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Quantification of Structurally Alert Mutagenic Impurities in Meropenem Trihydrate Drug Substance by Liquid Chromatography with High Resolution Mass Spectrometer (LC-HRMS)

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Abstract

Potential mutagenic impurities in Active Pharmaceutical Ingredient, Meropenem Trihydrate were assessed and a novel analytical method for their quantification was developed and validated. This Liquid Chromatographic method using High Resolution Mass Spectrometer (LC-HRMS) technique is proved to be suitable for simultaneous quantification of all ten identified impurities with required specificity, sensitivity, resolution, precision, accuracy, and other method characteristics as per ICH Guidelines. The acceptable limit of less than 2.9 µg/g was considered for evaluations, based on drug substance dosage and duration of treatment. The method stands most sensitive with a Limit of Detection of 0.35 µg/g, considering the challenge full acceptance criteria as per current regulatory standards.

Keywords

Mutagenic Impurities, LC-HRMS, Meropenem Trihydrate, Method Validation

1. Introduction

Meropenem drug is approved for use in complicated intra-abdominal infection (cIAI), complicated skin and skin structure infection (cSSSI) and bacterial me-

ningitis (in paediatric patients aged ≥ 3 months) in USA and in most other countries for nosocomial pneumonia, cIAI, septicaemia, febrile neutropenia, cSSSI, bacterial meningitis, complicated urinary tract infection (UTI), obstetric and gynecological infections, in cystic fibrosis patients with pulmonary exacerbations, and for the treatment of severe community-acquired pneumonia (CAP) [1]. Meropenem is the second carbapenem antibiotic available in the United States. It has a broad spectrum of therapeutic activity that includes moderate activity against gram-positive bacteria and excellent gram-negative aerobic and anaerobic activity [2]. This drug is marketed under the brand name MERREM IV (Meropenem for Injection), which is approved in the year 1996 in USA [3]. As this drug is indicated for bromo pulmonary infections in cystic fibrosis [4], the acceptable intake based on cumulative exposure will be >1 - 12 months duration, hence 20 μg/day acceptable intake for individual mutagenic impurity is applicable. The chemical structure of Meropenem Trihydrate is shown in **Figure 1**.

Some chemical species 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 [5]. In the pharmaceutical industry, a molecule bearing a structurally alert moiety is normally flagged by the most commonly used *insilico 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 [6]. The TTC-based general acceptable intake of 1.5 μg/day is protective for a lifetime of daily exposure. It is noted that established cancer risk assessments are based on lifetime exposures [6]. To address Less than lifetime exposure, for mutagenic impurities the use of a numerical cancer risk value (1 in 100,000) and potential risk has been identified for an impurity, an appropriate control strategy understanding as per ICH M7 [7] and analytical controls should be developed to ensure that mutagenic impurity is at or below the acceptable cancer risk level. The following mutagenic impurities are identified during risk assessment of Meropenem Trihydrate drug substance, and the impurities chemical names and chemical structures are given in **Table 1**.

Methyl vinyl phosphate and Meropenem side chain are used as raw materials in the preparation of Meropenem trihydrate synthesis. Methyl vinyl phosphate (MVP) is obtained from the coupled reaction of compounds MGI-4, MGI-10 and MGI-9, after that multiple syntheses steps gives the MVP, during this process MGI-2, MGI-6, MGI-8 and MGI-1 intermediates are formed. Further,

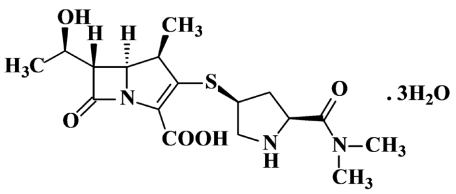
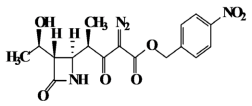
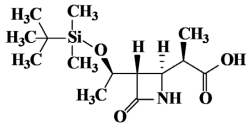
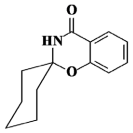
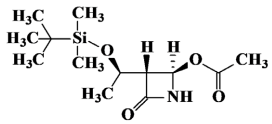
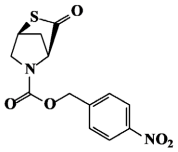
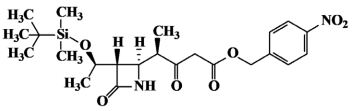
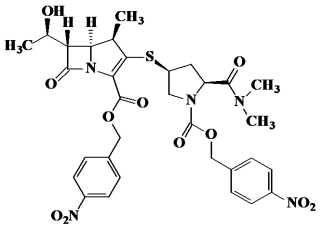
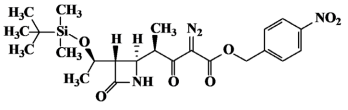
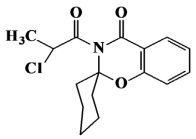
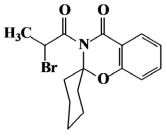


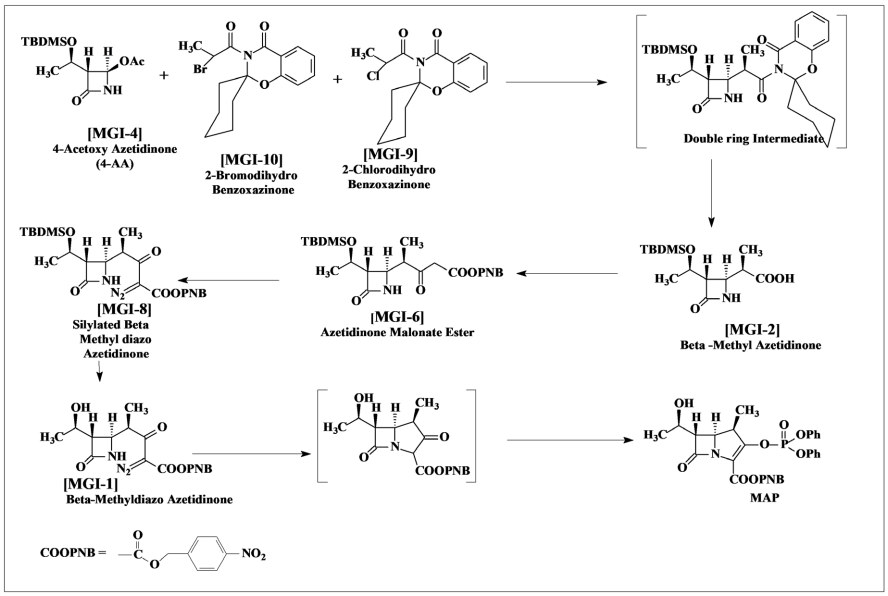
Figure 1. Chemical structure of Meropenem Trihydrate.

Table 1. Chemical structures of Mutagenic Impurities.

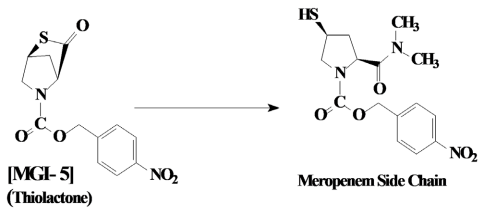
Impurity Code	Impurity Name	Chemical Name	Chemical Structure	RT in LCMS
MGI-1	*β*-Methyldiazo Azetidinone	(4-Nitrophenyl)methyl (*γ*R,2R,3S)-*α*-diazo-3-[(1R)-1-hydroxyethyl]-*γ*-methyl-*β*,4-dioxo-2-azetidinebutanoate		19.668
MGI-2	*β*-Methyl Azetidinone	(R)-2-((2S,3S)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl)propanoic acid		20.237
MGI-3	Dihydrobenzoxazinone	Spiro[benzo[e][1,3]oxazine-2,1'-cyclohexan]-4(3H)-one		22.050
MGI-4	4-Acetoxy Azetidinone	(2R,3R)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl acetate		23.850
MGI-5	Thiolactone	(1S,4S)-5-(4-nitrobenzyloxy-carbonyl)-2-thia-5-azabicyclo[2.2.1]heptane-3-one		24.515
MGI-6	Azetidinone Malonate Ester	(4-nitrophenyl)methyl (*γ*R,2R,3S)-3-[(1R)-1-[[(1,1-dimethylethyl) dimethylsilyl]oxy]ethyl]-*γ*-methyl-*β*,4-dioxo-2-azetidine butanoate		32.387
MGI-7	Diprotected Meropenem	4R,5S,6S)-4-nitrobenzyl 3-((3S,5S)-1-((4-nitrobenzyloxy)carbonyl)-5-(dimethyl carbamoyl)pyrrolidin-3-ylthio)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylate		32.550
MGI-8	Silylated *β*-Methyl diazo Azetidinone	4-nitrobenzyl (R)-4-((2R,3S)-3-((R)-1-((tert-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl)-2-diazo-3-oxopentanoate		34.096
MGI-9	2-Chlorodihydro Benzoxazinone	3-(2-chloropropanoyl)spiro[benzo[e][1,3]oxazine-2,1'-cyclohexan]-4(3H)-one		34.734
MGI-10	2-Bromodihydro Benzoxazinone	3-(2-bromopropanoyl)spiro[benzo[e][1,3]oxazine-2,1'-cyclohexan]-4(3H)-one		35.561

MGI-3 is byproduct during this process. In other hand, MGI-5 is key intermediate for the preparation of Meropenem side chain, source of mutagenic impurities is shown in Figure 2. MGI-7 (diprotected meropenem) is intermediate for the preparation of Meropenem, according to Expert, knowledge-based software (*i.e.* Derek Nexus) toxicity predictions [8], these 10 impurities are found to be mutagenic.

To develop and validate the possible potential mutagenic impurities in Meropenem drug substance, acceptance criteria 2.92 µg/g has been considered, this is derived from the maximum daily dose of 6g (*i.e.* equivalent to 6.84 g of Meropenem trihydrate) and 20 µg/day (*i.e.* consideration of short-term duration (>1 - 12 months). In view of TTC strategy, we have developed a new method for the quantification of ten potential genotoxic impurities in meropenem drug substance, Literature studies discloses that, research publications have tended to publish low threshold level determination of low molecular alkyl halides and nitro group compounds by GCMS & LCMS analysis [9] [10]. The main achievement of this LCMS method, all of ten impurities are well separated and validated, this validations are carried out as per ICH guideline [11] for regulatory requirements.



(a)



(b)

Figure 2. (a): Source of Mutagenic impurities from MVP; (b): Source of Mutagenic impurities from Meropenem side chain.

2. Experimental

2.1. Chemicals

The following reagent chemicals are used for analysis, Formic acid (LCMS grade), Ammonium formate (LCMS grade), Ammonium formate (LCMS grade), Methanol (LCMS grade). Water (LCMS grade).

2.2. Reference Standards and Samples

Meropenem trihydrate drug substances and impurities *β*-Methyldiazo Azetidinone (MGI-1), *β*-Methyl Azetidinone (MGI-2), Dihydrobenzoxazinone (MGI-3), 4-Acetoxy Azetidinone (MGI-4), Thiolactone (MGI-5), Azetidinone Malonate Ester (MGI-6), Diprotected Meropenem (MGI-7), Silylated *β*-Methyl diazo Azetidinone (MGI-8), 2-Chlorodihydro Benzoxazinone (MGI-9) and 2-Bromodihydro Benzoxazinone (MGI-10)are gifted from APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad).

2.3. LCMS Equipment & Parameters

Liquid Chromatography with High Resolution Mass Spectrometry (LC-HRMS) an Q-Exactive plus Orbitrap Mass Spectrometer, Make: Thermo Fisher Scientific, Model: Q Exactive plus connected to Vanquish UHPLC system with Chromeleon 7.2.10 software was used fwith Heated Electrospray Ionization (Hrobe operated in positive polarity. The source parameters are, Spray voltage: 3.8 K, Capillary temp: 250˚C, Sheath Gas: 55, Aux gas: 25, Probe Heater temp: 350˚C and S-Lens: 55, The MS parameters are, Polarity: Positive, Inclusion: ON, Microscans: 3, Resolution: 70000, AGC target: 2e5, maximum IT: 100 ms, MSX count: 1, Isolation window: 1.5 m/z, Isolation offset: 0.0 m/z, for ions monitoring, *β*-MethyldiazoAzetidinone, *β*-Methyl Azetidinone, Thiolactone, 4-Acetoxy Azetidinone and Dihydrobenzoxazinone (N)CE/stepped (N)CE value is 10, 25, 35 and for Azetidinone Malonate Ester, Diprotected Meropenem, Silylated *β*-Methyl diazo Azetidinone, 2-Chlorodihydro Benzoxazinone and 2-Bromodihydro Benzoxazinone impurities, (N)CE/stepped (N)CE value is 10, 35. Further, Divert valve A parameters are Used: True, Start 1 - 2: False, Switch count: 2, Element 1: At 15.0 min (switch to 1 - 6) and Element 2: At 40.0 min (switch to 1 - 2), the mass ionization inclusion are as follows:

Mass (m/z)	Formula	Polarity	Start (min)	End (min)	(N)CE	ID
391.12483	$C_{17}H_{18}N_4O_7$	Positive	15.00	28.00	10	*β*-MethyldiazoAzetidinone
302.17821	$C_{14}H_{27}NO_4Si$		15.00	28.00	10	*β*-Methyl Azetidinone
218.11756	$C_{13}H_{15}NO_2$		15.00	28.00	35	Dihydrobenzoxazinone
310.14451	$C_{13}H_{25}NO_4Si$		15.00	28.00	25	4-Acetoxy Azetidinone
309.05397	$C_{13}H_{12}N_2OS$		15.00	28.00	10	Thiolactone
479.22080	$C_{23}H_{24}N_2O_7Si$		28.00	38.00	35	Azetidinone Malonate Ester

Continued

698.21265	$C_{32}H_{35}N_5O_{11}S$	28.00	38.00	35	Diprotected Meropenem
505.21130	$C_{23}H_{32}N_4O_7Si$	28.00	38.00	10	Silylated *β*-Methyl diazo Azetidinone
308.10480	$C_{16}H_{18}ClNO_3$	28.00	38.00	10	2-Chlorodihydro Benzoxazinone
352.05428	$C_{16}H_{18}BrNO_3$	28.00	38.00	10	2-Bromodihydro Benzoxazinone
391.12483	$C_{17}H_{18}N_4O_7$	15.00	28.00	10	*β*-Methyldiazo Azetidinone
302.17821	$C_{14}H_{27}NO_4Si$	15.00	28.00	10	*β*-Methyl Azetidinone

2.4. Chromatographic Conditions

Mobile phase A consists of Ammonium formate solution (*i.e.* Dissolved 1.26 g of Ammonium Formate in 1000 ml of water and sonicate to dissolve and add 1.0 ml of Formic acid) and both Mobile phase B and diluent as Methanol.Analytical column: Kinetex Biphenyl 2.6 µm (150 mm × 4.6 mm) (Make: Phenomenex) is used for chromatographic separations. The following are test parameters, Pump mode: Gradient, Flow rate: 0.500 ml/min, Injection volume: 10 µl, Data aquation time: 45 min, Column temperature nominal: 40˚C and Sampler module Temperature nominal: 5˚C. The pump gradient program is follows:

Time (min)	Mobile Phase A (%, *v/v*)	Mobile Phase B (%, *v/v*)
$T_{0.0}$	50	50
$T_{5.0}$	50	50
$T_{35.0}$	5	95
$T_{40.0}$	5	95
$T_{40.1}$	50	50
$T_{45.0}$	STOP	--

2.5. Solutions

2.5.1. Standard Stock Solution A

Standard stock solution A was prepared by makingup to final concentration of about 0.2 µg/ml by using *β*-Methyldiazo Azetidinone, *β*-Methyl Azetidinone, 4-Acetoxy Azetidinone, Thiolactone, Dihydrobenzoxazinone, Diprotected Meropenem, Azetidinone Malonate Ester, Silylated *β*-Methyl diazo Azetidinone, 2-Chlorodihydro Benzoxazinone and 2-Bromodihydro Benzoxazinone impurity standards.

2.5.2. Standard Stock Solution B

Transfer 1 ml of Standard stock solution-A into 100 mL clean, dry volumetric

flask containing about 30 mL of diluent, mix and make up to volume with diluent (concentration made up to 0.002 mg/ml).

2.5.3. Standard Solution (Concentration of 2.8 µg/g w.r.t Sample)

Dilute 0.70 mL of Standard Stock Solution B to 100 ml with diluent mix well with hand then filter the solution using 0.45 µm PTFE syringe filter.

2.5.4. Sensitivity Solution (Concentration of 0.35 µg/g w.r.t Sample)

Dilute 1.25 mL of Standard Solution to 10 mL with diluent mix well with hand then filter the solution using 0.45 µm PTFE syringe filter.

2.5.5. Sample Solution

Weigh 50 mg of Meropenem drug substance into a 15 ml torson tube and add 10 ml of diluent mix well and vortex for 5 minutes and filter the solution using 0.45 µm PTFE syringe filter (concentration made up to 5 mg/ml).

Blank solution: Diluent (*i.e.* Methanol) is used as blank.

2.5.6. Injection Order in Test Procedure

The injection sequence is proceeded in the following order, Blank (use diluent), sensitivity solution, Standard solutions (six times before and after samples sequence).

2.5.7. System Suitability Requirement

System suitability is established from the standard solution as injected in the procedure. The area of an interference peak for the analytes in the blank injection, if present, should be not more than 5% of the peak areas of analytes in the standard solution. S/N ratio of analyte peaks obtained from sensitivity solution should be not less than 3. The % RSD of the peak areas for analyte for the first six injections of standard solution should be not more than 10%.The cumulative % RSD of the peak areas for analyte should be not more than 15%. (Cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard). Further MGI-1 to MGI-10 impurities are about 19.6 min, 20.3 min, 22.1 min, 23.9 min, 24.5 min, 32.4 min, 32.6 min, 34.1 min, 34.7 min and 35.6 min respectively, once system suitability requirement is fulfilled, the following m/z is monitored for impurity quantification respectively.

β-MethyldiazoAzetidinone (**MGI-1**), **m/z** 136.0394, *β*-Methyl Azetidinone **(MGI-2)**, **m/z** 302.1782 Dihydrobenzoxazinone **(MGI-3)**, **m/z** 200.1071, 4-Acetoxy Azetidinone **(MGI-4)**, **m/z** 250.1227, Thiolactone **(MGI-5)**, **m/z** 237.0685, Azetidinone Malonate Ester **(MGI-6)**, **m/z** 136.0393, Diprotected Meropenem **(MGI-7)**, **m/z** 352.0945, Silylated *β*-Methyl diazo Azetidinone **(MGI-8)**, **m/z** 305.0866, 2-Chlorodihydro Benzoxazinone **(MGI-9)**, **m/z** 201.0911, 308.1048 and 2-Bromodihydro Benzoxazinone **(MGI-10)**, **m/z** 122.9739, 201.0911.

3. Results and Discussion

3.1. Method Development and Optimization

Optimization of LCMS spectrometer conditions: Ten impurities stock solutions were prepared individually in methanol and diluted to get final concentration of about 2.8 $\mu g \cdot g^{-1}$. In the proposed method, we performed positive mode, which has been revealed to be more prone to analytical problems such as, elevated baseline, matrix effects. However, we did not find any of difficulties during method validation process and not found any significant matrix effect for this method. Selection of UPLC column: The big task of the method development at trace level determination of all these impurities is separation of impurities with meropenem drug substance, related substances, and their responses in chromatographic system. Optimization for separation and response, several attempts were made with different columns viz., X-terra ms C18 column (250 mm × 4.6 mm, 5.0 µm) and acquity UPLC BEH C18 column (100 mm, × 2.1 mm and 1.7 µm) using isocratic and gradient elution, but finally Kinetex Biphenyl 2.6 µm (150 mm × 4.6 mm) (Make: Phenomenex) was selected. Based on outcomes of-various trails, buffer, chromatographic conditions, and selection of ionization parameters have been selected.

3.2. Methodvalidation

By considering the routine application of this LC-HRMS test procedure, it has been validated to show that the test procedure is suitable to yield consistent and reproducible results across the range of ten mutagenic impurity limits in Meropenem trihydrate drug substance.

The validation experiments involved the demonstration of Specificity, LOD (Limit of Detection) & LOQ (Limit of Quantification), Linearity, Precision (System precision and Method precision), Accuracy, Range and System suitability.

3.3. Specificity

Sample solutions of Meropenem (Control sample), sample spiked with ten impurities *i.e.* MGI-1 to MGI-10 at standard concentration level (Spiked Sample), samples spiked with ten impurities *i.e.* MGI-1 to MGI-10 at standard concentration level along with all related compounds were prepared and injected into LCMS study for evaluating specificity.In control sample and control sample spiked with all the related compounds, no interference was observed at the Retention time of specific MGI compounds in chromatogram. It has been proven that the developed method was specific w.r.t other related substances as per listed in meropenem drug substance. Also, it is observed that, Standard and Spiked sample Results are comparable with respect to Retention Time and selected m/z. Specificity related experiments results are shown in **Table 2** and typical chromatograms are shown in **Figure 3**.

Table 2. Specificity experiment results.

Analyte	Standard	Test sample	Control sample	Spiked with RS Impurities sample
MGI-1	19.668	19.668	ND	ND
MGI-2	20.237	20.236	ND	ND
MGI-3	22.050	22.050	ND	ND
MGI-4	23.850	23.849	ND	ND
MGI-5	24.515	24.514	ND	ND
MGI-6	32.387	32.386	ND	ND
MGI-7	32.550	32.549	ND	ND
MGI-8	34.096	34.095	ND	ND
MGI-9	34.734	34.733	ND	ND
MGI-10	35.561	35.560	ND	ND

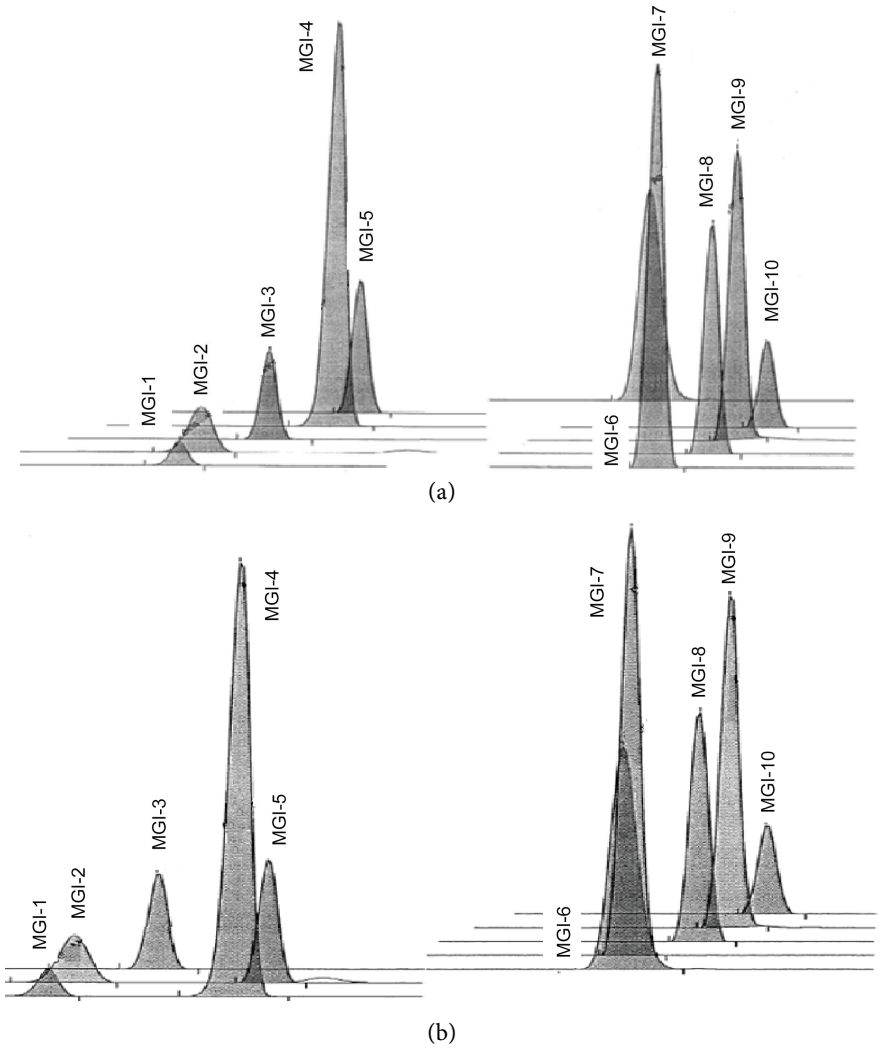


Figure 3. Specificity experiments—Standard and Spiked sample. (a): Specificity experiments—Standard; (b): Specificity experiments—Spiked sample.

3.4. LOD and LOQ

The Limit of detection and Limit of quantification values of analyte mutagenic impurities were verified for precision, by preparing the solutions at the proposed concentrations and injecting six times into LCMS with the test method conditions as described under methodology section. The minimum S/N ratio requirement is for each impurity is 3 for LOD and 10 for LOQ from the experiments. From the six injections, % RSD for each impurity acceptance criteria are not more than 33.0% for LOD and not more than 20.0% for LOQ. The experiments results are shown in Table 3.

Table 3. LOD and LOQ experiment results.

Statistical evaluation	**MGI-1**		**MGI-2**		**MGI-3**	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Mean	16,418	39,234	38,086	92,208	51,597	125,365
SD	1278.79	3034.56	1304.44	2902.04	3313.27	2534.77
% RSD	7.8	7.7	3.4	3.1	5.8	2.0
Conc. (µg/g)	0.3514	0.7028	0.3553	0.7105	0. 3623	0.7245
Signal to noise ratio*	3.6	11.7	3.8	13.6	3.9	17.1
Statistical evaluation	**MGI-4**		**MGI-5**		**MGI-6**	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Mean	303,316	653,576	93,326	193,022	213,968	460,142
SD	3247.2	6945.38	3247.2	3189.92	3587.03	11558.12
% RSD	1.1	1.1	1.1	1.7	1.7	2.5
Conc. (µg/g)	0.3523	0.7046	0.3570	0.7140	0. 3385	0. 6769
Signal to noise ratio*	6.5	14.5	4.2	12.4	6.2	17.1
Statistical evaluation	**MGI-7**		**MGI-8**		**MGI-9**	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Mean	260,406	572,832	155,934	335,530	194,521	409,438
SD	8354.9	14047.91	5922.81	5615.76	5236.3	8652.19
% RSD	3.2	2.5	3.8	1.7	2.7	2.1
Conc. (µg/g)	0.3406	0.6811	0.3512	0.7098	0.3549	0.7098
Signal to noise ratio*	5.2	14.9	7.1	12.6	6.3	18.8
Statistical evaluation	**MGI-10**					
	LOD	LOQ				
Mean	59466	121377				
SD	2723.45	4022.74				
% RSD	4.6	3.3				
Conc. (µg/g)	0.3521	0.7042				
Signal to noise ratio*	7.8	13.2				

LOD: Limit of Detection
LOQ: Limit of Quantification

4. Linearity

A series of solutions were prepared using MGI-1 to MGI-2 impurities .at concentration levels from LOQ to 150% of specification level and each solution was injected into LCMS as per test procedure and the correlation coefficient is more than 0.99 is obtained for each impurity. Hence the response of each impurity is linear from LOQ to 150% of specification level indicates the developed method is linear. The linearity experiment results are shown in Table 4.

5. Precision

System precision: Standard solution was prepared as per test method and injected six times into LCMS.

Method precision: Six sample solutions were prepared individually, with a one sample of Meropenem drug substance spiked with MGI-1 to MGI-10 impurities at Specification level and injected into LCMS as per methodology. RSD for the results of each impurity obtained from the analysis of six individual spiked sample preparations is not more than 20.0%.

Intermediate precision: Standard solution and six individual spiking in with MGI-1 to MGI-10 impurities at specification level and injected each solution into LCMS as per the methodology by different analyst, different Column and on a different day. RSD for the results of each impurity obtained from the analysis of six individual spiked sample preparations is not more than 20.0% and overall RSD from both experiments method precision and Intermediate precision is not more than 25.0%. % RSD values are shown in Table 5.

Table 4. Linearity experiment results.

	MGI-1	**MGI-2**	**MGI-3**
Slope	64623.587	160459.909	193079.453
Intercept	11565.6	24621.344	30117.941
Correlation coefficient	0.9993	0.9992	0.9986
	MGI-4	**MGI-5**	**MGI-6**
Slope	1047083.088	282921.488	707065.617
Intercept	175423.784	31715.255	54814.876
Correlation coefficient	0.9978	0.9991	0.9996
	MGI-7	**MGI-8**	**MGI-9**
Slope	1021985.017	509649.102	628013.724
Intercept	295256.853	48424.785	56506.467
Correlation coefficient	0.9970	0.9994	0.9990
	MGI-10		
Slope	186662.579	Concentration levels: LOQ to 150%	
Intercept	26673.315		
Correlation coefficient	0.9992		

Table 5. % RSD results from Precision.

Name	System precision	Method precision	Overall
MGI-1	2.7	3.8	6.5
MGI-2	2.1	2.0	3.1
MGI-3	1.6	2.7	3.1
MGI-4	1.1	0.8	2.0
MGI-5	2.4	3.3	4.2
MGI-6	1.3	0.7	2.5
MGI-7	2.4	0.4	2.8
MGI-8	1.8	1.3	2.1
MGI-9	1.2	1.7	1.4
MGI-10	2.5	1.4	1.9

6. Accuracy

The Accuracy of the proposed LCMS method was checked by using standard addition technique. The experiment was performed by spiking known amounts of ten PG impurities at three different concentration levels ranging from LOQ, 100%, and 150% into Meropenem trihydrate drug substance. It was carried out each in triplicate injections at these level concentrations. The obtained values of the test compound were within the specified limits as per regulatory requirements. The complete accuracy experimental results were shown in Table 6. The recovery results indicated that the test method has an acceptable level of accuracy for the determination of each impurity content in Meropenem at LOQ level, 50% level and 100% level of specification.

7. Robustness

Standard solution as per method and Sample solution spiked with all of impurities at specification level were prepared as per test method and injected into LCMS at different deliberately varied conditions to evaluate the system suitability and method's ability to remain unaffected. The altered conditions include change in flow rate by (±10%), Column oven temperature (±2˚C), and before and after source cleaning, according to system suitability criteria these experimental results are complies, hence method is suitable w.r.t robustness conditions. Acceptance criteria results are shown in Table 7.

Further solution stability also checked during the validations studies, it has been found that Standard solution is stable up to 80 hours at temperature (~5˚C) and Sample solution is stable up to 57 hours at temperature (~5˚C), this information is useful for during routine sample analysis to know the standard and sample nature w.r.t stability.

Table 6. % Recovery values from accuracy experiments.

Name	Mean (%) Recovery		
	LOQ Level	50% Level	150% level
MGI-1	84.6	101.2	104.1
MGI-2	82.7	103.7	105.3
MGI-3	87.3	102.8	107.2
MGI-4	92.0	104.0	108.7
MGI-5	86.8	96.9	96.6
MGI-6	94.7	105.2	106.7
MGI-7	91.0	105.4	118.1
MGI-8	91.6	101.7	104.0
MGI-9	88.9	100.5	101.0
MGI-10	86.5	98.6	96.2

Table 7. Robustness experiment acceptance criteria.

	PGI-1		PGI-2	
Name	**S/N ratio**	**% RSD**	**S/N ratio**	**% RSD**
Low flow	6.9	2.4	7.9	0.8
High flow	9.3	3.1	6.8	1.6
Low temperature	6.6	2.4	3.7	1.9
High temperature	6.6	2.7	3.8	0.6
Before—Source cleaning	3.6	2.7	3.9	2.1
After—Source cleaning	3.8	3.9	3.4	1.6
	PGI-3		**PGI-4**	
Name	**S/N ratio**	**% RSD**	**S/N ratio**	**% RSD**
Low flow	6.3	0.5	7.4	1.0
High flow	8.1	1.2	6.1	1.5
Low temperature	6.5	0.8	5.4	1.9
High temperature	5.1	1.1	5.1	0.7
Before—Source cleaning	6.6	1.1	4.1	2.4
After—Source cleaning	6.0	0.6	4.4	0.7
	PGI-5		**PGI-6**	
Name	**S/N ratio**	**% RSD**	**S/N ratio**	**% RSD**
Low flow	8.3	1.9	7.4	1.5
High flow	7.6	1.3	4.8	1.0
Low temperature	5.8	2.2	8.6	1.0
High temperature	5.8	2.0	5.2	1.2
Before—Source cleaning	4.0	1.7	5.6	2.4
After—Source cleaning	4.2	1.1	4.4	2.5

Continued

	PGI-7		PGI-8	
Name	**S/N ratio**	**% RSD**	**S/N ratio**	**% RSD**
Low flow	8.5	1.3	8.0	2.6
High flow	6.0	0.8	6.8	1.8
Low temperature	6.9	0.8	6.1	0.7
High temperature	6.1	1.3	6.5	1.2
Before—Source cleaning	7.1	1.3	8.0	1.8
After—Source cleaning	5.3	0.8	6.0	1.3
	PGI-9		**PGI-10**	
Name	**S/N ratio**	**% RSD**	**S/N ratio**	**% RSD**
Low flow	5.0	1.1	5.0	2.5
High flow	4.3	1.6	3.9	1.6
Low temperature	7.4	1.3	8.5	1.6
High temperature	4.2	1.1	5.1	1.5
Before—Source cleaning	6.9	1.2	8.6	2.5
After—Source cleaning	5.4	2.0	6.8	3.6

8. Conclusions

Liquid chromatography with high resolution mass spectrometer (LC-HRMS) method was successfully developed and validated with respect to quantification of ten potential genotoxic impurities in Meropenem trihydrate drug substance with low level specification limits. Further this method has been adopted for routine analysis to comply with regulatory requirements in pharmaceutical industry as this testing procedure is selective, linear, accurate, robust and precise as per experimental data.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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