IJPSR (2020), Volume 11, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 October 2019; received in revised form, 04 March 2020; accepted, 22 March 2020; published 01 October 2020

DEVELOPMENT AND VALIDATION OF GC-MS METHOD FOR THE TRACE LEVEL DETERMINATION OF STRUCTURALLY ALERT ALKYL HALIDE IMPURITIES IN CILASTATIN SODIUM DRUG SUBSTANCE

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Keywords:

Cilastatin sodium, Genotoxic impurities, GCMS, Alkyl halides, Structural alert

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ABSTRACT: A Simple and trace level-sensitive Gas chromatography with mass spectrometer (GCMS) method was developed, optimized and validated for the determination of seven potentially genotoxic impurities which are having structural alerts *i.e.,* 1-Bromo-5-chloropentane (*PGI-1*), Ethyl-6-chloro-2-oxo hexanoate (*PGI-2*), Methyl-7-chloro-2-oxo heptanoate (*PGI-3*), Ethyl-7-chloro-2-oxo heptanoate (*PGI-4*), Ethyl-7-bromo-2-oxo heptanoate (*PGI-5*), Ethyl-8-chloro-2-oxo octanoate (*PGI-6*), Ethyl-2-acetyl-7-chloro heptanoate (*PGI-7*) contents in Cilastatin sodium drug substance. The chromatographic separations of seven genotoxic impurities were achieved on ZB-5 MS [5% Polysilarylene, 95% Poly-dimethylsiloxane copolymer capillary column of 30 m length, 0.32 mm internal diameter, film thickness 1.0 µm]. The analytic method has been demonstrated through method validation experiments w.r.t ICH guidelines, like specificity, linearity, Limit of Detection, Limit of Quantification, method precision and accuracy (recovery) parameters with allowable TTC limit. The achieved limit of detection (LOD) values was 3 µg/g. This developed method was found to be linear with correlation coefficient is greater than 0.999. Developmental and validation experiments were discussed in detail in this paper.

INTRODUCTION: Cilastatin sodium is the inhibitor of the renal dipeptidase, dehydro-peptidase, which is used to reduce the development of drug-resistant bacteria and should be used only to treat or prevent infections that are proven and strongly suspected to caused by bacteria.

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DOI: 10.13040/IJPSR.0975-8232.11(10).5017-26

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5017-26

The chemical name of Cilastatin sodium drug substance is (Z)-7-[[(*R*)-2-amino-2-carboxyethyl] thio]- 2- [(*S*)-2, 2-dimethylcyclopropane carboxa-mido]-2-heptenoate, which has formulated with Imipenem and marketed as PRIMAXIN I.V and it is initially approved in the year 1985. The latest medical prescribed label appeared in year 2016 [1].

In this formulated product, Imipenem has the widest spectrum of antimicrobial activity of currently available beta-lactam agents and, in contrast to other beta-lactam antibiotics [2]. Cilastatin may be a promising agent for inhibiting various forms of drug-induced nephrotoxicity mediated

via megalin in the clinical setting [3]. Imipenem and Cilastatin sodium for injection is used for the treatment of serious infections caused by susceptible strains of the designated micro-organisms with shorter duration (*i.e.* administered less than 1 month). Imipenem/cilastatin and meropenem have been studied in comparative clinical trials establishing their efficacy in the treatment of a variety of infections including complicated intra-abdominal infections, skin and skin structure infections [4]. The chemical structure of Cilastatin sodium has shown in **Fig. 1**.

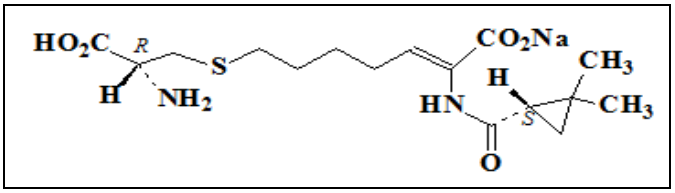

FIG. 1: CHEMICAL STRUCTURE OF CILASTATIN SODIUM DRUG SUBSTANCE

Ethyl-7-chloro-2-oxo heptanoate (*PGI-4*) is one of the key starting materials for the preparation of Cilastatin sodium drug substance. Based on PGI-4 route of synthesis, PGI-1 is a raw material and PGI-7 is intermediate, and the remaining possible analogue and homologue impurities were considered.

The seven potential genotoxic impurities bearing structurally alert alkyl halides *i.e.* 1-Bromo-5-chloropentane (*PGI-1*), Ethyl-6-chloro-2-oxo hexa-noate (*PGI-2*), Methyl-7-chloro-2-oxo heptanoate (*PGI-3*), Ethyl-7-chloro-2-oxo heptanoate (*PGI-4*), Ethyl-7-bromo-2-oxo heptanoate (*PGI-5*), Ethyl-8-chloro-2-oxo octanoate (*PGI-6*), Ethyl-2-acetyl-7-chloro heptanoate (*PGI-7*). The synthesis of PGI-4 is given in **Fig. 2**, the chemical structures of seven impurities are shown in **Fig. 2**, and these PG impurities are shown in Figure **Fig. 3**.

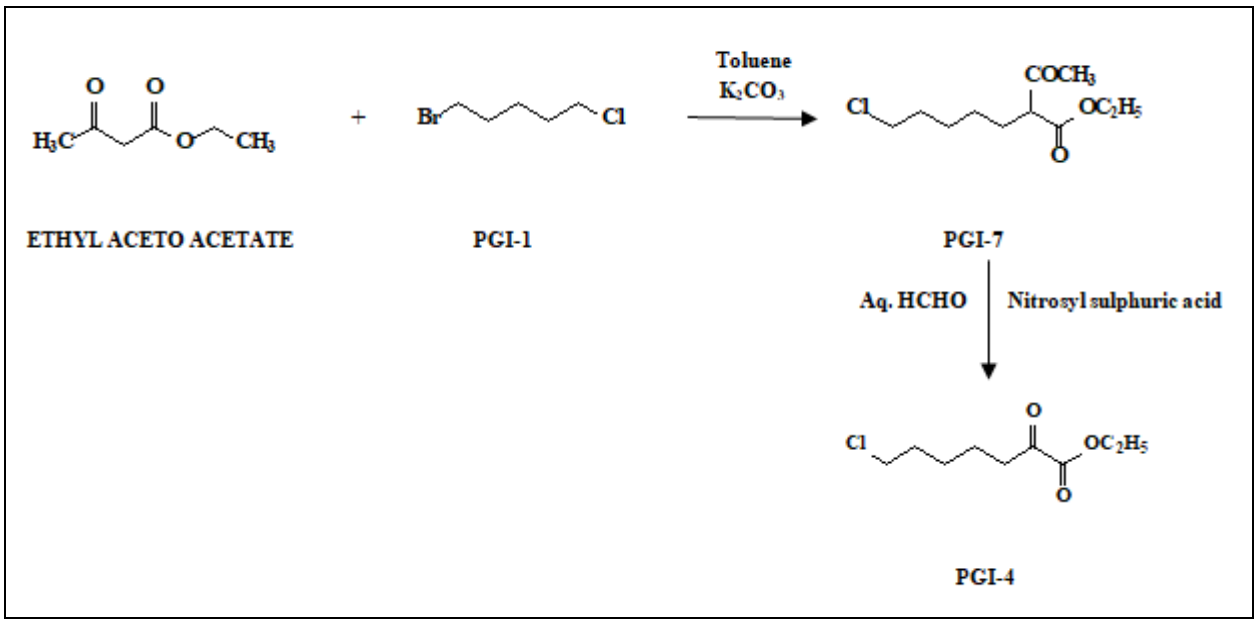

FIG. 2: SYNTHETIC PROCESS OF PREPARATION OF PGI-4

The toxicological assessment of genotoxic impurities is important in the regulatory framework for the pharmaceutical industry check. In this perspective, the application of promising computational methods (*e.g.* Quantitative Structure-Activity Relationships (QSARs), Structure-Activity Relationships (SARs) and/or expert systems) for the evaluation of genotoxicity is required, especially when very limited information on impurities is available. Derek Nexus (a knowledge-based, expert decision support system for the prediction of toxicity) and Sarah Nexus (A statistical software tool for the prediction of mutagenicity) software applications were used for the confirmation of mutagenic activity for selected impurities according to regulatory submissions under the ICH M7 guideline [5]. Alkyl halides are electrophilic species that are capable of directly alkylating DNA. Consequently, many compounds are mutagenic in the Ames test in the presence and absence of S9 mix, notably in Salmonella typhimurium strains TA100 and TA1535 [6-7].

In the pharmaceutical industry, a molecule bearing an alkyl chloride moiety is normally flagged by the most commonly used *in-silico systems*, and consequently, an Ames assay test is carried out. If the compound shows mutagenic activity, potential genotoxic carcinogenicity is assumed; further staged TTC concept is applied [4]. The TTC-based acceptable intake of 1.5µg/day is considered to be protective for a lifetime of daily exposure. It is noted that established cancer risk assessments are based on life time exposures [8].

The alert has demonstrated from the predicted ICH M7 classification report, and these PGI's are classified as Class-3 with respect to mutagenic, carcinogenic potential and leads to resulting control actions against Cilastatin sodium drug moiety. According to ICH M7, Class-3 impurities have to be controlled at low or below acceptable limit (as per appropriate TTC limit). The details of seven impurities and their ICH M7 Classification report are noticed in **Table 1**.

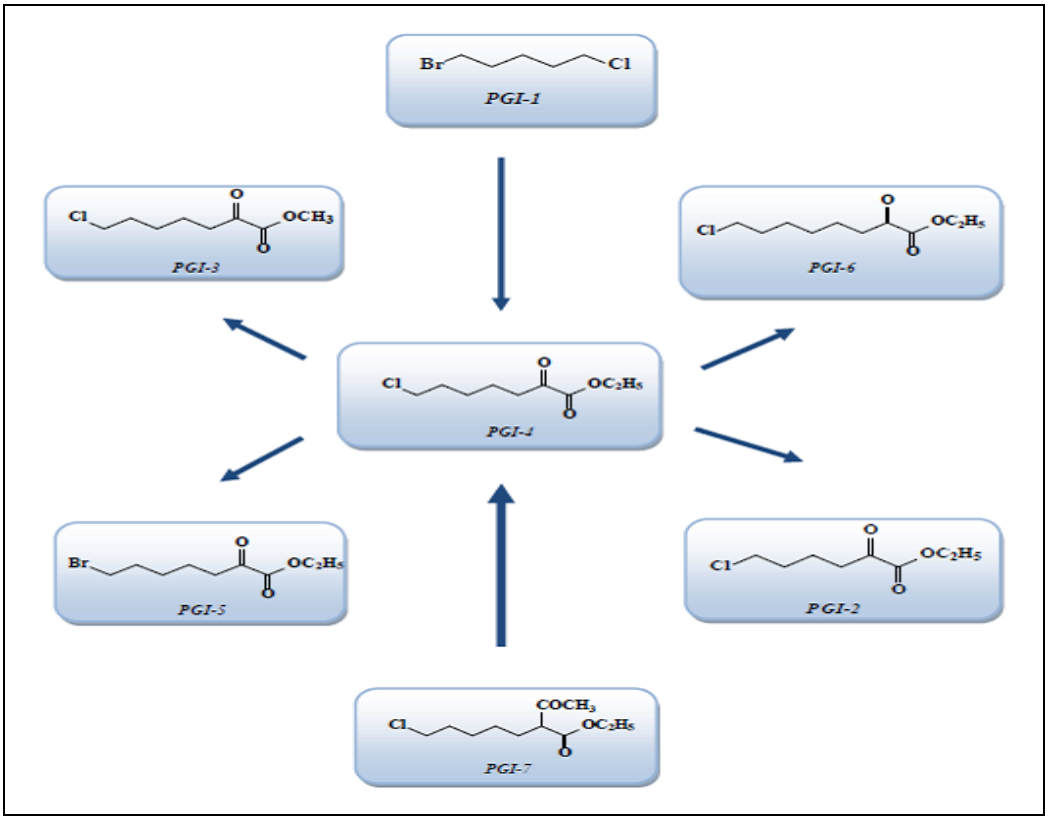

FIG. 3: CHEMICAL STRUCTURES OF SEVEN PG IMPURITIES

TABLE 1: PGI'S DETAILS AND ICH M7 CLASSIFICATION REPORT

Impurity	Impurity name	ICH Prediction[1]					Specification level (µg/g)[2]
		Derek	Sarah	Experimental Data	Similarity to API	Class	
PGI-1	1-Bromo-5-chloropentane	Plausible: Alkylating agent	Positive (27%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-2	Ethyl-6-chloro-2-oxo hexanoate	Plausible: Alkylating agent	Negative (30%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-3	Methyl-7-chloro-2-oxo heptanoate	Plausible: Alkylating agent	Negative (28%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-4	Ethyl-7-chloro-2-oxo heptanoate	Plausible: Alkylating agent	Negative (30%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-5	Ethyl-7-bromo-2-oxo heptanoate	Plausible: Alkylating agent	Equivocal	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-6	Ethyl-8-chloro-2-oxo octanoate	Plausible: Alkylating agent	Negative (30%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30
PGI-7	Ethyl-2-acetyl-7-chloro heptanoate	Plausible: Alkylating agent	Negative (61%)	Carc: Unspecified Ames: Unspecified	Alert(s) not found in API	Class 3	Maximum 30

[1]Predicted from Lhasa limited, Nexus: 2.2.1 version, Derek Nexus: 6.0.1 version, [2]By considering LTL exposure as per ICH M7

Historically, research scientists have tended to rely on the volatility of alkyl halides and developed GC methods with FID (Flame ionization detector). But now a days, for more sensitivity purpose, combination with high sensitive and selective detection techniques like GC-MS, LC-MS or GC with ECD (Electron capture detector) *etc*., are using for the determination of genotoxic impurities. There are some literature are available for existing methodologies to determine alkyl halides by different techniques namely, HPLC UV derivitization [9], supercritical fluid Chromatography (SFC) [10], gas chromatography with electron impact ionization detector [11] and some other review articles [12]. In view of importance of toxicology concept, Gas Chromatography with Mass spectrometer (GC-MS) was chosen to develop a new single method and trace level analysis for seven PGI's in Cilastatin sodium drug substance. To the best of our knowledge, determination of these impurities by GC-MS has not been reported in literature till date. This research paper describes the development, optimization, and validation of GC-MS method for these impurities.

EXPERIMENTAL:

Chemicals, Reagents and Samples: Analytical grade reagents Undecane, Dodecane, *tert*-butyl methyl ether (or) Methyl *tert*-butyl ether, Sodium chloride, Methanol, Ethanol, Acetone, Mesityl oxide, Ethyl acetoacetate, Toluene, Methylene chloride, Diacetone alcohol, Acetonitrile, tertbutyl-amine, Methyl chloride, and Benzene were procured from Merck, India. Water (HPLC grade) was used in this work.

Cilastatin sodium drug substance and impurities namely 1-Bromo-5-chloropentane (*PGI-1*), Ethyl-6-chloro-2-oxo hexanoate (*PGI-2*), Methyl-7-chloro-2-oxo heptanoate (*PGI-3*), Ethyl-7-chloro-2-oxo heptanoate (*PGI-4*), Ethyl-7-bromo-2-oxo heptanoate (*PGI-5*), Ethyl-8-chloro-2-oxo octa-noate (*PGI-6*), Ethyl-2-acetyl-7-chloro heptanoate (*PGI-7*) were gifted from APL Research Centre-II (A division of Aurobindo Pharma Ltd., Hyderabad).

Gas Chromatograph System with Mass Spectrometer (GCMS): The method was developed and validated on gas chromatograph system with mass spectrometer (GCMS), Agilent Technologies 7890B coupled with 5977A quadrupole mass selective detector (MSD) and GC sampler 80 (Auto sampling unit) (Make: Agilent Technologies, Santa clara, CA, USA). The data handling system was MASS HUNTER version 0704 to monitor the output signals and for processing.

Chromatographic Conditions and Methodology: Column: ZB-5 MS [5% Polysilarylene and 95% Polydimethylsiloxane copolymer capillary column of 30 m length, 0.32 mm Internal diameter and film thickness 1.0 µm], Detector-Mass spectrometer, Carrier gas-Helium, Purge gas - Nitrogen, Column flow-1.0 ml/min, Split- 1:20, Run time-40 min, Capillary injector-230 ℃, Injection volume-1.0 µL.

Column oven temp. : 80°C (5min) $\xrightarrow{10°C/min.}$ 240°C (19 min.)

MS Parameters: MS source temperature-230°C, MS quad temperature-150 ℃, MSD transfer line temperature- 240 °C, Detector voltage- delta EMV, ionization mode -EI. SIM time segments for GCMS, dwell time-20ms, resolution mode-low. In order to MS sensitivity point, to decrease overload of sample matrix on detector, MS diversion of detector maintained on-time 7 min and off-time 21 min. The quantification* and qualifier** ions w.r.t each analyte, internal standards, standard analytes fragment ions and mass spectrums of respected impurities are shown in **Fig. 4**.

SIM TIME SEGMENTS

	PGI-1	PGI -2	PGI -3	PGI -4	PGI -5	PGI -6	PGI -7	Undecane	Dodecane
Quantification ion*	69	119	133	133	69	147	130	71	71
Qualifier ion**	104	91	69	69	177	83	110	85	85

Preparation of Solutions:

0.2M Sodium Chloride Solution: Accurately weighed and transferred about 1.16 g of Sodium chloride into a 100 ml clean, dry volumetric flask, dissolved and made up to volume with water.

Internal Standard Solution: Accurately weighed and transferred about 0.075 g of Undecane and 0.030 g of Dodecane into a 20 ml clean, a dry volumetric flask containing about 10 ml of Methyl *tert*-butyl ether, and made up to volume with

methyl *tert*-butyl ether. Diluted 1.0 ml of this solution to 25 ml with methyl *tert*-butyl ether. Further diluted 2.0 ml of this solution to 250 ml with methyl *tert*-butyl ether.

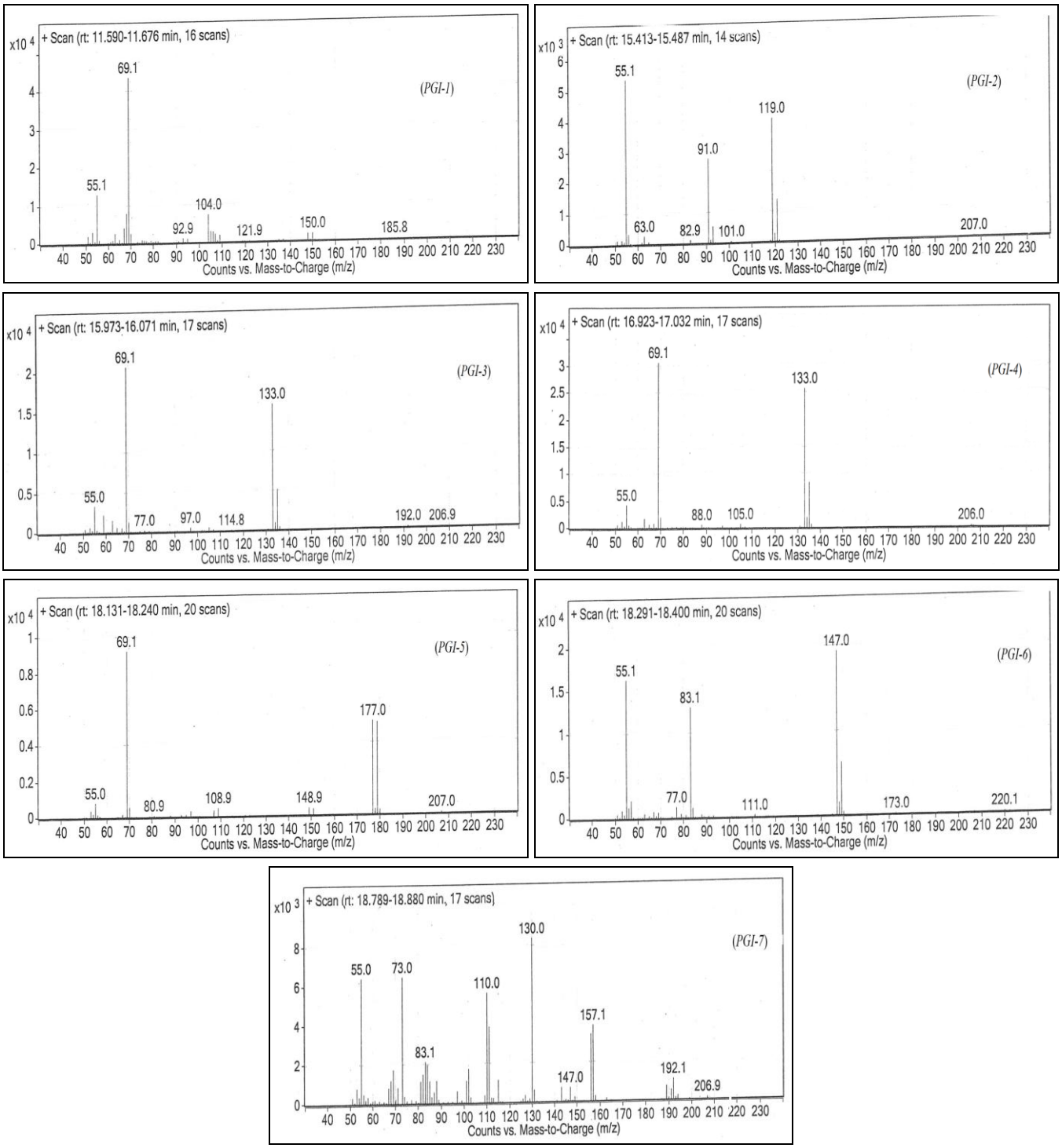

FIG. 4: MASS SPECTRUMS OF RESPECTED IMPURITIES

Blank Solution: Transferred 3.0 ml of 0.2M Sodium chloride solution into a clean and dry glass centrifuge tube. Added 2.0 ml of internal standard solution and vortex the centrifuge tube for 1 min. Allowed the two phases to separate. Collected the upper layer (Methyl *tert*-butyl ether layer) for injection.

Standard Solution: (Prepare in Six Replicate): Accurately weighed and transferred about 0.050 g each of 1-Bromo-5-chloropentane, Ethyl-6-chloro-2-oxo hexanoate, Ethyl-8-chloro-2-oxo octanoate, Ethyl-2-acetyl-7-chloro heptanoate, Ethyl-7-bromo -2-oxo heptanoate, Ethyl-7-chloro-2-oxo hepta-noate and Methyl-7-chloro-2-oxo heptanoate into a

20 ml clean, dry volumetric flask containing about 10 ml of internal standard solution, mixed and made up to volume with an internal standard solution. Diluted 1.5 ml of this solution to 50 ml with an internal standard solution. Further diluted 1.0 ml of this solution to 50 ml with an internal standard solution. (Note: The weight of each standard is to be adjusted to get the final concentration about 1.5 µg/ml, based on the purity of the reference standard used).

Transferred 3.0 ml of 0.2M Sodium chloride solution into a clean and dry glass centrifuge tube. Added 2.0 ml of Standard solution and vortex the centrifuge tube for 1 min. Allowed the two phases to separate. Collected the upper layer (Methyl *tert*-butyl ether layer) for injection.

Sample Solution: Accurately weighed and transferred about 0.1 g of sample into a clean and dry glass centrifuge tube. Added 3.0 ml of 0.2M Sodium chloride solution and 2.0 ml of internal standard solution and vortex the centrifuge tube for 1 min. Allowed the two phases to separate. Collected the upper layer (Methyl *tert*-butyl ether layer) for injection.

System Suitability Criteria: RSD for the ratio of peak area of 1-Bromo-5-chloropentane, Ethyl-6-chloro-2-oxo hexanoate, Ethyl-8-chloro-2-oxo octanoate, Ethyl-7-chloro-2-oxo heptanoate, Ethyl-7-bromo-2-oxo heptanoate, and Methyl-7-chloro-2-oxo heptanoate to the peak area of internal standard (Undecane) for six injections of the standard solution is not more than 10.0%. RSD for the ratio of peak area of Ethyl-2-acetyl-7-chloro heptanoate to the peak area of internal standard (Dodecane) for six injections of the standard solution is not more than 10.0%.

RESULTS AND DISCUSSION:

Method Development and Optimization: Solubility profile of cilastatin sodium has checked as this is essential requirement for initiating method development activity. As the material is highly hygroscopic, the solubility profile is complicated in selected solvents (*i.e.* methanol, methylene chloride, methyl tert-butyl ether, acetonitrile, cyclohexane, acetone, dimethyl sulfoxide, *N*-methyl-2-pyrrolidine, and water) and landed to give background noise. Further during decomposition in gas chromatography due to inlet high temperature was affected the method development. As a result the separation and reproducibility was not achieved properly. To avoid such type of difficulties in development, liquid-liquid extraction (LLE) technique has been chosen. To implement this technique, water was selected as cilastatin sodium is very soluble in water. Methyl *tert*-butyl ether has chosen as another solvent for extraction technique.

Initially for extraction technique, studies were performed with cyclohexane and methylene chloride solvents. Where, they found to be low response in cyclohexane and difficulty of extracting with methylene chloride for analyte peaks from aqueous layer observed. In the view of these observations, water and methyl *tert*-butyl ether has been finalized for the liquid-liquid extraction technique. The selection of solvents for liquid-liquid extraction technique based on their solubility difficulties, experimental observations, and advantages are given in **Table 2**.

TABLE 2: SOLVENTS SELECTION FOR THE CILASTATIN DRUG SUBSTANCE IN LIQUID-LIQUID EXTRACTION TECHNIQUE

Solvent	Solubility	Observation	Selection
Water	Soluble	Good solubility with drug substance and extracted by other solvents like cyclohexane, methyl *tert* -butyl ether and methylene chloride *etc*.,	✓
Methanol	Soluble	Background noise is may high(with sample) and cannot be extracted by other solvents like water, Methyl *tert*-butyl ether and methylene chloride etc.,	✗
Methylene chloride	Not soluble	The extraction procedure is difficult due to pippetout lower layer, as this solvent extracted by aqueous medium	✗
Methyl tert- butyl ether	Not soluble	The lower layer is aqueous medium, the upper layer is organic media (i.*e*. Methyl *tert*-butyl ether) and easily pippetout media, the solvent can be extracted by aqueous medium and not observed any (with sample) background noise in chromatograms	✓
Acetonitrile	Not soluble	Due to non-extractability of this solvent with water, it is not suitable for liquid-liquid extraction technique	✗
Cyclohexane	Not soluble	The lower layer is aqueous medium and upper is organic media (i.e. Cyclohexane), but all seven analytes responses are very low when compared to Methyl *tert*-butyl ether and Methylene chloride solvents extraction.	✗

Isotopic analogs of the analytes are preferred as internal standards for quantitative analysis. Due to unavailability isotopic analogs of each analyte and higher economic values of these isotopes, undecane and dodecane have been chosen as internal standards as these two hydrocarbon alkanes not have any active groups like hydroxyl, amino, nitro etc., and therefore no discernible problems in chromatographic systems and found symmetric peaks with good shapes; also no interference observed.

Further different GC columns have been used for better separation of analytes *i.e.* HP 50+ (Make: Agilent J&W) column [*i.e.* 50%-phenyl)-methyl-polysiloxane phase, 30m length, 0.53mm Internal diameter and film thickness 1.0µm] has been used for trails as this column is generally used for polar compounds analysis. By using optimized GC parameters with this column some of the method development trails have been conducted to separate all analytes. Unfortunately out of seven analytes, few analytes show low response and remaining were not eluted. In virtue of targeted resolution, ZB-5 MS, capillary column (*i.e.* 5% Polysilary-lene, 95% Polydimethylsiloxane copolymer capillary column of 30m length, 0.32mm Internal diameter and film thickness 1.0 µm (Make: Phenomenex, Torrance, CA, USA) has been chosen and trail experiments revealed that all the analytes were well separated and got good responses w.r.t chromatographic norms. Finally, GC chromato-graphic conditions, solution concentrations, stationary phase, and liquid-liquid extraction solvents were optimized and finalized method has been validated in accordance with ICH and FDA guidelines [13].

Method Validation: The optimized method was established through the validation experiments per the ICH guidelines, individually in terms of specificity or selectivity, LOD, LOQ, linearity, accuracy, and precision (system precision and method precision).

Specificity: Specificity is the ability of the method to determine the individual analyte in the presence of other related substances of the drug substance. For specificity determination, all individual analyte solutions were prepared and injected individually to confirm retention time and solutions of Cilastatin sodium drug substance (Control sample), Cilastatin sodium drug substance spiked with analytes at specification level (Spiked sample) and Cilastatin sodium drug substance spiked with analytes and all other known residual solvents at specification level (All spiked sample) were injected to confirm any interference of other solvent peaks with analyte peaks. From the chromatogram of individual injections of analytes, Control sample (Cilastatin sodium drug substance), Spiked sample (Cilastatin sodium drug substance spiked with analytes), All spiked sample (Cilastatin sodium drug substance spiked with analytes and all other known residual solvents), it is observed that there is no interference from the other solvent peaks at the retention time of analyte peaks indicated that the method is selective and specific. The specificity experiment chromato-grams are given in **Fig. 5a, 5b and 5c**.

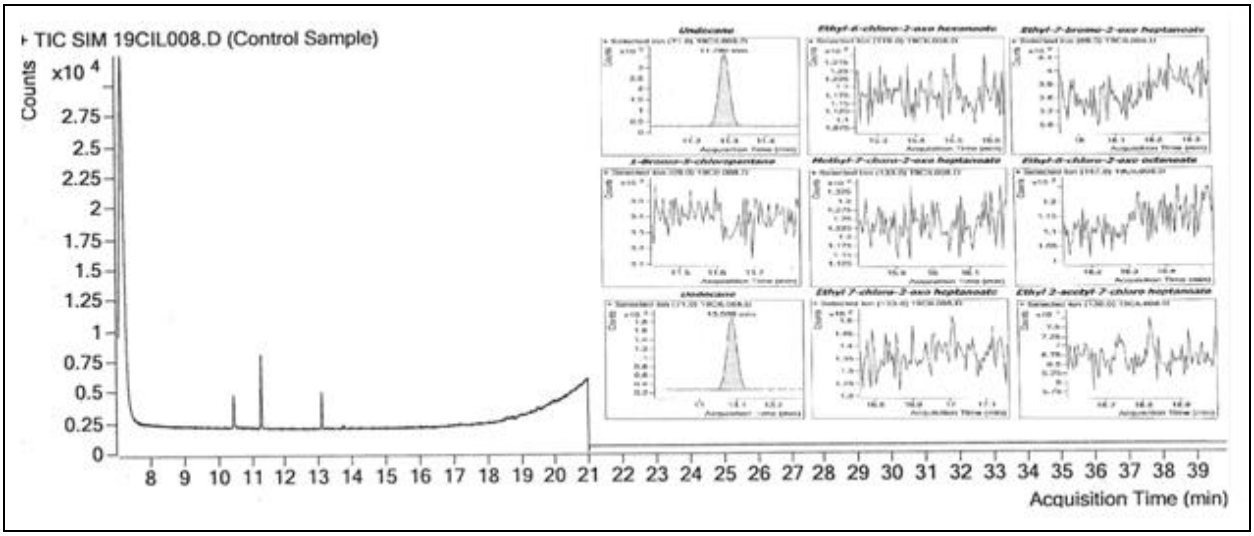

FIG. 5A: A TYPICAL GC-MS CHROMATOGRAM OF CONTROL SAMPLE

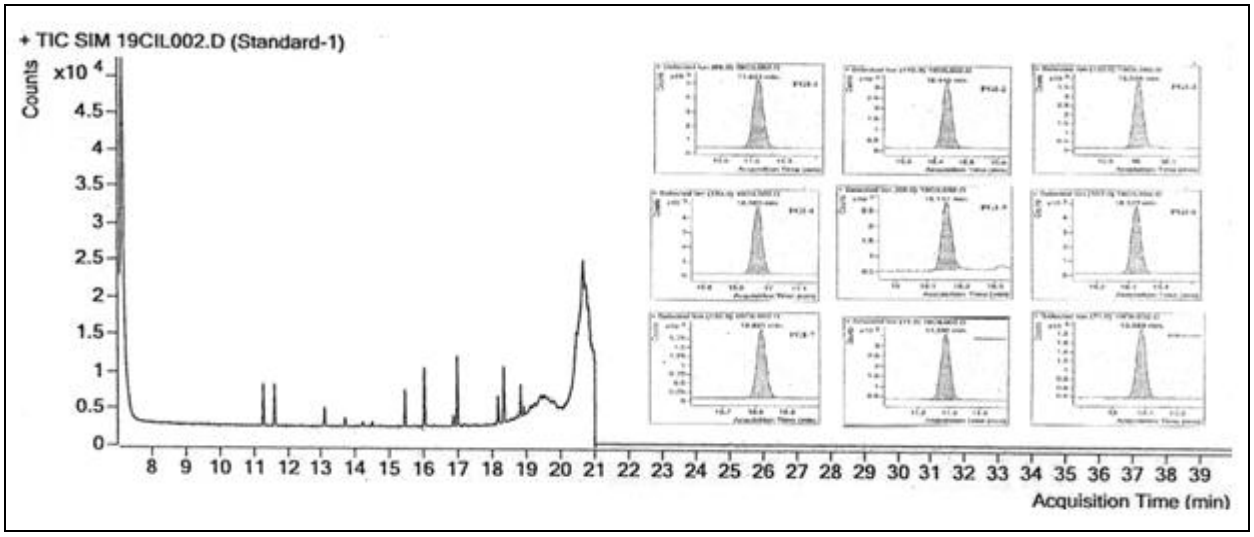

FIG. 5B: A TYPICAL GC-MS CHROMATOGRAM OF STANDARD

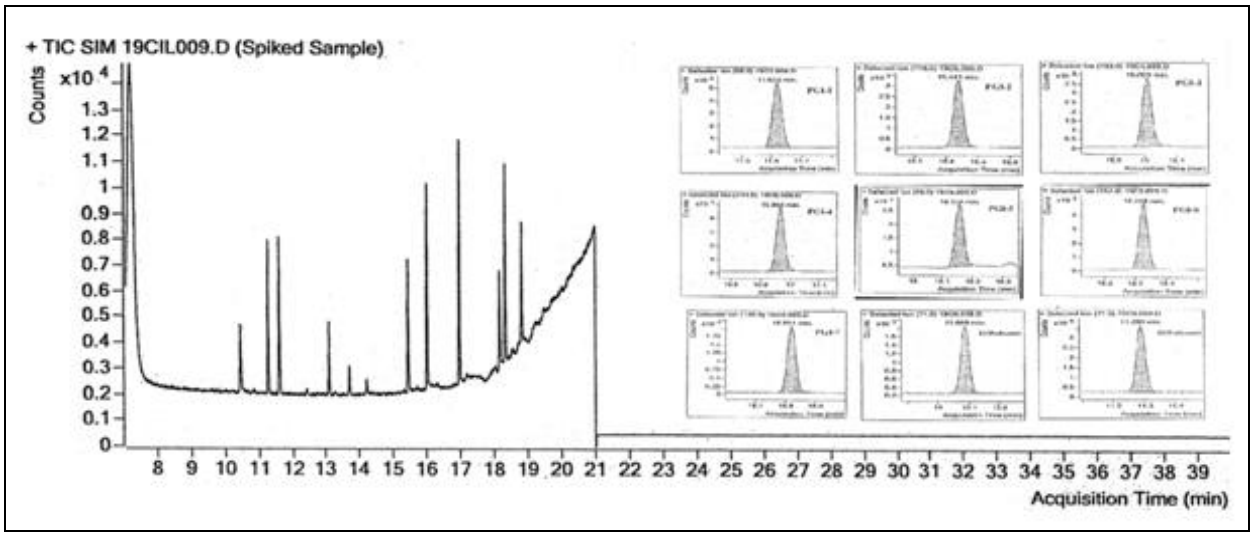

FIG. 5C: A TYPICAL GCMS CHROMATOGRAM OF SPIKED SAMPLE

LOD and LOQ: For determining the limit of detection (LOD) and limit of quantification (LOQ), the visual evaluation method was adopted and predicted the LOD and LOQ values. Each predicted concentration was verified for precision by preparing the solutions at about these predicted concentrations and injected each solution six times into the GCMS. Obtained LOD and LOQ values are reported in **Table 3**. Based on obtained results, the method is precise for the quantification of analytes in cilastatin sodium drug substance.

Linearity: The linearity of the detector was determined by preparing a series of solutions using all analytes at concentration levels from about LOQ level to 45 µg/g, *i.e.*, 150% of specification level. The data were subjected to statistical analysis by using a linear regression model. The statistical evaluations like slope, intercept, STEYX, and correlation coefficient values of linearity data is given in **Table 3**. Obtained correlation coefficient values were found to be more than 0.990 for all analytes. Hence, the responses of analytes are linear from LOQ to 150% of specification levels.

Accuracy: Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries were determined by spiking analytes at four different levels at LOQ level, 15, 30, and 45 µg/g into Cilastatin sodium drug substance. These samples were prepared and analyzed in triplicate, and the percentage of recoveries were calculated. The % recovery values for analytes were calculated and revealed that the GCMS method is accurate for the determination of analytes in cilastatin drug substances from LOQ level to 150% of the specification level. The fully validated accuracy results are shown in **Table 3**.

Precision: System precision was demonstrated by preparing the standard solutions of individual analytes and injected six times into GCMS. % RSD values of the ratio of peak areas of analytes against their internal standards for six injections of the standard solution was calculated and found to be less than 2.8 for all analytes.

Hence, the system is precise. Method precision was demonstrated by preparing six sample solutions individually using a single batch of cilastatin sodium drug substance spiked with analytes at specification level (about 30 µg/g) and injected each solution and determined the content of analytes and found to be less than 2.3 for all analytes. Hence, the GCMS method is precise. Achieved results for system precision and method precision are reported in **Table 3**.

Range: Based on obtained results from linearity, method precision and accuracy experiments, it is revealed that the GCMS method works from LOQ level to 150% of the specification level.

TABLE 3: SUMMARY OF METHOD VALIDATION EXPERIMENTS

Impurity	Specificity RT (min)			LOD, LOQ & Linearity			Precision		Recovery (spiked with analytes)				
	Standard[1]	Spiked[2]	All spiked[3]	LOD (µg/g)	LOQ (µg/g)	Correlation Coefficient[4]	System precision[5]	Method Precision[6]	LOQ[7]	50%[7]	100%[7]	150%[7]	Mean (50% - 150%)
PGI-1	11.621	11.622	11.621	3	9	0.9998	0.8	0.7	100.4	101.3	98.2	97.9	99.2
PGI-2	15.440	15.441	15.441	3	9	0.9998	1.9	1.4	98.5	107.8	106.7	110.5	108.3
PGI-3	16.008	16.009	16.008	3	9	0.9995	2.1	1.6	97.3	105.5	105.0	109.7	106.7
PGI-4	16.969	16.969	16.970	3	9	0.9997	2.2	0.9	105.1	109.9	107.5	110.0	109.1
PGI-5	18.157	18.158	18.158	2.8	8.4	0.9996	2.4	2.2	101.2	106.6	106.1	110.8	107.8
PGI-6	18.327	18.328	18.327	3	9	0.9996	2.6	1.4	93.2	101.6	103.1	109.2	104.6
PGI-7	18.821	18.821	18.822	3	9	0.9998	2.7	1.4	90.3	100.7	100.8	106.0	102.5
Remarks	*There is no interference from the other solvent peaks at the retention time of analyte peaks & retention time of analytes obtained with Standard and test were comparable. [1]Includes IS (undecane and dodecane), undecane RT 11.290 min & dodecane RT 13.089min [2]Drug substance spiked with analytes [3]Drug substance spiked with analytes and other known solvents			*Experimental RSD values are obtained below 10.0% for LOQ and 33.0% for LOD. *The responses of analytes are linear from LOQ to 150% of specification levels. [4]Obtained from linearity Plot (Concentration *vs.* Area ratio).			*Experimental RSD values are obtained acceptable value below 10.0% [5]Standard solution six injections. [6]Six sample solutions (drug substance spiked with analytes at specification level)		*%Recovery values are obtained in the below acceptable range. (70.0% and 130.0% for LOQ level and 80.0% and 120.0% for other levels.) [7] Average from triplicate preparations				

CONCLUSION: The GC-MS method was developed, optimized and validated for the determination of 1-Bromo-5-chloropentane, Ethyl-6-chloro-2-oxo hexanoate, Ethyl-8-chloro-2-oxo octanoate, Ethyl-2-acetyl-7-chloro-heptanoate, Ethyl-7-bromo-2-oxo heptanoate, Ethyl-7-chloro-2-oxo heptanoate and Methyl-7-chloro-2-oxo heptanoate contents in Cilastatin sodium drug substance and the results of various validation parameters demonstrated that the methods are specific, sensitive, linear, precise and accurate and the method can be utilized into routine use.

ACKNOWLEDGEMENT: The authors gratefully acknowledge the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.), for allowing us to carry out the present work. The authors are also thankful to the colleagues of the Analytical Research Department and Chemical Research Department for their co-operation.

CONFLICTS OF INTEREST: There are no conflicts of interest.

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How to cite this article:
Raja KD, Ramana VSV, Babu KR, Babu BK, Kumar VJ, Kumar KSRP and Sharma HK: Development and validation of GC-MS method for the trace level determination of structurally alert alkyl halide impurities in cilastatin sodium drug substance. Int J Pharm Sci & Res 2020; 11(10): 5017-26. doi: 10.13040/IJPSR.0975-8232.11(10).5017-26.