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Research Article

A rapid UPLC-UV method development and validation for the quantitative determination of Formaldehyde using derivatization technique

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ABSTRACT

The Objective of the current study was to develop and validate a Rapid UPLC-UV method for the quantitation of Formaldehyde. Formaldehyde (HCHO) is reacted with 2,4-dinitrophenylhydrazine (DNPH) to form a Schiff base (HCHO-DNPH derivatization product). The chromatographic conditions were developed and optimized using a mixture of DNPH reagent and HCHO-DNPH derivatization product. The chromatographic separation was achieved on Acquity BEH C8, 100 mm x 2.1 mm, 1.7µ particle size column. Using Water and Acetonitrile (55:45, v/v) as a mobile phase with 0.4 mL/min flow rate in Isocratic mode, the column temperature was maintained at 35°C, detection wavelength was set at 360 nm and the injection volume was 5 µL. Acetonitrile was used as a diluent. The developed RP-UPLC method was validated according to ICH guidelines. In this method the LOD and LOQ values for Formaldehyde are 0.6 ppm and 2.0 ppm respectively. The percentage recovery was 96.3 to 97.0. The solution was observed to be stable up to 48 h at room temperature. The validated method produced good results of precision, linearity, accuracy, robustness and ruggedness. The proposed method was found to be Rapid and suitable for quantification of Formaldehyde which can be further extended in Drug product and its excipients analysis.

Keywords: 2,4, Dinitrophenyl Hydrazine (DNPH); Derivatization technique; Formaldehyde (HCHO); Ultra Performance Liquid Chromatography (UPLC); Validation.

INTRODUCTION

The pharmaceutical industry is trying hard to satisfy patients therapeutically needs, apart from active ingredients, inactive excipients play a major role in formulation development. Pharmaceutical excipients are substances other than the pharmacologically active drug or prodrug which are included in the manufacturing process are contained in a finished pharmaceutical product dosage form.

Although the excipients are considered as inactive material but it has shown some interactions with active drug substances to affect the safety and efficacy of drug products (Weiner M. L. et al., 1999). Therefore, it is more important to have an awareness of the necessity to understand the interactions between formulation excipients and the active pharmaceutical substances in Drug product or finished dosage forms. Many of the reported drug excipient reactions involved in the hydrolysis, oxidation and specific interactions of drugs with reactive impurities in excipients.

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Polyethylene Glycol and Poly sorbates are generally used as a pharmaceutical excipients, Formaldehyde could be formed from the breakdown of the polymeric chain of these excipients (Galstrup J, 1996; Hamburger R et al., 1975; Waterman K et al., 2008; Sakharov A. M. et al., 2001;).

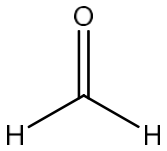


Figure 1: Structure of Formaldehyde

Formaldehyde (Fig. 1) is a colorless gas with a characteristic pungent odor. It is a volatile organic compound having a molecular weight of 30 amu and low boiling point of -21°C. It is not on the ICH guideline list of solvents and thus a control limit cannot be found. As per World Health Organizations guideline WHO/SDE/WSH/05.08/48, formaldehyde is carcinogenic by inhalation but is not carcinogenic by oral route. In the gastro intestinal tract, formaldehyde is rapidly oxidized to form formic acid, a class 3 solvent as per ICH. The US Environmental protection agency (EPA) has established a maximum daily dose reference (RfD) of 0.2 mg/kg per day for Formaldehyde (Water U.S. Environmental Protection Agency, 2006). Formaldehyde presents in excipients have been implicated in the degradation of several drug products, where in it forms

adduct with Primary and Secondary amine groups (Zhong Li et al., 2006).

Formaldehyde is a volatile molecule and having no choromophore in its structure. It is not easily amenable to GC-FID and also not easily ionisable with Mass spectrometry. Still there are few literatures on static headspace GC-MS for Pharmaceutical excipients (Padmaja Prabhu, 2011), Cosmetic products (Sarthchandraprakash N. K. et al., 2014), in water (Naeko Sugaya et al., 2001). HPLC-UV method for determination of formaldehyde in low level, this technique has been reported for analysis of Drug substance (Soman A, et al., 2008), analyses of Cosmetics (Wu P, et al., 2003); tap water (Lehotay J, et al., 1994). Fish-paste products (Kido K, et al., 1980), natural gas and oil combustion products (Goetze H. J. et al., 1989), aqueous extracts and model mixtures stimulating foods (Pertsovskii A.L. et al., 1985).

MATERIALS AND METHODS

Reagents and Chemicals

Highly pure water was prepared with the Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA) and HPLC grade acetonitrile (Rankem, Mumbai India), AR grade Formaldehyde (37-41%w/v) (Merck, Mumbai, India), Supra pure grade Glacial acetic acid (Merck, Mumbai, India) and 2,4 Dinitro phenyl hydrazine AR grade (Merck, Mumbai, India) were used.

Apparatus

The UPLC method development and validation were done using Waters E2695 series UPLC system with Photo diode array detector. The data were collected with Empower software and peak purity was also evaluated using LC-MS/MS for checking the purity and the integrity of mass values with respect to DNPH and HCHO-DNPH derivative product after method development. The LC-MS used for this study is Waters Xevo Q-TOF with mass lynx software.

Chromatographic conditions

Chromatographic separation was achieved on Acquity BEH C_8 100 X 2.1 mm, 1.7µ (Waters, USA) under isocratic mode of elution. The mobile phase was a mixture of Water and Acetonitrile (55:45, v/v). The mobile phase was freshly prepared, filtered through a Millipore filter (pore size 0.45µm) and sonicated for 15 minutes. Separation was performed at 35˚C using a 0.4 mL /min flow-rate and the run time was 5 minutes. The injection volume was 5µL and the detection wavelength was set at 360nm. The chromatographic and the integrated data were recorded in a computer system using Empower data acquiring software (Waters, USA).

ESI-MS–MS conditions

Waters Xevo Q-TOF instrument with UPLC separation module pumps, auto sampler device and TUV detector was used for the analysis of degradation products. The ionization was carried out in Electro spray ionization method in positive mode of detection. Nitrogen was the nebulizer and curtain gas. The ion source conditions were set as follows: Sampling cone, 10.0 volts; Extraction cone, 5.0 volts; Capillary volts, 2.1 kilo volts; Source temperature, 100°C; Desolvation temperature, 350°C; Cone gas flow, 50 liters/hour; Desolvation gas flow, 700 liters/hour.

Preparation of 2, 4, Dinitrophenyl hydrazine DNPH reagent solution (0.3 mg/mL)

A solution (1 mg/mL) of DNPH was prepared by dissolving known amounts of the components in Acetonitrile. Then further diluted to (0.3 mg/mL) the solutions were adequately diluted with the mobile phase to study accuracy, precision, linearity, limits of detection and quantification.

Preparation of Formaldehyde Stock solution (0.002 mg/mL)

Around 27.8mg of Formaldehyde (37-41% w/v) solution is diluted to 100ml with water to obtain 0.1mg/mL. The 0.1mg/mL solution is further diluted to 0.002 mg/mL.

Preparation of Formaldehyde standard solution (20 ppm)

1ml of Formaldehyde Stock solution (0.002 mg/mL) is transferred into 5mL of volumetric flask, added 1.0 ml of 2, 4, Dinitrophenyl hydrazine DNPH reagent solution (0.3 mg/mL) and 0.2 ml of Glacial acetic acid into that flask and placed lid and sealed with para film and heated over water bath at 60°C for 10 minutes, then dissolved and diluted to volume with Acetonitrile.

RESULTS AND DISCUSSION

The present study was aimed at developing a chromatographic system capable of separation and quantitative determination of Formaldehyde in presence of DNPH reagent. Also to evaluate the sensitivity of this method to quantify at lower levels.

Method development

In this method, Derivatization process (Figure 2) plays a major role in the development. Different conditions like strength of acid, solvent, reagent concentration, temperature and different time of heating were studied. Initially, different concentrations of DNPH reagent in the range of 0.01 to 0.3 mg/mL were studied to understand and optimize the reagent concentration. Then different types and concentrations of acids like acetic acid and perchloric acid were studied. The heating temperatures were studied in the range of 40°C to 70°C. Heating time also varied from 5minutes to 30 minutes to see the maximum derivatization time. The response for Derivatized product is more in Acetic acid when compared to Perchloric acid. The response was not influenced with excess amount of Acid and extended heating. The 2,4 DNPH concentration increased

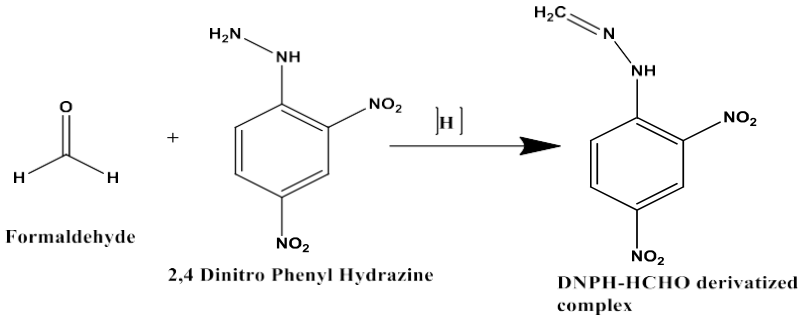
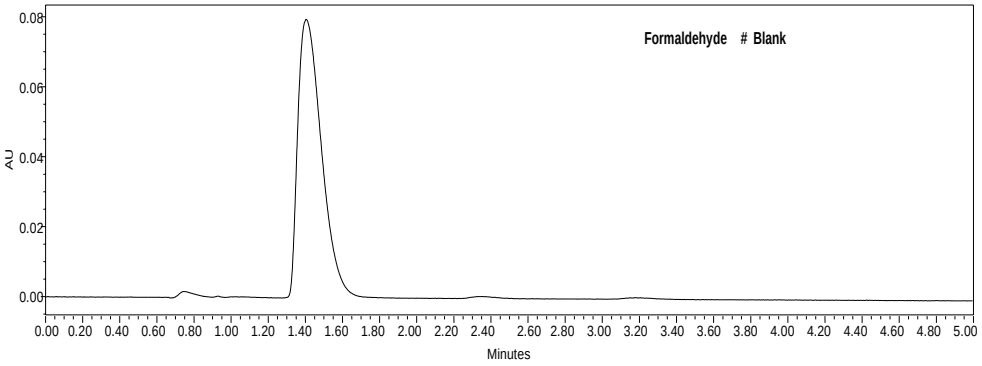
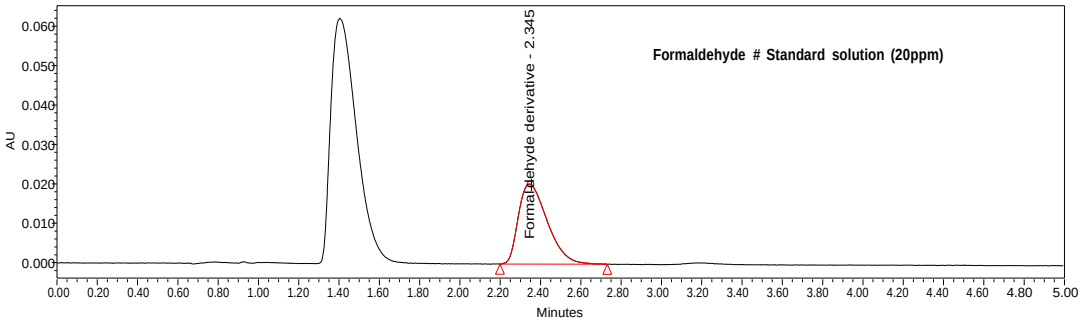


Figure 2: Derivatization of DNPH-HCHO complex reaction scheme



(a)



(b)

Figure 3: Typical HPLC chromatograms of a) Blank Solution b) Formaldehyde at 20ppm

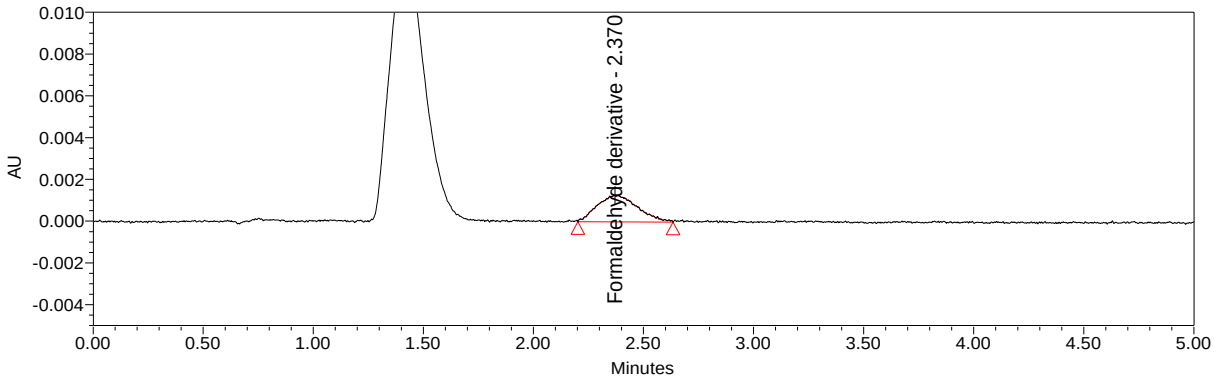


Figure 4: HPLC chromatogram of Limit of Quantitation at 2ppm

from 0.01mg/mL to 0.3 mg/mL, the stable area response was observed in 0.2mg/mL and 0.3mg/mL.

Optimization of the derivatization process

The derivatization process parameters are 0.3mg/mL DNPH reagent solution, 0.2ml of acetic acid solution and heating temperature was 60°C for 10 minutes. The developed method was validated in terms of accuracy, precision, linearity and robustness as per ICH guidelines (International conference on Harmonization, 2005).

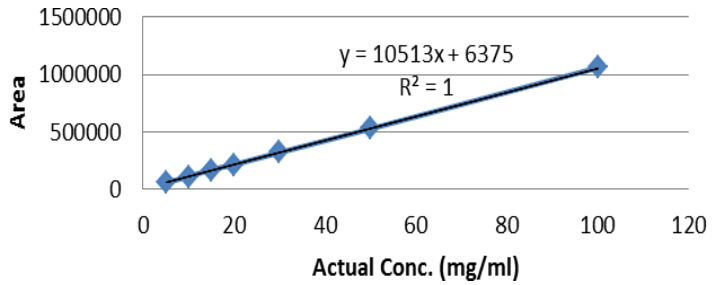
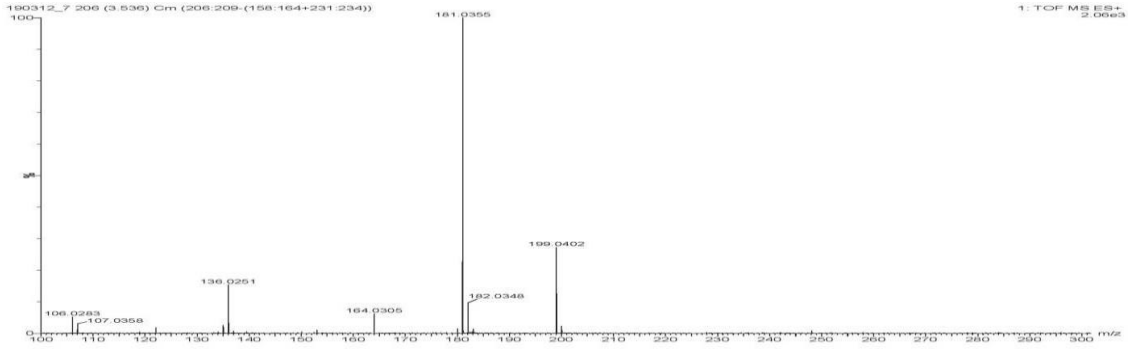
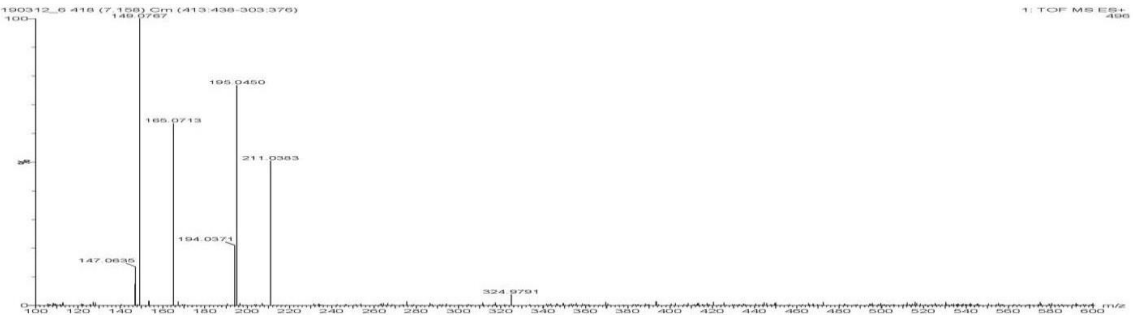


Figure 5: Linearity graphs for Formaldehyde



(a)



(b)

Figure 6: Positive ion ESI-MS spectra of a) DNPH b) HCHO-DNPH derivative product

Table 1: Results of System Suitability at 20ppm level of formaldehyde derivative

System precision at 20ppm level	
Injections	Area
1	217582
2	217219
3	217972
4	218054
5	217836
6	217904
Avg.	217761
SD	310.56
%RSD	0.14

Validation of the method

Specificity

Specificity is the ability of the method to measure the analyte response in presence of all other impurities and compounds. The DNPH-HCHO complex is well separated from DNPH reagent. The peak integrity was also confirmed with LC-MS analysis (Fig 6).

System suitability

The system suitability was checked by making five replicate injections of DNPH-HCHO complex (20ppm) for

Table 2: Results of Precision at LOQ level for Formaldehyde derivative peak

Precision at LOQ level	
Injections	Area
1	16052
2	16025
3	16017
4	16032
5	16045
6	15928
Avg.	16017
SD	45.21
%RSD	0.28

Table 3: Results of %RSD of Formaldehyde derivative peak by different analyst for 12 injections (6 injections from method precision and 6 injections from intermediate precision)

Injections	Area
Analyst-1, Injections-1	204055
Analyst-1, Injections-2	204905
Analyst-1, Injections -3	204392
Analyst-1, Injections -4	204848
Analyst-1, Injections -5	204154
Analyst-1, Injections -6	204329
Analyst-2, Injections -1	205181
Analyst-2, Injections -2	205135
Analyst-2, Injections -3	205021
Analyst-2, Injections -4	204121
Analyst-2, Injections -5	204774
Analyst-2, Injections -6	204048
Avg.	**204580**
SD	**439.8287**
%RSD	**0.21**

Table 4: Results of %Recovery of Formaldehyde derivative peak

S.No.	Concentration Taken (ppm)	Concentration Found (ppm)	% Recovery	Average % Recovery
Accuracy -10ppm-P1	10	9.6	96.0	**97.0**
Accuracy -10ppm-P2	10	9.8	98.0	
Accuracy -20ppm-P1	20	19.1	95.5	**96.3**
Accuracy -20ppm-P2	20	19.4	97.0	
Accuracy -50ppm-P1	50	48.2	96.4	**96.6**
Accuracy -50ppm-P2	50	48.4	96.8	

Quantitative determination (Fig 3) The system was deemed to be suitable for use %RSD for five replicate injections is <2.0 (Table-1).

Accuracy

The accuracy for Quantification of DNPH-HCHO derivative complex was determined by spiking at three different levels ranging from 10 ppm to 50 ppm at the specified level (20 ppm). The recovery range was found to be 96.3–97.0% (Table 4).

Precision

The precision of the method for Quantification was tested by six (n = 6) injections of HCHO-DNPH derivative product and the %R.S.D. of peak areas was determined. The R.S.D. was found to be 0.25%. The inter-

Table 5: Results of Solution stability of Formaldehyde derivative peak in the 20ppm standard solution

Solution stability of the Formaldehyde (20ppm)		
Time interval	Area in the Standard	%Recovery with initial
Initial	213704	-
2hrs	213563	99.9
6hrs	213787	100.0
10hrs	213363	99.8
14hrs	213675	100.0
18hrs	213694	100.0
24hrs	213494	99.9
48hrs	213595	99.9
S.D	135.41	0.07
Average	213609.38	99.9
%R.S.D	0.06	0.07

Table 6: Robustness experiments and conditions

Robustness experiment conditions			
Experiment No.	Ratio of water: Acetonitrile	Heating condition (°C)	Heating time (min)
Original conditions	600:400	60	10
1	620:380	60	10
2	580:420	60	10
3	600:400	65	10
4	600:400	55	10
5	600:400	60	11
6	600:400	60	9

Table 7: Results of Robustness of Formaldehyde derivative peak in the 20ppm standard solution

Results of System suitability # Robustness						
Injection	**Robustness-Experiment**					
	1	2	3	4	5	6
Standard Injection-1	213938	213476	213529	210632	213417	213520
Standard Injection-2	213845	213489	213506	210341	213954	213271
Standard Injection-3	213940	213594	213524	210156	213280	213059
Standard Injection-4	213521	213756	213573	209882	213305	213335
Standard Injection-5	213862	213389	213849	210136	213213	213574
Standard Injection-6	213446	213407	213669	210201	213619	213722
Mean	213758.6	213518.5	213608.3	210224.6	213464.6	213413.5
SD	217.91	137.10	131.70	249.00	278.74	238.50
%RSD	0.10	0.06	0.06	0.12	0.13	0.11

mediate precision is also determined on different system, different analyst and different column. The R.S.D. values were found to be 0.35%, indicating a good repeatability. The overall R.S.D. for both the method precision and Intermediate precision was found to be 0.21% (Table 3).

Linearity

The linearity of DNPH-HCHO derivatization complex was also studied by preparing standard solutions at 5 different levels ranging from 5ppm to 100 ppm. The data were subjected to statistical analysis using a linear-regression model. The regression equations and coefficients (r^2) are given in Fig. 5. The proposed linearity range was useful to quantify formaldehyde at the lower level around 5ppm to 100ppm. Thus, the proposed method meets the requirement of ICH guidelines and the results indicated a good linearity.

Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by meas-

uring the magnitude of analytical background by injecting a blank and calculating the signal-to-noise ratio for each compound by injecting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. The chromatogram for LOQ is shown in Fig. 4. The lowest limit of quantization (LOQ) was 2ppm and the lowest limit of detection (LOD) was 0.6ppm, indicating the suitability of the developed method for quantification of formaldehyde at lower levels.

Precision at LOQ levels

Precision at LOQ levels at 2ppm were studied. The method was tested by six (n = 6) injections and the %RSD was found to be 0.28 (Table 2).

Solution stability

The solution stability of DNPH-HCHO derivative complex was carried out by leaving a solution in a tightly capped volumetric flask at room temperature (25±2°C) for 48 h. The % Recoveries was calculated with initial peak area and solution is found to be stable (Table 5).

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the developed method, the experimental conditions were altered and the system suitability was evaluated. The effect of the mobile phase composition modified to different concentrations. The effect of heating temperatures and heating time was studied. In all these three parameters i.e. Mobile phase composition, Heating temperature and Heating time the % RSD in system suitability are calculated and meets the requirement. The details of parameters and system suitability results are mentioned in Table 6 and Table 7 respectively.

CONCLUSIONS

This report presents the development and validation of rapid and simple isocratic method suitable for the quantification analysis of Formaldehyde at lower levels using UPLC. It was demonstrated as specific, accurate, precise and robust. Excellent linearity was observed in the range of 5ppm to 100ppm. The Limit of Quantification and Limit of Detection levels are 2ppm and 0.6ppm respectively. The HCHO-DNPH derivative complex solution was found to be stable up to 48Hrs at room temperature. The derivatization process and detection at 360nm are applicable and extended to the drug product and excipient analysis. Normally drug product and excipients does not show absorbance above 350nm.

REFERENCES

Edition of the Drinking water standards and Health Advisories (EPA 822-R-06-013), Office of the Water U.S. Enviromental Protection Agency Washington DC. 2006, 5.

Glastrup J. Degradation of polyethylene glycol. A study of the reaction mechanism in a model molecule: tetraethylene glycol. *Polymer Degradation stability,* 52, 1996, 217-222.

Goetze H J, Harke S. Determination of aldehydes and ketones in natural gas combustion in the ppb range by high performance liquid chromatography. *Fresenius journal of Analytical chemistry,* 335(3), 1989, 286-288.

Hamburger R, Azaz E, Donbro M. Autoxidation of polyoxyethylenic nonionic surfactants and polyethylene glycols. *Pharma Acta Helv,* 50, 1975, 7-10.

ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, November 2005.

Kido K, Sakuma T, Watanabe T. Gas chromatographic analysis of Formaldehyde in fish paste products. *Eisei Kagaku,* 26(5), 1980, 224-228.

Lehotay J, Hromulakova K. HPLC determination of trace levels of aliphatic aldehydes C1-C4 in river and tap water using online preconcentration. *Journal of Liquid Chromatography,* 17(3), 1994, 579-588.

Naeko Sugaya, Tomoo Nakagawa, Katsumi Sakurai, Masatoshi Morita, Sukeo Onodera. Analysis of Aldehydes in water by Head space GC/MS. *Journal of Health Science,* 47(1), 2001, 21-27.

Padmaja Prabhu. Detection and Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine in Pharmaceutical Excipients by Static Headspace GC/MS. *Perkin Elmer Application Notes,* 2011, 1-5

Pertsovskii A L, Kremko L M. Gas chromatographic determination of trace amounts of formaldehyde in aqueous extracts and model mixtures stimulating foods. *Zh. Anal. Chem.,* 40(6), 1985, 1115-1118.

Sakharov AM, Mazaletskaya LI, Skibida IP. Catalytic oxidative deformylation of polyethylene glycols with the participation of molecular oxygen. *Kinet. Catalysis.,* 42, 2001, 662-668.

Sarthchandraprakash N.K., Jampala Balaji, Krishna Manral. A simple Headspace Gas Chromatography method to quantify the formaldehyde content in Cosmetic products. *e- Library Science Research Journal,* 3(2), 2014, 1-6.

Soman A, Qiu Y, Chan Q Li. HPLC-UV Method Development and Validation for the Determination of Low Level Formaldehyde in a Drug Substance. *Journal of Chromatographic science,* 46, 2008, 461-465.

Waterman K, Arikpo WB, Fergione MB, Graul TW, Johnson BA, MacDonald BC, et al. N-methylation and N-formylation of a secondary amine drug (Vare-

nicline) in a osmotic tablet. *Journal of Pharmaceutical Science,* 97(4), 2008, 1499-1507.

Weiner M. L., Kotkoskie L. A., Marcel Dekker. *Excipient Toxicity and Safety*, New York, 1999.

Wu P, Chang C, Shou S. Determination of formaldehyde in cosmetics by HPLC method and acetyl acetone method. *Journal of Food and Drug Analysis,* 11(1), 2003, 8-15.

Zhong Li, Laura K. Jacobus, Peter W. Wuelfing, Mary Golden, Gregory P. Martin and Robert A. Reed. Detection and Quantification of Low molecular weight Aldehydes in Pharmaceutical Excipients by Headspace Gas Chromatography. *Journal of Chromatography,* Volume A 1104, 2006, 1-10.