The advancement in pharmaceutical quality depends on different analytical techniques which are used at different levels by pharmaceutical professionals to assure as well as ensure the quality, performance of a raw material, finished drug products and continuously obtain the same accuracy from the equipment. From the first entry in a manufacturing unit till the final stage of packaging, each and every raw material packaging material and finished drug product has to undergo a series of tests like purity, assay, impurity detection, bursting strength, etc. to prove their effectiveness. With the upcoming generation of new molecules and therapeutic substances, the analytical chemists in pharmaceutical sector are also continuously pacing up with the new trends and providing analytical methods that have an ability to detect samples at low concentration levels. Another challenge of analytical method development involves identification and quantification of impurities for different raw materials and finished goods. Regulatory agencies around the world such as European Commission, 2001; McDowall 2005; US Food & Drug Administration which are now enforcing a number of prerequisites for developing an analytical method which needs to be satisfied as:

1. Qualified and Calibrated Instruments
2. Documented Methods
3. Reliable Reference Standards
4. Qualified Analysis
5. Sample Selection and Integrity
6. Change Control

While developing a new analytical method or improving an existing one, the most important aspect is to seek its scope and intended purpose. In addition, the robustness of the method is also evaluated. The life cycle of any analytical method is a step wise procedure as shown in Figure 1.1.
Whereas the development of analytical method needs the attention with respect to undertakes the following steps:

1. Identifying of a method for characterization
2. Literature survey
3. Deciding method requirements
4. Instrumental setup and preliminary studies on the selected samples
5. Optimization of parameters
6. Documentation of analytical data
7. Evaluation of the method developed with different known samples
8. Determination of percent recovery of samples
9. Demonstration of quantitative sample analysis

It is always a challenging task for pharmaceutical and healthcare professionals to establish an accurate and consistent analytical method for identification, potency assay evaluation and trace analysis during batch to the batch manufacture of the product in industries. Identification and
quantification of contamination and purity give the final assurance of drug safety to the patients and therefore avoids counterfeit drugs in the generic market. The manufacturing process of Pharmaceuticals and Healthcare Industries are strictly regulated by government agencies to ensure that the manufactured drug possesses correct identity, strength, quality, purity, and potency. Analytical method validation provides documented evidence that the method is used to analyze raw material, in the process, cleaning samples and the finished product will give consistent results.

There are typical method validation parameters recommended by FDA, USP and ICH [1][2] which should satisfy:
1. Specificity
2. Linearity and Range
3. Precision
   (a) Method Repeatability
   (b) Intermediate Precision (Ruggedness)
4. Accuracy
5. Stability
6. Limit of Detection (LOD)
7. Limit of Quantitation (LOQ)
8. Robustness

The limit of validation lies in the fact that it elucidates the unpredicted or unknown dilemma during the course of routine usage. For the drug development or accuracy of any analytical method or presence of any contaminant, Pharmaceutical, Healthcare, and Chemical companies are mainly dependent on Chromatographic techniques which include:

- Paper Chromatography
- Thin Layer Chromatography (TLC)
- Gas Chromatography (GC)
- Liquid Chromatography (LC)
- High-Performance Liquid Chromatography (HPLC)
- Ion Exchange Chromatography (IEC)
- Gel Permeation Chromatography (GPC)
Gas chromatography (GC) and High-performance liquid chromatography (HPLC) is the most common techniques that are used in industrial labs. GC is used as part of various research and industrial quality control facilities for different studies. Moreover, to this, it also helps in the identification and quantification of components in a mixture. [3]

Another most commonly used technique i.e. HPLC is a form of advanced liquid chromatography which is used to separate complex mixture of molecules encountered in a variety of systems. The year 1980 was a breakthrough when the first time the HPLC was used for the assay of active pharmaceutical drug materials. [4]

But, in due course of time, it has been realized that these techniques are time to consume as they use several hazardous solvents and toxic reagents during the analysis. Even the chemists that are using these techniques are under the threat of these hazardous chemicals. To establish and sustain global challenges, there is a huge scope of research to develop greener analytical techniques during the routine quantification, impurity identification for a drug substance and product which has a direct and indirect impact on environmental sustainability and running cost of experiments used in the laboratory analytical work.

The data shows that one liquid chromatogram generates more than 1 liter of organic waste daily wherein approximately 34 trillion liters of organic waste is generated annually [5]. This type of pollution needs to be avoided and this came into play by 1990. Since then, a new concept of Green chemistry has been introduced, as American Chemical Society (ACS) promoted sustainability, Green Chemistry, Green Engineering and emphasized on the embracing of sustainable technologies and new authoritarian strategies. The term Green Chemistry/ sustainable chemistry was coined by Paul Anastas in 1991 within the framework of US Environmental Protection Agency (EPA) programme[6]. Green chemistry approach towards the development of modern analytical techniques that can be applied in pharmaceuticals and healthcare industries in a cost-effective way. The relationship between green chemistry and analytical chemistry has led to a tremendous relief in environmental pollution. Conventional methods of chemical analysis need solvents and reagents which are thereby released as waste. In 1998, Anastas and Warner documented the principles of Green chemistry which were directly related to analytical chemistry are as follows [7]
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- Avoidance of waste generation
- Safer Solvents and Auxiliaries
- Design for energy effectiveness
- Safer Chemistry to reduce the probability of chemical accidents

Since, Ultra performance liquid chromatography (UPLC) is a quite new technique unraveling new potential in liquid chromatography, especially concerning with the reduce of estimation analytical time and solvent consumption. UPLC chromatographic system is designed in a special way to resist high system back-pressure. That’s why quality control analysis technique of various pharmaceutical formulations is now slowly shifting from HPLC to UPLC system. The elution on UPLC is performed under high pressure (up to 100 MPa) yet it has no negative impact on an analytical column or different segments of the chromatographic framework. Elution proficiency is kept up or even enhanced by UPLC. There is a wide scope for optimization of analytical conditions in UPLC like using solid phase extraction (SPE) methods to reduce the sample preparation time and the hazardous solvent used in existing traditional complex sample preparation approach used in GC & HPLC methods on a routine basis. Moreover, UPLC system supports the concept of JIT (Just in time), where the process is completed in a short duration and the results are in a form of ready to serve. New generic analytical methods can be developed to quantify the drugs in trace analysis, normal assay estimations by rapid resolution in short period of time and reducing the usage of toxic, hazardous gases and solvent in experimental analysis. Here Green chemistry principles help in developing appropriate approaches. Therefore, UPLC methods are now being acknowledged in Pharmaceutical and Healthcare Industries. The proposed research work is focussed on the use of green chemistry principles towards the development of modern analytical greener methodologies using UPLC/UPCC. This study is proposed for pharmaceutical industries by replacing the conventional chromatographic methodologies so as to have a positive effect on the environment and output. Green chemistry approach towards the use of less hazardous (safer) solvents (called green solvent) in analytical techniques will be beneficial for the environment and also the usage of rapid chromatography will reduce the time for drug estimations.
Hence, UPLC/UPCC method will not only provide a cost-effective analytical method but also constitute a greener technique by reducing usage of hazardous solvents in the laboratory i.e. usage of Class -1 solvents.

In pharmaceuticals, there are many drugs that are used for the influenza virus and also used as antifungal agents. Respiratory viruses such as influenza A, rhinoviruses and respiratory syncytial viruses are transmitted from person to person primarily by coughing virus-contaminated saliva and throat droplets. Moreover, such droplets can stay on surfaces, cups, and doorknobs and thereby infect another person.

Most of the scientific consideration has been directed towards synthetic inhibitors which block the replication of viruses intra-cellularly and two such classes of drug(s) have been discovered with activity against influenza A, namely M2 blockers and the neuraminidase inhibitors. In-vitro inactivation of respiratory viruses is proved by throat treatment lozenge formulation of Amylmetacresol and 2, 4-Dichlorobenzyl alcohol at low pH, dissolved in synthetic saliva (McDonnell & Russell, 1999) [8]. The combination of these two antiseptics (2, 4-Dichlorobenzyl alcohol- 1.2 mg, Amylmetacresol-0.6 mg) in the sugar-based formulation (throat infection treatment lozenge) provides dual relief from a sore throat and irritating coughs. [9]

Further, in pharmaceuticals, several azole groups of compounds are used as antifungal agents in the form of ointment and cream. These major azole compounds are Miconazole Nitrate and Clotrimazole whereas Chlorocresol is a chlorinated phenol which is used as an antibacterial and additive during the formulation of these antifungal ointments and cream. These formulations are checked for their effectiveness and potency by validated analytical methods. Several HPLC methods, spectroscopy evolutions, and titration methods are available to estimate these drugs before administration to the patient so as to comply with the regulatory authority and ensure the safety of the patient[10]. Chlorocresol method development and validation is a real challenge in the overall study due to the low concentration used in formulations and more sensitivity thereof [11]. Literature study and pharmacopeia monograph BP/EP/USP [11] showed that Chlorocresol is estimated by gas chromatography for the assay (http://library.njucm.edu.cn/yaodian/ep/EP5.0/16). During any developmental process; safety, novelty, quality, and efficacy should always be taken care of.
In the present study, the main concern is directed towards the development of greener analytical techniques for analysis of drugs like Clotrimazole, Miconazole Nitrate, Lozenges which are already been analyzed by harmful conventional methods.

1.1 Challenge for the future study:

Literature search and study of the gaps in different cases show that there is a huge scope of development of the greener analytical methodologies in the above mentioned pharmaceutical formulations testing which can help the industries to adopt greener approaches for trace analysis, assay in routine product releases, shelf life of drug product and also quality control estimation to improve the previously used methods, the research work has been aimed at:

1. To study about trace analysis for contamination identification and quantification in pharmaceutical and healthcare manufacturing, applying, cleaning, validation, and acceptance during product changeover.

2. To study about the assay quantification of Amylmetacresol and Dichlorobenzyl alcohol present in lozenges used for throat relief infection by using UPLC techniques with SPE to avoid GC methods under technology change.

3. Analytical method development and validation for assay method used to estimate Miconazole Nitrate and Chlorocresol in antifungal cream formulation via UPLC.

4. To study about the estimation of Chlorocresol and its unspecified impurity obtained during degradation studies by TOF/MS characterization in the azoles ointment used for fungal infection.

5. To study about the impurity detection (2, 4, Dichlorobenzaldehyde) in lozenges developed during the study of the shelf life of drug product by usage of UPCC applications.

6. Green analytical techniques are used by UPLC for the quantification of Paracetamol, Phenylephrine, Dextromethorphan and Cetirizine hydrochloride in Liquid Cough Syrup.

7. ROI comparison for all quantified activity with traditional and innovative analytical methods, including wastage reduction of samples, hazardous solvents /chemicals which are used in experiments during drug testing.
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All above studies will give a productive and rapid solution to industries in terms of compliance with regulations and green chemistry principles such as:

- Cost effective procedure
- Rapid techniques and continuous improvement
- Environmental friendly analytical methods
- Sustainable chemistry
- Productivity enhancement