Eurasian Journal of Analytical Chemistry, 2016, **11**(2), 89-99

Trace Level Determination of *p*-Toluidine in Penem Drug Substances by Gas Chromatography

Siva K. Nachaka, Sreenivasulu Boju, Jagadeesh K. Vundavilli, Raju V.S.N. Kadiyala, Pavan Kumar S.R. Kothapalli & Hemant K. Sharma
Aurobindo Pharma Limited Research Centre-II, Telangana, INDIA
Annapurna Nowduri & Venkateswara R. Battula
Andhra University College of Engineering, Andhra Predesh, INDIA

•*Received 19 September 2013* •*Revised 11 February 2013* •*Accepted 21 April 2014*

A Gas chromatographic method using flame ionization detector (GC-FID) has been developed and validated for the quantitative determination of *p*-Toluidine in Meropenem trihydrate, Imipenem monohydrate, Doripenem monohydrate and Ertapenem monosodium pharmaceutical drug substances. The chromatographic method was achieved on fused silica capillary column (Rtx-5) with 5% diphenyl and 95% dimethyl polysiloxane stationary phase. The detection and quantitation limits obtained for *p*-Toluidine were 0.9 µg g^{-1} and 2.7 µg g^{-1} respectively. The relative standard deviation for analyte response was found as 0.9% for six replicate injections of standard equivalent to 100 µg g^{-1} of *p*-Toluidine. The average recoveries of *p*-Toluidine in Meropenem trihydrate, Imipenem monohydrate, Doripenem monohydrate and Ertapenem monosodium were obtained 99.6%, 99.2%, 96.7% and 107.9% respectively. The method was found to be linear in the range between 2.7 and 150 µg/g with a correlation coefficient 0.9999. The developed method was found to be robust for the determination of *p*-Toluidine in Penem drug substances.

Keywords: *p*-toluidine by GC, meropenem trihydrate, imipenem monohydrate, doripenem monohydrate, ertapenem monosodium

INTRODUCTION

p-Toluidine is an aryl amine that chemical structure is similar to aniline except a methyl group is substituted on the benzene ring. It is the by-product in the manufacturing process of Penem's like Meropenem trihydrate, Imipenem monohydrate, Doripenem monohydrate and Ertapenem monosodium. Tried to search references on the determination of *p*-Toludine from the API such as Meropenem trihydrate, Imipenem monohydrate, Doripenem monohydrate and Ertapenem monosodium , but did not found any references. Hence to determine *p*-Toludine from such API, we add 1N Sodium hydroxide (NaOH) to neutralize the API and make that API available in a free base, extracted in polar solvents such

Correspondence: Siva K. Nachaka
Aurobindo Pharma Limited Research Centre-II, Survey No: 71 & 72, Indrakaran village, Sangareddy mandal, Medak district, 502329, Telangana, INDIA
E-mail: sivakrishna.sri2008@gmail.com
doi: 10.12973/ejac.2016.124a

ISSN: 1306-3057

Methylene chloride (MDC). In this process *p*-Toludine can easily be extracted and quantified using developed Gas Chromatography method.

Meropenem trihydrate [MER] is member of the carbapenem class of β-lactam antibiotic drug [1], which is a semi synthetic β-methyl carbapenem and exhibits an extremely broad spectrum of anti bacterial activity and stable against renal dehydropeptidase (DHP-I), during formulations MER is stable for long time at room temperature, but it under goes degradation by free water. This is very active against streptococci, methicillin-sensitive staphylococci, neisseria, haemophilus, anaerobes, and the common aerobic gram-negative nosocomial pathogens including Pseudomonas [2,3]. MER does not require being taken with the renal enzyme inhibitor, with its activity being similar to that of Imipenem monohydrate [4] [IMP]. Its low propensity for inducing seizures means that it is suitable for treating bacterial meningitis and is the only carbapenem approved in this indication. Thus, MER continues to be an important option for the empirical treatment of serious bacterial infections in hospitalized patients [5].

Imipenem monohydrate [IMP] also known as *N*-formimidoyl thienamycin, a new carbapenem β-lactam broad-spectrum antibiotic, is highly active in vitro against most aerobic and anaerobic gram-positive and gram-negative bacteria isolated from infectious diseases of human beings [6]. IMP is administered intravenously in combination with cilastatin, a renal dipeptidase inhibitor that increases urinary excretion of active drug. In vitro studies have demonstrated that IMP has an extremely wide spectrum of antibacterial activity against gram-negative and gram-positive aerobic and anaerobic bacteria, even against many multi resistant strains of bacteria. It is very potent against species which elaborate beta-lactamases [7].

Doripenem monohydrate [DOR] is a synthetic carbapenem antibiotic that is structurally related to β-lactam antibiotic [8] with in vitro activity against gram-positive, gram-negative, and anaerobic organisms. DOR has been developed to date for intravenous use. It has been described as having the favourable attributes of both IMP and MER against both gram-positive and gram-negative bacteria. It is stable against a wide variety of β-lactamases, including extended-spectrum and AmpC β-lactamases [9]. The half life of DOR is higher than that of IMP or MER [10].

Ertapenem monosodium [ERT] is a long-acting, β-methyl parenteral group 1 carbapenem antibiotic that has a broad antibacterial spectrum and once-a-day dosing supported by clinical studies. ERT is active against both gram-positive and gram-negative bacteria, including Enterobacteriaceae, Streptococcus pneumoniae and most species of anaerobic bacteria [11]. Usage of ERT does not affect the resistance of gram-negative bacteria to group 2 carbapenems [12]. The chemical structures of Penem's and *p*-Toluidine are shown in Figure 1.

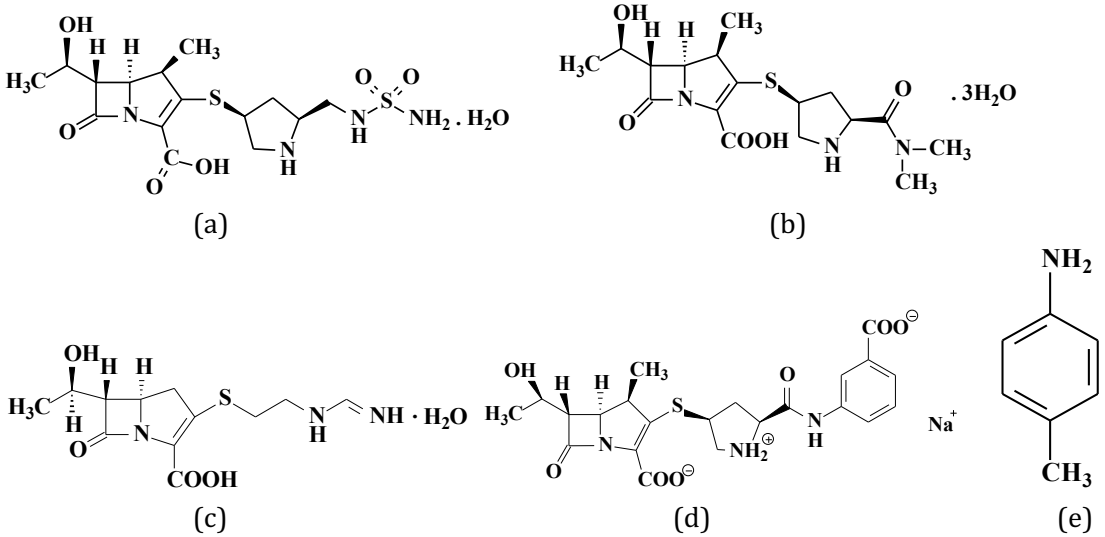

Figure 1. Chemical structures of (a) MER, (b) IMP, (c) DOR, (d) ERT and (e) *p*-Toluidine

EXPERIMENTAL

Gas Chromatography System and Column

Agilent 6890N gas chromatograph (GC) equipped with a flame ionization detector and a GERSTEL Multipurpose sampler used in the research work. Data acquisition and processing were conducted using the HPCHEM station. [Make:Agilent Technologies (Santa Clora, CA, USA)]

Shimadzu 2010 Gas chromatograph (GC) equipped with a flame ionization detector and a Combipal auto sampler GC solution process softwere was used in the research work. [Make:Shimadzu Corporation, Kyoto Japan]

The Rtx-5 column (30m length, 0.53mm internal diameter, 5% diphenyl, 95% dimethylpolysiloxane stationary phase with 5.0µm film thickness) was procured from Restek (Tokyo, Japan).

Reagents, Chemicals and Standards

p-Toluidine, Dodecane (Internal standard), Decane, N,N-Dimethylformamide, Mesityloxide, Methanol, Ethanol, Diacetone alcohol, 1-Methyl-2-pyrrolidinone, N,N-Diisopropylethylamine, Acetone, Isopropyl alcohol, Acetonitrile, *ter*-Butanol, Methyl acetate, tert-Butyl chloride, Valeric acid, 2,6-Lutidine, Ethyl acetate, Tetrahydrofuran, N-Methylmorpholine, 4-(Dimethylamino)pyridine, Acetic acid, Methyl chloride, Ethyl chloride, Isopropyl chloride, Methyl valerate and Benzene were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical grade of Methylene chloride, Sodium Hydroxide pellets, Sodium Sulphate anhydrous and HPLC water were procured from (Mumbai, India).

MER, IMP, DOR and ERT penem drug substances were gifted from Aurobindo Pharma Limited Research Centre (Hyderabad, India).

Chromatographic conditions

The column used was Rtx-5, 30m x 0.53 mm I.D, 5.0µm film thickness from Agilent with Helium as carrier gas. It provided baseline separation for the p-toluidine and internal standard(Dodecane) using Gas chromatograph condition given below in the Table 1.

Table 1. Gas chromatograph condition

Gas chromatograph (GC) condition:	
Column	Rtx-5, 30m x 0.53 mm I.D, 5.0µ film thickness
Oven temperature programming	60°C (hold for 4 mins) to 130°C @ 6°C min^{-1} to 220°C @ 10°C min^{-1} (hold for 5.33 mins)
Injection temperature	180°C
Detector	Flame Ionisation detector (FID)
Detector temperature	250°C
Carrier Gas	Helium
Carrier flow	40Kpa
Split ratio	1:3
Injection volume	2.0µl
Run time	30 min

Standard and Sample Solutions Preparations

1N Sodium Hydroxide Solution

Accurately weigh and transfer about 8000 mg of Sodium hydroxide pellets into a 200 mL, volumetric flask containing about 50 mL water, dissolve, and then dilute to volume with water.

Internal standard solution (IS)

Accurately weigh and transfer 700 mg of Dodecane into a 25 mL volumetric flask containing about 15 mL of Methylene chloride, then dilute to volume with Methylene chloride. Dilute 2.5 mL of this solution to 500ml with Methylene chloride (140 mg ml^{-1}).

Preparation of Blank Solution

Transfer 3 mL of internal standard solution into a 15 mL centrifuge tube and add 4 Ml of 1 N sodium hydroxide solution and centrifuge for 5 min at 3000 rpm. Collect the lower layer (Methylene chloride layer) carefully through an auto pipette and pass through anhydrous sodium sulphate.

Preparation of Standard Solution (17 mg ml^{-1})

Accurately weigh and transfer 21 mg of *p*-Toluidine into a 25 mL volumetric flask containing about 15 mL of internal standard solution, then dilute to volume with internal standard solution. Dilute 1.0 mL of this solution to 50 mL with of internal standard solution.

Transfer 3 mL of standard solution into a 15 mL centrifuge tube and add 4 mL of 1 N sodium hydroxide solution and centrifuge for 5 min at 3000 rpm. Collect the lower layer (Methylene chloride layer) carefully through an auto pipette and pass through anhydrous sodium sulfate.

Preparation of Sample Solution

Accurately weigh and transfer 500 mg of the sample into a 15 mL centrifuge tube, add 4 mL of 1 N sodium hydroxide solution. Shake the contents for approximately 2 min mechanically. Transfer 3.0 mL of the internal standard solution into the centrifuge tube. Again, shake the contents for approximately 2 min mechanically and centrifuge for 5 min at 3000 rpm. Collect the lower layer (Methylene chloride layer) carefully through an auto pipette and pass through anhydrous sodium sulphate.

RESULTS AND DISCUSSION

Method development and optimization

The challenge is to achieve the detection and quantitation at a low level using the gas chromatograph with a flame ionization detector (GC-FID) for obtaining good separation and the desired sensitivity. Due to high boiling point of *p*-Toluidine (i.e:200°C), the peak is not eluted in head-space technique. So development trials were initiated in direct-liquid injection technique using the stationary phase, 6% cyanopropyl, 94% dimethyl polysiloxane (DB-624; Make: J & W Scientific). The sample solution was prepared by dissolving the sample in Methanol, filtering, and injecting into the GC. Background interference was encountered in this trial. After cleaning the inlet port (to avoid ghost peaks), a broad peak shape of AA was observed, which suggests another type of sample preparation to reduce the

interference from the sample matrix for quantification and proper peak shape purposes.

During optimization procedure we have tried with few of diluents i.e chloroform, diethyl ether and ethyl acetate. Finally, Methylene chloride extraction is used for sample preparation. Sample was dissolve in 1N NaOH solution and extracted with Methylene chloride has given satisfactory results.

To minimise detector response variations w.r.t. standard peak, we have introduced Internal standard [IS]. Initially we have selected Decane as a IS, but unfortunately Decane peak was co-eluted with *p*-Toluidine peak, so in aspect to specificity experimental design, Decane solvent has not suitable for the evalution. When Dodecane is used for IS, this interference has not observed and also reproducibility of peak response is very good. Hence, Dodecane is selected for IS.

The effect of injection volume is considered for the quantification at low level of the solvents was investigated by injecting between 1µl to 2µl of the standard solution. The results shows the low detection levels are very difficult to achieve when ever using 1µl injection volume and 300mg sample concentration. After increasing injection volume (2µl) it seems that there is no interference between sample matrix and our analyte peaks in entire experiments. Further experimental studies were not done, to determine initial column temperature. An initial temperature of 60°C was chosen finally, which allowed baseline separation of the *p*-Toluidine from interfering peaks in the sample solvent.

In case of ERT drug substance, one unknown peak eluting very near retention time to *p*-Toluidine from sample matrix, we avoid these problem we use following column and temperature programme.

Rtx-5, 60 m x 0.53 mm x 5.0 µm. The GC oven temperature was 60°C for 2 min, further the temperature ramp set 5°C/min to 80°C, for 5 min and further the temperature ramp at 10°C/min to attain 200°C, and then hold for 12min, the chromatograph stop time was set to 35 min. Helium was used as a carrier gas at a constant pressure of 70 Kpa. The inlet temperature was kept at 180°C. The detector temperature was set at 260°C.

Method Validation

The developed and optimized method was validated for specificity, sensitivity [limit of detection (LOD) & limit of quantitation (LOQ)], linearity, precision [system precision, method precision & intermediate precision], accuracy, and robustness as per ICH guideline [13].

Specificity

Specificity is the ability of the method to measure the *p*-Toluidine response in the presence of all other residual solvents, which are used in the process of penem's preparation. For this specificity experimental design, blank solution, control samples (i.e sample as such preparation by using each penem drug substance), spiked samples (i.e. sample spiked with known concentration of *p*-Toluidine by using each penem drug substance) and all spiked sample (i.e. sample spiked with known concentrations of *p*-Toluidine and residual solvents by using each penem drug substance) were prepared and injected into GC. From the chromatograms, it was found that, all the solvent peaks were well separated from our interest of *p*-Toluidine peak in all spiked samples also *p*-Toluidine content was determined from the both spiked and all spiked solutions and the % differences and retention times of all solvents were tabulated in Table 2. It indicates that the test method is selective and specific for the determination of *p*-Toluidine in penem drug substances. The

typical GC overlay chromatograms of blank, control sample, spiked sample and all spiked sample of each penem drug substance were presented in Fig.2

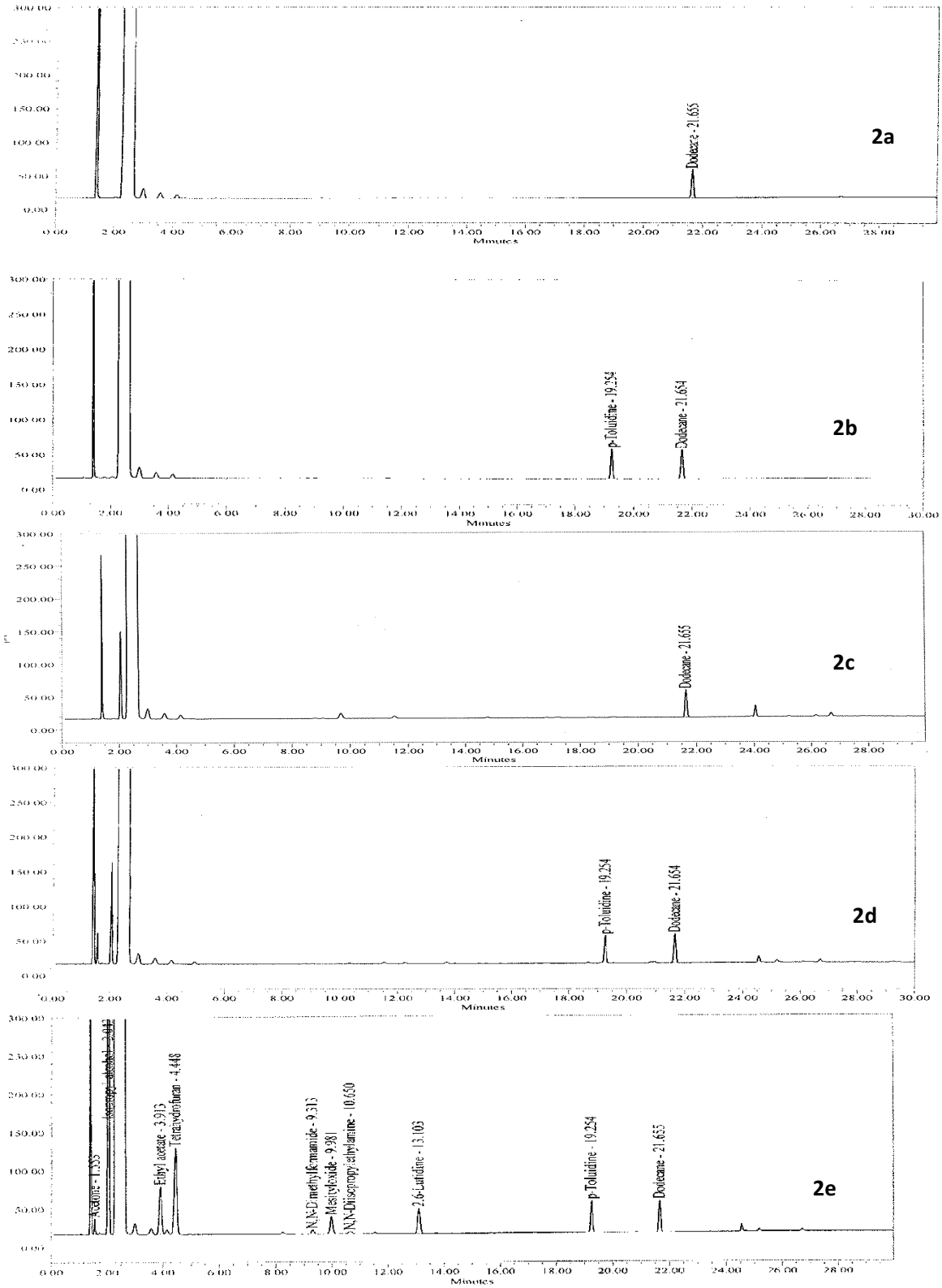

Figure 2. Typical GC chromatograms of 2(a) Chromatogram of Blank, 2(b) Chromatogram of Standard. 2(c) Chromatogram of MER drug substance, 2(d) Chromatogram of MER drug substance spiked with *p*-Toluidine and 2(e) Chromatogram of MER drug substance spiked with *p*-Toluidine including other known residual solvents

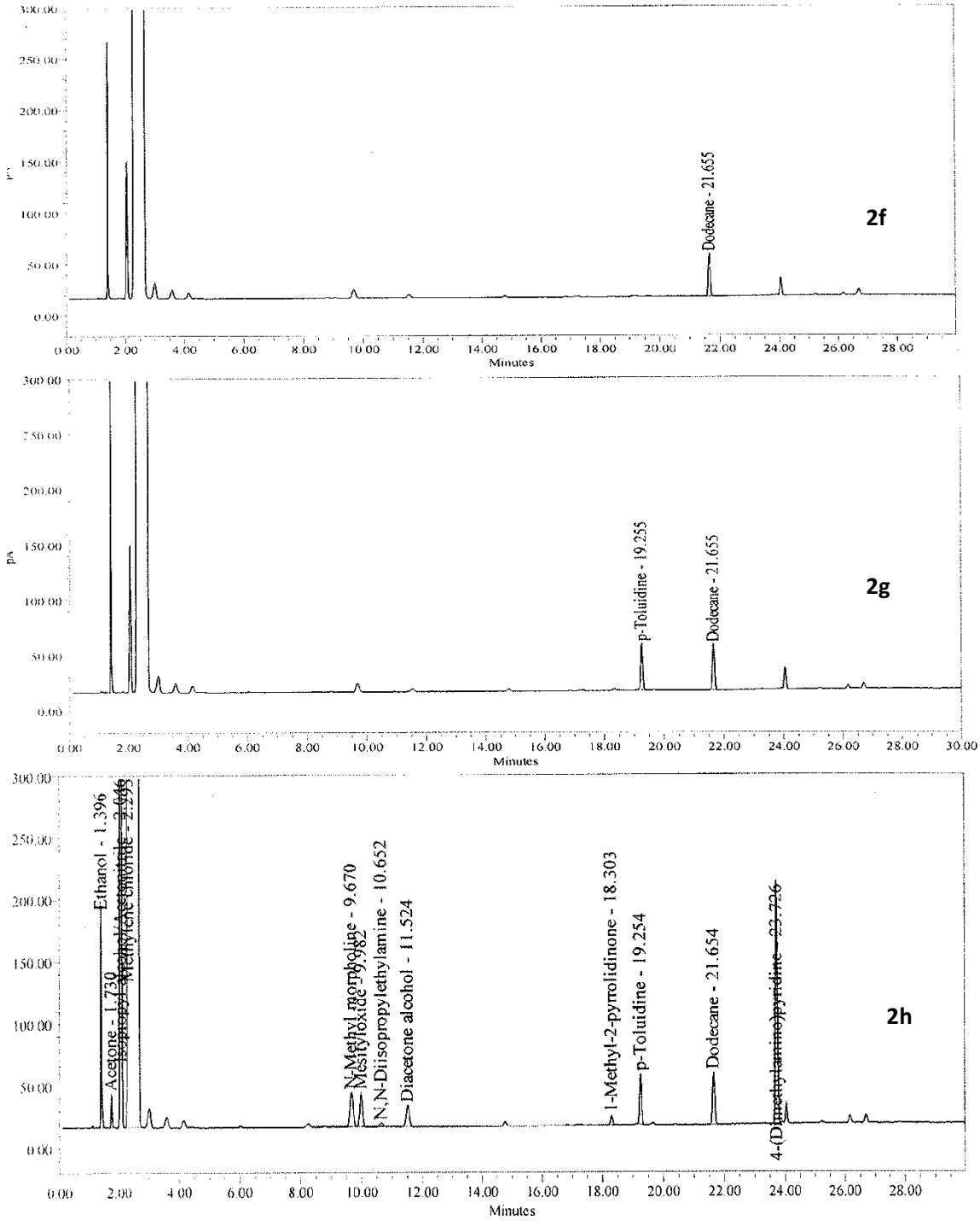

Fig. 2. Typical GC chromatograms of 2(f) Chromatogram of IMP drug substance, 2(g) Chromatogram of IMP drug substance spiked with *p*-Toluidine and 2(h) Chromatogram of IMP drug substance spiked with *p*-Toluidine including other known residual solvents

3.2.2. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) values for *p*-Toluidine were determined by the signal-to-noise ratio (s/n) method. The minimum concentration at 3:1 s/n was considered as the LOD and the concentration at 10:1 s/n was considered as the LOQ. The LOD and LOQ values obtained for *p*-Toluidine were 0.9 and 2.7 μg g^{-1}, respectively, with respect to the sample concentration, which corresponds to 0.15 and 0.45 μg mL^{-1}. Precision was verified by preparing the solutions at about the LOD and LOQ concentrations and injecting each solution six

times into the GC. Experimental results were presented in Table 2. along with Linearity experiment details.

Table 2. Retention Times of solvents from all spiked sample solutions in Penem drug substances – Specificity experiment.

Solvent Name	Retention Times from all spiked sample solutions.			
	Solvents used in MER	Solvents used in IMP	Solvents used in DOR	Solvents used in ERT
Methanol	-	-	1.395	3.203
Ethanol	-	1.396	1.395	-
Acetone	1.553	1.730	-	-
Isopropyl alcohol	2.043	2.046	2.036	4.572
Acetonitrile	-	2.046	-	-
Methylene chloride	-	2.293	2.275	5.162
t-Butanol	-	-	$	-
Methyl acetate	-	-	$	$
Ethyl acetate	3.913	-	3.914	-
Tetrahydrofuran	4.448	-	4.451	8.440
Benzene	$	$	$	$
n-Propanol	-	-	-	5.875
Acetic acid	-	$	$	-
Valeric acid	-	-	$	-
Isopropyl chloride	-	$	-	-
t-Butyl chloride	-	-	$	-
N,N-Dimethylformamide	9.313	-	9.317	15.430
N-Methyl morpholine	-	9.670	9.668	15.905
Mesityl oxide	9.981	9.982	-	-
N,N-Diisopropylethylamine	10.650	10.652	10.667	-
Methyl valerate	-	-	10.819	-
Diacetone alcohol	-	11.524	-	-
1-Methyl-2-Pyrrolidinone	-	18.303	-	-
2,6-Lutidine	13.103	-	-	19.307
p-Toluidine	19.254	19.254	19.253	24.889
Dodecane♦	21.655	21.654	21.654	27.554
4-(Dimethylamino)pyridine	-	23.726		-

♦ Internal standard
- Solvents not used in respective drug substance synthesis
$ Solvents retention times not eluted in respective drug substance

Table 3. *p*-Toluidine determination from spiked and all spiked samples – Specificity experiment.

	***p*-Toluidine determination (ppm)**			
	in MER	**in IMP**	**in DOR**	**in ERT**
Spiked sample	91.8	98.6	98.7	98.8
All spiked sample	96.7	94.7	95.1	96.9
% Difference	5.3	3.9	3.6	1.9

Linearity

The linearity was evaluated by measuring the area ratio for *p*-Toluidine to internal standard over the range of 0.45 to 25 µg/mL [2.7 to 150 µg/g with respect to sample concentration] and the obtained data were subjected to statistical analysis using a linear regression model. The statistical results such as correlation coefficient, slope, intercept, STEYX are reported in Table 4.

Accuracy

Accuracy of the method was verified through performing recovery experiments by spiking known concentration of *p*-Toluidine at four different levels i.e LOQ (2.7 µg/g), 50%, 100% and 150% w.r.t standard concentration in all penem drug

substances. These sample solutions were prepared and injected in triplicate into GC. The % recoveries for *p*-Toluidine ranged between 94.7 and 101.1% in MER, 94.1 to 102.2% in IMP, 96.4 to 97.7% in DOR and 103.3 to 108.3% in ERT penems. Details of % recoveries were calculated and tabulated in Table 5.

Table 4. Statistical data of LOD, LOQ and Linearity experiments

Statistical Parameters	Experimental Results
LOD and LOQ Experiments:	
Limit of Detection (LOD) (ppm)	0.9
Limit of Quantification (LOQ) (ppm)	2.7
Precision for LOD (%RSD) (n=6)	1.3
Precision for LOQ (%RSD) (n=6)	1.3
Linearity Experiments:	
Correlation coefficient	0.99993
Concentration range (ppm)	2.7 - 150
Intercept	-0.0026
Slope	0.0090
STEYX	0.0064

Table 5. Statistical data of Accuracy for *p*-Toluidine

Name of the drug substance		Level-1 (at LOQ level)	Level-2 (at 50% level)	Level-3 (at 100% level)	Level-4 (at 150% level)
MER	Mean	94.7	100.7	97.0	101.1
	SD	0.17	0.23	0.29	0.06
	%RSD	0.2	0.2	0.3	0.1
IMP	Mean	94.1	95.9	99.4	102.2
	SD	1.10	0.57	0.31	1.00
	%RSD	1.2	0.6	0.3	1.0
DOR	Mean	97.7	96.4	96.8	96.8
	SD	1.40	0.23	0.06	0.10
	%RSD	1.4	0.2	0.1	0.1
ERT	Mean	103.3	107.7	107.7	108.3
	SD	2.89	0.31	0.12	0.21
	%RSD	2.8	0.3	0.1	0.2

Precision

The system precision was demonstrated by injecting the standard solution of *p*-Toluidine six times into GC and calculating the area ratios using the areas obtained from *p*-Toluidine and Dodecane. The method precision of the method was established by preparing six individual sample preparations by spiking *p*-Toluidine to all penem drug substances separately, injecting into the GC, and calculating the *p*-Toluidine content. The ruggedness of the method was evaluated by preparing six individual sample preparations [the same samples which were used in the method precision experiment] by spiking *p*-Toluidine to all penem drug substances separately, injecting into the GC, and calculating the p-Toluidine content using two different columns, different makes of instruments (i.e. Agilent and Shimadzu), and by different analysts on different days. The achieved precision experimental results are reported in Table 6.

Robustness

This study was performed by making deliberate variations in the method parameters i.e pressure, column oven temperature and ramp temperature. The pressure was changing ± 10 Kpa w.r.t initial value, column oven temperature was verified ± 2°C and ramp temperatures also verified by modifying ± 2°C /min. By

using all of these modified parameters, system suitability verified. We have observed *p*-Toluidine and Dodecane peaks were well separated with a good resolution and all experimental results were presented in Table 7.

Table 6. Statistical data of Precision

Name of the drug substance		System Precision Ratio of Areas *	Method Precision Conc.(µg/g) *p*-Toluidine	Ruggedness Conc.(µg/g) *p*-Toluidine
MER	Mean	0.8795	97.7	95.7
	SD	0.0075	0.22	0.31
	%RSD	0.9	0.2	0.3
DOR	Mean	0.8705	99.0	99.1
	SD	0.0012	0.11	0.24
	%RSD	0.1	0.1	0.2
IMP	Mean	0.8894	98.8	103.2
	SD	0.0025	0.36	0.69
	%RSD	0.3	0.4	0.7
ERT	Mean	1.0778	98.4	99.8
	SD	0.0030	0.35	0.60
	%RSD	0.3	0.4	0.6

**p*-Toluidine /Dodecane

Table 7. Robustness Parameters

Parameter Variations	Pressure	Temperature			System
	[kPa]	Oven [°C]	Ramp-1[°C/min]	Ramp-2[°C/min]	Suitability♠
Initial as per Method	40	60	8	10	18.8
Pressure [-10%]	36	60	8	10	18.3
Pressure [+10%]	42	60	8	10	18.8
Temperature [-2°C]	40	58	6	8	21.9
Temperature [+2°C]	40	62	10	12	16.7

♠ *Resolution between p-Toludine and Dodecane peaks. (Requirement : Not less than 15)*

CONCLUSIONS

In this research article, method development and validation for the determination of *p*-Toluidine in penems drug substances was established. During the validation procedure, carried out according to ICH guidelines, specificity, precision, accuracy, limits of detection and quantitation and robustness were evaluated. All set up criteria were fulfilled. The method is specific, accurate, linear and shows a satisfactory level of precision. Hence, the validated GC method can be employed in the routine analysis for the quantification of *p*-Toluidine in penem drug substances.

Acknowledgements

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

References

1. GoyalVK, Rajput SS (2014) Meropenem: Current perspective. International Journal of Medical Science Research and Practice; 1(1): 03-05.

2. Takeuchi Y, Takebayashi Y, Sunagawa M, Isobe Y, Hamazume Y, Uemura A & Noguchi T (1993) The stability of a novel carbapenem antibiotic, meropenem (SM-7338), in a solid state formulation for injection. *Chem Pharm Bull*, 41: 1988-2002.
3. John FM (2008) Update on the efficacy and tolerability of Meropenem in the treatment of serious bacterial infections. *Clinical infectious Diseases*, 47: S41-51.
4. Martínez Lacasa J, Garau J. (1997) The role of carbapenems in the treatment of nosocomial infection Enferm Infect. *Microbiol Clin*, 15(1): 78-85.
5. Baldwin CM, Lyseng-Williamson KA, Keam SJ (2008) Meropenem: a review of its use in the treatment of serious bacterial infections. *Drugs*, 68(6): 803-38.
6. Park SY, Parker RH. (1986) Review of Imipenem. *Infect Control*, 7(6): 333-7.
7. Clissold SP, Todd PA, Campoli-Richards DM. (1987) Imipenem/cilastatin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs*, 33(3): 183-241.
8. Raritan, NJ: Orth-McNeil. (2007) Doribax (doripenem for injection) [package insert]. Pharmaceutical Inc; http://www.doribax.com/doribax/interactive pi.html.
9. Matthews S J, Lancaster J W. (2009) Doripenem monohydrate, a broad-spectrum carbapenen antibiotic Database of Abstracts of Reviews of Effects (DARE): Quality-assessed Reviews. *Clinical Therapeutics*; 31(1): 42-63.
10. Fiona Walsh (2007) Doripenem: A new carbapenem antibiotic a review of comparative antimicrobial and bactericidal activities. *Ther Clin Risk Manag*, 3(5): 789–794.
11. Wexler HM. (2004) In vitro activity of ertapenem: review of recent studies. *J Antimicrob Chemother*, 53(2): 11-21.
12. Falagas ME, Tansarli GS, Kapaskelis A, Vardakas KZ. (2013) Ertapenem use and antimicrobial resistance to group 2 carbapenems in Gram-negative infections: a systematic review. *Expert Rev Anti Infect Ther*, 11(1): 69-78. doi: 10.1586/eri.12.149.
13. International Conference on Harmonization (ICH) (2005) of Technical requirements for registration of pharmaceuticals for human use, Topic Q2 (R1): Validation of Analytical procedure: Text and methodology, Geneva.

◈ ⊙ ◈