



# INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications

Journal Home Page: [www.pharmascope.org/ijrps](http://www.pharmascope.org/ijrps)

## Ion-associative complex formation for estimation of piperacillin

Giri Prasad G<sup>1</sup>, Venkata Nadh R\*<sup>2</sup>, Kiran Kumar K<sup>3</sup><sup>1</sup>Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522510, India<sup>2</sup>GITAM University, Bengaluru, Karnataka-562163, India<sup>3</sup>KBN College, Vijayawada-1, India

Article History:	ABSTRACT	
Received on: 10.09.2018 Revised on: 06.12.2018 Accepted on: 09.12.2018	<p>A simple, validated and affordable visible spectrophotometric method was developed for the determination of piperacillin present in bulk and tablet formulation. The proposed method involves the development of a coloured ion-association complex between piperacillin cation and tropaeolin-ooo anion, which can be further extracted in chloroform. In the formation of cation from piperacillin, site of protonation is oxygen of amide group. Two secondary amides of piperacillin are protonated to form a doubly charged cation. Maximum absorption was observed at 529 nm for the coloured complex. Regression analysis (<math>r = 0.9999</math>) shows that the plotted calibration curve exhibits good linearity in the studied range of concentration (<math>4.0\text{--}24.0 \mu\text{g mL}^{-1}</math>). As per the existing guidelines of ICH, various parameters (interday precision, intraday precision, accuracy, ruggedness, LOD, LOQ) of the method were tested for validation. Low values of relative standard deviation (<math>&lt; 2\%</math>) were observed indicating that the proposed method is reproducible, accurate and precise. The proposed method was extended to assay piperacillin powder for injection formulation.</p>	
<p><b>Keywords:</b></p> Validation, Piperacillin, Tropaeolin-ooo, Ion associative complex, Assay		

\* Corresponding Author

Name: Venkata Nadh R  
 Phone: +91-9441021705  
 Email: doctornadh@yahoo.co.in

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v10i1.1787>

Production and Hosted by

Pharmascope.org

© 2018 Pharmascope Publications. All rights reserved.

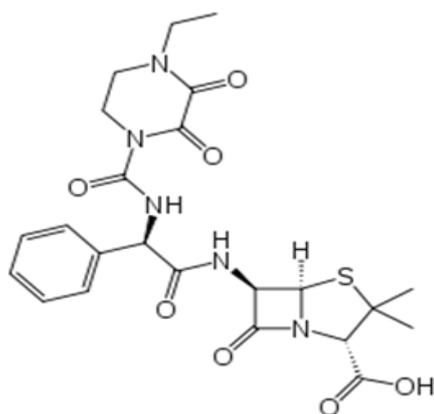
### INTRODUCTION

Piperacillin is one of  $\beta$ -lactam antibiotic which is widely useful in the dealing of polymicrobial infections and infections that are hospital-acquired (Tamma *et al.*, 2015; Tsai *et al.*, 2016). Analytical methods were reported in literature for its determination using expensive instruments like LCMS/MS (Florian Scheer *et al.*, 2014; Sebastiano Barco *et al.*, 2014), HPLC MS/MS (Zhiping *et al.*, 2012), HPLC (Rama Krishna Veni *et al.*, 2013; Pai

PNS *et al.*, 2006; Ramalingam *et al.*, 2014; Lakshmana Rao *et al.*, 2011; Augey *et al.*, 1996; Arzuaga *et al.*, 2005; Veillette *et al.*, 2016), UV and visible (Sangeetha *et al.*, 2017; Ines Toral *et al.*, 2012; TORAL *et al.*, 2012; Xiaoping *et al.*, 2003; Zhang *et al.*, 2004; Prasad *et al.*, 2019). But, no visible spectrophotometric method is available using TPooo. Chemical structure of piperacillin shows the presence of five amide groups (Figure 1) which will be useful for the formation of an ion-associative complex with Tropaeolin-ooo. Therefore, the present method describes the visible spectrophotometric determination of piperacillin by the formation of an extractable ion-associative complex.

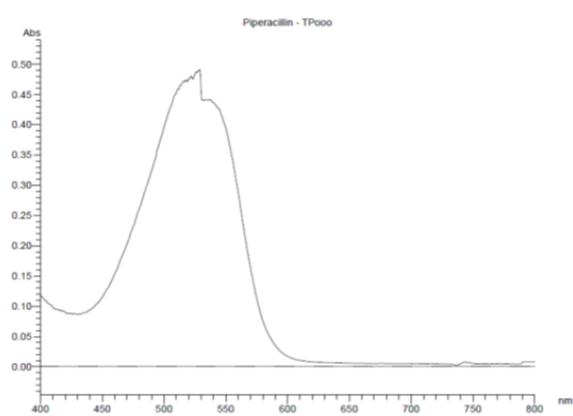
### MATERIALS AND METHODS

During the entire study, analytical grade purity chemicals and double distilled water were used. The absorbance was noted with the help of UV-Visible Spectrophotometer (TECHOMP double beam and model - UV 2310 loaded with the software of HITACHI version 2.0) by using 10 mm path length containing quartz cuvettes. Weighing balance (Make: Shimadzu and Model: AUX-220) and digital pH meter (Elico make and model: LI-120) were



**Figure 1: Piperacillin chemical structure**

used respectively for sample weighting and measurement of pH. Measurement of absorbance values was done in the range of  $30 \pm 2$  °C, i.e., at room temperature.



**Figure 2: Visible spectrum of piperacillin-TPooO ion association complex**

### Preparation of solution

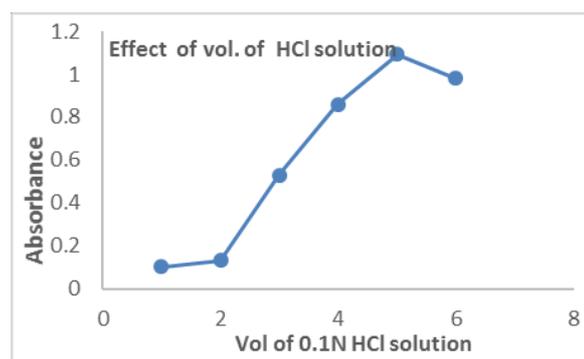
**Tropaeolin-ooo solution (0.2% w/v):** 200 mg of Tropaeolin-ooo (TPooO) is dissolved in 100 mL of distilled water.

**Standard solution of piperacillin:** Accurately weighed about 100 mg of piperacillin and transferred into a volumetric flask (100 mL). The powder was dissolved in methanol (25 mL) and completed the dissolution by using sonication. Then the same solvent was used to make up to the mark. After well mixing those contents, filtration was done using Nylon 6,6 membrane of make Ultipor®. The obtained standard stock solution ( $1000 \mu\text{g mL}^{-1}$ ) was used further.

## RESULTS AND DISCUSSIONS

Out of the many available methods for quantitative determination of pharmaceutical compounds, the popular one involves the formation of coloured ion-association complex. This technique can be adopted for compounds containing nitrogen, capable of forming cation by protonation. The developed complex between the above cation and

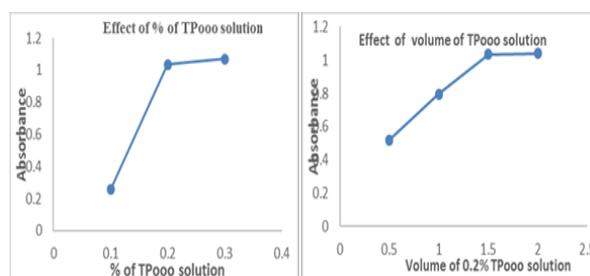
anionic dye is extractable in to organic solvents and visible spectrophotometer was used to measure absorbance of organic phase. (Kiran *et al.*, 2013; Prasad and Nadh, 2019; Giri and Venkata, 2019). An additional benefit of this extractable ion-associative extract method is its applicability in the estimation of specific compound even in the existence of other ingredients of formulations. This instigated us to develop a procedure to determine piperacillin in bulk drug and powder for injection formulation, centred on the idea of ion-association complex formation using a chromogen like Tropaeoline-ooo (TPooO). The absorption spectrum of coloured complex shows  $\lambda_{\text{max}}$  as 529 nm (Fig. 2).



**Figure 3: Effect of concentration of HCl solution**

### Optimization of reactions conditions and stoichiometry

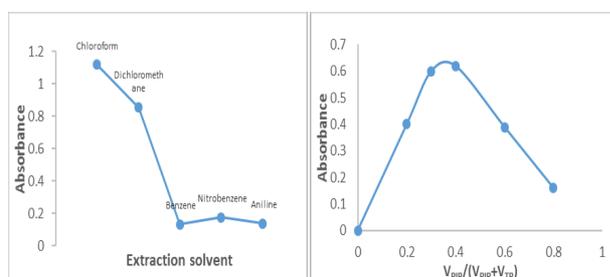
Optimization of reaction conditions was carried out at room temperature ( $30 \pm 2$ °C). The maximum intensity of colour was observed with 5 mL of 0.1 N hydrochloric acid solution (Fig. 3). As absorbance decreases beyond 5 mL HCl, it will be better to avoid higher volume to prevent the possibility of hydrolysis of amides. Optimum concentration and volume of TPooO were found to be 0.2% (w/v) and 1.5 mL respectively (Fig. 4).



**Figure 4: Effect of (a) percentage and (b) volume of TPooO solution**

Instantaneous colour development was observed after mixing the reactants, and minimum duration for the stability of colour intensity was found to be two hours. Different solvents including  $\text{CHCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_6\text{H}_6$ ,  $\text{C}_6\text{H}_5\text{NO}_2$  and  $\text{C}_6\text{H}_5\text{NH}_2$  were used to identify the best extracting solvent and chloroform

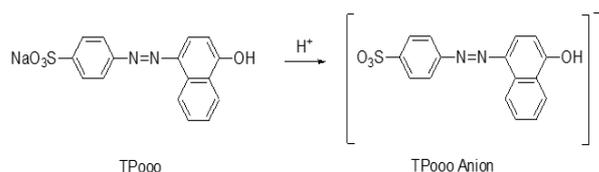
was found to be it (Fig. 5.a.). Usage of 10 mL of chloroform for 15 ml of aqueous layer yielded constant absorbance values which are also maximum. Hence, 2:3 volume ratio of organic to aqueous phase was fixed with a contact time of 2 minutes. Effective order of reactants addition was piperacillin + hydrochloric acid + TPooo. Job's continuation method was used to establish the ion-association complex stoichiometry (Job, 1928). Equal concentrations of piperacillin and TPooo were mixed in different molar ratio, but the total aqueous medium volume was maintained as 15 mL. The above extraction method was used, and absorbance values were measured. Plotted a graph between absorbance and  $V_{PIP} / (V_{PIP} + V_{TP})$  to show the formation of 1:2 ion-association complex between protonated piperacillin cation and TPooo anion (Fig.5.b.). Scheme-1 shows the formation of a coloured ion association pair between two TPooo anions and one piperacillin cation.



**Figure 5: (a) Effect of extraction solvent and (b) Continuous variation graph between piperacillin and TPooo**

### Optimized Method procedure

In a sequential order, added suitable aliquots of the above prepared standard piperacillin solution (0.2 to 1.2 mL of 200  $\mu\text{g mL}^{-1}$ ), HCl solution (5 mL of 0.1 N), TPooo solution (1.5 mL of 0.2% w/v) to a series of separating funnels (125 mL). Distilled water was added to separating funnels to make the aqueous phase volume to 15 mL. Shaken for two minutes after the addition of 10 mL of chloroform to each funnel. Anhydrous sodium sulphate was used to dry the chloroform layer after separation from the aqueous layer. Measured the absorbance of organic phase against similar reagent blank.

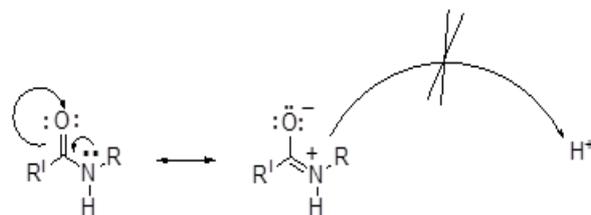


**Figure 6: Formation of TPooo anion**

### Chromophore Formation and Chemistry

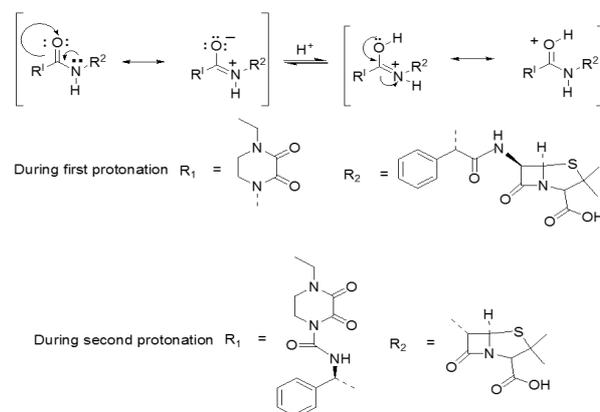
4-(4-Hydroxy-1-naphthylazo)benzenesulfonic acid sodium salt is the chemical name of Tropaeolin OOO.  $\alpha$ -Naphthol Orange, Acid Orange 20 and

Orange I are other names of it. In aqueous medium, the sodium sulfonate group of azo dye (TPooo) undergoes dissociation to form a negatively charged anion (Fig.6).



**Figure 7: Non-availability of the lone pair of electrons on nitrogens for protonation in amides**

Formation of cation from piperacillin involves three questions. (1) What is the site of protonation out of oxygen and nitrogen? (2) Out of tertiary and secondary amides, which participate in protonation? (3) Whether protonation takes place on single or multiple amide groups? A lot of deliberations were recorded regarding the site of protonation site in an amide. It is a known fact that amides are less basic than corresponding amines. Because the lone pair of electrons present on amide nitrogen undergoes delocalization with the oxygen on adjacent carbonyl group to give a more stabilized resonance species. But at the same time, diminishes the basicity due to less availability of a lone pair of electrons. The nature of formed cation can be interpreted in terms of electronic, steric and resonance. Five amide groups (two secondary and three tertiary amides) are available on piperacillin. Question is whether protonation takes place on nitrogen or oxygen. Basic nature of nitrogen in amides can be ruled out because lone pair of electrons present on it is delocalized on to the conjugated system (carbonyl group) and hence not much available to share with proton (Fig.7).



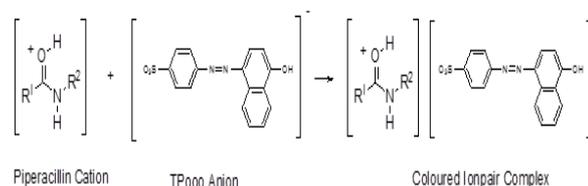
**Figure 8: Formation of piperacillin cation with protonation at oxygen of amide group**

Though amides exhibit neither acidity nor basicity in physiological conditions, it can be either proto-

nated or unprotonated in suitable chemical conditions like strong acidic or basic. Loss of hydrogen present on the nitrogen atom of 2° amides is possible as resonance stabilizes the resulting form. Therefore, 2° amides are alike to carboxylic acids in the sense of proton donation (DeRuiter, 2005). Hence, protonation on the nitrogen atom of 2° amides can be ruled out. At the same time, it is unambiguous that O-protonation is more favoured compared to N-protonation in ordinary amides. But N-protonation is preferred in molecules subjected to strain. Such strain effects were thoroughly studied by Cho *et al.* (1997) with the help of *ab initio* calculations and electron correlation effect which is crucial to know the protonation sites in the case of strained amides. According to them, O-protonation is also preferred in the strained molecule (N-foamylazetidine) whereas it is N-protonation in the case of the highly strained molecule (N-foamylazetidine). O-protonated and N-protonated strained amides exhibit planarity and non-planarity respectively. It means that in  $\beta$ -lactam of the bicyclic ring, O-protonation is more likely. Protonation of carbamide group containing urea and uronium compounds was studied by Wen and Brooker (1993), and according to these studies, protonation point is carbonyl oxygen. Protonation of urea, thiourea, and their derivatives was studied by NMR and IR spectra, then confirmed protonation at oxygen or sulphur (Redpath *et al.*, 1962). In the case of protonation of urea, *ab initio* calculations were done and confirmed those results with Raman spectroscopy (Wen and Brooker, 1993). Uronium nitrate (urea nitrate) was studied by neutron diffraction studies (Worsham and Busing, 1993). Hence, it is clear that the site of protonation is on the oxygen of the amide group (Fig.8).

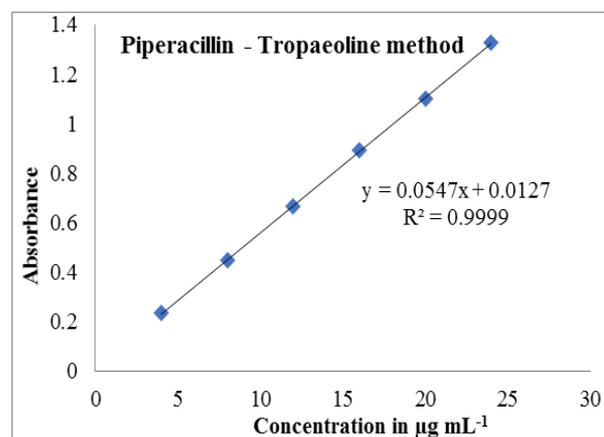
We can rule out the protonation of any one of the three tertiary amides present in cyclic rings by taking into consideration of different parameters including ring strain. Two tertiary amides are present in 2,3-dioxopiperazine moiety and one more in 6-aminopenicillansure. Embracing a coplanar confirmation is the requirement to take place resonance phenomenon. The bicyclic ring structure of beta-lactam antibiotics prevents the existence of the amide group in the same plane. Hence, due to lack of co-planarity, no delocalization is possible and hence no resonance stabilization in beta-lactam antibiotics. Therefore, amide nitrogen in the bicyclic ring is relatively more basic compared to those having delocalization (DeRuiter, 2005). Hence, protonation on the oxygen of its amide group is competed by nitrogen. Similarly, in 2,3-dioxopiperazine moiety, the electron withdrawing nature of adjacent carbonyl group for both the amide oxygen atoms prevents protonation.

The leftover possibility of protonation is on either one or two secondary amides present in piperacillin, which are substituted forms of urea and acetamide. Formation of 1:2 ion-association complex between protonated piperacillin cation and TP000 anions shows that protonation takes place at both secondary amides. Urea is relatively a stronger base compared to acetamide because lone pair of electrons on the nitrogen of acetamide are unavailable due to delocalization on to carbonyl group, whereas, in the case of urea, the lone pair of electrons present on second nitrogen is available for hydrogen. It is also clear from the pK<sub>b</sub> values of acetamide and urea, which are 14.51 and 13.89 respectively (Walter, 2012). However, their pK<sub>b</sub> values are nearer. Hence, the first protonation takes place on the oxygen of uronium oxygen followed by substituted acetamide (Fig.8).



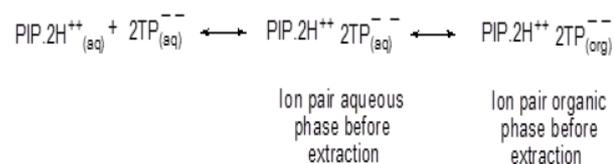
**Figure 9: Formation of ion-pair complex**

Then, an associative complex formation takes place between the oppositely charged TP000 anion and piperacillin cation (Fig.9). Electrostatic forces of attraction help to hold together these two. Chloroform was used as a solvent to extract the coloured complex. Its  $\lambda_{\text{max}}$  is at 529 nm.



**Figure 10: Calibration graph of piperacillin**

### Validation of Method



**Figure 11: Formation of coloured ion association pair**

**Table 1: Calibration values of piperacillin**

Concentration ( $\mu\text{g mL}^{-1}$ )	Absorbance*
4	0.236
8	0.446
12	0.665
16	0.892
20	1.102
24	1.328

\* Average of three determinations

**Table 2: Optical, regression and validation parameter values**

S. No.	Parameter	Observation
Optical characteristics		
1.	Apparent molar absorptivity	$2.90 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
2.	Sandell's sensitivity	$0.0178 \mu\text{g cm}^{-2}\text{A}^{-1}$
Regression analysis		
1.	Slope	0.054
2.	Intercept	0.0127
3.	Regression coefficient ( <i>r</i> )	0.9999
Validation parameters		
1.	$\lambda_{\text{max}}$	529 nm
2.	Beer's Law Limit (Linearity)	4-24 $\mu\text{g mL}^{-1}$
3.	Limit of detection	0.15 $\mu\text{g mL}^{-1}$
4.	Limit of quantitation	0.50 $\mu\text{g mL}^{-1}$
5.	Stability period	12 hours

**Table 3: Recovery of piperacillin**

Level of recovery (%)	The theoretical amount of piperacillin ( $\mu\text{g mL}^{-1}$ )	The practical amount of piperacillin ( $\mu\text{g mL}^{-1}$ )	Statistical evaluation	% Recovery
50	12	11.98	Mean:11.96	99.83
	12	11.94	SD: 0.017	99.50
	12	11.97	%RSD:0.142	99.75
100	16	15.96	Mean:15.97	99.75
	16	15.98	SD: 0.010	99.88
	16	15.97	%RSD:0.051	99.81
150	20	19.94	Mean:19.95	99.70
	20	19.97	SD: 0.15	99.85
	20	19.95	%RSD:0.062	99.75

Nominal amount of piperacillin taken (a) = 8  $\mu\text{g mL}^{-1}$ ; Added amount of piperacillin (b) = 4, 8 and 12  $\mu\text{g mL}^{-1}$  per 50%, 100% and 150% respectively; Theoretically total amount of piperacillin = a+b

**Table 4: Precision data**

Concentration of Piperacillin ( $\mu\text{g mL}^{-1}$ )	Concentration*			
	Intraday (Mean $\pm$ SD) ( $\mu\text{g mL}^{-1}$ )	% RSD	Inter-day (Mean $\pm$ SD) ( $\mu\text{g mL}^{-1}$ )	% RSD
4	4.009 $\pm$ 0.031	0.775	4.009 $\pm$ 0.031	0.999
12	11.870 $\pm$ 0.086	0.724	11.925 $\pm$ 0.122	1.027
24	24.954 $\pm$ 0.104	0.453	23.936 $\pm$ 0.122	0.511

\* Average of six determinations

**Linearity and range**

For each concentration in the range of 4 – 24  $\mu\text{g mL}^{-1}$ , measured the absorbance thrice and the average value was noted down (Table 1). Linearity was observed for the calibration curve in the studied range (Fig. 10). Equation of linear regression is

$y = 0.0547x + 0.0127$  with high correlation coefficient ( $> 0.9999$ ). Hence, a proposed analytical method is tested for its linearity. Various parameters (optical as well as regression) are given in Table 2.

**Table 5: Ruggedness data of piperacillin**

Test Concentration of Piperacillin ( $\mu\text{g mL}^{-1}$ )	Concentration*	
	Analyst change	
	Mean $\pm$ SD ( $\mu\text{g mL}^{-1}$ )	% RSD
4	4.046 $\pm$ 0.042	1.039
12	11.870 $\pm$ 0.115	0.970
24	23.863 $\pm$ 0.133	0.559

\* Average of six determinations

**Table 6: Estimation of piperacillin from its formulation**

Formulation	Labelled amount (g)	Amount found*(g)	% Drug Recovered	%RSD
Pipracil®	2	1.9778 $\pm$ 0.0031	98.89	0.172

\* Average of three determinations

### Accuracy

Proposed method's accuracy can be ascertained from % recovery values. To carry out these studies, 8  $\mu\text{g mL}^{-1}$  of piperacillin was taken as the nominal amount and added to it, different amounts of the same drug (4, 8 and 12  $\mu\text{g mL}^{-1}$  for 50%, 100% and 150% recovery levels respectively) so that the total theoretical amount of piperacillin is maintained within the linearity range. Table 3 shows 99.50 – 99.88 as the range of values of % recovery. Acceptable level of accuracy is manifested from low values of SD and %RSD.

### Precision

Precision studies (both intraday and inter-day) were conducted by selecting 3 different piperacillin concentrations within the linearity range (4 – 24  $\mu\text{g mL}^{-1}$ ). Table 4 is a compilation of observed values which were measured on the same day and successive days by considering six readings for each study. The method is proved to be satisfactory in terms of precision as its %RSD values of intraday, and inter-day studies are observed below 1% which are within an acceptable limit.

### Ruggedness

The proposed method's ruggedness is evident from the reproducible assay results of the selected 3 different piperacillin concentrations within the linearity range (4 – 24  $\mu\text{g mL}^{-1}$ ) from values of 2 separate analysts conducted experiments on dissimilar days (Table 5).

### Determination of detection limits

By considering the signal to noise ratio, the proposed method's sensitivity was determined by calculating both limits for detection and quantification (Sethi PD, 2001). As per the current ICH guidelines, LOD and LOQ of the present method were calculated from the values of S (slope of the calibration curve) and  $\sigma$  (standard deviation of the response) (ICH guidelines, 2005).

$$\text{LOD} = 3.3 \times \sigma / S = 0.15 \mu\text{g mL}^{-1} \text{ and}$$

$$\text{LOQ} = 10 \times \sigma / S = 0.50 \mu\text{g mL}^{-1}$$

### Pharmaceutical Formulations Analysis

Sonicated a mixture of hydrochloric acid solution (0.5 M), a suitable volume of water and piperacillin powder for injection (Pipracil®) for ten minutes to extract API present in it. Then estimated the API by following the above-proposed method by measuring the absorbance values of chromophores derived from the extracts and using the above-constructed calibration curve (Table 6). Excellent drug recovery values indicate the absence of any intervention of common excipients, and hence, successful application of the above method can be extended for determination of piperacillin in pharmaceutical formulations. In most of the QC laboratories of pharmaceutical industries located in developing countries, spectroscopic method is preferred for routine analysis (Sudhir *et al.*, 2013; Kiran *et al.*, 2014; Prasad and Nadh 2018). Hence, an attempt was made in the present study to propose and develop a sensitive visible spectrophotometric method to determine the amount of piperacillin in pure and powder for injection formulations using an extractive ion association complex-forming agent like TPooo.

### CONCLUSIONS

An affordable visible spectrophotometric method was developed for the determination of piperacillin present in bulk and tablet formulation by the formation of an extractive ion-associative complex between piperacillin cation and TPooo anions. In the formation of cation from piperacillin, site of protonation is oxygen of amide group. Two secondary amides of piperacillin are protonated to form a doubly charged cation. The proposed method was validated as per the existing guidelines of ICH. Due to its simplicity, the proposed method can be used in routine analysis of piperacillin (bulk drug and powder for injection formulation) in quality control laboratories, as alternatives to the expensive instrumental methods.

## REFERENCES

- Arzuaga, A., Isla, A., Gascón, A.R., Maynar, J., Martín, A., Solinís, M.A., Toral, D. and Pedraz, J.L. 2005. Quantitation and stability of piperacillin and tazobactam in plasma and ultrafiltrate from patients undergoing continuous venovenous hemofiltration by HPLC. *Biomedical Chromatography*, 19:570-8.
- Augey, V., Grosse, P.Y., Albert, G., Audran, M. and Bressolle, F. 1996. High-performance liquid chromatographic determination of tazobactam and piperacillin in human plasma and urine. *Journal of Chromatography B: Biomedical Sciences and Applications*, 682:125-36.
- Cho, S.J., Cui, C., Lee, J.Y., Park, J.K., Suh, S.B., Park, J., Kim, B.H. and Kim, K.S. 1997. N-protonation vs O-protonation in strained amides: Ab initio study. *The Journal of Organic Chemistry*, 62:4068-71.
- DeRuiter, J. 2005. Amides and related functional groups. *Principles of Drug Action*, 1:1-6.
- Florian Scheer. and Irene Krämer. 2014. A liquid chromatography assay for the simultaneous quantification of piperacillin and ciprofloxacin in human plasma and dialysate in critically III patients undergoing continuous renal replacement Therapy. *International Journal of Analytical Mass Spectrometry and Chromatography*, 2:43-51.
- Giri, G.P. and Venkata, R.N. 2019. Determination of mianserin using Fe<sup>3+</sup>-phenanthroline by visible spectrophotometry *Research Journal of Pharmacy and Technology*, 12(1).
- ICH guidelines. 2005. Validation of analytical procedures: Text and Methodology. Q2 (R1): 8-13
- Ines, Toral, M., Francisca., Nova-Ramirez and Fallon Nacaratte. 2012. Simultaneous determination of piperacillin and tazobactam in the pharmaceutical formulation tazobactam by derivative spectrophotometry. *Journal of Chilean Chemical Society*, 57:1189-93.
- Job, P. 1928. Formation and stability of inorganic complexes in solution. *Annali di Chimica*, 9: 113.
- Kiran, K.K., Nadh, R.V. and Nagoji, K.E.V. 2013. Extractive spectrophotometric determination of nicergoline through ion-pair complexation reaction. *Oriental Journal of Chemistry*, 29(1):263-269, DOI:10.13005/ojc/290142.
- Kiran, K.K., Nadh, R.V. and Nagoji, K.E.V. 2014. Determination of bendamustine hydrochloride in pure and dosage forms by ion-associative complex formation reaction. *Oriental Journal of Chemistry*, 30:905-910, DOI:10.13005/ojc/300272.
- Lakshmana Rao, A., Sai Krishna, K., Kiran Kumar, C.h. and Raja, T. 2011. Simultaneous determination of piperacillin and tazobactam in bulk and pharmaceutical dosage forms by RP-HPLC. *International Journal of Farmaceutica Journal of Farmaceutica Science*, 3: Suppl 2.
- Pai, P.N.S., Rao, G.K., Murthy, M.S. and Prathibha, H. 2006. Simultaneous estimation of piperacillin and tazobactam in injection formulations. *Indian Journal of Pharmaceutical Science*, 68:799-801.
- Prasad, G.G and Nadh, R.V. 2019. Determination of mianserin using tropaeolin-ooo by ion-pair formation. *International Journal of Applied Pharmaceutical*, 11(1) 168-73. DOI: 10.22159/ijap.2019v11i1.30125.
- Prasad, G.G. and Nadh, R.V. 2018. Oxidative Coupling: A tranquil approach for determination of selexipag by visible spectrophotometry, *Oriental Journal of Chemistry*, 34(6) 3112-7. DOI: 10.13005/ojc/340656.
- Prasad, G.G., Nadh, R.V. and Kiran, K.K. 2019. Piperacillin estimation by ion-associative complex formation, *Asian Journal of Pharmaceutical Clinical Research*, 12(3) 1-5. DOI:10.22159/ajpcr.2019.v12i3.29289.
- Rama Krishna Veni, P., Sharmila, N., Narayana, K.J.P., Hari Babu, B. and Satyanarayana, P.V.V. 2013. Simultaneous determination of piperacillin and tazobactam in pharmaceutical formulations by RP-HPLC method. *Journal of Pharmaceutical Research*, 7:127-131.
- Ramalingam, P. and Rajendran, K. 2014. Detection of stability and degradation of piperacillin and tazobactam in injectables from In-patient Wards and pharmacy by RP-HPLC method. *Gulf Medicine Journal*, 3(S2): S132-S141.
- Redpath, C.R. and Smith, J.A. 1962. Proton magnetic resonance studies of amides. Part 4.—Salts of urea, thiourea, and formamidine disulphide. *Transactions of the Faraday Society*, 58:462-9.
- Sangeetha, S., Kumar, M., Kumudhavalli, M.V., Alexandar, S. and Jaykar, B. 2017. Development and validation of UV spectrophotometric area under curve method for quantitative estimation of piperacillin and tazobactam. *Journal of Chemistry Technische Research*, 10:988-94.
- Sebastiano Barco., Francesco Maria Risso., Matteo Bruschetti., Roberto Bandettini., Luca, A., Ramenghi., Gino Tripodi., Elio Castagnola. and Giuliana Cangemi. 2014. A validated LC-MS/MS method for the quantification of piperacillin/tazobactam on dried blood spot. *Bioanalysis*, 6:2795-802.

- Sethi, P.D. 2001.HPLC quantitative analysis of pharmaceutical formulations. CBS publications, India.
- Sudhir, M.S. and Venkata Nadh, R. 2013. Simple and validated ultraviolet spectrophotometric method for the estimation of febuxostat in bulk and pharmaceutical dosage forms. *Oriental Journal of Chemistry*, 29:1507-1514, DOI:10.13005/ojc/290429.
- Tamma, P.D., Han, J.H., Rock, C., Harris, AD., Lautenbach, E., Hsu, A.J., Avdic, E. and Cosgrove, S.E., 2015. Carbapenem therapy is associated with improved survival compared with piperacillin-tazobactam for patients with extended-spectrum  $\beta$ -lactamase bacteremia. *Clinical Infectious Diseases*, 60:1319-25.
- TORAL, M., Nova-Ramírez, F. and Nacaratte, F.2012. Simultaneous determination of piperacillin and tazobactam in the pharmaceutical formulation Tazonam® by derivative spectrophotometry. *Journal of Chilean Chemical Society*, 57:1189-93.
- Tsai, D., Stewart, P., Goud, R., Gourley, S., Hewagama, S., Krishnaswamy, S., Wallis, S.C., Lipman, J. and Roberts, J.A. 2016. Pharmacokinetics of piperacillin in critically ill Australian Indigenous patients with severe sepsis. *Antimicrobial agents and chemotherapy*, AAC-01657.
- Veillette, J.J., Winans, S.A., Forland, S.C. and Maskiewicz, V.K. 2016. A simple and rapid RP-HPLC method for the simultaneous determination of piperacillin and tazobactam in human plasma. *Journal of pharmaceutical and biomedical analysis*, 131:80-86.
- Walter, H. editor. 2012. Titrations in nonaqueous solvents. Elsevier, Dec 2.
- Wen, N. and Brooker, M.H. 1993. Urea protonation: Raman and theoretical study. *The Journal of Physical Chemistry*. 97:8608-16.
- Worsham, J.E. and Busing, W.R.1969. The crystal structure of uronium nitrate (urea nitrate) by neutron diffraction. *Acta Crystallographica Section B*, 25:572-8.
- Xiaoping, B., Yongli, B.X, and Xiaolei, W.2003. Content Determination of sodium piperacillin and sodium tazobactam for Injection by double wavelength UV spectrophotometry. *Journal of Chinese Pharmaceutical Affairs*, 8:027.
- Zhang, A.P., Bai, X.H. and Tong, L.G.2004. Determination of piperacillin in compound injection by double wavelength ultraviolet spectrophotometry. *Journal of Shanxi Medicine University*, 6:015.
- Zhiping, Li., Qin, L.i., Wang, Y.i., Cao, D.i. And Chao Chen.2012. Determination of free and total piperacillin-tazobactam in plasma by HPLC-MS-MS an adapted method for neonates. *Chromatography*, 75:533-39.