectively. The retention times of ITP and GCZ were 2.951 and 7.735 min respectively. The method had the requisite accuracy, precision and robustness for simultaneous determination of ITP & GCZ in API matrix. The percentage assays of ITP & GCZ were found out to be 100.80% and 99.76% respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of ITP & GCZ in bulk drug and in its pharmaceutical dosage forms.

Keywords: Itopride (ITP), Gliclazide (GCZ), RP-HPLC

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INTRODUCTION

Itopride is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide (Figure 1). It is a first-line diuretic drug[1] of the Thiazide class that acts by inhibiting the kidneys ability to retain water. It is a calcium-sparing diuretic[2, 3]; frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones. GCZ is an oral antidiabetic drug in the biguanide class. It is the first-line drug[1] of choice for the treatment of Type II diabetes, in particular, in overweight and obese people and those with normal kidney function. Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor. GCZ works by suppressing glucose production[4, 5] by the liver.

It's chemically, 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-p-tolylsulphonylurea (Figure 2). Surveys of pertinent literature revealed few LC-MS/MS[6,-8], HPLC [9-12] & Spectrophotometric methods[13-15] have been reported individually for the estimation of ITP & GCZ in pharmaceutical dosage forms at the time of commencement of these investigations. As the novelty of the combination drug is under clinical trials II & III, the authors were fascinated in carrying out these investigations for a better new approach in routine pharmaceutical analysis. Detailed accounts of all analytical methods existing for the drug are made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC system. The results of this labor of love are set forth by developing a simple, precise and accurate RP-HPLC method for the estimation of ITP & GCZ in bulk matrix.

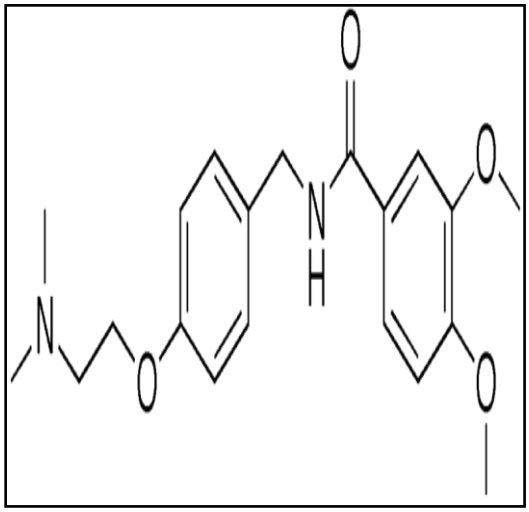

Figure 1: Itopride

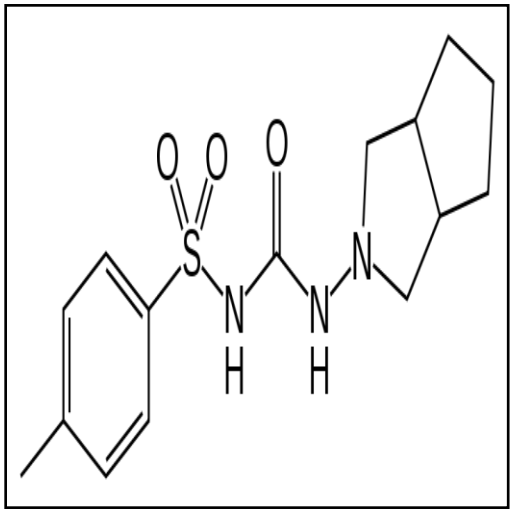

Figure 2: Gliclazide

MATERIALS AND METHOD

Pure ITP & GCZ (99.97%) used as working standards, were gifted from Glenmark Pharmaceuticals Limited, Mumbai. Methanol & Water (HPLC grade) were purchased from Rankem Chemicals, Mumbai. All other chemicals and reagents employed were of analytical grade and purchased from Merck, India.

Instrumentation

The chromatographic system comprised & achieved by using Younglin HPLC pump spectra ACME-9000 system with GRACE C_{18} [250 x 4.6 mm, 5 µ particle size] 10 µm column and UV-730D detector connected to AUTO-3000 Software was used for the data integration studies. A Bandline Sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum DI 707 digital pH meter was used for the adjustments.

Optimized Chromatographic Conditions

The HPLC system was operated isocratically with the column temperature maintained at ambient control, using a mobile phase composition of Phosphate Buffer: Methanol (40:60 v/v) with pH of phosphate buffer adjusted to 3.5 using Orthophosphoric acid (OPA) at a flow rate of 0.8 ml/min within a runtime of 20 minutes. Prior to the use the mobile phase was degassed by ultrasonic bath and filtered by Millipore vacuum filter system equipped with a 0.45 µm high vacuum filter. Both the drugs were detected and quantified at 238 nm by UV spectrophotometry as shown in Figure: 3.

Preparation of Standard Solutions

The standard solutions were prepared by transferring 100 mg of ITP & GCZ working standards into 100 mL volumetric flasks. To each, 30 mL methanol was added, and the mixture was sonicated to dissolve and make up the volume with methanol. Aliquots of these standard solutions were transferred using A – grade bulb pipettes into 100 mL volumetric flasks and the solutions made up to the volume with mobile phase to give final concentrations of 10 – 100 µg/mL & 5 – 50 µg/mL of ITP & GCZ respectively. The chromatograms of blank and individual chromatogram of ITP & GCZ were depicted in Figures 3-6.

METHOD VALIDATION

The method was validated in accordance with ICH guidelines[16, 17]. The parameters assessed were Linearity, Accuracy, Limit of detection (LOD), Limit of Quantification (LOQ), Precision, Reproducibility, Robustness and System Suitability tests.

Linearity

Aliquots of standard ITP & GCZ stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of ITP & GCZ were in the range of 10 - 100 µg/mL & 5 – 50 µg/mL respectively. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with UV-730D detector at 260 nm and the calibration graph was obtained by plotting peak area versus concentration of ITP & GCZ. The plot of peak areas of each sample against respective concentration of ITP & GCZ was found to be linear in the range of 10 - 100 µg/mL & 5 – 50 µg/mL with correlation coefficient of 0.999 for both the matrix samples. Linear regression least square fit data obtained from the measurements are given in **Table 1**. The respective linear regression equation being Y= 2353.655x + 121036.4018 for ITP & Y = 7863.21x + 29436.6011 for GCZ. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Accuracy

Accuracy was evaluated in triplicate by addition of three different amounts of ITP & GCZ to a previously analyzed samples and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 80, 100, and 120% of the amount originally present. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in Table: 2. the accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of an analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked for 24 h by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (% RSD) for ten determinations of peak areas of ITP (12.5 µg/mL) and GCZ (50 µg/mL), performed on the same day. For both intra- and inter-assay variation, standard solutions of ITP (40, 60 and 80 µg/mL) and GCZ (20, 30 and 40 µg/mL) were injected in triplicate. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in Table: 1 respectively.

Limit of Detection and Quantitation

Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical

ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

Method Applicability & System Suitability

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The present developed method was evaluated by applying to pharmaceutical dosage forms for the estimation of ITP & GCZ by our research group. To ensure the validity of the analytical procedure, a system suitability tests were established. The following parameters like theoretical plate number (N), tailing factor, retention time, resolution, area and % peak area were analyzed by using 20 µL of the working standard solution containing ITP (12.5 µg/mL) & GCZ (50 µg/mL) injecting six times into HPLC system.

Specificity of the Analyzed Method

Specificity was measured as ability of the proposed method to obtain well separated peak for ITP & GCZ without any interferences from component of the API matrix. The specificity of the method was also checked for the interference in the analysis of a blank solution (without any sample) and then a drug solution (API) was injected into the column, under optimized chromatographic conditions to demonstrate the separation of both ITP & GCZ from any form of the interference, if present. With the analyzed chromatographs obtained, it clearly depicts there were no interferences and also no change in the retention times, thus enhancing a benchmark that the method found was specific and also confirmed with the results of analysis with the standard. The mean retention time for ITP & GCZ were found out to be 2.951 and 7.735 min respectively.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

In response to lack of simple, reliable and easy-to-use method for the determination of ITP & GCZ concentrations in pharmaceutical matrices, an isocratic RP- HPLC method was developed for quantification of above mentioned, API. Nevertheless, there is need to consider the successive steps for the development of the method. In fact, the problems relating to the standardization of sample preparation and selection of mobile phase needs to be emphasized. The authors examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, Acetonitrile-Water, and Acetonitrile-Methanol were tested. Phosphate Buffer: Methanol (40:60 v/v), and the pH of phosphate buffer was adjusted to 3.5, using Ortho Phosphoric acid (OPA)

which was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were temperature, flow rate, detection wavelength and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.01M on the basis of theoretical plate number. At 260 nm, UV responses of all two active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram of ITP & GCZ has been shown in Figure: 7. the system suitability tests were carried out on freshly prepared standard stock solutions of ITP & GCZ. Parameters that were studied to evaluate the suitability of the system were discussed and presented in Table II.

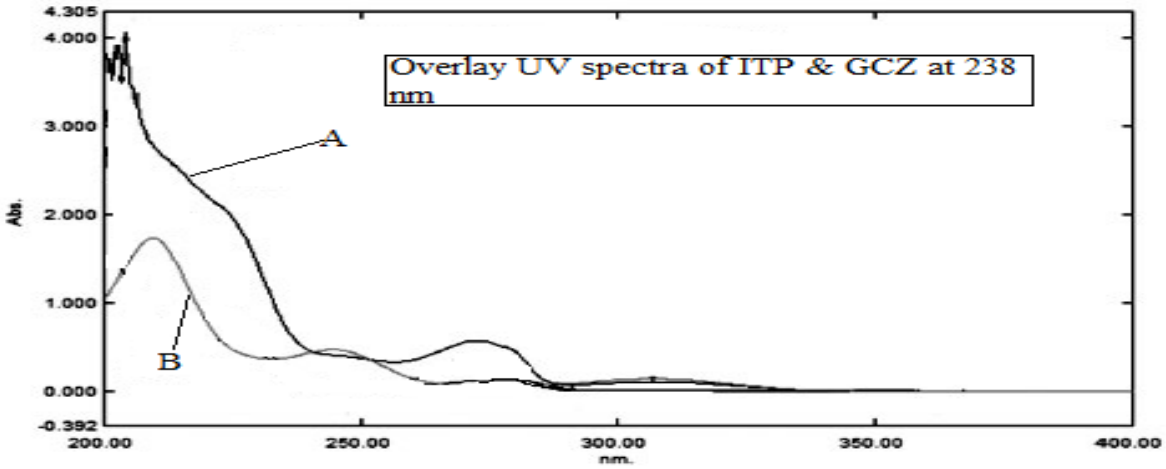

Figure: 3: Overlay spectra of ITP & GCZ

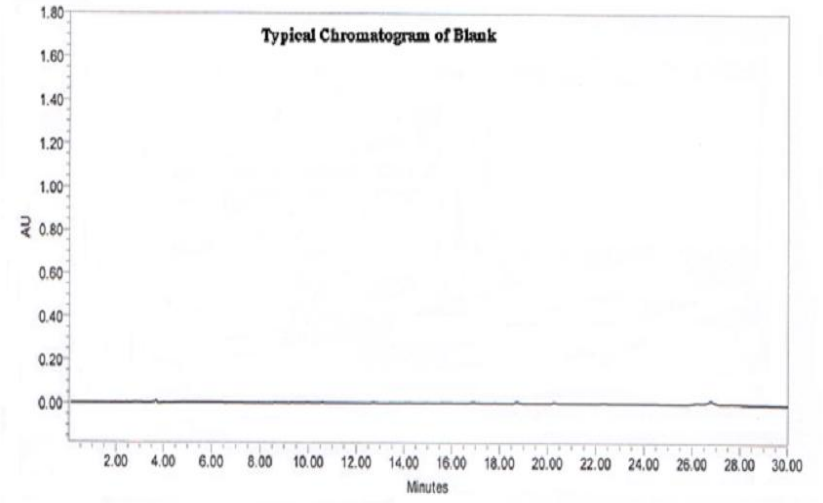

Figure 4: Typical chromatograph of the API (ITP & GCZ) in blank

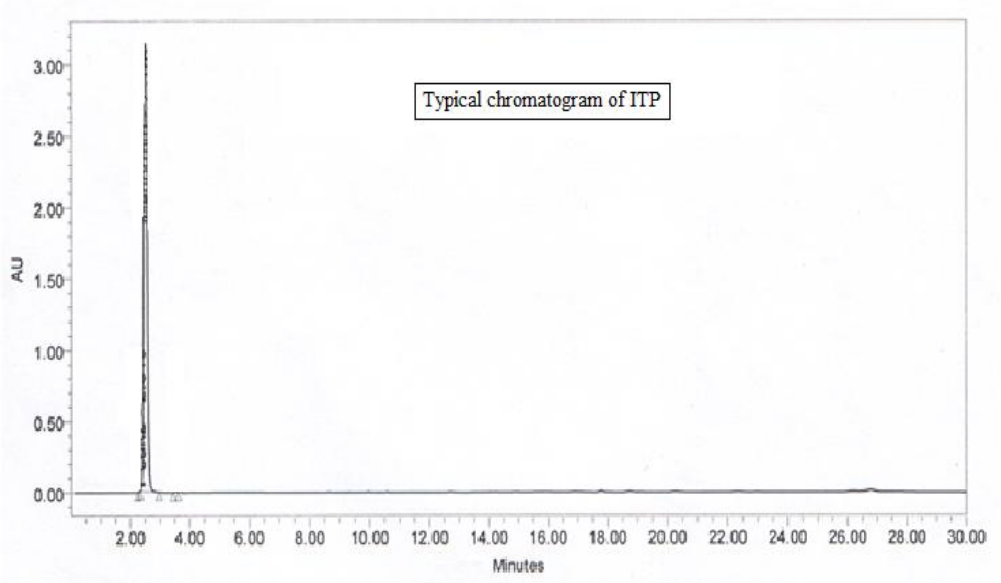

Figure 5: Typical Chromatogram of ITP

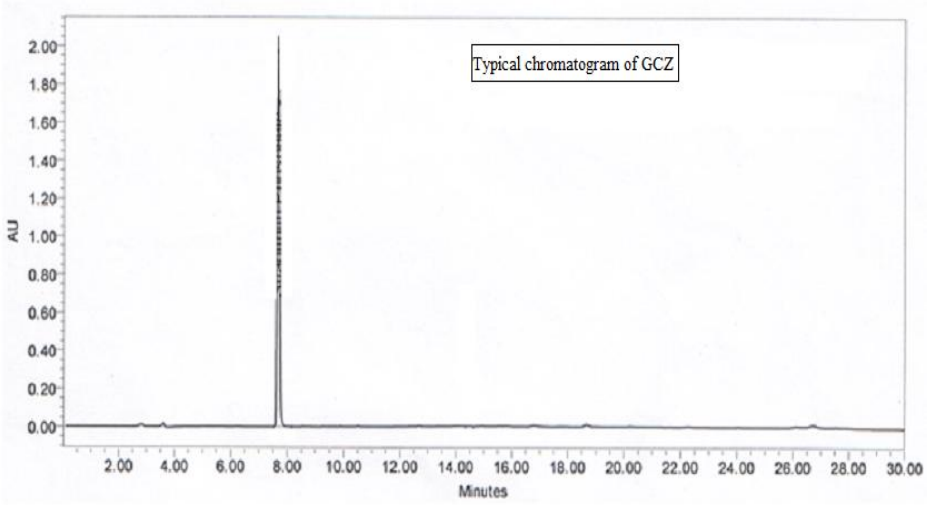

Figure 6: Typical Chromatogram of GCZ

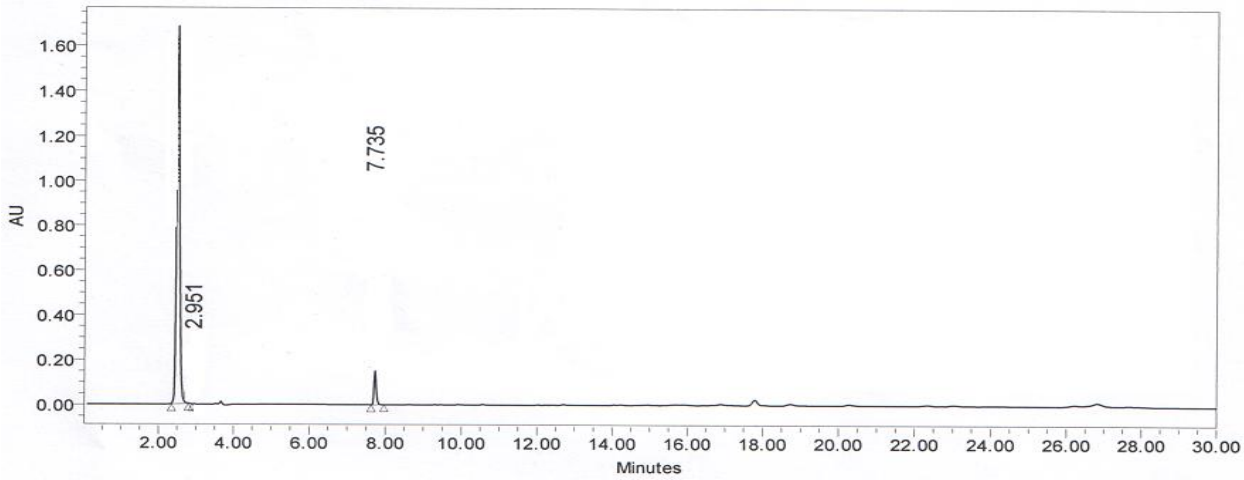

Figure 7. Typical chromatogram of Itopride (ITP) and Gliclazide (GCZ)

Method Validation Test

Recommended method[10] validation characteristics including method precision (RSD, %), method accuracy (Recovery % and RSD, %), linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

Linearity

The plot of peak areas of each API against respective concentrations were found to be linear, in the range of 10 –100 µg/mL & 5–50 µg/mL for ITP & GCZ with correlation coefficient of 0.999 for ITP & 0.998 for GCZ (Table: 1). Linear regression least square fit data obtained from the measurements are given in Table: 1. The respective linear regression equation being Y= 2353.655x + 121036.4018 for ITP & Y = 7863.21x + 29436.6011 for GCZ respectively. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1. These results show that there was an excellent correlation between peak areas and analyte concentration.

Table 1: Linear Regression Data of Calibration Curve & Proposed Methods

Parameter	Itopride (ITP)	Gliclazide (GCZ)
Concentration range (µg/mL)	10 - 100	5 - 50
Slope (m)	2353.655	7863.21
Intercept (Y)	11266.7521	34567.2387
Standard error of estimate (c)	121036.4018	29436.6011
Correlation coefficient (r)	0.999	0.999
Linear regression coefficient (r^2)	0.998	0.997
%RSD	0.6	0.4

Accuracy

Recovery of the individual substances (API) at 80%, 100%, and 120% of specified concentrations were between 99.01-101.50%, which proves the accuracy of the method. From these data obtained, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (Table:1)

Precision

The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (Table: 1).

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The Limit of Detection (LOD) & The Limit of Quantification (LOQ) analyzed were found to be 0.7 & 2.1 µg/mL for ITP & 1.3 & 3.9 µg/mL for GCZ respectively. These values reflect the high

sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

Table 2: Validation Summary / System Suitability:

Parameter	ITP	GCZ
Theoretical Plates(N)	8755	6748
Tailing factor	2.03	2.55
Retention time (min)	2.951	7.735
Resolution	-----	14.87
Area	15451077	23578923
% Peak Area	99.87	96.58
LOD (µg/mL)	0.7	2.1
LOQ (µg/mL)	1.3	3.9

Specificity

No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical API formulations were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms in the nearby future.

CONCLUSION

A simple and easily available HPLC method was developed in this study, for the quantification of ITP & GCZ in pharmaceutical API matrices specifically. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of ITP & GCZ can be used for routine analysis in pharmaceutical quality control within a short time.

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