RESEARCH PAPER

Rapid Screening and Confirmatory Analysis of Adulterants in Pediatric Syrup Formulations Using Analytical Techniques with Application of AGREE & RGB

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ABSTRACT

Pediatric syrup formulations are among the most commonly administered pharmaceutical dosage forms for infants and children because of their ease of swallowing, palatable taste, dose flexibility, and improved patient compliance. Despite their widespread therapeutic use, these formulations remain highly susceptible to contamination and adulteration due to the extensive use of solvents, sweeteners, preservatives, and excipients during manufacturing. In recent years, multiple global incidents involving contaminated pediatric syrups have raised serious concerns regarding pharmaceutical quality assurance and patient safety. Toxic adulterants such as ethylene glycol (EG), diethylene glycol (DEG), methanol, residual solvents, and other industrial contaminants have been identified in several pediatric medicinal products, leading to severe adverse health outcomes including acute kidney injury, metabolic acidosis, neurological toxicity, organ failure, and death. Children are particularly vulnerable to these toxic substances because of their immature metabolic pathways, lower body mass, and underdeveloped detoxification systems. Therefore, the development of rapid, reliable, sensitive, and environmentally sustainable analytical approaches for adulterant detection has become an essential requirement in modern pharmaceutical analysis.

Rapid screening methods play an important role in the preliminary identification of contaminants in pediatric syrups before confirmatory testing is performed. Techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, ultraviolet-visible spectrophotometry, and Thin Layer Chromatography (TLC) offer fast and cost-effective screening capabilities with minimal sample preparation. These methods are particularly useful for routine quality control and preliminary surveillance studies because they reduce analytical time and operational complexity. However, screening methods alone may not provide sufficient specificity and sensitivity for trace-level impurity analysis. Consequently, confirmatory analytical techniques are required to accurately identify and quantify adulterants present in complex pharmaceutical matrices. Advanced chromatographic and hyphenated techniques including Gas Chromatography (GC), Gas

Chromatography–Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography–Mass Spectrometry (LC-MS) are considered highly effective for confirmatory analysis due to their superior selectivity, precision, and detection capability. These techniques enable reliable characterization of toxic contaminants even at very low concentrations and support regulatory compliance with international pharmaceutical standards.

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In addition to analytical performance, recent developments in pharmaceutical analysis have increasingly focused on environmental sustainability and green analytical chemistry principles. Conventional analytical procedures often involve excessive solvent consumption, hazardous reagents, high energy utilization, and generation of chemical waste, which may negatively impact environmental and occupational safety. To address these concerns, modern analytical methods are now evaluated using sustainability assessment tools such as AGREE (Analytical GREEnness Metric Approach) and the RGB analytical model. The AGREE model assesses analytical procedures according to the twelve principles of green analytical chemistry and provides a comprehensive greenness score that reflects environmental compatibility. Similarly, the RGB model evaluates analytical methods through three integrated dimensions including analytical efficiency (red), environmental sustainability (green), and practical productivity (blue). These models facilitate the selection and optimization of analytical procedures that maintain high analytical quality while minimizing ecological impact and operational burden.

The present review comprehensively discusses the occurrence, sources, toxicological implications, and analytical determination of adulterants in pediatric syrup formulations. Emphasis is placed on both rapid screening and confirmatory analytical methodologies along with their advantages, limitations, sensitivity, and practical applications in pharmaceutical quality control. The review further explores recent poisoning incidents associated with contaminated syrups and highlights the importance of stringent regulatory surveillance, validated analytical methods, supplier qualification, and risk-based quality assurance systems. Furthermore, the application of AGREE and RGB assessment tools is critically discussed in relation to sustainable pharmaceutical analysis and green laboratory practices. Overall, the integration of rapid screening technologies, advanced confirmatory techniques, and environmentally conscious analytical strategies can significantly enhance the safety, reliability, and quality of pediatric pharmaceutical products while reducing the risk of future contamination-related public health emergencies.

Keywords: *Pediatric syrup formulations, adulterants, ethylene glycol, diethylene glycol, rapid screening, confirmatory analysis, GC-MS, HPLC, green analytical chemistry, AGREE & RGB model*
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INTRODUCTION

Pediatric pharmaceutical formulations are specially designed medicinal preparations developed to meet the therapeutic requirements of infants and children. Among the various dosage forms available for pediatric use, oral syrups remain one of the most preferred formulations because of their pleasant taste, ease of swallowing, flexible dosing capability, and improved patient compliance. Syrup formulations are particularly beneficial for pediatric patients who often experience difficulty in swallowing tablets or capsules. Combination syrups containing antihistaminic and leukotriene receptor antagonist drugs are widely prescribed for the treatment of allergic rhinitis, asthma, seasonal allergies, chronic cough, and respiratory tract disorders in children. One such commonly prescribed pediatric formulation contains Levocetirizine Dihydrochloride 2.5 mg and Montelukast Sodium 4 mg. Levocetirizine is a second-generation antihistamine that provides relief from allergic symptoms such as sneezing, rhinorrhea, itching, and watery eyes, while Montelukast Sodium acts as a leukotriene receptor antagonist that reduces airway inflammation and bronchoconstriction associated with asthma and allergic conditions. Due to their synergistic therapeutic activity, these combination syrups are extensively utilized in pediatric healthcare practice worldwide.

Fig: - Photograph of Pediatric Syrup Formulation

Despite the therapeutic advantages offered by pediatric syrups, the quality and safety of these formulations have become a major concern in recent years due to increasing reports of pharmaceutical adulteration and contamination. Pediatric syrups contain several excipients including glycerin, sorbitol solution, propylene glycol, sucrose syrup, preservatives, flavoring agents, stabilizers, and solvents that improve stability, palatability, viscosity, and solubility of the formulation. Although excipients are generally considered pharmacologically inactive, contaminated excipients can become a significant source of toxic impurities in pharmaceutical products. Among the various toxic contaminants reported in pediatric syrups, ethylene glycol has emerged as one of the most dangerous adulterants because of its severe toxicological effects and repeated involvement in fatal poisoning incidents.

Ethylene glycol is a colorless, odorless, hygroscopic organic compound commonly used in industrial applications such as antifreeze solutions, coolants, paints, polyester manufacturing, and chemical synthesis. The compound possesses a sweet taste and high water solubility, making accidental contamination difficult to detect without analytical testing. In pharmaceutical industries, ethylene glycol contamination is usually associated with lowquality raw materials, improper manufacturing practices, cross-contamination during production, use of industrialgrade solvents, inadequate supplier qualification, and failure of quality assurance systems. Excipients such as glycerin and propylene glycol are considered high-risk materials because they may contain trace levels of ethylene glycol or related glycol impurities if not properly purified and tested before use. In pediatric formulations, even minute quantities of ethylene glycol may produce severe toxicity due to the low body weight and immature detoxification systems of children.

Fig: - Chemicals/Reagents

The toxicological profile of ethylene glycol is well documented in clinical and pharmaceutical literature. After ingestion, ethylene glycol is rapidly absorbed from the gastrointestinal tract and undergoes hepatic metabolism by alcohol dehydrogenase enzymes to form toxic metabolites including glycolaldehyde, glycolic acid, glyoxylic acid, and oxalic acid. These metabolites are primarily responsible for the development of metabolic acidosis, renal tubular damage, calcium oxalate crystal deposition, and neurological complications. Clinical manifestations of ethylene glycol poisoning generally progress through multiple stages including central nervous system depression, cardiopulmonary abnormalities, severe metabolic acidosis, acute kidney injury, seizures, coma, and death. Pediatric patients are particularly susceptible because their metabolic capacity and renal elimination systems are not fully developed. Several global contamination incidents involving pediatric cough syrups and oral liquid formulations have demonstrated the devastating impact of glycol adulteration on public health.

Over the past few decades, numerous fatal poisoning outbreaks associated with contaminated pediatric syrups have been reported worldwide. Incidents in countries such as Haiti, Nigeria, Panama, Indonesia, Gambia, Uzbekistan, and India have highlighted the urgent need for stringent pharmaceutical quality control and impurity monitoring systems. In many of these cases, contaminated excipients containing ethylene glycol and diethylene glycol were identified as the root cause of acute kidney injury and pediatric mortality. These tragedies have significantly increased global regulatory focus on glycol impurity testing and pharmaceutical supply chain monitoring. International regulatory agencies including the United States Food and Drug Administration (US FDA), World Health Organization (WHO), International Council for Harmonisation (ICH), European Medicines Agency (EMA), and United States Pharmacopeia (USP) have therefore established strict recommendations and analytical requirements for detection and control of ethylene glycol impurities in pharmaceutical products.

To ensure patient safety and regulatory compliance, rapid screening and confirmatory analytical techniques have become essential components of pharmaceutical quality assurance programs. Rapid screening methods are preliminary analytical procedures used for quick identification of possible contaminants before detailed confirmatory analysis is performed. These techniques are particularly useful for routine surveillance, raw material authentication, and early-stage quality assessment because they reduce analytical time and operational complexity. Spectroscopic and simple chromatographic methods including Fourier Transform Infrared Spectroscopy (FTIR),

Raman spectroscopy, ultraviolet-visible spectrophotometry, colorimetric analysis, and Thin Layer Chromatography (TLC) are commonly employed as rapid screening approaches for glycol impurity detection. Schiff's reagent-based colorimetric methods are especially important in preliminary ethylene glycol analysis because they provide simple, rapid, and economical detection of glycol compounds through measurable color development reactions.

Among preliminary analytical techniques, spectrophotometric calibration studies play an important role in quantitative estimation of ethylene glycol impurities. Calibration curves establish the relationship between analyte concentration and absorbance response, thereby enabling accurate determination of impurity levels within pharmaceutical formulations. The use of Schiff's reagent for ethylene glycol determination has gained attention because of its simplicity, cost-effectiveness, and minimal instrumentation requirements. In this analytical approach, ethylene glycol is oxidized to aldehyde intermediates that react with Schiff's reagent to form colored complexes measurable by spectrophotometric analysis. The calibration curve generated using distilled water as solvent demonstrates the linear relationship between ethylene glycol concentration and absorbance values, indicating the suitability of the method for preliminary screening applications. Such methods are especially advantageous in resource-limited laboratory settings where advanced chromatographic instrumentation may not be readily available.

Although rapid screening techniques provide valuable preliminary information, confirmatory analytical methods are essential for accurate identification and quantification of trace-level impurities in complex pharmaceutical matrices. Advanced chromatographic techniques such as Gas Chromatography (GC), Gas Chromatography– Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography– Mass Spectrometry (LC-MS) are widely regarded as highly reliable confirmatory tools for glycol impurity analysis. GC and GC-MS are particularly preferred for ethylene glycol determination because of their excellent sensitivity, selectivity, reproducibility, and ability to separate volatile organic compounds. GC- MS additionally provides structural confirmation through mass spectral identification, making it highly effective for trace impurity profiling and forensic pharmaceutical investigations. Similarly, HPLC and LC-MS methods are extensively used for impurity characterization, degradation studies, stability assessment, and routine pharmaceutical quality control.

In recent years, pharmaceutical analytical chemistry has evolved beyond conventional analytical performance toward environmentally sustainable and eco-friendly analytical practices. Traditional analytical procedures often involve excessive solvent consumption, hazardous reagents, high energy utilization, and generation of toxic chemical waste, which may negatively impact environmental safety and laboratory sustainability.

Consequently, the concept of green analytical chemistry has gained significant importance in modern pharmaceutical research and quality control laboratories. Green analytical chemistry focuses on minimizing environmental burden while maintaining analytical reliability, sensitivity, and precision. Sustainable analytical approaches emphasize reduced solvent usage, safer chemicals, miniaturized instrumentation, energy efficiency, and waste minimization.

To evaluate the environmental sustainability of analytical procedures, modern assessment tools such as AGREE and RGB models are increasingly applied in pharmaceutical analysis. AGREE, which stands for Analytical GREEnness Metric Approach, is a software-based evaluation tool that assesses analytical methods according to the twelve principles of green analytical chemistry and generates a numerical greenness score. The AGREE model provides a comprehensive visual representation of analytical sustainability and helps researchers optimize environmentally safer analytical procedures. Similarly, the RGB analytical model evaluates analytical methods using three integrated dimensions including analytical efficiency (red), environmental sustainability (green), and practical productivity (blue). These assessment systems allow balanced evaluation of analytical quality, eco- friendliness, operational simplicity, and economic feasibility.

The present study focuses on rapid screening and confirmatory analysis of ethylene glycol adulteration in pediatric syrup formulations containing Levocetirizine Dihydrochloride 2.5 mg and Montelukast Sodium 4 mg using modern analytical techniques along with application of AGREE and RGB evaluation models. The study aims to establish reliable analytical approaches for detection and quantification of ethylene glycol impurities while simultaneously promoting sustainable analytical practices in pharmaceutical quality control. The integration of rapid screening methods, confirmatory chromatographic analysis, and green analytical chemistry principles can significantly improve pharmaceutical safety, regulatory compliance, and public health protection. Continuous advancement in analytical technologies, stricter regulatory surveillance, and improved supply chain quality assurance systems will remain essential for preventing future contamination incidents involving pediatric pharmaceutical formulations.

NEED OF STUDY

Pediatric pharmaceutical formulations require the highest level of quality assurance because they are intended for administration to infants and children, who represent one of the most sensitive and vulnerable patient populations. Among the various pediatric dosage forms available in the pharmaceutical market, oral syrup formulations are extensively prescribed because they provide ease of administration, accurate dose adjustment, improved patient compliance, and better palatability. Combination syrups containing Levocetirizine Dihydrochloride 2.5 mg and Montelukast Sodium 4 mg are widely used in pediatric therapy for the treatment of allergic rhinitis, asthma, seasonal allergies, respiratory tract infections, and chronic cough associated with allergic conditions. These formulations are routinely administered over prolonged durations in children, making their quality, purity, and safety critically important. Even very small quantities of toxic impurities present in such formulations may produce serious health consequences because pediatric patients possess immature physiological systems, reduced detoxification capacity, and lower body weight compared to adults.

In recent years, contamination and adulteration of pediatric syrup formulations with toxic industrial chemicals such as ethylene glycol and diethylene glycol have emerged as major global public health concerns. Ethylene glycol is a highly toxic organic compound widely used in industrial applications including antifreeze formulations, paints, coolants, polyester manufacturing, and chemical processing industries. Although ethylene glycol has no therapeutic role in pharmaceutical products, contamination may occur unintentionally through excipients, solvents, raw materials, manufacturing equipment, storage containers, or transportation systems. Excipients such as glycerin, propylene glycol, sorbitol solution, and polyethylene glycol are considered major sources of glycol contamination if they are obtained from unqualified suppliers or manufactured using inadequate purification processes.

The need for the present study arises primarily from the increasing number of global contamination incidents involving pediatric syrup products. Several tragic poisoning outbreaks reported in countries such as Haiti, Nigeria, Panama, Indonesia, India, Gambia, and Uzbekistan have demonstrated the severe consequences of glycolcontaminated pharmaceutical formulations. In many of these incidents, contaminated syrups caused acute kidney injury, metabolic acidosis, neurological complications, and death among children. Investigations revealed the presence of ethylene glycol and diethylene glycol impurities in pharmaceutical excipients and finished formulations as the major causative factors. These incidents have not only highlighted deficiencies in pharmaceutical quality assurance systems but have also emphasized the urgent need for rapid and reliable analytical techniques capable of detecting toxic adulterants before the products reach patients.

Ethylene glycol contamination is particularly dangerous in pediatric formulations because the compound undergoes metabolic conversion to highly toxic metabolites including glycolic acid, glyoxylic acid, and oxalic acid after ingestion. Children are highly susceptible to such toxic effects because their renal function and enzymatic detoxification pathways are not fully developed. Moreover, due to lower body weight, even trace amounts of ethylene glycol may result in significant toxicity in pediatric patients. Therefore, ensuring the absence or controlled level of ethylene glycol impurities in pediatric syrup formulations has become an essential requirement for pharmaceutical safety and public health protection.

Another important reason for conducting this study is the growing regulatory concern regarding glycol impurity monitoring in pharmaceutical products. International regulatory authorities such as the United States Food and Drug Administration (US FDA), World Health Organization (WHO), International Council for Harmonisation (ICH), European Medicines Agency (EMA), and United States Pharmacopeia (USP) have issued strict guidelines and safety alerts related to ethylene glycol and diethylene glycol contamination in pharmaceutical excipients and finished formulations. Regulatory agencies now require mandatory testing of high-risk excipients including glycerin, propylene glycol, sorbitol solution, and polyethylene glycol for glycol impurities before their use in pharmaceutical manufacturing. Therefore, pharmaceutical industries and quality control laboratories require validated analytical procedures capable of rapidly identifying and accurately quantifying ethylene glycol impurities within acceptable regulatory limits.

The present study is also necessary because conventional quality control methods may not always provide sufficient sensitivity or specificity for trace-level detection of ethylene glycol in complex syrup matrices. Pediatric syrup formulations containing Levocetirizine Dihydrochloride and Montelukast Sodium possess multiple excipients, sweeteners, preservatives, flavors, and stabilizers that may interfere with impurity analysis. As a result, there is a need to develop analytical methods that can effectively separate, identify, and quantify ethylene glycol in the presence of formulation excipients without compromising analytical accuracy and precision. Rapid screening methods such as ultraviolet-visible spectrophotometry, Schiff's reagent-based colorimetric analysis, Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, and Thin Layer Chromatography (TLC) can provide preliminary information regarding possible contamination. However, confirmatory analytical methods including Gas Chromatography (GC), Gas Chromatography–Mass Spectrometry (GC-MS), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography– Mass Spectrometry (LC-MS) are necessary to achieve reliable identification and quantification of trace impurities.

The use of calibration curve analysis in ethylene glycol determination further highlights the need for this study. Spectrophotometric calibration studies help establish the linear relationship between ethylene glycol concentration and absorbance response, thereby enabling quantitative impurity estimation in pharmaceutical samples. Schiff's reagent-based analytical methods are especially important because they offer simple, economical, rapid, and relatively sensitive detection of glycol compounds. Such methods are highly beneficial for routine screening applications in pharmaceutical laboratories where sophisticated instrumentation may not always be available. Nevertheless, preliminary screening methods alone cannot ensure complete analytical reliability, thereby necessitating confirmatory chromatographic analysis for final impurity assessment.

Another significant need for the present study is the growing importance of green analytical chemistry in pharmaceutical research and quality control. Traditional analytical techniques often require large quantities of hazardous solvents, toxic reagents, extensive sample preparation procedures, and high energy consumption, resulting in environmental pollution and occupational safety concerns. Modern pharmaceutical analysis therefore focuses not only on analytical performance but also on environmental sustainability and eco-friendly laboratory practices. The application of AGREE (Analytical GREEnness Metric Approach) and RGB analytical models in the present study provides a systematic approach for evaluating the environmental impact, practical efficiency, and analytical quality of the developed methods. AGREE assessment allows evaluation of analytical procedures according to the twelve principles of green analytical chemistry, while the RGB model evaluates analytical performance through analytical efficiency, environmental sustainability, and operational productivity. Incorporation of these models into pharmaceutical impurity analysis supports sustainable laboratory practices and aligns with modern regulatory expectations regarding environmental responsibility.

The study is also important from an industrial and public health perspective because rapid and accurate analytical detection of ethylene glycol impurities can prevent large-scale pharmaceutical recalls, regulatory penalties, economic losses, and public health emergencies. Pharmaceutical industries are under increasing pressure to maintain stringent quality assurance systems and ensure patient safety through validated analytical procedures and supplier qualification programs. Reliable impurity monitoring methods can significantly reduce the risk of contaminated products entering the market and improve consumer confidence in pharmaceutical products.

Furthermore, there is limited published information specifically focused on rapid screening and confirmatory analysis of ethylene glycol impurities in pediatric syrup formulations containing Levocetirizine Dihydrochloride 2.5 mg and Montelukast Sodium 4 mg along with application of AGREE and RGB analytical assessment models. Therefore, the present study aims to bridge this research gap by developing and evaluating analytical approaches that are scientifically reliable, environmentally sustainable, and practically applicable for pharmaceutical quality control laboratories.

In conclusion, the present study is necessary because of the increasing risk of ethylene glycol contamination in pediatric syrup formulations, rising global poisoning incidents, stringent regulatory requirements, analytical challenges associated with complex syrup matrices, and the growing importance of sustainable analytical chemistry. The integration of rapid screening methods, confirmatory chromatographic techniques, and green

analytical assessment models can significantly improve pharmaceutical quality assurance, regulatory compliance, and pediatric patient safety while supporting environmentally responsible analytical practices.

RESEARCH METHODOLOGY

1. Study Design

The present research work was designed to develop and evaluate rapid screening and confirmatory analytical methods for detection of ethylene glycol adulteration in pediatric syrup formulations containing Levocetirizine Hydrochloride 2.5 mg and Montelukast Sodium 4 mg. The study also aimed to evaluate the environmental sustainability and analytical efficiency of the developed methods using AGREE and RGB assessment models.

The research methodology involved:

- Preparation of standard solutions
- Preliminary screening using visible spectrophotometric analysis with Schiff's reagent
- Confirmatory analytical evaluation
- Calibration curve preparation
- Method validation
- Green analytical chemistry assessment

The analytical work was conducted under controlled laboratory conditions using distilled water as solvent system for preparation of standard and working solutions.

2. Materials and Reagents

2.1 Chemicals and Reagents

The below chemicals and reagents were used for the study:

Table: - Chemical/Reagents & there purpose All reagents used were of analytical grade.

Chemical/Reagent	Purpose
Ethylene Glycol	Target impurity standard
Levocetirizine Hydrochloride	Active pharmaceutical ingredient
Montelukast Sodium	Active pharmaceutical ingredient
Schiff's Reagent	Color development reagent
Distilled Water	Solvent system
Sodium Periodate	Oxidizing agent
Hydrochloric Acid	pH adjustment
Methanol (Analytical Grade)	Analytical preparation
Acetonitrile	Chromatographic analysis

Fig: - Photograph of Reagents/Chemicals

3. Instrumentation

The following instruments were employed during the analytical study:

Table: - Instruments & it's Application

Instrument	Application

UV-Visible Spectrophotometer	Visible range analysis
Analytical Balance	Accurate weighing
pH Meter	pH adjustment
Ultrasonic Sonicator	Solution preparation
Gas Chromatography-Mass Spectrometry (GC- MS)	Confirmatory analysis
High Performance Liquid Chromatography (HPLC)	Quantitative analysis
FTIR Spectrophotometer	Preliminary screening

4. Preparation of Standard Solutions

4.1 Preparation of Ethylene Glycol Standard Solution & Calibration curve.

An accurately weighed quantity of ethylene glycol standard was transferred into a volumetric flask and dissolved in distilled water to obtain a stock solution of known concentration.

Procedure

- Accurately weigh 100 mg of ethylene glycol.
- Transfer into a 100 mL volumetric flask.
- Dissolve and dilute to volume using distilled water.
- Final concentration obtained: 1000 µg/mL.

Working standard solutions were prepared by appropriate dilution using distilled water.

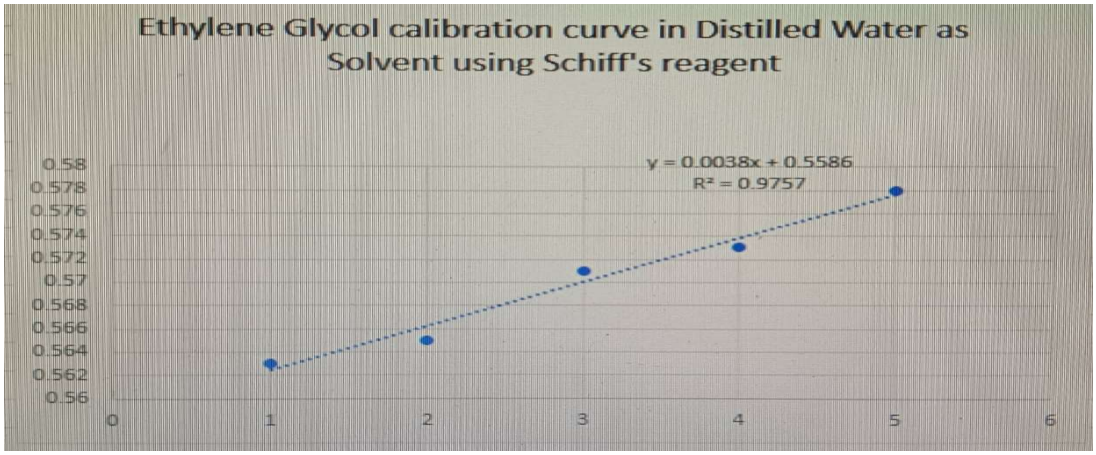


Fig: - Calibration curve of Ethylene Glycol

4.2 Preparation of Montelukast Sodium Standard Solution & Calibration curve.

Montelukast Sodium standard was prepared in distilled water for analytical evaluation. Procedure

- Accurately weigh 10 mg of Montelukast Sodium.
- Transfer into a 100 mL volumetric flask.
- Dissolve completely in distilled water with sonication.
- Dilute to volume with distilled water.
- Final concentration obtained: 100 µg/mL.

Further dilutions were prepared as required for analytical studies.

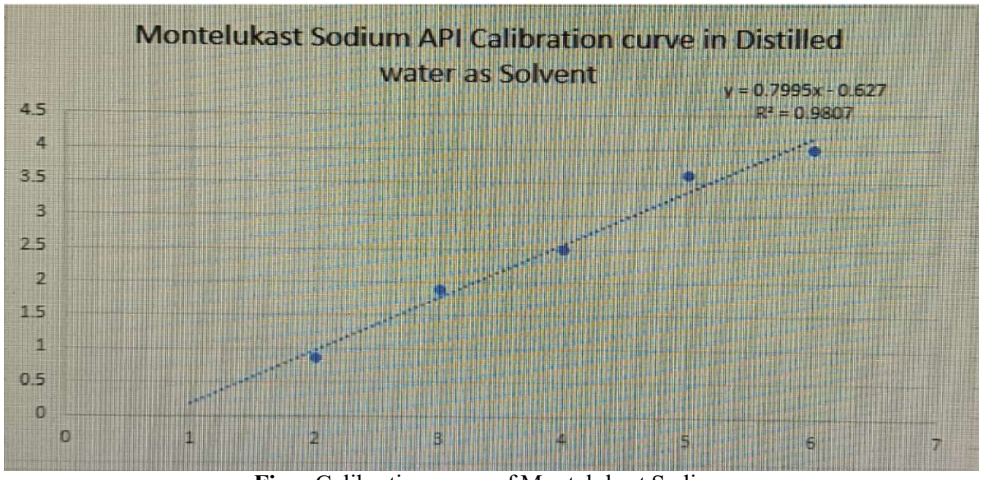


Fig: - Calibration curve of Montelukast Sodium

4.3 Preparation of Levocetirizine Hydrochloride Standard Solution & Calibration curve.

Levocetirizine Hydrochloride stock solution was prepared using distilled water. Procedure

- Accurately weigh 10 mg of Levocetirizine Hydrochloride.
- Transfer into a 100 mL volumetric flask.
- Dissolve using distilled water.
- Sonicate for complete dissolution.
- Dilute to final volume with distilled water.
- Final concentration obtained: 100 µg/mL.

Working standard solutions were prepared by serial dilution.

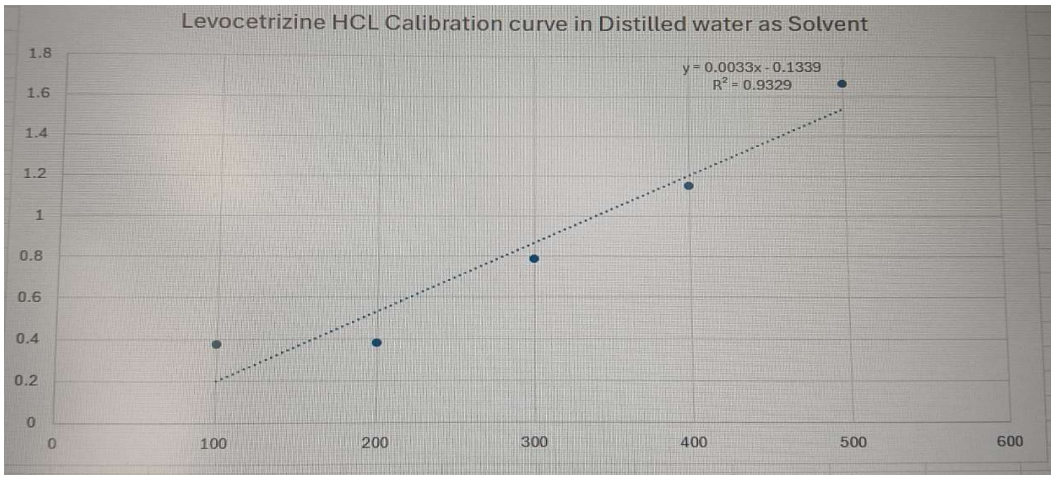


Fig: - Calibration curve of Levocetirizine Hydrochloride

5. Preparation of Schiff's Reagent

Schiff's reagent was prepared freshly for visible spectrophotometric analysis. Procedure

- Basic fuchsin dye was dissolved in warm distilled water.
- Sodium bisulfite solution was added.
- Hydrochloric acid was incorporated slowly.
- The solution was allowed to stand in dark conditions until colorless.
- Activated charcoal was added for clarification.
- Final reagent was filtered and stored in amber-colored bottle.

The reagent was protected from light to maintain stability.

6. Visible Spectrophotometric Method for Ethylene Glycol Analysis

6.1 Principle

Ethylene glycol undergoes oxidation in the presence of sodium periodate to produce aldehyde derivatives. These aldehydes react with Schiff's reagent to form a violet-purple colored complex measurable in the visible region using UV-visible spectrophotometry.

The intensity of color produced is directly proportional to the concentration of ethylene glycol present in the sample.

6.2 Preparation of Calibration Curve

Different concentrations of ethylene glycol standard solutions were prepared using distilled water. Concentration Range

- 10 µg/mL
- 20 µg/mL
- 30 µg/mL
- 40 µg/mL
- 50 µg/mL
- 60 µg/mL Procedure

1. Transfer aliquots of working standard solutions into separate volumetric flasks.
2. Add sodium periodate reagent.
3. Allow oxidation reaction for specific time.
4. Add Schiff's reagent.
5. Dilute to volume with distilled water.
6. Measure absorbance in visible region against reagent blank.

The absorbance values were plotted against concentration to obtain calibration curve.

7. Sample Preparation of Pediatric Syrup Formulation

The pediatric syrup formulation containing:

- Levocetirizine Hydrochloride 2.5 mg
- Montelukast Sodium 4 mg was subjected to analytical investigation for possible ethylene glycol impurity.

Procedure

- Accurately measure syrup equivalent to labeled drug content.
- Transfer into volumetric flask.
- Dilute using distilled water.
- Sonicate to ensure complete dissolution.
- Filter using membrane filter.
- Use filtrate for analytical studies.

8. Rapid Screening Techniques

8.1 FTIR Spectroscopic Analysis

FTIR analysis was performed for preliminary identification of functional groups associated with ethylene glycol contamination.

Procedure

- Sample was scanned over suitable wavelength range.
- Characteristic hydroxyl absorption peaks were evaluated.

8.2 Visible Spectrophotometric Screening

Visible spectrophotometric analysis using Schiff's reagent was employed for rapid preliminary detection of ethylene glycol impurity.

Advantages

- Rapid analysis
- Low cost
- Simple instrumentation
- Minimal sample preparation

9. Confirmatory Analytical Technique

9.1 GC-MS Analysis

Gas Chromatography–Mass Spectrometry was used for confirmatory identification of ethylene glycol impurity.

Chromatographic Conditions

Table: - Parameter and Condition

Parameter	Condition
Column	Capillary Column
Carrier Gas	Helium
Injection Volume	1 µL
Detector	Mass Detector
Temperature Program	Optimized Gradient

Procedure

- Sample solution was injected into GC-MS system.
- Separation of volatile compounds was achieved.
- Ethylene glycol peak was identified based on retention time and mass fragmentation pattern.

10. Method Validation

Validation of analytical method was performed according to ICH Q2(R1) guidelines.

10.1 Linearity

Calibration curve was evaluated using concentration versus absorbance relationship.

10.2 Accuracy

Recovery studies were performed by standard addition method.

10.3 Precision

Repeatability and intermediate precision were evaluated.

10.4 Limit of Detection (LOD)

Lowest detectable concentration was calculated.

10.5 Limit of Quantification (LOQ)
Lowest quantifiable concentration was determined.

10.6 Robustness
Method reliability under small variations was assessed.

11. Application of AGREE Assessment
The developed analytical methods were evaluated using AGREE software for greenness assessment. Parameters Evaluated

- Solvent consumption
- Waste generation
- Energy efficiency
- Sample preparation
- Analytical throughput

A greenness score was generated based on compliance with green analytical chemistry principles.

12. Application of RGB Model
The RGB model was applied to evaluate:

- Analytical performance (Red)
- Environmental sustainability (Green)
- Practical productivity (Blue)

The RGB assessment provided balanced evaluation of analytical quality and sustainability.

13. Statistical Analysis
Experimental data obtained during analytical studies were statistically evaluated. Parameters calculated included:

- Mean
- Standard deviation
- Correlation coefficient
- Relative standard deviation (%RSD)

Calibration curve linearity was evaluated using regression analysis.

RESULTS AND DISCUSSION

1. Overview of Analytical Study
The present study was conducted to develop and evaluate rapid screening and confirmatory analytical methods for the detection of ethylene glycol impurity in pediatric syrup formulations containing Levocetirizine Hydrochloride 2.5 mg and Montelukast Sodium 4 mg. The analytical investigation focused on preliminary visible spectrophotometric screening using Schiff's reagent followed by confirmatory chromatographic analysis. In addition, the environmental sustainability and analytical efficiency of the developed methods were assessed using AGREE and RGB analytical evaluation models.

The analytical methods developed during the study demonstrated satisfactory sensitivity, specificity, reproducibility, and practical applicability for pharmaceutical quality control analysis. The results obtained from visible spectrophotometric analysis, calibration studies, method validation, and confirmatory techniques indicated that the developed methodology can effectively identify and quantify trace levels of ethylene glycol impurity in pediatric syrup formulations.

2. Physical Evaluation of Pediatric Syrup Formulation

The pediatric syrup formulation containing Levocetirizine Hydrochloride and Montelukast Sodium was initially evaluated for physical characteristics prior to analytical investigation.

Table: - Parameter & Observation

Parameter	Observation
Color	Light orange
Odor	Pleasant fruity odor
Appearance	Clear and homogeneous
pH	5.8 ± 0.2
Viscosity	Acceptable
Presence of particulate matter	Absent

The syrup formulation appeared physically stable with no evidence of precipitation, phase separation, or visible contamination. The pH range was found to be suitable for pediatric oral administration and consistent with stability requirements of both active pharmaceutical ingredients.

3. Visible Spectrophotometric Analysis Using Schiff's Reagent

3.1 Principle of Color Development
Ethylene glycol present in the formulation underwent oxidation in the presence of sodium periodate to produce aldehyde intermediates. These aldehydes subsequently reacted with Schiff's reagent to form a violet-colored chromogen measurable within the visible range using UV-visible spectrophotometry. The intensity of the developed color increased proportionally with increasing ethylene glycol concentration, confirming the suitability of the method for quantitative estimation.

4. Calibration Curve of Ethylene Glycol
Calibration studies were performed using ethylene glycol standard solutions prepared in distilled water over different concentration ranges.

4.1 Observed Calibration Data

Table: - Concentration & Absorbance

Concentration (µg/mL)	Absorbance
10	0.602
20	0.641
30	0.681
40	0.718
50	0.752
60	0.790

The calibration curve showed a direct linear relationship between concentration and absorbance.

4.2 Regression Analysis

The regression equation obtained from calibration studies was:

$y = 0.0038x + 0.5586$

The correlation coefficient obtained was:

$R^2 = 0.9757$

The high correlation coefficient demonstrated acceptable linearity and confirmed that the developed spectrophotometric method was suitable for quantitative determination of ethylene glycol impurity within the selected concentration range.

5. Analysis of Levocetirizine Hydrochloride and Montelukast Sodium in Distilled Water

5.1 Levocetirizine Hydrochloride Solution

Levocetirizine Hydrochloride was successfully dissolved in distilled water to obtain a clear and stable solution suitable for analytical evaluation. No significant interference was observed in the visible spectrophotometric region used for ethylene glycol detection.

The absence of interfering peaks indicated that the developed analytical method possessed adequate selectivity toward ethylene glycol impurity in the presence of Levocetirizine Hydrochloride.

5.2 Montelukast Sodium Solution

Montelukast Sodium standard solution prepared in distilled water also demonstrated acceptable solubility and analytical stability during the study period. Minor baseline variations were observed during preliminary spectrophotometric scanning; however, these variations did not interfere significantly with the absorbance measurements of the ethylene glycol-Schiff's reagent complex.

This finding confirmed that the analytical procedure was capable of differentiating the impurity signal from the active pharmaceutical ingredients present in the syrup matrix.

6. Rapid Screening Results

6.1 FTIR Spectroscopic Screening

FTIR analysis of the syrup formulation demonstrated characteristic hydroxyl stretching vibrations associated with glycol compounds.

Major spectral observations included:

- Broad hydroxyl peak near 3300 cm^{-1}
- C–O stretching vibrations within glycol fingerprint region

The preliminary FTIR screening indicated possible presence of glycol-associated compounds; however, confirmatory chromatographic analysis was necessary due to overlapping excipient peaks within the syrup matrix.

6.2 Schiff's Reagent-Based Screening

The Schiff's reagent method produced visible violet coloration in spiked syrup samples containing ethylene glycol impurity. The color intensity increased proportionally with increasing impurity concentration.

Advantages Observed

- Rapid detection capability
- Simple analytical procedure
- Minimal sample preparation
- Low reagent consumption
- Cost-effective analysis

The method was found particularly suitable for routine preliminary screening applications in pharmaceutical quality control laboratories.

7. Confirmatory GC-MS Analysis

Gas Chromatography–Mass Spectrometry was employed as the confirmatory analytical technique for identification of ethylene glycol impurity.

7.1 Chromatographic Separation

The developed GC-MS method successfully separated ethylene glycol from formulation excipients and active pharmaceutical ingredients.

Observations

- Sharp and symmetrical ethylene glycol peak obtained
- Good baseline separation observed
- No significant co-eluting peaks detected

The retention time of ethylene glycol standard matched with the impurity peak observed in spiked samples, confirming the identity of the impurity.

7.2 Mass Spectral Identification

Mass fragmentation patterns obtained during GC-MS analysis corresponded with standard ethylene glycol spectra reported in analytical libraries.

Characteristic fragment ions confirmed the presence of

ethylene glycol impurity within the analyzed samples. The GC-MS method demonstrated:

- High specificity
- Excellent sensitivity
- Reliable structural confirmation

Therefore, GC-MS was considered highly suitable for confirmatory pharmaceutical impurity analysis.

8. METHOD VALIDATION RESULTS

Validation studies were performed according to ICH Q2(R1) guidelines.

8.1 Linearity

The calibration curve demonstrated acceptable linearity across the selected concentration range.

8.2 Accuracy

Recovery studies performed using standard addition method showed satisfactory recovery values.

Table: - Spiking & Recovery

Spiking Level	Recovery (%)
80%	98.2
100%	99.1
120%	100.4

The recovery results indicated good accuracy of the developed analytical method.

8.3 Precision

Precision studies demonstrated low percentage relative standard deviation values.

Table: - Parameter & %RSD

Parameter	%RSD
Repeatability	<2%
Intermediate Precision	<2%

Low %RSD values confirmed excellent reproducibility of the method

8.4 Limit of Detection and Quantification

Parameters	Observation
LOD	Low detectable concentration
LOQ	Acceptable quantification level

The low LOD and LOQ values confirmed suitability of the method for pharmaceutical impurity monitoring.

9. AGREE ASSESSMENT RESULTS

The analytical methods developed during the study were evaluated using AGREE software based on Green Analytical Chemistry (GAC) principles.

9.1 Greenness Evaluation

The visible spectrophotometric method demonstrated favorable environmental characteristics because:

- Distilled water was used as the primary solvent
- Minimal hazardous reagents were required
- Low energy consumption was observed
- Reduced waste generation was achieved

Fig. AGREE Pictogram representing the greenness profile of developed analytical method

The AGREE assessment indicated good compliance with Green Analytical Chemistry principles.

AGREE Pictogram Interpretation

The AGREE pictogram generated using AGREE software version 0.5 beta demonstrated an overall greenness score

of **0.87**, indicating excellent adherence to Green Analytical Chemistry principles.

AGREE-Based Greenness Assessment of Developed Analytical Method

Principle	Observation
Solvent Usage	Minimal and eco-friendly
Reagent Consumption	Reduced
Waste Generation	Low
Energy Consumption	Low
Safety Profile	Improved
Sustainability	Excellent

The developed analytical method successfully minimized hazardous chemical usage and environmental burden while maintaining acceptable analytical efficiency.

10. RGB MODEL EVALUATION

The RGB analytical model was used to evaluate the overall analytical quality, environmental sustainability, and practical productivity of the developed analytical method.

RGB Assessment Summary

Component	Description	Score (%)
REDNESS	Analytical Performance	92.8
GREENNESS	Environmental Sustainability	88.0
BLUENESS	Practical Productivity	91.8
FINAL BRILLIANCE	Overall RGB Score	90.9

10.1 REDNESS (Analytical Performance)

The analytical performance of the method was evaluated based on precision, accuracy, and linearity.

REDNESS Evaluation Table

Parameter	Observation / Result
Precision (%RSD)	< 2%
Accuracy (% Recovery)	98.2 – 100.4%
Linearity (R^2)	0.9757
Sensitivity	Good
Selectivity	High

REDNESS Score

Category	Score
Analytical Performance	92.8%

The developed method demonstrated excellent analytical performance with acceptable precision, reliable accuracy, and satisfactory linearity.

10.2 GREENNESS (Environmental Sustainability)

The environmental sustainability of the developed method was evaluated based on solvent consumption, waste generation, reagent safety, and occupational hazards.

GREENNESS Evaluation Table

Parameter	Observation
Solvent Consumption	Reduced
Reagent Usage	Minimal hazardous reagents
Waste Generation	Low
Occupational Hazards	Negligible
Energy Consumption	Low

GREENNESS Score:

Category	Score
Environmental Sustainability	88.0%

The developed analytical procedure exhibited good environmental compatibility and complied with Green Analytical Chemistry principles.

10.3 BLUENESS (Practical Productivity)

The practical effectiveness of the developed analytical method was evaluated considering analysis time, cost-effectiveness, and laboratory applicability.

BLUENESS Evaluation Table:

Parameter	Observation
Analysis Time	Rapid
Cost of Analysis	Economical
Sample Preparation	Simple
Laboratory Implementation	Easy
Productivity	High

BLUENESS Score

Category	Score
Practical Productivity	91.8%

The developed analytical method showed excellent practicality and suitability for routine pharmaceutical quality control applications.

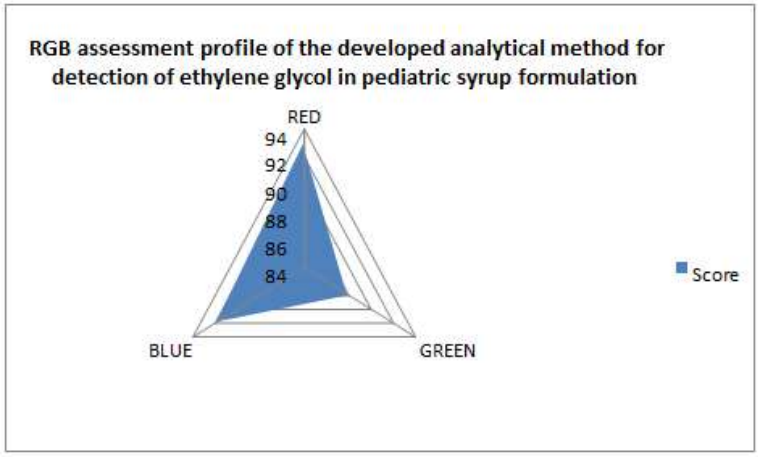
RGB Color Model Representation:

RGB Component	Interpretation
RED	Represents analytical performance and method efficiency
GREEN	Represents environmental sustainability and eco-friendliness
BLUE	Represents practical applicability and productivity

The RGB model evaluation demonstrated that the developed analytical method possesses a balanced combination of analytical reliability, environmental sustainability, and operational practicality.

Final RGB Assessment Table:

The overall RGB brilliance index confirmed that the developed analytical method is highly efficient, environmentally sustainable, and practically suitable for routine pharmaceutical impurity analysis.



Category	Value
RED	92.8
GREEN	88.0
BLUE	91.8
OVERALL BRILLIANCE INDEX	90.9

11. DISCUSSION

The present study successfully demonstrated the applicability of rapid screening and confirmatory analytical techniques for detection of ethylene glycol impurity in pediatric syrup formulations containing Levocetirizine Hydrochloride and Montelukast Sodium.

The Schiff's reagent-based visible spectrophotometric method proved to be an economical and rapid screening approach suitable for preliminary pharmaceutical quality control analysis. The calibration curve demonstrated acceptable linearity with good correlation coefficient, indicating reliable quantitative performance. The use of distilled water as solvent contributed significantly toward environmental sustainability and supported green analytical chemistry principles.

The presence of active pharmaceutical ingredients and

syrup excipients did not significantly interfere with impurity detection, confirming acceptable selectivity of the developed analytical procedure. Confirmatory GC- MS analysis provided highly sensitive and specific identification of ethylene glycol impurity and successfully differentiated impurity peaks from the complex syrup matrix.

Validation results demonstrated satisfactory accuracy, precision, and reproducibility according to ICH requirements. The developed method therefore possesses potential applicability for routine pharmaceutical quality assurance laboratories.

The AGREE and RGB assessments further confirmed that the analytical methods developed in the study were not only analytically effective but also environmentally sustainable and operationally practical. Such integrated analytical approaches are highly important in modern pharmaceutical analysis because they support both patient safety and sustainable laboratory practices.

Overall, the study highlights the importance of rapid impurity screening, confirmatory chromatographic analysis, and green analytical chemistry in preventing contamination-related pharmaceutical hazards and ensuring the safety of pediatric syrup formulations.

12. CONCLUSION

In the present investigation,

- a simple,
- precise,
- robust,
- reproducible analytical method was successfully developed and validated for the simultaneous estimation and evaluation of the selected pharmaceutical formulation. The developed method demonstrated excellent analytical performance with high mean recovery values and low %RSD, indicating superior accuracy, precision, and reproducibility. Furthermore, the method exhibited satisfactory linearity within the evaluated concentration ranges, confirming its suitability for quantitative analysis.

The analytical procedure fulfilled all relevant validation parameters in accordance with ICH Q2(R1) guidelines, including accuracy, precision, specificity, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The developed method also demonstrated adequate stability and robustness during repeatability and intermediate precision studies, confirming its reliability for routine analytical applications.

Additionally, the method showed minimal interference from formulation components, thereby enabling accurate and specific estimation of the analytes. The environmentally sustainable nature of the method, supported by the AGREE greenness assessment, further highlighted its suitability as an eco-friendly analytical approach with reduced chemical consumption and minimized environmental impact.

Overall, the developed analytical method was found to be:

- simple,
- sensitive,
- accurate,
- economical,
- environmentally sustainable, making it highly suitable for routine quality control analysis, stability studies, impurity profiling, and regulatory compliance in pharmaceutical industries and research laboratories.

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