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# Determination of *Ketotifen Fumarate* in Syrup Dosage Form by High Performance Liquid Chromatography

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***ABSTRACT***

***The goal of the current study was to establish and authenticate an isocratic reverse-stage High-Performance Liquid Chromatography (HPLC) method for quantifying ketotifen fumarate (KF) in pharmaceutical liquid dosage compositions. Easy, quick, accurate, exact, and accurate reverse-stage high-performance liquid chromatography was advanced for the simultaneous assessment of ketotifen fumarate in the liquid syrup dosage type. The HPLC system using isocratic elution method with reverse-phase Inertsil ODS-(250 mm × 4.6 mm, 3 µm) column was detected by ultraviolet absorbance at 297 nm with no interference from widely using excipients, the mobile phase (A) is a mixture of triethylamine and water (175 µl in 500 ml of water), and the mobile phase (B) is a mixture of triethylamine and methanol (175 µl in 500 ml of methanol) at a flow rate of 1.5 mL/min (mobile phase A 40 %:mobile phase B 60%) at column temperature using 40 ° C, the retention time for ketotifen fumarate was 6.4±0.5 min. The concentration curves were linear in the range of 10.0 to 35.0 µg / ml (R2 = 0.9999). The developed method was tested for the specificity, precision, linearity, precision, reliability, robustness, and consistency of the solution. The regeneration of ketotifen fumarate in formulations was found to be 99.75 %, 99.91 %, and 100.05 %***

***respectively. The percent RSD for percent recovery was found to be 0.21 and 0.17 and 0.10 for ketotifen fumarate. In the conclusion, the suggested technique was successfully used for the quantitative determination of ketotifen fumarate in formulations.***



## 1. INTRODUCTION

Ketotifen is described as a non-competitive, moderately selective histamine (H1-receptor) antagonist and a mast cell stabilizer. Mediators produced from mast cells and included in hypersensitivity reactions are blocked by the medication. Ketotifen has also indicated a decline in eosinophilic activation and chemotaxis. Its properties, including inhibition of the development of airway hyperreactivity related to stimulation of platelets by PAF (Platelet Activating Factor), inhibition of PAF-induced aggregation of eosinophils and platelets in the airways, inhibition of priming of eosinophils via recombinant human cytokines, and bronchoconstriction antagonism due to leukotrienes, may lead to its output. Ketotifen is often used in clinical practice to treat asthma, rhinitis, allergic reactions, and anaphylaxis[1].

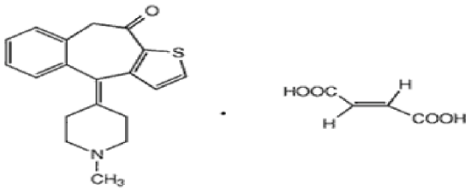


**Fig. 1: Structure of Ketotifen fumarate**

Kétotifene can be used for non-medical use and is toxicologically important in overdoses[2], regardless of adverse events (hypnotic potential, sedatives, other anti-allergy, and alcohol). Because ketotifen medicines have a relatively high demand in the pharmaceutical market, good quality control of these medicines is undoubtedly desirable. The development of precise, express, and functional procedures of quantitative determination of the medicine ingredient in drug dosage types of ketotifen is therefore very significant in new medical and toxicological research According to literature evidence, some techniques of ketotifen research are being developed. One research reported the use of a PVC membrane electrode to detect ketotifen fumarate, while ketotifen tetraphenylborate has been utilized as an ion exchanger. These are utilized for the determination of ketotifen by potentiometric titration in real specimens and their medicinal preparations [3].

The way of chemiluminescence has been identified. The reaction of potassium hexacyanoferrate (III) with a combination of calcination and ketotifen is formed. A flow injection developed for the analysis of ketotifen in tablets [4] has also been advanced. For the simple determination of ketotifen fumarate, additional research methods utilizing chemiluminescence detection have been established. The method relies on the catalytic activity of ketotifen fumarate in the chemiluminescence reactions of tris (1, 10 phenanthrols) ruthenium (II), Ru (phen) 3 2 + and Ce (IV) in the sulphuric acid medium [5].

Liquid chromatography with UV detection is most often utilized in traditional laboratories due to its wide accessibility and appropriateness. Nevertheless, most of the methods mentioned with UV detection included complex chromatographic conditions including gradient elution or common for large periods, rendering them unsuitable for routine analysis. Several analysis techniques for GC and GC / MS have also been identified [6-11].

The goal of this thesis was to progress and evaluate a new, clear, quick, and stable HPLC

technique for the determination of ketotifen in syrup and medicinal formulations, including syrup. Stabilization-indicating HPLC tests are used in the protection and stability study of medicinal compounds and drug products.

## 2. METHODS AND MATERIALS

***Determination of Ketotifen Fumarate by High performance Liquid chromatography***

UPLC analysis was performed utilizing Ultimate TM 3000 Variable Pump, ISO (Isocratic Pump), LPG (Low-Pressure Gradient Quaternary Pump), DGP (Dual Gradient Pump), and HPG (High-Pressure Gradient Binary Pump) with UV-Viss detectors and large detection flexibility. For Ketotifen Fumarate analysis, mobile phase A consisted of mixed 175 µl trimethylamine R and 500 ml water (v/v)also mobile phase B consisted of 175 µl trimethylamine and 500 ml methanol R (v/v) Before analysis, that both the mobile phase and the specimen solutions were thoroughly rinsed using a sonicator and processed through a 0.45µm filter mobile stage with a concentration of 1.5 mL/min using a different ratio (mobile phase A 40 percent: mobile phase B 60 percent) as a result, the run time was 6.4±0.5 min. The stationary step was the water symmetry column C18 (4.6 * 150 mm, particle size 5µm). Degassing was attained by filtration with a 0.45 µm Millipore filter membrane and then by sonication. The volume of the injection was 20 µl and the finding wavelength was chosen at 297 nm and the temperature of the column at 40 ° C. Tests were performed in an air-conditioned atmosphere at a temperature of 25 ± 2 ° C.

Ketotifen Fumarate standard was a Pioneer Company gift and all chemicals and reagents utilized were HPLC grade. Trimethylamine was obtained from Merk Germany. Methanol, (CHROMASOLVE®, Sigma -Aldrich Chemise GmbH, Germany) are as described, and -A Milli-Q Reagent Grade water system was utilized to deionize and purify the water. Preparation Ketotifen Fumarate standard and sample solution was done using in a diluent in a mixture of water, methanol (1:1) (v / v %) to obtain a concentration of 0.2 mg/ml for both standard and sample solution. Thereafter a calibration curve was got by diluting the stock concentration with the described diluent to achieve concentrations of 0.01, 0.015, 0.02, 0.025, and 0.03 mg/ml Ketotifen Fumarate using the same method as mentioned above.

***Applied Method and Investigation of Syrup Measurement Forms***

The material content of two (ASMAFORT Syrup1mg/5ml B.N 0077 Syrup) was combined and a correspondingly calculated quantity of 5 ml of ketotifen fumarate of approximately 30 ml of diluent was applied to the 50 ml volumetric flask dissolve and the flask was stored in an ultrasonic bath for 10 minutes and formerly made up to volume with the diluent to prepare the specimen stock solution. Passed to 0.45 Mille-Q filter paper used until processing.

The suitability of the system was evaluated via six replicate analyzes of Ketofen Syrup1mg/5ml at a concentration of 0.02mg / ml. The acceptance criterion for the percentage relative standard deviation (percent RSD) used for maximum absorption and DS retention time was ±2 percent. The precision of the HPLC process was tested in order to make sure there was no contamination of the active ingredients found in the compounds.

Linearity is the capacity to achieve findings that are directly proportional to the concentration of the analyte. Three injections of 6 different ketotifen concentrations (50, 75, 100, 125, and 150 µg / ml) were calculated. The highest peak areas have been measured against concentrations. Linearity was assessed using the calibration curve to measure the coefficient of association, slope, and interception. In general, the value of the correlation coefficient ( r2) > 0.9999 is known to be proof of fitness for the regression line results. The precision of the analytical method is the proximity of the predicted value to the calculated value. This is done by measuring the recovery percentage (R percent) of the analyte recovered. In this case, a follow-up study (n=3) of three different concentrations (75 mg/ml, 100 mg/ml, and 125 mg/ml) of standard ketotifen solution was performed to determine the accuracy of the current procedure, and a follow-up analysis (n=3) of (3) different concentrations (75 mg/ml, 100 mg/ml, and 125 mg/ml) of standard ketotifen solution was performed and the protocol was used. The data from

the experiment were analyzed statistically using the formula [Percent Recovery = (Recovered conc./Injected conc.) ×100] to evaluate the recovery and validity of the technique proposed. The agreed average recovery would be between 98-102 percent.

The accuracy of an observable procedure is the level of agreement between proctored exams if the technique is continuously used to evaluate several replicates on three separate occasions. Intraday accuracy was calculated by measuring the calibration curves of six replicates of the different doses of Ketotifen on the same day. Interday accuracy was determined via observing six replicates of different concentrations of Ketotifen on three different days. The overall system accuracy was defined as the relative standard deviation (RSD percentage). Precision was measured in the present development and validation protocol system via (6) replicate analyzes at a concentration of 0.02 mg/ml of standard Ketotifen solution that uses proposed technique, and a percentage of RSD ≤ 2% was approved.

## 3. RESULTS

***Linearity of Ketotifen by Ultra -Performance Liquid Chromatography***

The linearity of the procedure was evaluated from 0.01 to 0.03 mg / ml of Ketotifen at five separate concentration levels (50 %, 75 %, 100 %, 125 % and 150 %), respectively. and then the average area was calculated by average of (3) repeat dimensions. The slope was 397.812373, intercept 0.007519258, correlation (r) 0.999967577 and coefficient of correlation ($R^2$) 0.9999 [Table 1]. The maximum peak areas are plots toward their amounts through the value of the correlation coefficient ($R^2$) 0.9999 and are viewed as proof of the appropriate fitness of the regression line results (Figure 2).

**Table 1**: Linearity of Ketotifen and Level of Concentration

| level | Concentration mg/ml | Area | Average Area | | |
|---|---|---|---|---|---|
| **50%** | 0.0101330 | 4.0661 | 4.06544 | Slope | 0.397812373 |
| | | 4.0555 | | Intercept | 0.007519258 |
| | | 4.0713 | | R | 0.999967577 |
| | | 4.0664 | | r2 | 0.9999 |
| | | 4.0679 | | | |
| **75%** | 0.0151995 | 6.025 | 6.0285 | | |
| | | 6.0251 | | | |
| | | 6.0254 | | | |
| | | 6.0403 | | | |
| | | 6.0267 | | | |
| **100%** | 0.0202660 | 8.0593 | 8.06608 | | |
| | | 8.0629 | | | |
| | | 8.0586 | | | |
| | | 8.0753 | | | |
| | | 8.0743 | | | |
| **125%** | 0.0253325 | 10.0742 | 10.06128 | | |
| | | 10.0538 | | | |
| | | 10.062 | | | |
| | | 10.0605 | | | |
| | | 10.0559 | | | |
| **150%** | 0.0303990 | 12.096 | 12.12664 | | |

| |
|---|
| 12.1177 |
| 12.1337 |
| 12.1524 |
| 12.1334 |

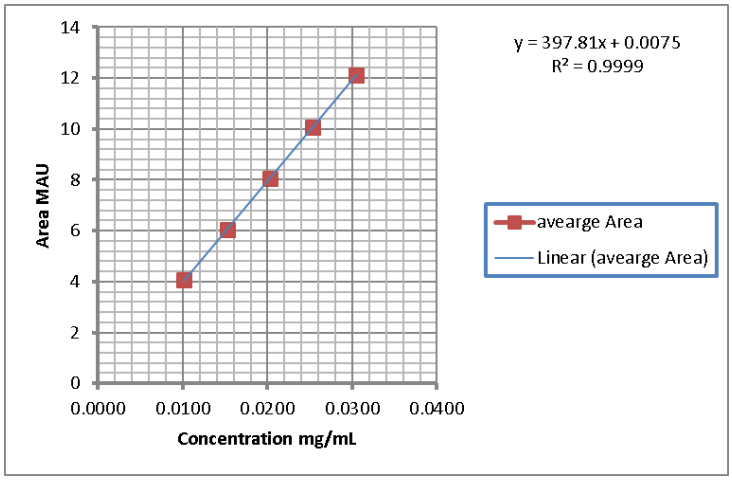


**Figure 2**: Representative linearity plot of Ketotifen

***Determination and Recovery of Ketotifen***

The accuracy of the analytical technique reflects the friendship of the contract between a set of dimensions got from multiple sampling of the same homogeneous sample underneath the prescribed time .I was prepared placebo for accuracy according to ASMAFORT Syrup without Ketotifen fumarate the excipients contain (Lycasin ,sorbitol ,citric acid ,sodium citrate ,glycerol, methyl paraben ,propyl paraben ,propylene glycol ,povidone ,banana flavor and purified water). The result displays that in repeatability, the % RSD of peak area of Ketotifen standard concentration of 75 percentages, 100 percentages, and 125 percentages was found to be 0.21%, 0.17%, and 0.10%, correspondingly (Table 2).

The process was found to be highly reliable and reproducible. Validation of an analytical procedure is a method for proving that an analytical procedure is appropriate for its intended intent. The obtained results from the validation method analysis could be used to determine the efficiency, reliability, and accuracy of the analytical results. A new UPLC approach has been advanced and validated for Ketotifen.

**Table 2:** Determination of Ketotifen Fumarate recovery

| Conc. mg/mL | Area | Average Area | SD | RSD% |
|---|---|---|---|---|
| 20.2660 | 8.0399<br>8.0648<br>8.0558<br>8.0615<br>8.0530 | 8.0550 | 0.01 | 0.1% |

| Accuracy - recovery of Ketotifen | | | | | | |
|---|---|---|---|---|---|---|
| **Level** | **Amount spiked µg/mL** | **Area** | **Amount recovered µg/mL** | **% recovery** | **Average Recovery** | **%RSD** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 6.0367 | 15.19 | 99.92% | | |
| 75% | 15.1995 | 6.0299 | 15.17 | 99.81% | 99.75% | 0.21% |
| | | 6.0124 | 15.13 | 99.52% | | |
| | | 8.0632 | 20.29 | 100.10% | | |
| 100% | 20.2660 | 8.0405 | 20.23 | 99.82% | 99.91% | 0.17% |
| | | 8.0392 | 20.23 | 99.80% | | |
| | | 10.0634 | 25.32 | 99.95% | | |
| 125% | 25.3325 | 10.0743 | 25.35 | 100.05% | 100.05% | 0.10% |
| | | 10.0826 | 25.37 | 100.14% | | |
| | | **Average** | | | 99.90% | |

Analytical technique validation is a procedure to ensure that an analytical technique is acceptable for its original purpose. The data from the validation method analysis could be used to determine the accuracy, reliability, and consistency of the experimental data. A mixture of triethylamine and water (175 µl in 500 ml of water) has been tested and the column has been successfully separated using mobile stage (A) and the mobile stage (B) is a mixture of triethylamine and methanol (175 µl in 500 ml of methanol) has been found to provide the best quantitative separation. Optimized blank chromatography The retention time of the Ketotifen peak norm was found to be 6.4 min, as shown in (Figure 3). The chromatogram of the sample Asmafort Syrup 1mg/5ml is shown in (Figure 4), the blank chromatogram is shown in (Figure 5) and the placebo chromatogram of Asmafort is shown in (Figure 6).

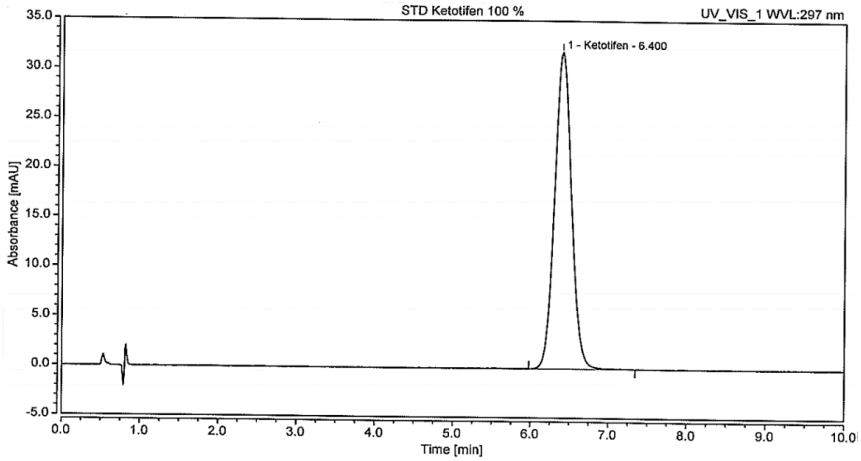


**Figure 3:** Chromatogram for standard Ketotifen 20 µg / mL at 297 nm.

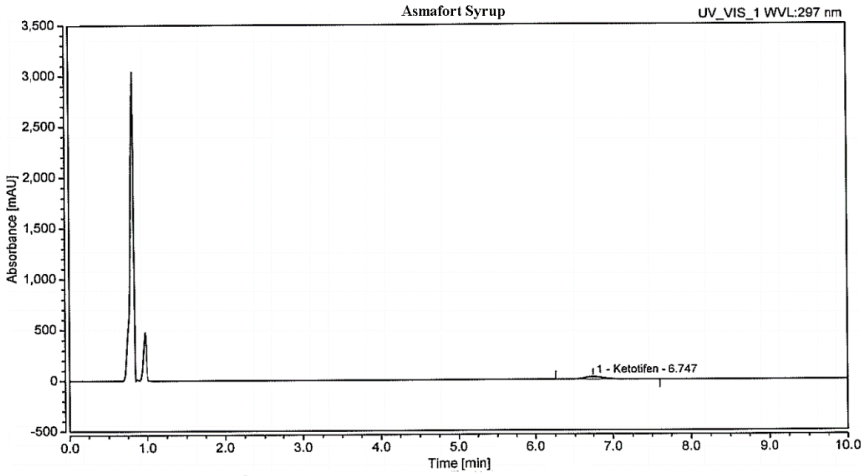


**Figure 4:** chromatogram for Sample Asmafort Syrup 20 µg / mL at 297 nm**.**

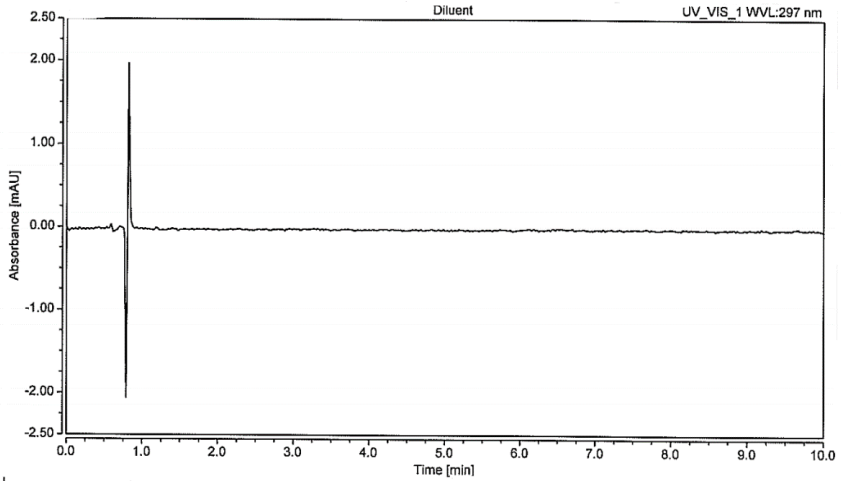


**Figure 5:** Chromatogram for blank.

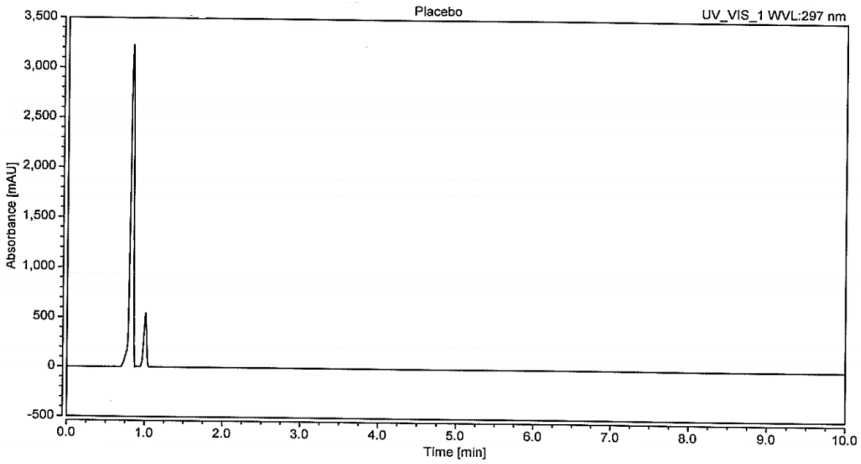


**Figure 6:** placebo chromatogram, at 297 nm.

***Precision and Accuray***

Over-day (in-day) exactness and accurateness of the technique were analysed at (3) concentration levels for ketotifen compounds using (3) replication determinations for each concentration for (1) day. Likewise, between-day (inter-day accuracy and precision were tested via an analysis of the same (3) concentrations of ketotifen compounds using (3) duplicate determinations replicated over three days. The recovery was calculated utilizing the corresponding regression equations and was satisfactory. The percentage of the relative standard deviation (RSD percent) and the percentage of the relative error (Er percent) shall not increase by 2.0 percent, as shown by the high repeatability and precision of the proposed technique for estimating the analyte in its solid materials (Table 3).

**Table 3:** Intraday and inter day Precision.

| Compound | Type of analysis | Level | Nominal value (mg/mL) | Found (mg/mL) | RSD% |
|---|---|---|---|---|---|
| **Ketotifen** | Within-day | 75% | 0.015199 | 0.01519 | 0.23 |
| | | 100 % | 0.02027 | 0.02029 | 0.17 |
| | | 125% | 0.02533 | 0.02532 | 0.10 |
| | Between-day | 75% | 0.0152 | 0.01513 | 0.25 |
| | | 100 % | 0.02027 | 0.02023 | 0.20 |
| | | 125% | 0.02533 | 0.02537 | 0.13 |

## 4. DISCUSSION

As described earlier, few high-performance liquid chromatography approaches have previously been reported for the determination of Ketotifen hydrogen fumarate in medicinal dosage forms.

A ketotifen fumarate chromatogram is seen. With a retention time of 6.4 min, the substance is well resolved using a mobile phase (A) mixture of triethylamine and water (175 µl in 500 ml of water) and a mobile stage (B) mixture of triethylamine and methanol (175 µl in 500 ml of methanol) as a mobile stage. The flow rate was 1.5 ml per minute. The wavelength of the UV detector was set at 297 nm and the particle size of the column was 5 µm and 4.6 mm × 150 mm. Ketotifen hydrogen fumarate was regularly eluted at 6.4 minutes. Drug substances were tested in HPLC-grade water directly injected with 20 µl HPLC analysis and resolution (peak areas). Solutions of each drug in HPLC-grade water were injected directly for HPLC analysis and reactions (peak areas) were reported. There was no disturbance from the mobile phase or baseline disruption, and all the analytes were well absorbed in 297 nm.

The approach has been statistically checked for its predictability, consistency, robustness, and precision, as discussed.

System validation the method was tested according to ICH criteria for linearity, accuracy, precision, repeatability, selectivity, and specificity. Validation tests were conducted by replicating sample treatments and normal solutions to the chromatograph. Linearity was measured by plotting the peak area against the normal concentration of ketotifen. The calibration graphs obtained were linear for the following concentrations: 0.01-0.03mg / ml. The linear correlation coefficients describing the calibration plots for ketotifen were: $y = 397.82x + 0.0075$($n = 5$, $(r2) = 0.9999$).

The accuracy of the model was calculated in compliance with the ICH guidelines by observing recovery at three different concentrations: 75%, 100%, and 125 percentages. The re-analyzed sample solution was substituted for each concentration and the quantity of the product substance was calculated. Details of the accuracy analysis are given in [Table 2]. All findings have been within reasonable limits, i.e. CV and S.D. < 2.0 percent. < 1.0. 1.0. It was also clear that the device allowed a very accurate quantitative assessment of ketotifen in the form of a syrup dose.

The standard deviation ( SD) of the intercepts (response) was designed from the linearity curve equation. The drug detection limit (LOD) was determined using the following equation as indicated by the International Conference on Harmonization (ICH) recommendation:

LOD=3.3 * S / S

Where, where,

5-007 = The standard deviation of the answer

S = slope of the curve of calibration

The limit of quantitation (LOQ) of the drug was determined using the following equation as set out in the International Conference on Harmonization (ICH) recommendation:

LOQ = 10 * 5-007 / S

where,

5-007 = the standard deviation of the answer

S = slope of the curve of calibration.

In comparison with chemiluminescence (CL) detection method the recoveries were 96.3-99.8%, accuracies for the medications in plasma were not less than 98.0% and this method not accept for determination of ketotifen in syrup just only successfully for tablet [4] that means the proposed method has a good recovery and precision.

PVC membrane electrode method the recoveries were 97.3-102.0% all the outcomes were within the not acceptable limits according to ICH guidelines [3] comparison with methods to the cheap and accurate method precisions for the drugs in plasma were that means the proposed method has a good recovery and precision.

The repeatability of the method was evaluated from 12 replicate injections of the sample solution ASMAFORT Syrup at an analytical concentration of 0.02 mg/ml. The RSD for the active concept was found to be 1.19 percent and the R.S.D percent was found to be 089 percent.

## 5. CONCLUSION

In the conclusion, the suggested method in the current study has simple, fast, and sensitive analytical characteristics for Ketotifen Fumarate analysis and it has been advanced and validated with a short chromatographic runtime. Statistical analysis shows that the technique is suitable for the analysis of ketotifen in the drug formulations without any interference by the active ingredients. The established validated process was discovered to be quick, sensitive, reliable, time-saving and less costly for quantification of several samples in Syrup formulations and suggest that the proposed approach will be very useful as a mechanism for quality control of chemical drugs.

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