Determination of Process-related Genotoxic Impurities of Pentoxifylline by GC-HS Method

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Abstract

The synthesis of pentoxifylline contained three different kinds of genotoxic contaminants. While 6-chloro-2-hexanone is possibly genotoxic, 3,7-dimethyl-1-(5-oxohexyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione is not; structural alert for genotoxic mutagenicity and carcinogenicity is present. According to the structural alert and QSTR model consensus, 1-bromo-3-chloropropane and 3-chloro-1-propanol are both genotoxic. For most volatile and semi-volatile analytes, gas chromatography (GC) with direct liquid injection and a flame ionization detector (FID) is the appropriate method. The suggested approach was shown to be very effective, precise, linear, accurate, and specific in monitoring three genotoxic contaminants. Pharmaceutical examination of 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol. GC techniques were used to analyze these contaminants. The method is particularly sensitive to quantifying 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol contaminants at 0.3 ppm and 0.9 ppm, respectively, according to the results of the LOQ and LOD methods. The goal of this work is to determine threshold amounts and appropriate methodologies for quantifying the three genotoxic contaminants that may be present during pentoxifylline production.

Keywords: Genotoxic impurities, gas chromatography (GC, flame ionization detector (FID), chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol

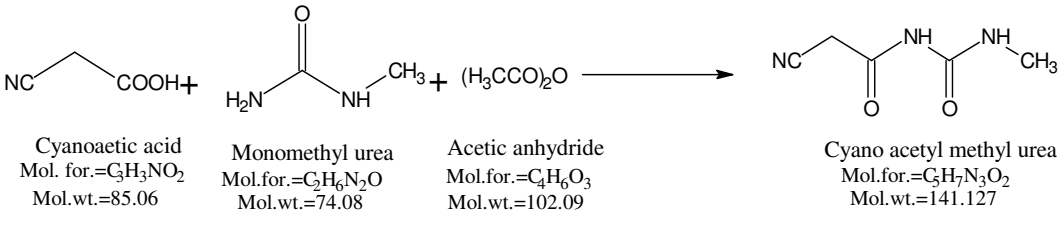
Introduction

Pentoxifylline, sometimes referred to as oxpentifylline, is a xanthine derivative that is prescribed to patients with peripheral artery disease to relieve their muscle pain. [1] It is marketed globally under numerous brand names and is generic. [2] It is primarily used in medicine to treat intermittent claudication, a type of muscle discomfort brought on by peripheral artery disorders, which causes pain, cramps, numbness, or weakness in the arms or legs. SIGN also recommends the use of pentoxifylline as a licensed supplement to compression bandaging for the treatment of persistent venous leg ulcers. [3] as it has been demonstrated to increase healing rates. [4] Additionally, it has been demonstrated that pentoxifylline helps with alcoholic hepatitis, with some studies demonstrating a reduction in the risk of hepatorenal syndrome.

***Mechanism of action*:** Pentoxifylline, one of the other methylated xanthine derivatives, is a competitive nonselective phosphodiesterase inhibitor [5] that also increases intracellular cAMP, activates PKA, suppresses leukotriene production and TNF [6-7], lowers innate immunity and inflammation. [8] Pentoxifylline also lessens blood viscosity, enhances red blood cell deformability (a hemorheological action), and lowers the risk of platelet aggregation and thrombus formation. [9] Additionally, pentoxifylline inhibits adenosine 2 receptors.[10] Pentoxifylline may be able to reduce the levels of several biomarkers in non-alcoholic steatohepatitis, according to some research [11], but not enough to say if this usage of the medication is safe and effective.[12] Research on the use of pentoxifylline for hearing loss and erectile dysfunction in animals has been done [13]. Studies on humans have been done for Peronei's disease.[14]

Pentoxifylline is preventive against osteoradionecrosis and repairs refractory osteoradionecrosis of the jaw when combined with tocopherol and clodronate.[15] The following was determined by a Cochrane systematic review on the use of pentoxifylline for intermittent claudication. The majority of the included studies had poor quality, and there was a lot of variation in the reported results regarding trial length[16], pentoxifylline dosages, and participant walking lengths at the beginning of the trials. The majority of the included studies lacked sufficient information to allow for the assessment of selective reporting, did not report on the blinding of outcome assessors, and did not discuss randomization procedures or how treatment allocation was hidden. Given all these factors, the role of pentoxifylline in intermittent claudication remains uncertain, although participants generally tolerated this medication well.

Manufacturing Process of Pentoxifylline: (Reaction Scheme)

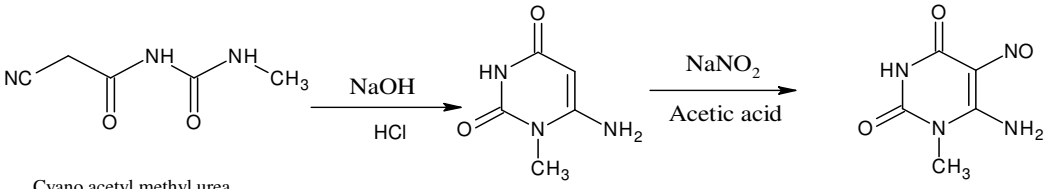


Cyanoaetic acid
Mol. for.=$C_3H_3NO_2$
Mol.wt.=85.06

Monomethyl urea
Mol.for.=$C_2H_6N_2O$
Mol.wt.=74.08

Acetic anhydride
Mol.for.=$C_4H_6O_3$
Mol.wt.=102.09

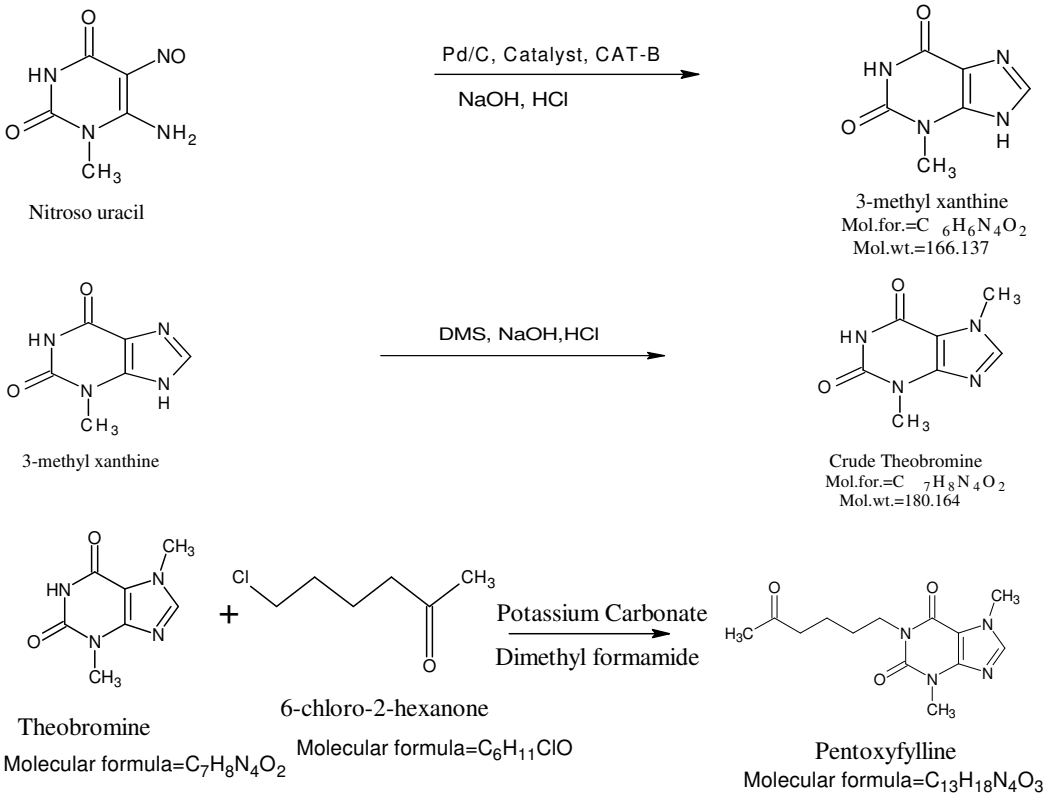
Cyano acetyl methyl urea
Mol.for.=$C_5H_7N_3O_2$
Mol.wt.=141.127



Cyano acetyl methyl urea

6-amino-1-methyluracil
Mol.for.=C $_5H_7N_3O_2$
Mol.wt.=141.127

Nitroso uracil
Mol.for.=C $_5H_6N_4O_3$
Mol.wt.=170.126



The literature review verified that, as of now, no techniques have been documented for identifying the genotoxic impurities of pentoxifylline, which include 1-bromo-3-chloropropane [17] (CAS no: 109-70-6) 3-chloro-2-hexanone [18] (CAS number: 627-30-5), and 6-chloro-2-hexanone [19] (CAS no: 10226-30-9). The development of FID-GC techniques for the identification of pentoxifylline and its contaminants is emphasized throughout the dissertation. Based on the genotoxic prediction report for Pentoxifylline's process impurities structures, which indicates that these impurities are genotoxic, the author felt it was necessary to identify these impurities using an appropriate chromatographic technique. As a result, this job was finished in two sections.

Genotoxicity prediction methods and confirmation

Objective: To predict the Genotoxic potential of given compounds.

***Method*:** Genotoxicity prediction is a consensus inference derived from three different methodologies.

a) Decision tree-based Alerts: It uses the fragment rule base which is validated by the results of the Joint Research Centre's European Chemicals Bureau hazard estimation based on the Benigni/Bossa [20] rule base for genotoxic carcinogenicity [21-22] and mutagenicity.

b) Toxicophores significance by ANOVA: This methodology makes use of a database of substances from Gold [23] and Zeiger that have TD50 values given for species of rats and mice.[24] ANOVA analysis is performed using carefully selected toxicophoric data from the

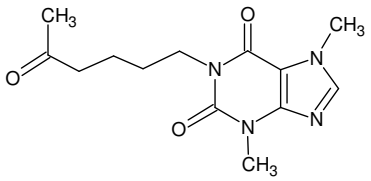
literature, and the related F-ratio and probability are computed. The fragment's relevance about its contribution to genotoxicity is stated if it is found in any of the test chemicals. Estimating the degree of a compound's toxicity is made easier by the relevance of its hazardous fragments.

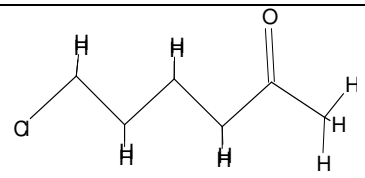
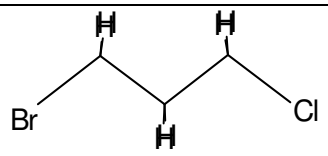
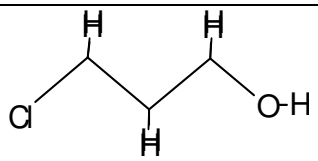
c) Predicting genotoxicity using SAR / QSAR models: The nearest neighbour method is used to create strong and trustworthy QSTR models for genotoxicity, mutagenicity [25–27], and carcinogenicity.[28] The classification model has been verified by OECD [29] Guidance and REACH rules. [30]

Materials And Methods

a) Materials and Reagents: Methylene Chloride (GC Grade) was obtained from Merck Specialty Chemicals, Mumbai, India. Reference materials 6-chloro-2-hexanone and 3-chloro-1-propanol were obtained from Tokyo Chemical Industry, Mumbai, India and 1-bromo-3-chloropropane was obtained from Alfa-Acer and Pentoxifylline was obtained from Supriya Lifescience Ltd, Mumbai.

Fig.1: Structure of Pentoxifylline



		
6-chloro-2-hexanone (CAS no: 10226-30-9)	1-bromo-3-chloropropane (CAS no: 109-70-6)	3-chloro-1-propanol (CAS no: 627-30-5)

b) Instrumentation: The gas chromatographic analysis was carried out using GC 7890B equipped with a flame ionization detector (FID) and 7683B auto-injector (Agilent Technologies, USA). The chromatographic data was recorded using Agilent data acquisition software.

c) Preparation of Solutions:

Blank Solution: Methylene chloride was used for the preparation of sample and standard solutions.

Standard Solution: Each of 10 mg of 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol diluted with 100 ml of methylene chloride into a 100mL volumetric flask.

Stock Solution: 1.0 mL of the above solution was transferred into a 100 mL volumetric flask and diluted up to the mark with methylene chloride. This corresponds to 1 ppm of each impurity w.r.t. the test concentration.

Pentoxifylline sample solution: 1.0 g of Pentoxifylline sample was accurately weighed and taken into a 100 mL volumetric flask and diluted with 100 ml of methylene chloride.

RESULTS AND DISCUSSIONS:

METHOD DEVELOPMENT: Since gas chromatography with direct liquid injection and a flame ionization detector (FID) works well with the majority of volatile and semi-volatile analytes, the author used it in their most recent work. The major difficulty was getting gas chromatography to produce the appropriate detection and quantification limit. Choosing the right solvent for pentoxifylline's solubility has turned into a problem in achieving the necessary concentration for a better FID signal. As a result, we tested several GC columns and dilution solvents in the current study to meet the goals and demands of the industry.

a*) Method Development Experiment-1*

Blank Solution: DMSO was used for the preparation of sample and standard solutions.

Standard Solution: Each of 10 mg of 6-chloro-2-hexanone, 1-bromo-3-chloropropane and 3-chloro-1-propanol diluted with 100 ml of DMSO into a 100mL volumetric flask.

Chromatographic conditions:

Column: DB-17 (Agilent), 30 m x 0.32 mm ID x 0.5µm capillary column or equivalent. . (50% phenyl – methyl polysiloxane); Injection mode: Split; Flow control mode: Constant flow; Column flow: 2 ml/min; Linear Velocity: 37 cm/sec; Purge flow: 3.0 ml/min; Split ratio: 3.0; Carrier gas: Nitrogen; Column Temperature: 100°C (hold 2 min.) to 230 ºC @ 30 ºC (hold 5 min.) Injector Temperature: 250 ºC; Detector Temperature: 260 ºC Injection Volume: 1µl; Detector: FID.

Observation: The two genotoxic impurities were only detected and also resolution was not getting proper at the threshold level i.e. 1ppm with the above experimental conditions.

b) Method Development Experiment-2

Blank Solution: Methylene chloride was used for the preparation of sample and standard solutions.

Standard Solution: Each of 10 mg of 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol diluted with 100 ml of Methylene chloride into a 100mL volumetric flask.

Observation: The three genotoxic impurities were detected and also resolution was not getting proper at the threshold level i.e. 1ppm with the above experimental conditions.

c) Final Chromatographic Conditions

The main target of this study was to develop a GC method to get better resolution and good response between Pentoxifylline and its three genotoxic impurities at 1 ppm level. Better results were obtained by using a DB-17 (30m × 0.32 mm internal diameter) column with a film thickness of 0.5µm and with an oven temperature program 100°C (hold 2 min.) to 230 ºC @ 30

ºC (hold 5 min.). Finally, the experiment was completed with the following chromatographic conditions.

Instrument: GC 7890B equipped with flame ionization detector (FID) and 7683B auto-injector (Agilent Technologies, USA). Carrier gas: Nitrogen Column flow

Column: DB-17 (Agilent), 30 m x 0.32 mm ID x 0.5µm capillary column or equivalent. . (50% phenyl – methyl polysiloxane) ; Injection mode: Split; Flow control mode: Constant flow; Column flow: 2 ml/min; Linear Velocity: 37 cm/sec; Purge flow: 3.0 ml/min; Split ratio: 3.0; Carrier gas: Nitrogen; Column Temperature: 100°C (hold 2 min.) to 230 ºC @ 30 ºC (hold 5 min.) ; Injector Temperature: 250 ºC; Detector Temperature: 260 ºC; Injection Volume: 1µl; Detector: FID.

Observation: It is observed that the two genotoxic impurities were well resolved, with good response and better peak shapes at the threshold level i.e. 1ppm. The advanced developed method was validated as per ICH guidelines [32-33]**.** The validation parameters included selectively/ specificity, the limit of detection, the limit of quantification, accuracy, precision, linearity, ruggedness, and robustness.

Specificity: The capacity to evaluate the analyte without a doubt in the presence of any other traces of components that could be anticipated to be present is known as specificity/selectivity.[34] To achieve this, genotoxic contaminants were added to the Pentoxifylline sample (1.0µg/mL blend with Pentoxifylline test concentration of 10 mg/ml). Every impurity was well separated from the others and provided superior separation.

Fig. 2: Chromatogram for Genotoxic impurities spiked with Pentoxifylline

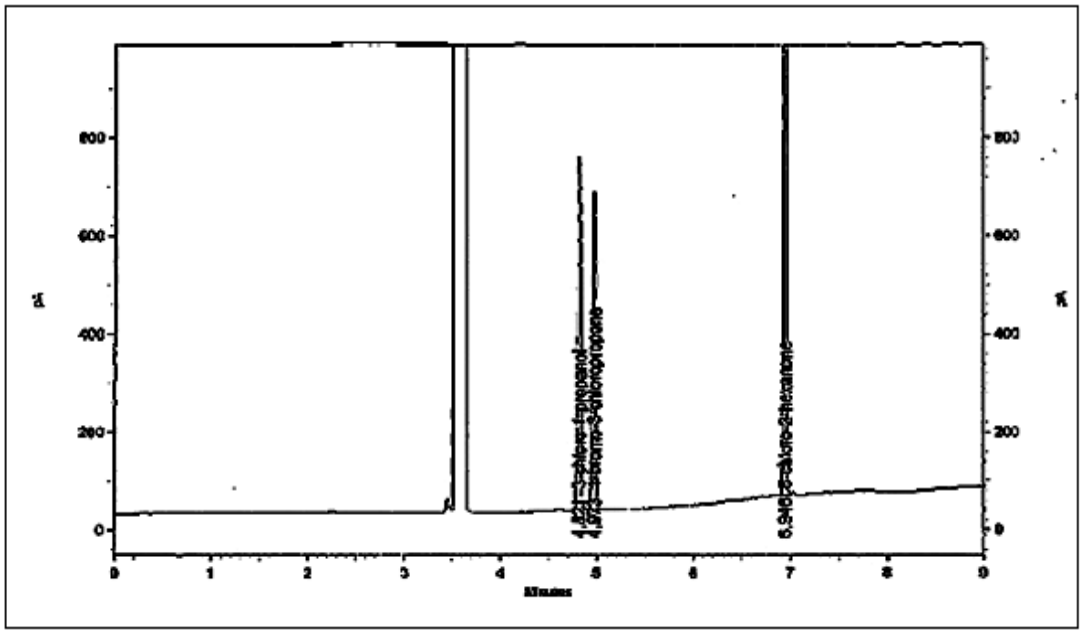
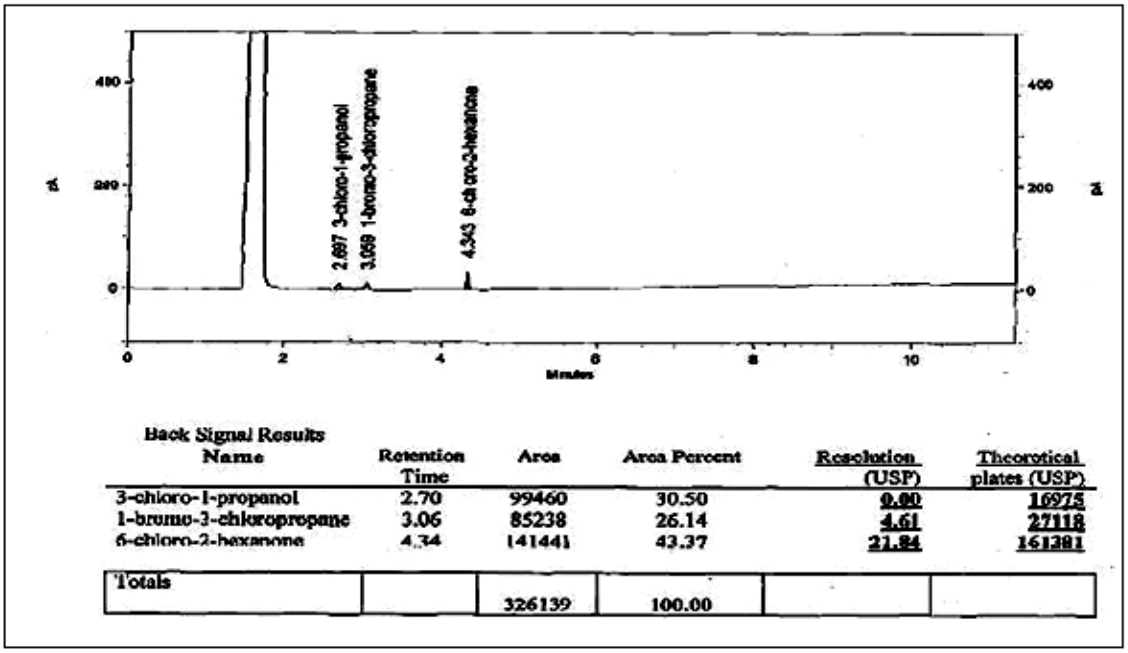


Fig. 3: Chromatogram for Genotoxic impurities spiked with Pentoxifylline

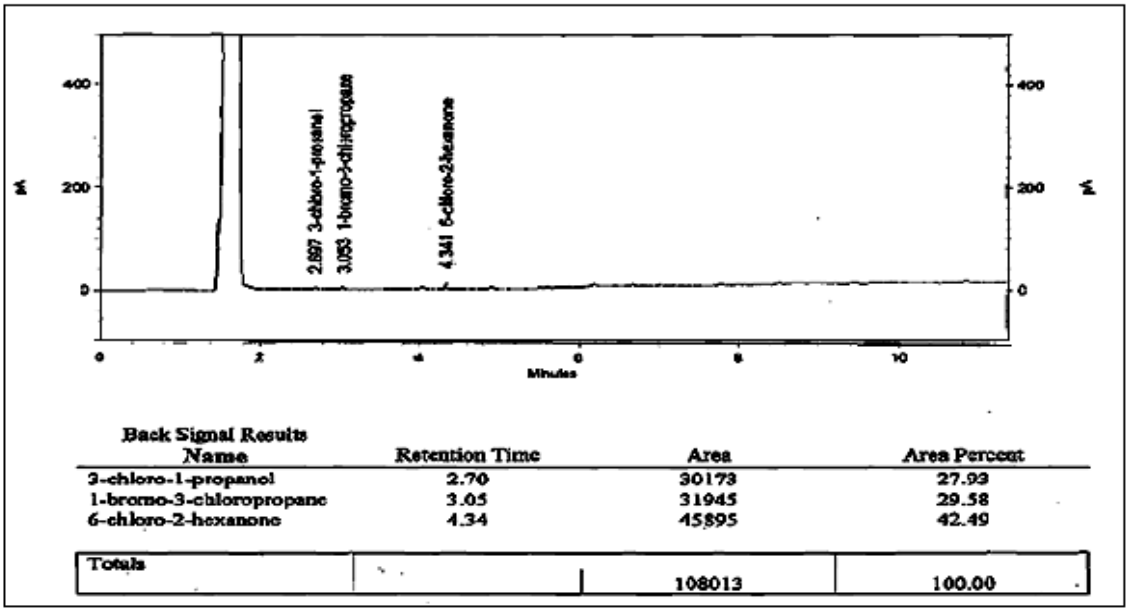


Back Signal Results

Name	Retention Time	Area	Area Percent	Resolution (USP)	Theoretical plates (USP)
3-chloro-1-propanol	2.70	99460	30.50	0.00	16975
1-bromo-3-chloropropane	3.06	85238	26.14	4.61	27118
6-chloro-2-hexanone	4.34	141441	43.37	21.84	161381
Totals		326139	100.00		

***THE DETECTION LIMIT (LOD) AND QUANTITATION LIMIT (LOQ)*:** To determine DL and QL values, the concentration of impurities [35] was reduced sequentially such that they yielded S/N of about 3 and 10 respectively thus preparing a series of solutions of 0.1, 0.2, 0.3, 0.4 and 0.5 ppm with mixed concentrations of 6-chloro-2-hexanone, 1-bromo-3-chloropropane and 3-chloro-1-propanol and injected into the GC as described in methodology. The method was carried out by performing six replications of QL concentration and %RSD was calculated. The results indicate that the method is very sensitive to quantifying 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol impurities at 0.3 ppm and 0.9 ppm respectively.

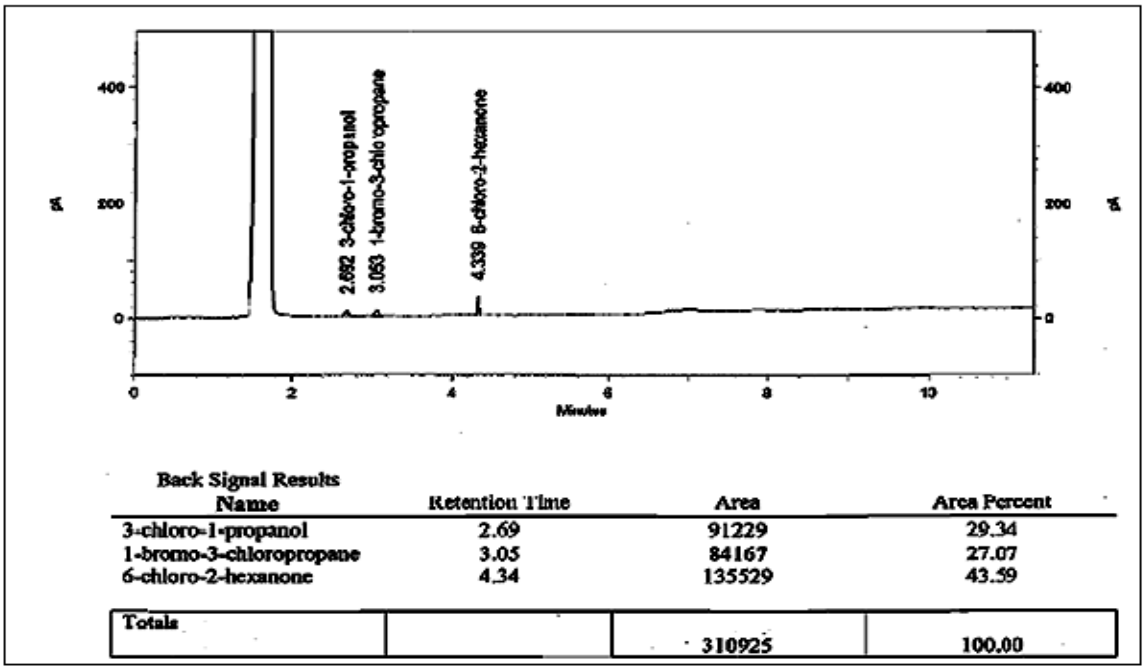
Fig. 4: Chromatogram of LOD for Genotoxic impurities



Back Signal Results

Name	Retention Time	Area	Area Percent
3-chloro-1-propanol	2.70	30173	27.93
1-bromo-3-chloropropane	3.05	31945	29.58
6-chloro-2-hexanone	4.34	45895	42.49
Totals		108013	100.00

Fig. 5: Chromatogram of LOQ for Genotoxic impurities



Back Signal Results

Name	Retention Time	Area	Area Percent
3-chloro-1-propanol	2.69	91229	29.34
1-bromo-3-chloropropane	3.05	84167	27.07
6-chloro-2-hexanone	4.34	135529	43.59
Totals		310925	100.00

Table 1: Precision of (LOD) for Pentoxifylline genotoxic impurities

Solution No	Concentration		Response (Area)		
	ppm	%	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
LOD Sol-1	0.3	0.0003	30173	31945	45895
LOD Sol-2			30908	29030	49625
LOD Sol-3			30283	30320	47731
LOD Sol-4			30871	30285	48970
LOD Sol-5			34472	31738	44014
LOD Sol-6			33917	28728	45472
Average			**91774**	**87213**	**31771**
Std. Dev.			**3295.88**	**4224.79**	**1909.10**
RSD			**3.59**	**4.84**	**6.01**

Table 2: Precision of LOQ for Pentoxifylline genotoxic impurities

Solution No	Concentration		Response (Area)		
	ppm	%	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
LOQ Sol-1	0.9	0.0009	91229	84167	135529
LOQ Sol-2			91467	89282	134226
LOQ Sol-3			91778	81423	137874
LOQ Sol-4			97498	87141	135351
LOQ Sol-5			87183	87569	133819
LOQ Sol-6			91489	93696	132977
Average			**91774**	**87213**	**134963**
Std. Dev.			**3295.88**	**4224.79**	**1717.03**
RSD			**3.59**	**4.84**	**1.27**

Table 3: System Precision of genotoxic impurities for Pentoxifylline

Sr. No.	Response (Area)		
	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
Inj. 1	99460	85238	141441
Inj. 2	106044	81213	141196
Inj. 3	103543	86298	142706
Inj. 4	103884	87128	140007
Inj. 5	98523	81752	143920
Inj. 6	98261	89543	143904
Average	**101619**	**85195**	**142196**
Std. Dev.	**3284.37**	**3211.56**	**158.85**
RSD	**3.23**	**3.77**	**1.11**

Table 4: Method Precision of genotoxic impurities for Pentoxifylline

Sample Injection	% Recovery		
	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
100% recovery -1	98.10	104.90	105.10
100% recovery-2	100.40	102.40	103.00
100% recovery -3	102.30	102.20	99.00
100% recovery -4	106.50	98.60	103.60
100% recovery -5	104.20	100.70	106.60
100% recovery -6	103.80	105.00	102.70
Average	**100.27**	**103.17**	**102.37**
RSD	**2.10**	**1.46**	**3.03**

PRECISION: The repeatability performed the precision under the same operating conditions over a short interval of time. It expresses the closeness of agreement between a series of measurements obtained from multiple sampling from the same homogeneous sample[36].

a) System precision: The system precision was determined by six replicate injections of a standard solution at 100.0% of the specified limit concerning the working concentration. Results of peak area for 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone for six replicate injections is summarized. The percentage relative standard deviation for the peak areas of 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone obtained in the range of 3.23%, 3.77%, and 1.11% at working concentration respectively.

b) Method precision: The precision of the method was determined by analyzing a sample of Pentoxifylline with 3-chloro-1-propanol, 1-bromo-3-chloropropane and 6-chloro-2-hexanone at 100% of the specification limit (six replicate spiked sample preparations). The results obtained are summarized. The percentage relative standard deviation for 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone at 100% of the specification level in six preparations should not be more than 5.0%. The percentage relative standard deviation for 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone are obtained at 2.10%, 1.46%, and 3.03 % at the working concentration. The Mean Recovery is within limits. Therefore, the

GC method for the determination of 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone in Pentoxifylline is accurate.

LINEARITY*:* Linearity solutions for the Pentoxifylline genotoxic impurities were prepared individually by diluting the stock solution at six concentration levels in the range of LOQ to 150% of the specification levels *viz.* DL, 0.3 ppm to 1.5 ppm. Tests were carried out and the slope, Y-intercept, and correlation coefficient of the calibration curve were calculated and tabulated. The peak area versus concentration was plotted. The results was linear within the range of 0.3 ppm to 1.5 ppm.

Fig. 6: Linearity plot for 3-chloro-1-propanol

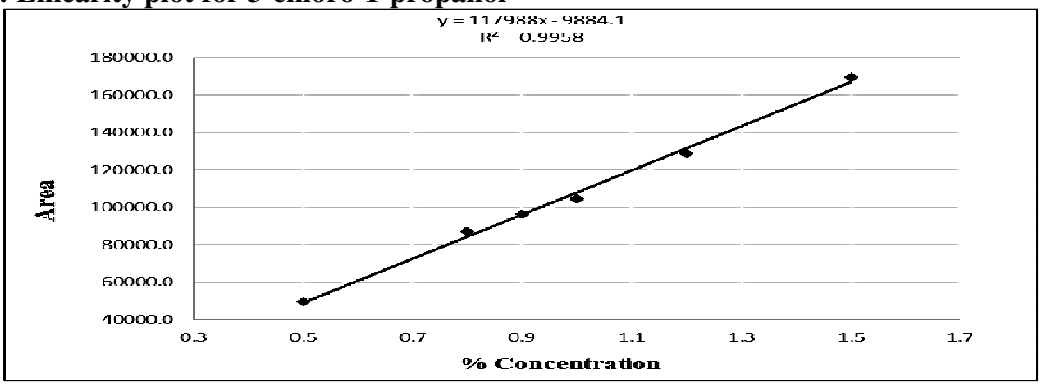


Fig. 7: Linearity plot for 1-bromo-3-chloropropane

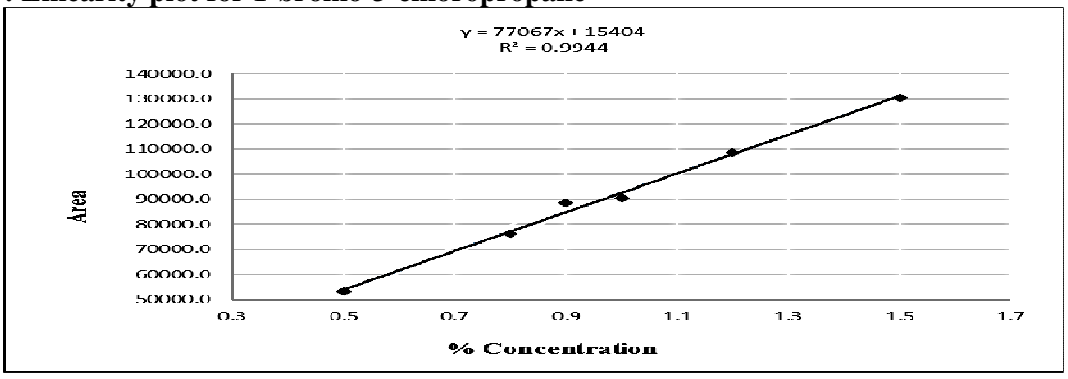


Fig. 8: Linearity plot for 6-chloro-2-hexanone

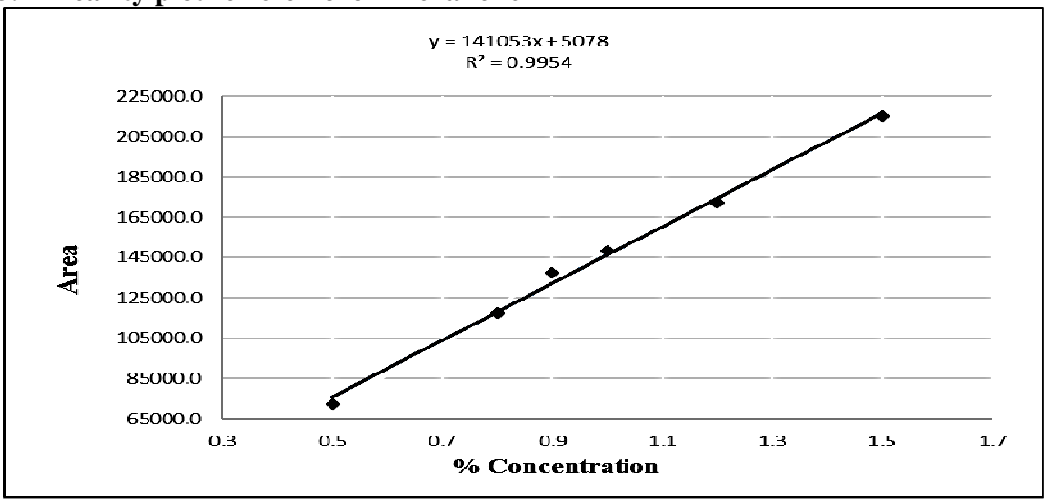


Table 5: Linearity summary for Pentoxifylline genotoxic impurities

Sample Name	Response Area		
	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
Linearity -50%	49555	53192	72089
Linearity - 80%	87365	76173	117673
Linearity -100%	104754	90445	148279
Linearity -120 %	128952	108466	172111
Linearity -150 %	169804	130406	215030
Correlation coefficient	**0.995**	**0.994**	**0.995**

***RECOVERY* (Accuracy) :** Pentoxifylline was spiked with 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone at three different levels of 50%, 100%, and 150% of the specifications in triplicate (in total twelve determinations) and preceded according to the Sample Preparation described in Methodology (table-6-8). Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels. The Mean Recovery for all components is within limits. Therefore, the GC method for the determination of 3-chloro-1-propanol,1-bromo-3-chloropropane, and 6-chloro-2-hexanone in Pentoxifylline is accurate[37].

Table 6: Recovery of 3-chloro-1-propanol

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 50% -1	0.04900	0.05163	105.40
Accuracy 50% -2	0.04900	0.04918	100.40
Accuracy 50% -3	0.04900	0.04776	97.50
Accuracy 100% -1	0.09800	0.09660	98.60
Accuracy 100% -2	0.09800	0.10094	103.00
Accuracy 100% -3	0.09800	0.09767	99.70
Accuracy 150% -1	0.14700	0.15319	104.20
Accuracy 150% -2	0.14700	0.15094	102.70
Accuracy 150% -3	0.14700	0.15849	107.80
	Mean	**102.14**	
	SD	**3.373**	
	% RSD	**3.302**	

Table 7: Recovery of f 1-bromo-3-chloropropane

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 50% -1	0.04950	0.04911	99.20
Accuracy 50% -2	0.04950	0.04930	99.60
Accuracy 50% -3	0.04950	0.05031	101.60
Accuracy 100% -1	0.09900	0.09595	96.90
Accuracy 100% -2	0.09900	0.09924	100.20
Accuracy 100% -3	0.09900	0.10172	102.70
Accuracy 150% -1	0.14850	0.14678	98.80
Accuracy 150% -2	0.14850	0.14456	97.30
Accuracy 150% -3	0.14850	0.15115	101.80
	Mean	**99.79**	
	SD	**1.996**	
	% RSD	**2.000**	

Table 8: Recovery of 6-chloro-2-hexanone

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Accuracy 50% -1	0.04850	0.05105	105.30
Accuracy 50% -2	0.04850	0.04663	96.10
Accuracy 50% -3	0.04850	0.05024	103.60
Accuracy 100% -1	0.09700	0.10043	103.50
Accuracy 100% -2	0.09700	0.09670	99.70
Accuracy 100% -3	0.09700	0.10024	103.30
Accuracy 150% -1	0.14550	0.15051	103.40
Accuracy 150% -2	0.14550	0.15165	104.20
Accuracy 150% -3	0.14550	0.15574	107.00
	Mean	**102.90**	
	SD	**3.202**	
	% RSD	**3.111**	

RUGGEDNESS Six Pentoxifylline sample preparations of the same lot of Pentoxifylline API are made and spiked with 3-chloro-1-propanol, 1-bromo-3-chloropropane and 6-chloro-2-hexanone at 100% levels by a different analyst, using different column on a different day and injected into a different GCHS using the method as described methodology, along with Standard preparation[38].

a) System Precision (Ruggedness): Six replicate injections of Pentoxifylline standard preparation will be made into the GC using the method as described under methodology.

Table 9: Ruggedness - System precision

Sr. No.	3-chloro-1-propanol		1-bromo-3-chloropropane		6-chloro-2-hexanone	
	RT	**Area**	**RT**	**Area**	**RT**	**Area**
1	2.70	105352	3.06	91450	4.34	154419
2	2.70	115964	3.06	92383	4.34	152895
3	2.70	111939	3.06	90118	4.34	151840
4	2.70	111810	3.06	92788	4.34	165708
5	2.70	114398	3.06	91124	4.34	155678
6	2.70	117426	3.06	91136	4.34	155946
Average	**2.70**	**112815**	**3.06**	**91500**	**4.34**	**156081**
Std. Dev.		**4269.83**		**961.76**		**4975.11**
RSD		**3.78**		**1.05**		**3.19**

Table 10: Ruggedness - 100% Spike of standard solution

Sample Injection	% Recovery		
	3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
100% recovery -1	105.40	101.20	104.80
100% recovery-2	102.30	101.90	105.30
100% recovery -3	100.50	103.40	103.40
100% recovery -4	102.10	103.80	107.80
100% recovery -5	102.80	100.40	107.80
100% recovery -6	105.50	103.70	103.90
Average	**102.73**	**102.17**	**104.50**
RSD	**2.41**	**1.10**	**0.94**

Table 11: Ruggedness - 100% Spike of standard solution (Analyst I &II)

Analyst No	Sample Injection	% Recovery		
		3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
Analyst I	100% recovery -1	98.10	104.90	105.10
	100% recovery-2	100.40	102.40	103.00
	100% recovery -3	102.30	102.20	99.00
	100% recovery -4	106.50	98.60	103.60
	100% recovery -5	104.20	100.70	106.60
	100% recovery -6	103.80	105.00	102.70
Analyst II	100% recovery -1	105.40	101.20	104.80
	100% recovery-2	102.30	101.90	105.30
	100% recovery -3	100.50	103.40	103.40
	100% recovery -4	102.10	103.80	107.80
	100% recovery -5	102.80	100.40	107.80
	100% recovery -6	105.50	103.70	103.90
	Overall Average	**102.83**	**102.35**	**104.42**
	Overall Std. Dev.	**2.4275**	**1.9233**	**2.4368**
	Overall RSD	**2.36**	**1.88**	**2.33**

The RSD of system precision (ruggedness) 3-chloro-1-propanol is 3.78%, 1-bromo-3-chloropropane is 1.05 % and 6-chloro-2-hexanone is 3.19 % respectively and it meets acceptance criteria. Therefore, the GC method for the determination of 3-chloro-1-propanol, 1-bromo-3-chloropropane and 6-chloro-2-hexanone in Pentoxifylline API is precise.

b) Method Precision (Ruggedness) : Five sample preparations of Pentoxifylline are to be prepared as the procedure given in the method and injected into the GC using the method as described under Methodology. Based on above study shows that the reported impurities of 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone in Pentoxifylline API is reproducible.

c) Intermediate Precision (Spike Study): Spike 3-chloro-1-propanol, 1-bromo-3-chloropropane, and 6-chloro-2-hexanone at a 100% concentration of specification level in Pentoxifylline substance and inject and process as per the methodology (Table-10-11). Mean recovery should be in the range of 90.0% to 110.0% for 100% levels. The Mean Recovery is within limits. Therefore, the GC method for the determination of 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol in Pentoxifylline is suitable to use in routine analysis. % recovery of all impurities for both Analyst are well with the defined specification for 6-chloro-2-hexanone is 2.33%, 1-bromo-3-chloropropane is 1.88% and 3-chloro-1-propanol is 2.36% hence the GC method for the determination of 6-chloro-2-hexanone, 1-bromo-3-chloropropane and 3-chloro-1-propanol in Pentoxifylline API is reproducible.

***ROBUSTNESS*:** Prepared samples at a 100% concentration of specification level in the sample and injected and processed as per the methodology. Separately standard preparations containing a concentration of 100% specification level of 6-chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol are also to be prepared and injected and used for quantification of impurities in the sample a) As such method, b) Change in flow rate (±2%)

Table 12: Robustness - 100% Spike of standard solution (With different Analyst and change in condition)

Analyst No	Sample Injection	% Recovery		
		3-chloro-1-propanol	1-bromo-3-chloropropane	6-chloro-2-hexanone
Analyst –I (as such method)I	100% recovery -1	98.10	104.90	105.10
	100% recovery-2	100.40	102.40	103.00
	100% recovery -3	102.30	102.20	99.00
	100% recovery -4	106.50	98.60	103.60
	100% recovery -5	104.20	100.70	106.60
	100% recovery -6	103.80	105.00	102.70
Analyst –II (change in flow)	100% recovery -1	92.80	95.00	102.50
	100% recovery-2	98.00	97.40	97.50
	100% recovery -3	106.50	98.50	96.60
	100% recovery -4	102.30	97.90	100.20
	100% recovery -5	104.90	92.50	101.60
	100% recovery -6	101.90	99.30	100.70
	Overall Average	**101.80**	**99.53**	**101.59**
	Overall Std. Dev.	**3.9903**	**3.7490**	**2.9629**
	Overall RSD	**3.92**	**3.77**	**2.92**

CONCLUSION: Three genotoxic impurities may be present in the synthesis of Pentoxifylline. The proposed method was found to be specific, accurate, linear, precise, and very useful for monitoring three genotoxic impurities -chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol in pharmaceutical analysis. The **%** recovery of all impurities for both Analyst with different chromatographic conditions are well with the defined specification for 6-chloro-2-hexanone is 2.92%, 1-bromo-3-chloropropane is 3.77% and 3-chloro-1-propanol is 3.92% hence the GC method for the determination of 6-chloro-2-hexanone, 1-bromo-3-chloropropane and 3-chloro-1-propanol in Pentoxifylline API is robust. The purpose of this study is to quantify and qualify suitable methods and threshold levels of the three genotoxic impurities that may be present in the synthesis of Pentoxifylline. A gas chromatographic technique with an FID detector was adopted for suitable quantification of these impurities. The proposed method was found to be specific, accurate, linear, precise, and very useful for monitoring three genotoxic impurities -chloro-2-hexanone, 1-bromo-3-chloropropane, and 3-chloro-1-propanol in pharmaceutical analysis. Genotoxic alerts on structures of these impurities were confirmed by the Genotoxic Predication report.

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