

# Improved Analytical Method for Dichlorobenzaldehyde Impurity Detection in Lozenges by Convergence Chromatography

Nandan Kumar Dhir<sup>1\*</sup>, Tulika Mishra<sup>2</sup> and Verinder Kaur<sup>1</sup>

<sup>1</sup>Department of Chemistry, Chandigarh University, Gharuan, Mohali – 140413, Punjab, India; dhirmandu@yahoo.co.in

<sup>2</sup>Department of Biotechnology, Chandigarh University, Gharuan, Mohali – 140413, Punjab, India

## Abstract

Lozenges are the commonly used throat relieving pharmaceutical product that contains DBA and AMC as their active pharmaceutical ingredients. Reports have shown that during the longer storage conditions DBA converts to toxic 2, 4, dichlorobenzaldehyde (DCBZ). Present study reports the development of novel method for detection of DCBZ with UPC<sup>2</sup> (Ultra performance convergence chromatography) instrument, that utilize the potential supercritical fluid chromatography, which is sustainable, reduced cost and green technology reducing the use of organic solvent. In this simple and robust method to detect DCBZ, runtime reduced to 2 minutes as compared to 10 min by Gas Chromatography with higher precision. By utilizing conventional method of Gas Chromatography, 2, 4, dichlorobenzaldehyde is detectable but with very low response and interference with other ingredients use in Lozenges. Method has facilitated faster detection and more sensitivity for DCBZ detection. The improved method provides less flow rate during the process, making it highly cost-effective. Use of non-polar solvents, high to low gradient is some of the advantages of the improved method of present investigation when compared to traditional method. The improved process of present invention proves to be 27 times more cost-effective.

**Keywords:** 2,4 - dichlorobenzaldehyde, Di Benzyl Alcohol, Gas Chromatography, Lozenges Ultra Performance Convergence Chromatography

## 1. Introduction

The use of Lozenges to smooth throat hoarseness dates back to 20<sup>th</sup> century.<sup>1</sup> Its popularity credits to the fact that it dissolves slowly in the mouth or throat that favors the drug delivery system to relieve sore throat. Its slow release promotes constant delivery of drug to oral cavity or to coat the throat tissues.<sup>2</sup> There are various types of Lozenges based upon their site of action (Local or systemic) or based upon their textures (Medicated, hard candy type etc). Now a days there are many medicated Lozenges that are very popular due to their easier

uptake, easy transport, compact packing, and moreover due to its characteristic of ease of drug delivery at constant rate. But, whatever kind of drug product is there, according to ICH guidelines it is believed that due to various sources in drug products there are always a chance for impurities.

In the pharmaceutical process quantification and identification of impurities plays a vital role. These impurities are the unwanted chemicals that remain with active pharmaceutical ingredients, or they develop during stability testing or develop during the formulation of drug substance and products. Whatever may be the cause these

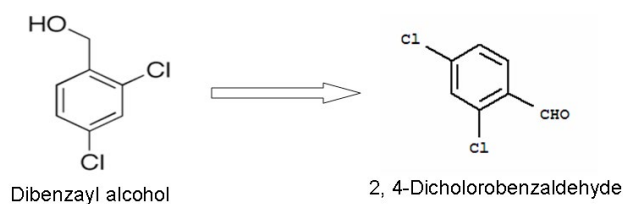
\*Author for correspondence

impurities may influence the efficacy and safety of pharmaceutical products.

In the present study our main concern is the impurity that originates from the degradation of drug substance during the shelf life period. In the medicines degradation product during storage or formulation or aging are very common. As per the ICH guidelines “degradation product is the molecule that results from chemical change brought about by overtime or due to action of light, pH, temperature etc.”<sup>3</sup>

In the lozenges, 2,4-Dichlorobenzyl Alcohol (DBA) and amylmetacresol (AMC) are present as active form of pharmaceutical ingredients (API). In the previous reports it is mentioned that 2,4-Dichlorobenzaldehyde arises by the oxidation of benzyl alcohol upon long term storage.<sup>4</sup>

2,4, dichlorobenzaldehyde (2,4-DCBZ) is a white crystalline solid with pungent smell. It has molecular formula  $C_{17}H_4Cl_2O$  and molecular weight 175.008 g/mol. The origin of 2,4-Dichlorobenzaldehyde by oxidation of Dibenzy alcohol is shown in Figure 1.



**Figure 1.** Degradation of DBA to 2DCBZ by oxidation is achieved by 30%  $H_2O_2$ .

In the conventional method commonly in practice by many pharmaceutical companies some of the impurities remain unidentified. Until now, Gas chromatography is in use for the detection of drug impurity and later coupled with even mass spectrometry.<sup>5</sup> During our previous study while developing novel method for DBA and AMC quantification it was observed that there is a presence of some other component also which was significantly different from Placebo peak.<sup>6</sup> While performing the Gas chromatography a very small peak was observed beneath the Placebo Peak and hence it was infer that Gas chromatography was not enough to detect and resolute this small peak from other ingredients used in formulation and thus ultra performance convergence chromatography was utilized.

This present study is an archetype shift from traditional analytical methodology where industries need to

introduce new technologies for operator safety, reduction of solvents with low risk, low wastage and to have minimum Hazardous exposure during laboratory testing activity. To sustain green chemistry applications and enhancement of new technology is required to survive in Global challenge and full fill the requirement of regulatory like EHS and EPA norms.

This is for the first time, impurities that can be originated by oxidation is being studied by ultra performance convergence chromatography as till now no literature has been found for such study. UPCC is a chromatographic technique which uses Gas Chromatography merged with the benefits of reversed-phase and normal-phase Liquid-Chromatography.

Wang et al., has studied the method of combination of two chromatographic techniques for the detection of those structures that was not detectable by the single technique.<sup>7</sup> The objective of present research is to develop innovate idea on analytical method by using Rapid technology (UPLC &UPC) to covert GC methods to liquid methods to avoid Complex preparation, Exposure of gases and user friendly green method environment. SPE was also consider for these applications with best and reliable recovery results value throughout the experiment. Most beautiful aspect of the present study is this that it has increased the sensitivity of DCBZ in a cost-effective manner. Economically, with the usage of ultra performance convergence chromatography the cost per run of the samples can be reduced to approximately 22 times with respect to GC analysis.

## 2. Materials and Methods

### 2.1 Gas Chromatography Analysis

The capillary gas chromatography was performed by Perkin, Clarus, GC-500, based upon the principle of separating and analyzing compounds that can be vaporized without decomposition. The standard solution of DCBZ, DCBA and AMC was prepared in Dichloromethane and was subjected to Gas chromatography. For the analysis the Reso mixture 51 ppm of DCBZ and DBA was taken and for the AMC 20  $\mu$ l of liquid was taken from liquid standard of 1000 ppm. The reso mixture was passed through the DB-5 Column of 15 meters, with initial temperature of 90° C and final as 150° C for 5 minutes. The detector used was FID and total analysis time was 13 min with Helium as carrier gas and 220° C temperature.

## 2.2 Ultra Performance Convergence Chromatography Analysis

Ultra performance convergence chromatography was performed by Acquity UPC<sup>2</sup> from Waters Corporation Milford, USA, based on the principles of normal-phase LC, with the ease-of-use of reversed-phase LC. For the analysis In the Reso mixture 51 ppm of DCBZ and DBA was taken and for the AMC 20 µl of liquid was taken from liquid standard of 1000 ppm and the final concentration was made to 10ppb.

The improved method of present study includes Ultra Performance Convergence Chromatography (UPCC) technique that involves Acquity UPC<sup>2</sup> Torus 30 x 150 mm, 1.7 µm by having the mobile phase A as carbon dioxide and mobile phase B as acetonitrile: isopropyl alcohol (ACN: IPA) in the ratio of 60:40, Flow: 1.1 ml / min with final column temp: 45°C for elution of impurity (2,4-dichlorobenzaldehyde) components. 2 µl was the sample injection that was used and the temperature of the sample was 15° C. The diluent used was n-Heptane. The samples were subjected to study at 220nm.

The data was analyzed by Empower Software platform.

## 2.3 Sample Solution

For the analysis 2 lozenges were dissolved in 40 ml of distilled water followed by addition of n-heptane as extraction solvent.

## 2.4 Chemicals

All the chemicals used were of High grade purchased from Sigma Chemical Co. (St. Louis, MO).

## 3. Results and Discussion

For the assessment of Organic Impurities Hyphenated techniques like Liquid chromatography-mass spectrometry (LC-MS), Gas Chromatography play a vital role in detection and identification of various impurities.<sup>8</sup>

The present study is based on different case studies and experimental study on pharmaceutical product testing on traditional analytical GC methods, where the objective is to work on green solvents and to use such methodology which will give best productivity with low risk on the safety of laboratory scientist. It also highlights JIT (Just In

Time) analytical process which is possible to introduce innovative idea on usage of conventional technology or Rapid analysis which is really helpful to the industry benefit for fast product release to consumer. To more precise these studies has been conducted in laboratory to evaluate the overall assessment and comparison of Traditional and conventional JIT method and opportunity to apply greener solvents and greener method for industrial applications.

During our previous study, developing novel, cost effective, rapid resolution method for DBA and AMC it was observed that there is a presence of interfering peak with Placebo Peak.<sup>6</sup> Further studies with Gas Chromatography showed the presence of 2,4-Dichlorobenzaldehyde as a interfering peak as shown in Figure 2 and Figure 3. In the Lozenges API includes 2,4-Dichloro Benzyl and Amylmetacresol, and later 2,4-Dichloro Benzyl Alcohol degrades into 2,4-Dichlorobenzaldehyde through oxidation reaction.<sup>9-11</sup> But, Performing Gas chromatography was quite expensive and time consuming. Thus, Present study was designed to develop improved method for DCBZ identification in lesser time and in a cost effective manner. Earlier, Zhou et al., obtained improved resolution of various triglycerols in Cow milk by UPCC which was not reported in earlier study.<sup>12</sup> Similarly, in our study also UPCC has clarified the data by showing prominent presence of 2, 4-Dichlorobenzaldehyde.

For the analysis standard chromatograms of 2,4-Dichloro Benzyl Alcohol, Amylmetacresol, 2,4-Dichlorobenzaldehyde and Resomix of three standards was obtained at 220nm as shown in Figure 4, Figure 5, Figure 6 and Figure 7 respectively. Standard Chromatograms when compared with Chromatograms of Sample Injection along with Placebo clearly indicates that the usage of UPCC has demonstrated the presence of all the three along with Placebo in a more defined manner and in a very less time (Figure 8). The reproducibility experiment was done by performing the inter-day experiment with six replicate injections of standards. Results as shown in Figure 8 and Table 2, undoubtedly indicates that this novel method has robust precision as compared to Gas chromatography. The peak of DBA, AMC and DCBZ was observed at 1.67 minutes, 1.59 minutes and 0.79 minutes respectively.

As shown in Table 1, the Retention Time for 2,4-Dichlorobenzaldehyde through gas chromatography was 1.65 which was reduced to 0.790 by UPCC that is 2

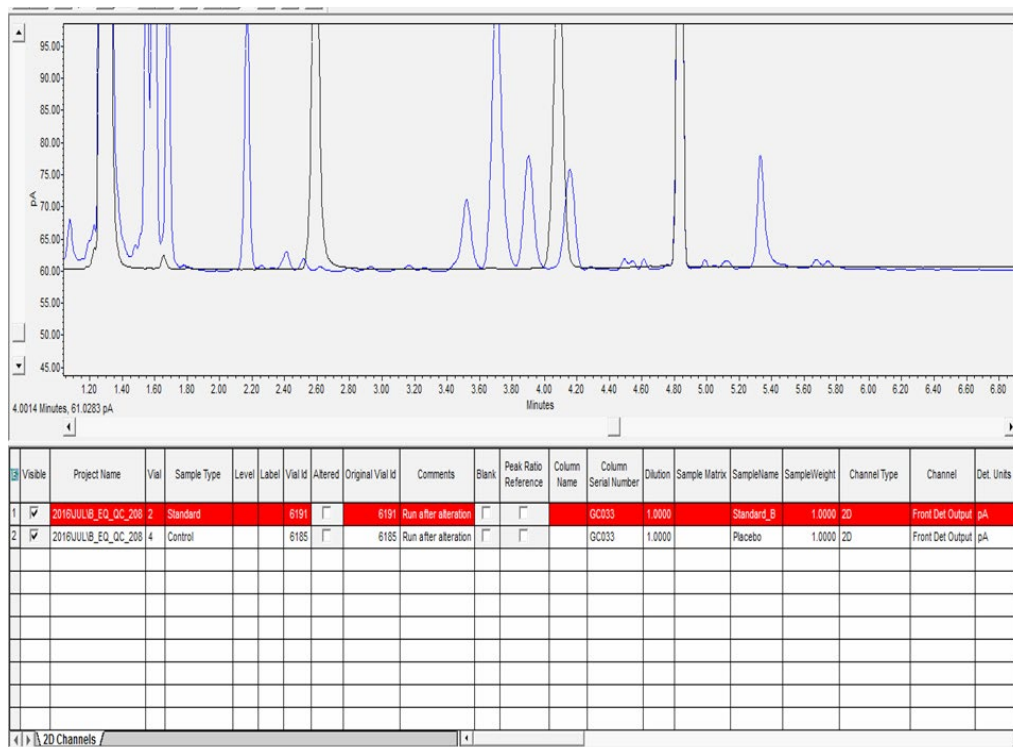


Figure 2. Co-elution of 2DBCZ in Gas Chromatography.

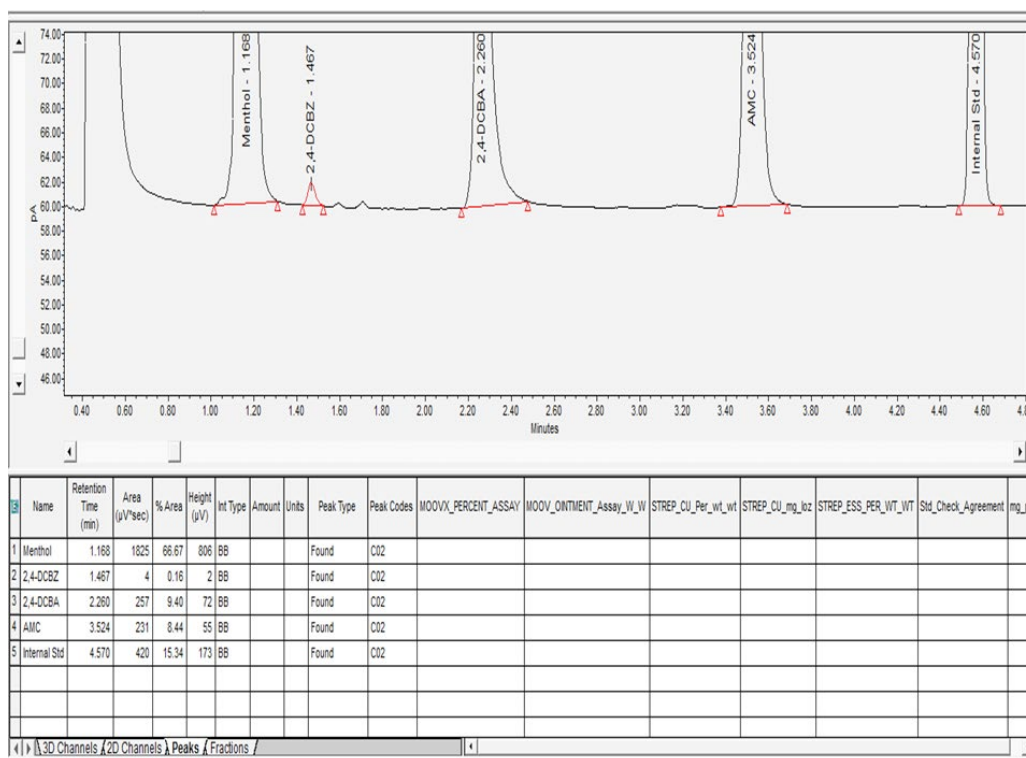
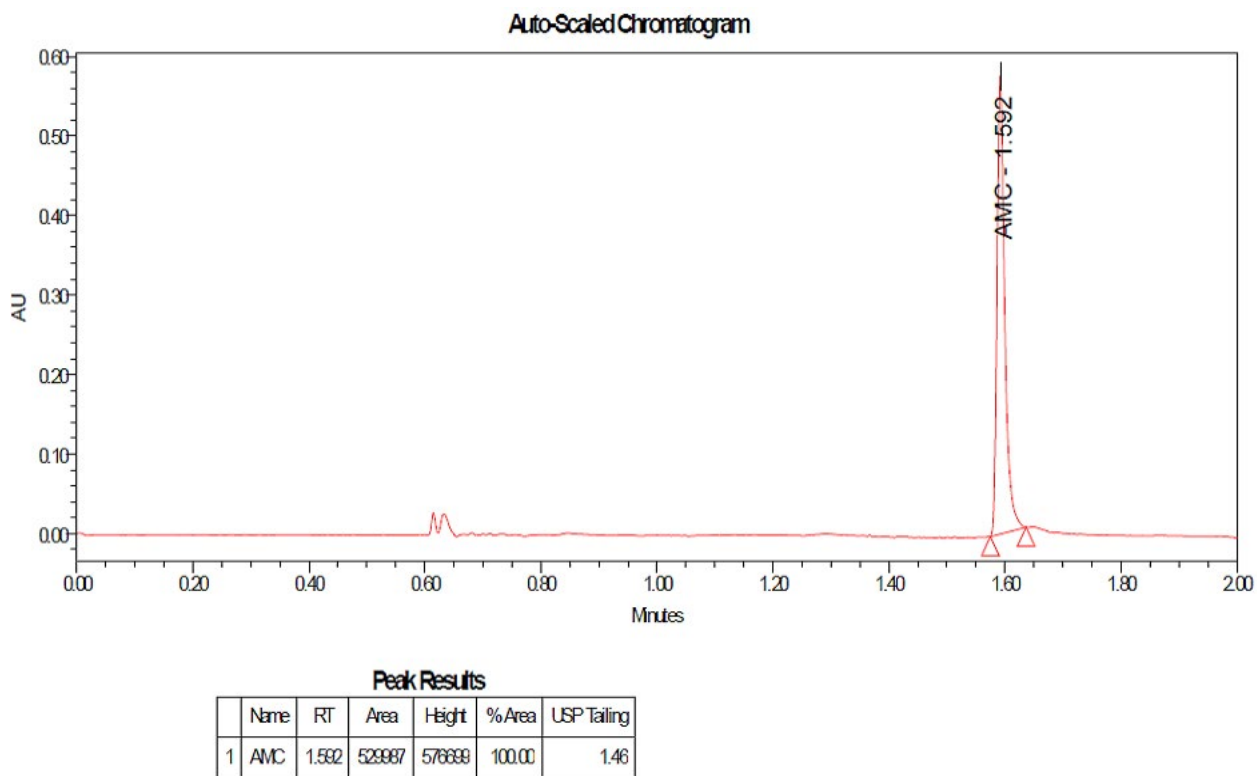
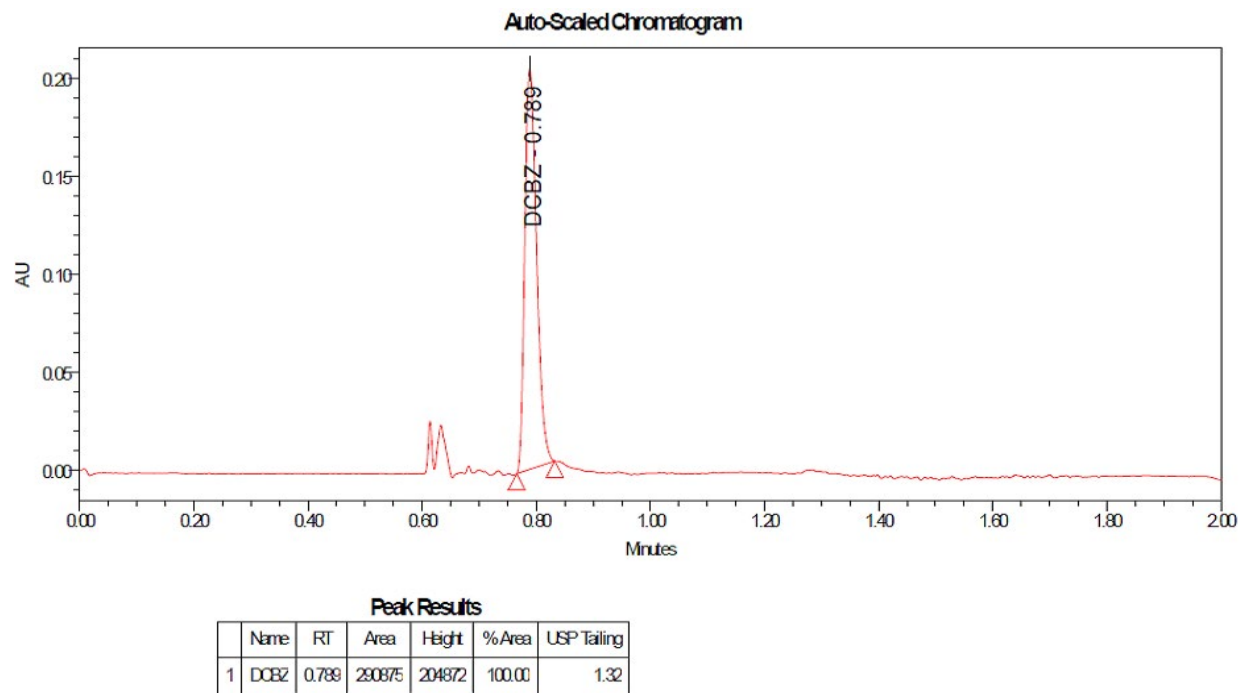


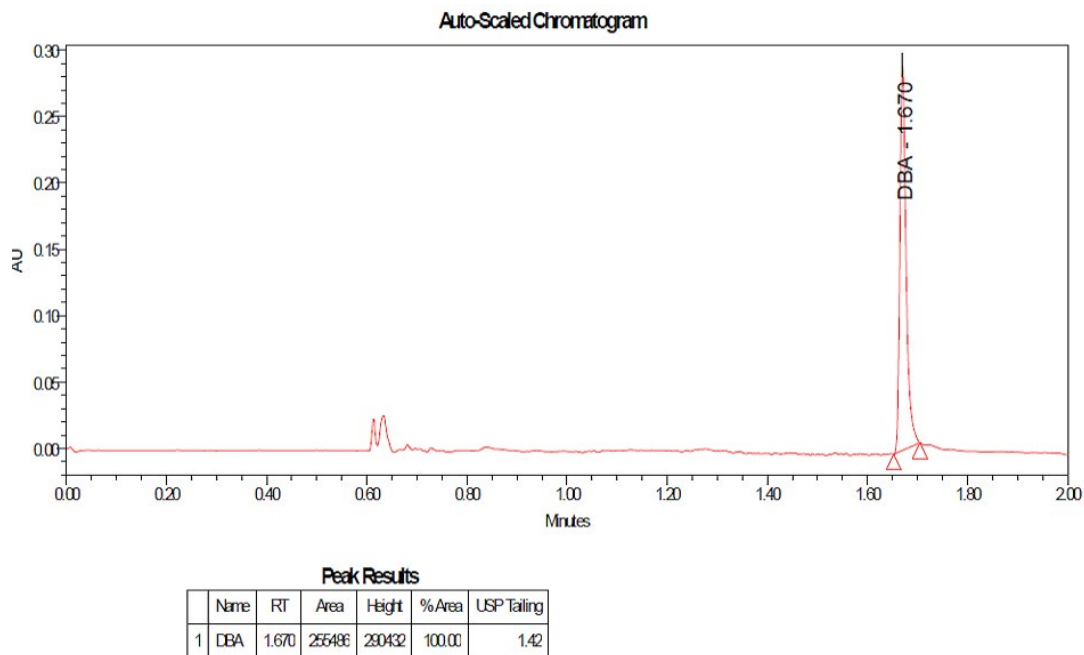
Figure 3. Standard Chromatogram in Gas Chromatography.



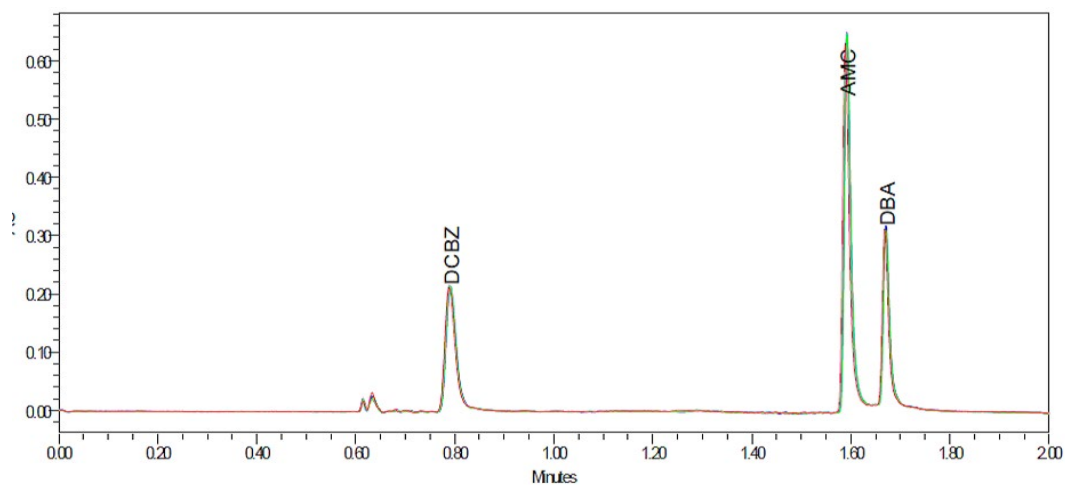
**Figure 4.** Standard Chromatogram of 2, 4-Dichloro Benzyl Alcohol assessed under UPCC.



**Figure 5.** Standard Chromatogram of Amylmetacresol, assessed under UPCC.



**Figure 6.** Standard Chromatogram of 2,4-Dichlorobenzaldehyde, assessed under UPCC.



**Figure 7.** Standard Chromatogram of Resomix assessed under UPCC.

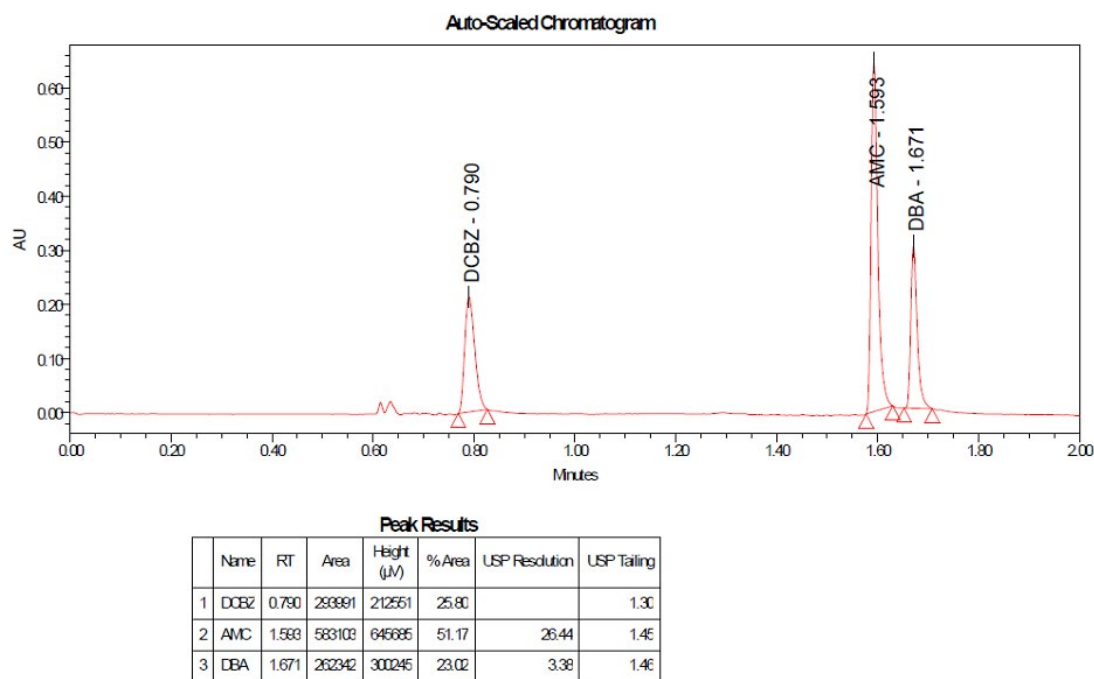
**Table 1.** Comparative data showing the difference between the conventional method (Gas Chromatography) and new improved method (Ultra Performance Convergence Chromatography)

2,4 dichlorobenzaldehyde	GC (Gas Chromatography)	UPCC (Ultra Performance Convergence Chromatography)	Comments
RT	1.65	0.790	2 time Faster Detection
Response /Detection	62pA	350000 pa*	High Throughput
Interferences	With Placebo	Separated from Placebo	SIM (Stability Indicating)
Peak height	181	212	High Response
Peak area	0.19	293	High Sensitivity
System precision	RSD >5.0%	0.6%	Repeatable
Run time	10 minute	2 minute	8 times faster
Standard Concentration	51 ppm	51 ppm	Similar

times faster detection. Our results have shown that in the Gas chromatography analytical method there was appearance of co elution of placebo peaks. Addition to this there was other disadvantages that included difficulty in quantifying 2, 4-Dichlorobenzaldehyde due to high precision and no specificity with respect to ingredient peak. The usage of highly toxic chemicals, reagents and gases also egg on to use some other better technique. Last but not the least the elution time of the sample was very high by Gas chromatographic techniques. This leads to the progress for new and better method for the quantification of Impurities in Lozenges samples by utilizing convergence Chromatography. Convergence Chromatography is the analytical tool that has lesser solvent and yet provides higher separation.<sup>13</sup> For impurity analysis Convergence Chromatography provides orthogonal approach aiding discovery of new impurities when compared with reversed phase liquid chromatography. This novel method is a greener method with low cost and high efficiency. With the usage of UPCC, utilization of MDC (Methylene dichloride) is completely omitted and instead CO<sub>2</sub> is used as primary mobile phase followed by acetonitrile as co solvent. In ultra performance Convergence Chromatography primary mobile phase is CO<sub>2</sub> which has lower viscosity, allowing for faster flow rate and use of smaller particle size, which increases separation efficiency. This supports our results showing better JIT method. Where total elu-

tion time has been reduced from 10 min to 2 minutes by UPCC as compare to Gas Chromatography.

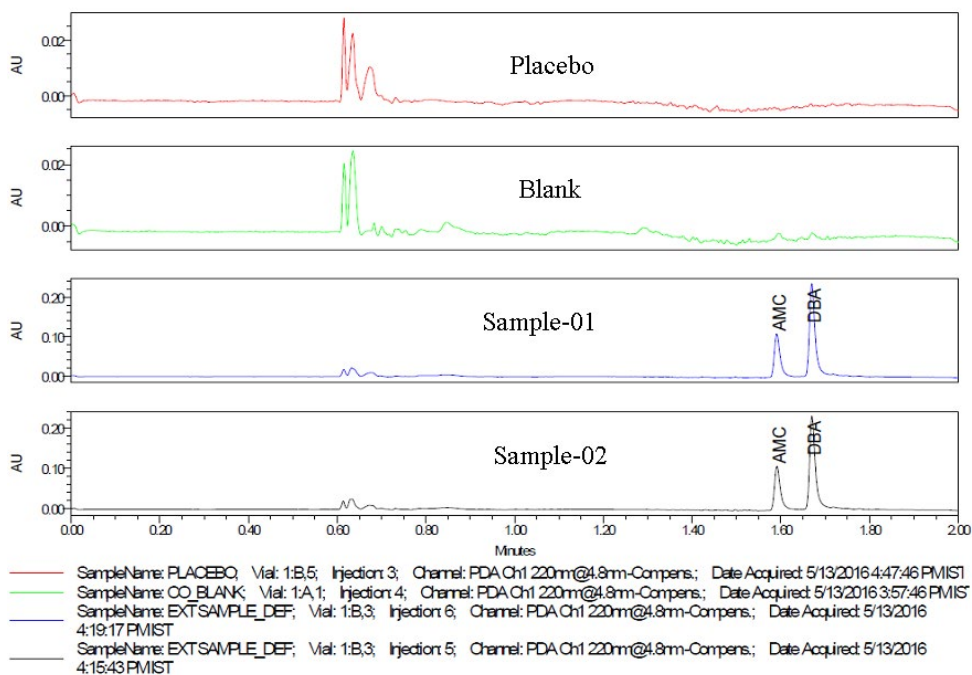
The comparative analysis of Gas Chromatography and UPCC as shown in Table 1 evidently highlights the benefits of this novel method. The results revealed that with the utilization of this novel method, problem of Placebo interference was overcome (Figure 7). Similarly, the peak height observed by Gas Chromatography was only 181 which was found to be 212 by Ultra Performance convergence chromatography (UPCC) and can be infer as high response. By developing new method through UPCC the sensitivity has also increased from 0.19 to 293 which is many folds higher. UPCC has also helped in preventing the unwanted loss of samples and reduce the use of costly solvents. The alternate HPLC method used by Poon et al., has also improved analysis of Tetrabutylammonium hydroxide in [18F] Fluorodeoxy-thymidine in a similar way.<sup>14</sup> They also found much better results with the modulation in the technique rather than using a conventional method. Also, the testing of Lozenges samples has shown absences of DCBZ (Figure 9). This improved analytical method provides cost-effectiveness in terms of gradient also as it uses gradient from high to low solvents. Use of buffer is also excluded and Ultra Performance Convergence Chromatography (UPCC) is used with the non-polar solvents which are much cheaper as compared to other expensive solvents.



**Figure 8.** Reproducibility chromatogram of six replicate injections of resomix standards.

**Table 2.** Component Summary Table for evaluation of precision

S.No	Sample Name	RT	Area
1	AMC	1.591	585694
2	AMC	1.592	586355
3	AMC	1.593	596635
4	AMC	1.592	590164
5	AMC	1.589	582780
6	AMC	1.593	583103
Mean		1.6	587456
Standard Deviation		0	5229
% RSD		0.1	0.9
1	DBA	1.670	258051
2	DBA	1.671	263029
3	DBA	1.671	268957
4	DBA	1.671	264717
5	DBA	1.669	262426
6	DBA	1.671	262342
Mean		1.7	263254
Standard Deviation		0	3558
% RSD		0.1	1.4
1	DCBZ	0.790	295310
2	DCBZ	.789	296966
3	DCBZ	.791	298426
4	DCBZ	.791	295671
5	DCBZ	.789	298038
6	DCBZ	.790	293991
Mean		0.8	296384
Standard Deviation		0	1705
% RSD		0.1	0.6



**Figure 9.** Chromatogram representing Placebo, blank and Test sample clearly showing the various peaks and absence of DBCZ in the test samples.



## 4. Conclusion

The present investigation emphasizes that the impurity which was detected by lesser response as well as have interference with other ingredients by traditional method of Gas chromatography can be detected by Novel modulated method of UPCC. When compared to traditional method the improved process of present investigation proves to be 27 times more cost-effective and provides 65% reduction in the sample run time thereby saving both time and funds. The method uses less amount of solvent which also makes it environment friendly and thus moves towards the greener Chemistry.

## 5. Acknowledgement

All the help and support provided by Mr. Arghya Acharjee, Senior Application specialist and Mrs. Dilshad, Application Executive, Waters, India is fully acknowledged.

## 6. References

1. Ramond CR, Astrazenca. Formulating fortunes the tale of medicated Lozenges. *Drug Discovery Today*. 2002; 7(5):286–7. [crossref](#)
2. Umashankar MS, Dinesh SR, Rinin R, Lakshmi KS, Damodharan N. Chewable Lozenge formulation-A Review. *Int. J. Pharmacy*. 2016; 7(4):9–16. [crossref](#)
3. European Medicines Agency. EMA/CHMP/ICH/353369/2013. ICH guideline Q3D on elemental impurities; 2016, p. 1–84.
4. Rizk M, Ibrahim F, Hefnawy M, Nasr JJ. Micellar liquid chromatographic analysis of benzyl alcohol and benzaldehyde in injectable formulations. *Acta Pharm*. 2007; 57:231–9. [crossref](#) PMID:17507319
5. Hong JY, Park NH, Yoo KH, Hong J. Comprehensive profiling and quantification of Sudan III dyes by gas chromatography/mass spectrometry. *J Chromato*. 2013;1297:186–95. [crossref](#) PMID:23726074
6. Dhir NK, Chakraborty D, Kaur V. Green analytical technique for drug quantification. Patent No. 4299/DEL/2015. 2015.
7. Wang LL, Zhang Y, Sun X, Chen S. Simultaneous quantitative analysis of main components in *linderae reflexae radix* with single one marker. *J Liq Chromatogr Relat Technol*. 2016; 39(8):422–7. [crossref](#) PMID:27366022 PMID:PMC4917913
8. Liu DQ, Sun M, Wu L. Eliminating pharmaceutical impurities: Recent advances in detection techniques. *Curr Opin Drug Discov Devel*. 2010; 13(6):748–57. PMID:21061235
9. Oxford JS, Lambkin R, Gibb I, Balasingam S, Chan C, Catchpole A. A throat lozenge containing amyl meta cresol and dichlorobenzyl alcohol has a direct virucidal effect on respiratory syncytial virus influenza A and SARS-CoV. *Antiviral Chem Chemother*. 2005; 16(2):129–34. [crossref](#) PMID:15889535
10. Official Report in Pharmacopial Monograph. AMC in BP-2015. (I) I-178.
11. Official Report in Pharmacopial Monograph. DBA in 2, 4 Dichlorobenzyl alcohol- EU Phur -2014 8.1, 3745.
12. Zhou Q, Gao B, Zhang XXY, Shi H, Yu LL. Chemical profiling of triacylglycerols and diacylglycerols in cow milk fat by ultra-performance convergence chromatography combined with a quadrupole time-of-flight mass spectrometry. *Food Chem*. 2014; 143:199–204. [crossref](#) PMID:24054231
13. White CM. 1988. *Modern Supercritical Fluid Chromatography*, Hüthig, Heidelberg (Ed.).
14. Poon J, Katsifis A, Fulham M. An Alternate HPLC Method for the Analysis of Tetrabutylammonium Hydroxide in [18F] Fluorodeoxythymidine (FLT). *J Liq Chromatogr. Relat Technol*; 2017. [dx.doi.org/10.1080/10826076.2017.1343733](#). [crossref](#)