WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 5, 1478-1491. Research Article ISSN 2277– 7105

STABILITY INDICATING RP-HPLC METHOD FOR THE QUANTITATIVE ESTIMATION OF IMIDOCARB DIPROPIONATE IN INJECTION DOSAGE FORM

Sayali Patil[1]*, Swati Parikh[2], Vijaya Kumar Munipalli[2], Raman Mohan Singh[2], Smita Nayak[1], Bhaskar Vaidhun[1]

[1]Gahlot Institute of Pharmacy, Plot No. 59, Sector- 14, Koparkhairane, Navi Mumbai-400709, Maharashtra, India.

[2]Central Drugs Testing Laboratory, Zonal FDA Bhavan, GMSD Compound, Belasis Road, Mumbai Central, Mumbai- 400008, Maharashtra, India.

Article Received on
01 March 2022,

Revised on 22 March 2022,
Accepted on 11 April 2022

DOI: 10.20959/wjpr20225-23834

***Corresponding Author**
Sayali Patil
Gahlot Institute of Pharmacy, Plot No. 59, Sector- 14, Koparkhairane, Navi Mumbai-400709, Maharashtra, India.

ABSTRACT

Objective: The primary aim of this study was a develop of simple, fast, isocratic and stability indicating reverse phase high-performance liquid chromatography (RP-HPLC) method for quantitative estimation of Imidocarb dipropionate (IMD) in its injection dosage form. **Method**: The chromatographic separation was achieved on Hemochrome/C18 column (250mm × 4.6mm, 5µm particle size) by using simple mobile phase containing 0.2 % Trifluroacetic acid (TFA): Acetonitrile (ACN) in the ratio of 80: 20 % (v/v). Diluent used was water and ACN in the ratio of 1: 1% v/v. The flow rate used was 1.0 ml/min and detection was carried out at 243 nm. **Result**: The Retention time for method was about 6.08 min. The reliability and analytical performance of proposed HPLC method was statistically validated with respect to linearity, ranges, precision, accuracy, robustness, detection and quantification limits. The method was linear in the range of 5-40 µg/ml, with correlation coefficient 0.9999. The limit of detection and limit of quantification was found to be 0.0015 and 0.0048 µg/ml, respectively. The forced degradation studies were performed by exposing drug to various conditions like acid, alkaline, thermal, water, oxidation and photolytic degradation conditions. The obtained results shows that the method is specific and no interferences due to excipients as well as degradation products and can be used for routine quality control of IMD in injection dosage form. **Conclusion**: The HPLC method developed for quantitative

estimation of IMD was successfully evaluated with respect to linearity, precision, accuracy, robustness, limit of detection and limit of quantification. The validated HPLC method can be applied for determination of IMD in injection dosage form.

KEYWORDS: Force degradation studies, IMD, Method development, RP-HPLC, Stability-indicating determination.

INTRODUCTION

Imidocarb dipropionate (IMD) is a urea derivative used in veterinary medicine as an antiprotozoal agent for the treatment of infection with Babesia and other parasites.[1] It is also a carbanilide derivative with antiprotozoal activity, marketed for veterinary use. Also IMD most commonly used for hepatozoon infection and trypanosoma brucei.[2] The mechanism of action of antiprotozoal agent are either drug interfering metabolic processes or drugs interfering with neuromuscular physiology of parasites.[3] The chemically it is known as 1,3-Bis(3-(2-imidazolin-2-yl)phenyl)urea;propionic acid.

Literature survey reveals that the monograph of IMD is not official in Indian pharmacopoeia, BP and U.S.P. Several instrumental methods like LC-MS[4-7], HPLC[8] and were reported for IMD, in one article IMD was determined in biological sample like bovine meat and milk samples by HPLC method. There is no validated HPLC method for determination of IMD in its injection form. Hence trails were made to develop simple, accurate, precise and robust and stability-indicating RP-HPLC method for the determination of IMD in injection dosage form.

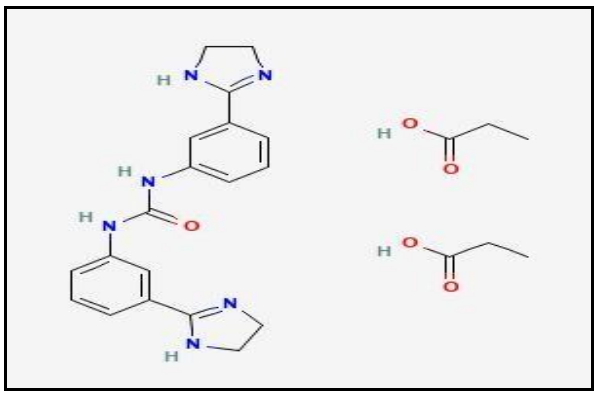

Fig. 1: Structure of Imidocarb dipropionate.

EXPERIMENT

Materials

A pure IMD working standard was obtained from Central Drug Testing Laboratory, Mumbai

with defined purity 96.7%. IMIX (Imidocarb dipropionate aqueous injection solution 120 mg/ml) injection manufactured by Intracin pharmaceutical pvt. Ltd., and was procured from the local market. ACN of HPLC grade, TFA of analytical grade from Rankem, and mili Q water used during method development.

Instrumentation

For all spectrophotometric measurements lab India UV/VIS spectrophotometer, with UV win lab software were used. For chromatography, Dionex ultimate 3000 using chromeleon 7.2.6 software with LC instrument control was used. HPLC equipped with PDA detectors (Waters 2996) detector used for forced degradation studies. HPLC column used for the estimation of IMD instil C18 (250mm × 4.6mm, 5µm) from Hemochrome was used.

Chromatographic conditions

The optimized mobile phase prepared by mixing 0.2 % TFA with ACN in the ratio of 80 : 20 % v/v was used. The mobile phase was filtered using a 0.45 µm filter and was degassed by sonication prior to use. The wavelength of 243 nm was chosen because drug has maximum absorption at this wavelength. The flow rate used was 1.0 ml/min. The injection volume was 20 µl, and temperature of column was 30 ^{0}C. The total run time of system was about 10 minute.

Preparation of diluent

Considering the chemical nature and solubility of the drug, water and ACN in the ratio of 1 : 1 % v/v was selected as diluent.

Preparation of standard solution

The standard solution for drug was prepared by dissolving exactly 10 mg into 25ml of volumetric flask. Sonicated, and then made volume with diluent and further dilutions were made to get a concentration of 20 µg/ml of IMD using mobile phase.

Preparation of sample solution

The sample solution was prepared by transferring 1 ml from IMIX (120mg/ml) sample to 200 ml volumetric flask and make up with diluent and further dilutions were made to get a concentration of about 18 µg/ml of sample in mobile phase.

Method development and optimization

The main objective of this study is to develop RP-HPLC method for IMD in injection dosage

form and also degradation products, Moreover the method should be simple enough for use in a routine quality control laboratory. Chemical structure of IMD reveals that it is a basic, non-polar molecule. Therefore the selection of column and mobile phase was done according to nature of molecule. Diluent were selected based on solubility of drug. IMD (10 µg/ml) spectra have sufficient absorption at 243 nm, (fig : 2) which was therefore chosen for entire study.

Consider chemical nature of molecule, base deactivated (BDS) column is first choice for determination of drug. Initial trails started with water and ACN in different proportions, but high tailing factor were observed. In another trail 0.1% Triethylacetate (TEA) pH 3 adjusted with Phosphoric acid (H_3PO_4) and ACN in different proportions were tried, but lower retention time was observed. Further trails were started with 0.2% TFA and ACN on Hemochrome column. Finally the mobile phase comprising of 0.2% TFA and ACN in ratio of 80 : 20% v/v on Hemochrome column produces good peak shape with acceptable system suitability testing parameters and are mention in table 3 and chromatographic conditions summarizes in table 1.

Table 1: Chromatographic conditions.

	Chromatographic condition
Column	Hemochrome intsil C18 (250×4.6 5U)
Mobile phase	Trifluroacetic acid: Acetonitrile: water (0.2 : 20 : 80 % v/v/v)
Flow rate	1 ml/min
Run time	10 min
Column temperature	30^0 C
Injection volume	20 µl
Detection wavelength	243 nm

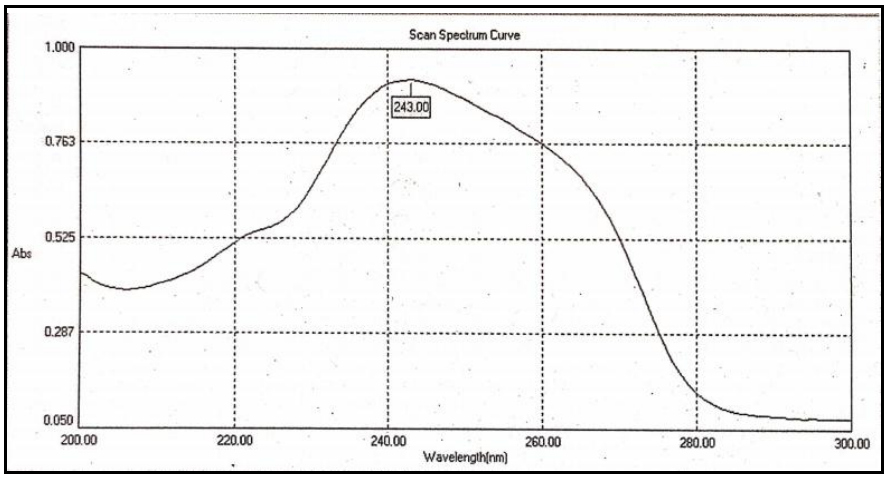

Fig. 2: Spectra of IMD.

METHOD VALIDATION

The present and optimized RP-HPLC method was validated according to ICH Q2 (R1)(International conference on harmonization) guideline to determine parameters like specificity, linearity, precision, accuracy, robustness, limit of detection(LOD) and limit of quantification(LOQ).[9-11]

Specificity

Forced degradation performed to establish degradation nature of drug substance and to establish stability indicating nature of a developed method[12-14], the drug was subjected to different conditions such as acidic, basic, water, photolytic and thermal conditions. And degraded samples were analyzed using PDA (photodiode array) system to analyze the peaks at different wavelength and to determine the peak purity.

Linearity

The linearity of the drug was carried out by preparing appropriate aliquots from standard stock solution of IMD to obtain concentration in the range of 5-40 µg/ml. The linear calibration was constructed by analysing the concentration over the selected range versus peak area of standard solution. The results was estimate by least-squares regression analysis, and the correlation coefficient and calibration equation was calculated and mentioned in fig 11.

Accuracy

Accuracy was performed by standard addition method (recovery study) of known amounts of IMD standard solution added to pre-analyzed sample solution. Method was established at three different level 110 %, 120% and 130 %. The results of mean recovery well within the acceptable limits and shown in table 4.

Precision

Method precision (Repeatability) was carried out by injecting replicates of IMD with concentration of 20 µl/ml and calculated % RSD and % assay for IMD. Intermediate precision was evaluated in terms of intraday and interday precision by analyzing different conditions like three different time intervals (i.e. at 10 am, 1 pm, 4 pm), or on three different days by three different analyst (table 5-6).

Robustness

The robustness of present method was examined by analysis of both reference and sample under variety of experimental conditions. For HPLC method, robustness was established by making delebrate modification include different wavelength (± 2 nm), different mobile phase composition (± 2 %), different flow rate (± 0.2 %) and different column temperature (± 2 ^{0}C) and results was calculated and shown in table 7.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and Quantification (LOQ) of obtimized method was determined using the calibration curve. Samples of IMD was prepared and injected six times. The LOD and LOQ was determined by using following formula and shown in table 8.

$$LOD = 3.3 \times \sigma/S$$

σ = Standard deviation,

S = Slop of calibration curve

$$LOQ = 10 \times \sigma/S$$

σ = Standard deviation,

S = Slop of calibration curve

RESULT

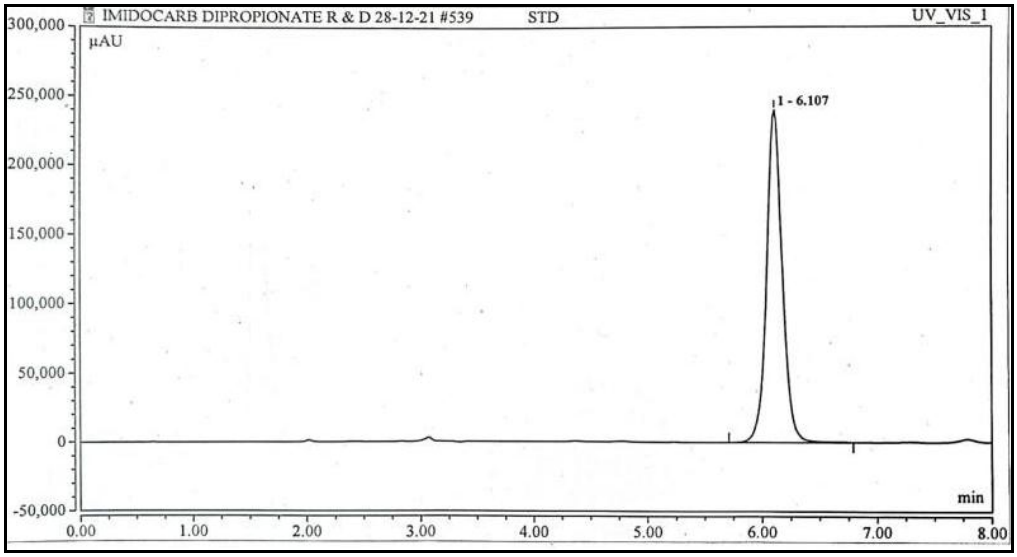

Fig. 3: Chromatogram of standard IMD solution using developed condition.

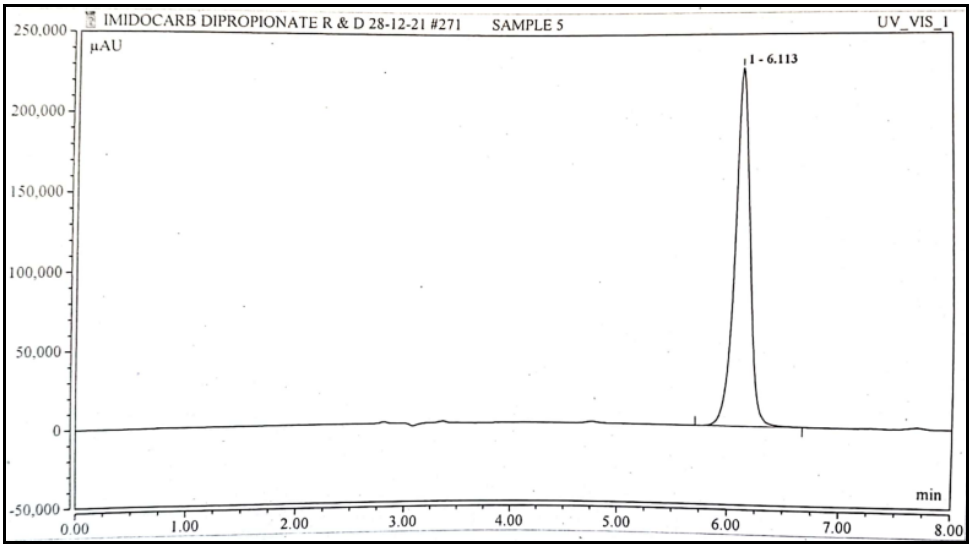

Fig. 4: Chromatogram of sample IMD solution using developed conditions.

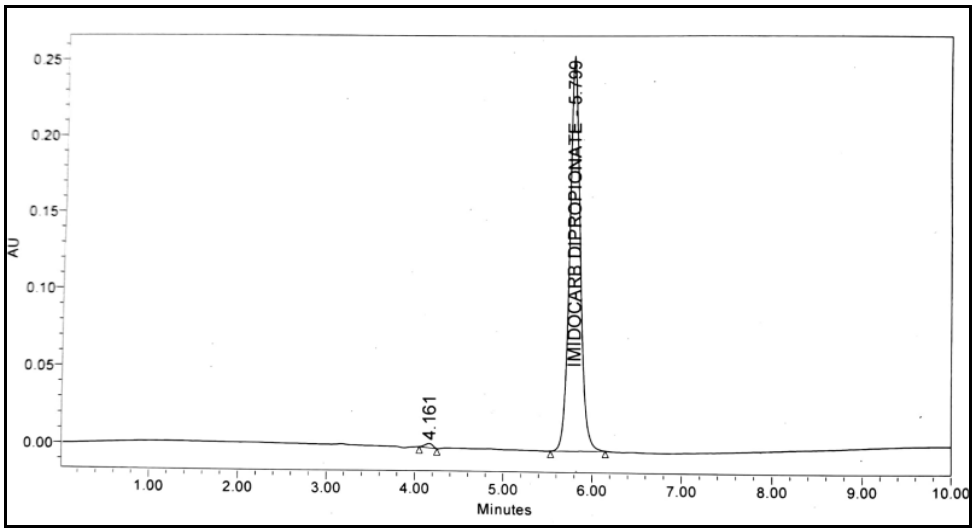

Fig. 5: IMD water degradation Chromatogram.

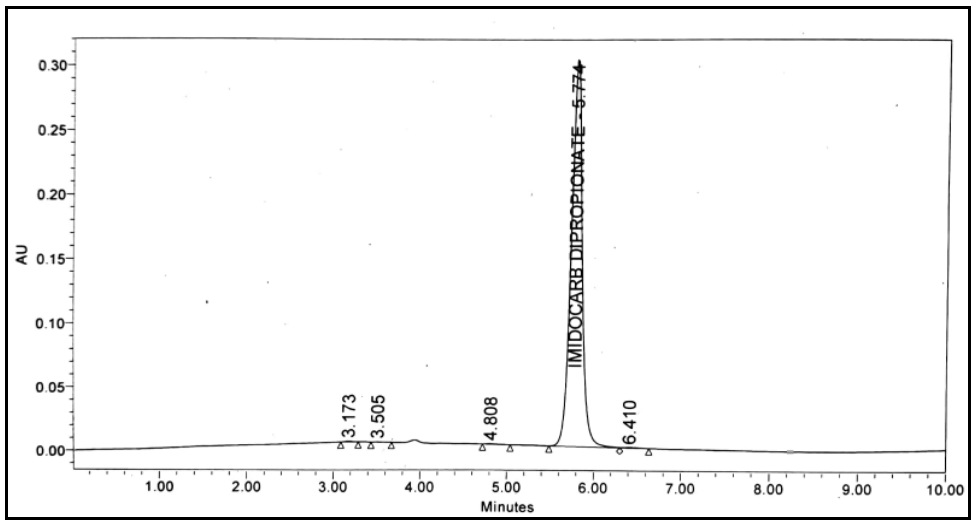

Fig. 6: IMD thermal degradation Chromatogram.

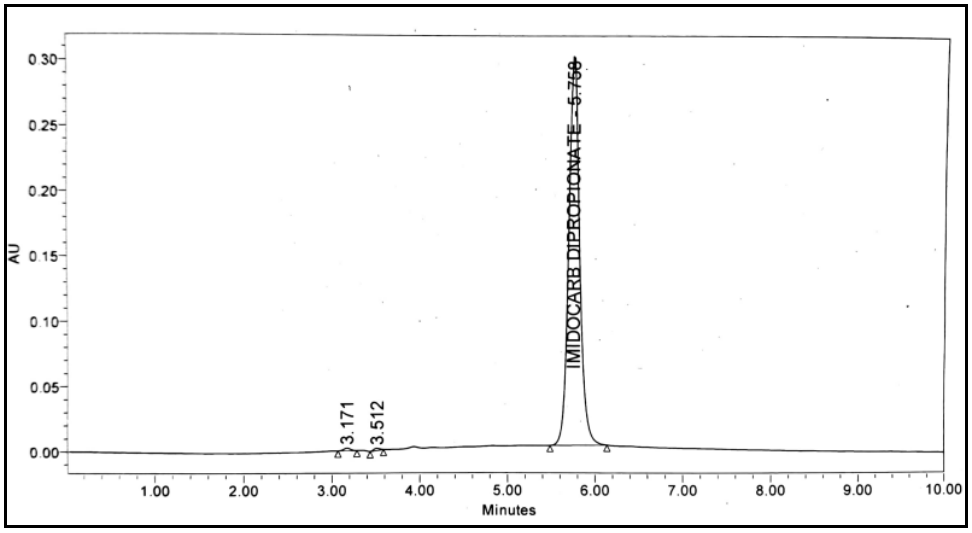

Fig. 7: IMD Photolytic degradation Chromatogram.

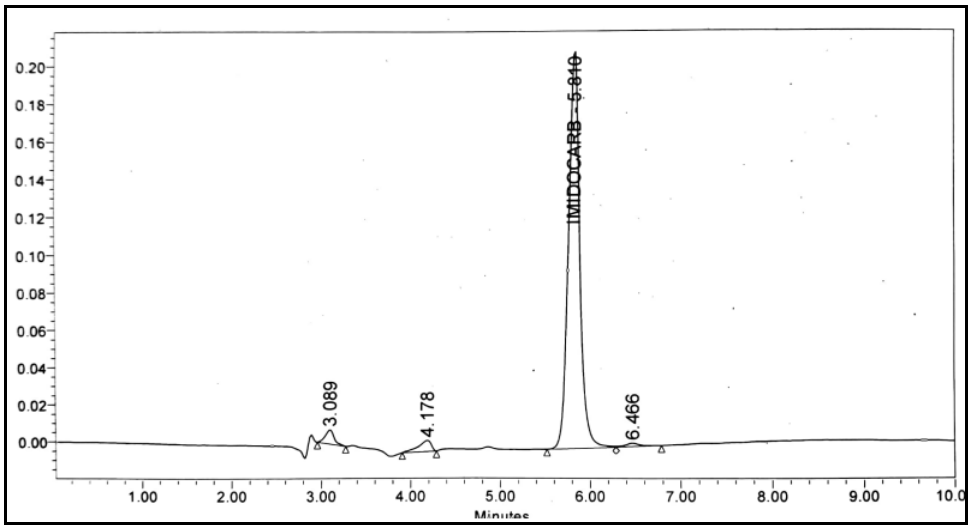

Fig. 8: IMD acidic degradation Chromatogram.

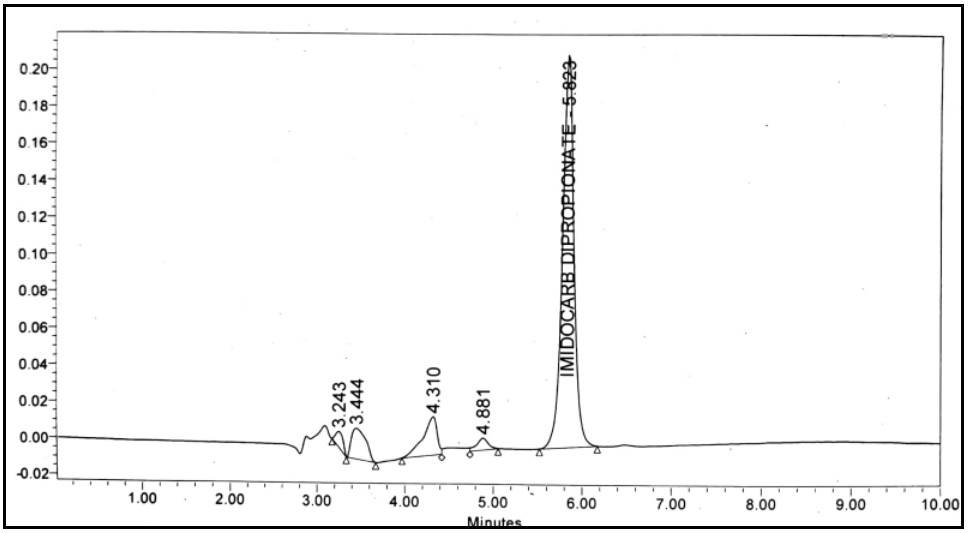

Fig. 9: IMD basic degradation Chromatogram.

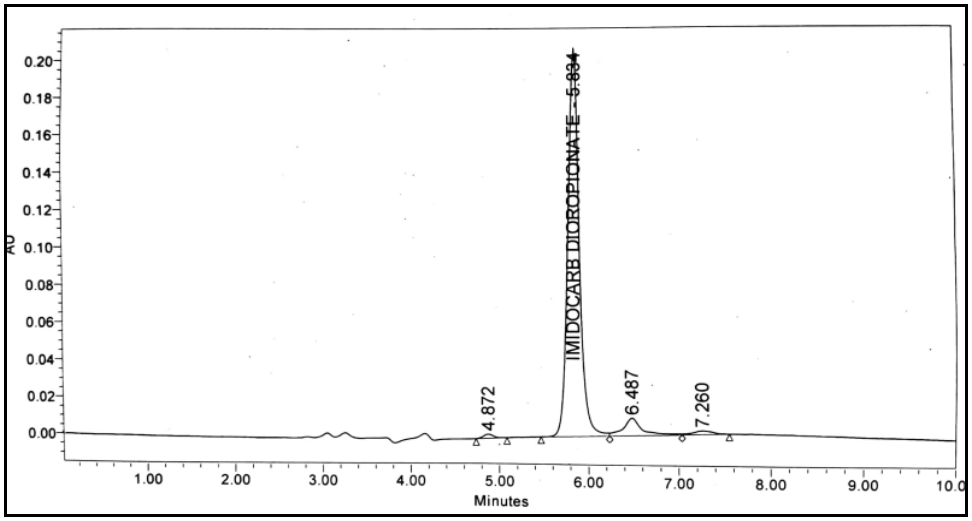

Fig. 10: IMD oxidation degradation Chromatogram.

Table 2: Degradation results of IMD.

Sr. no	Stress Condition	Duration (hrs)	Retention time of degradation products (Min)	% Residual Drug	Peak Purity (%)
1	Water	60 min	4.16	99.20	100.0
2	Thermal	60 min	3.17 ; 3.50 ; 4.80 ; 6.41	99.48	100.0
3	Photolytic	60 min	3.17 ; 3.51	99.27	100.0
4	Acidic (1M HCl)	60 min	3.08 ; 4.17 ; 6.46	93.50	100.0
5	Basic (1M NaoH)	30 min	3.24 ; 3.44 ; 4.31 ; 4.88	78.25	100.0
6	Oxidation(1%NaoH)	30 min	4.87 ; 6.48 ; 7.26	91.24	100.0

Table 3: Results of system suitability parameter of IMD.

Sr no.	Peak area	Capacity factor	Tailing factor (n=6)	Theoretical plates (n=6)
1	37261.89	6.08	0.98	10829
2	37324.72	6.08		
3	37154.17	6.09		
4	37262.61	6.08		
5	37317.82	6.09		
6	37238.19	6.09		
Average	37259.9	6.085		
SD	62.00	0.005		
% RSD	0.16	0.090		

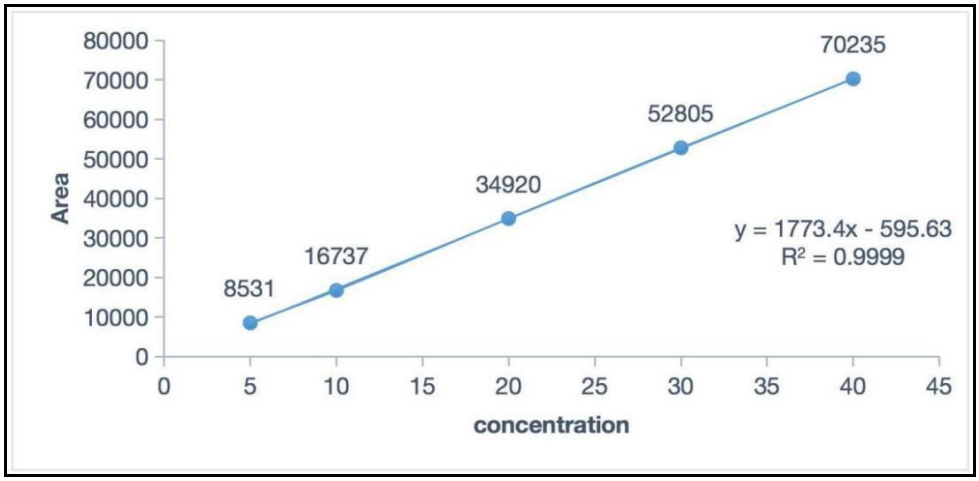

Fig. 11: Calibration curve of IMD.

Table 4: Average recoveries, % RSD value.

% Level	STD added (ml)	Amount Recovered (mg/ml)	% Recovery	SD	%RSD
110	1	147.06	101.96	0.0907	0.074
120	2	171.42	101.53	0.0971	0.069
130	3	195.31	101.32	0.1671	0.104
mean			101.60		

Table 5: Method Precision (Assay Repeatability) data of IMD.

Sr no.	Area
1	37420.43
2	37372.79
3	37360.42
4	37455.81
5	37371.00
6	37340.17
Average	37386.77
SD	42.92
% RSD	0.115

Table 6: Intermediate Precision (INTERDAY) data of IMD.

Sr no	Day 1	Day 2
1	102.12	101.33
2	101.29	101.33
3	100.51	100.96
4	102.97	100.26
5	101.18	101.81
6	101.81	101.26
Average	100.77	101.65
SD	0.549	0.454
% RSD	0.545	0.446

Table 7: Robustness data of IMD.

Parameter	Change in parameter	% Estimation	Mean	SD	% RSD
Wavelengh	241 nm	101.8	101.37	0.81	0.80
	243 nm	100.43			
	245 nm	101.88			
Flow rate	0.98 ml	101.38	101.14	0.65	0.64
	1 ml	100.43			
	1.02 ml	101.97			
Temperature	28 °C	101.26	101.26	0.77	0.76
	30 °C	100.43			
	32 °C	101.72			
Mobile phase (A : B)	83 : 17	101.64	101.04	0.85	0.84
	80 : 20	100.43			
	78 : 22	101.34			

Table 8: Limit of Detection and Quantitation (LOD & LOQ).

Sr no.	Area
1	37420.43
2	37372.79
3	37360.42
4	37455.81
5	37371.00
6	37340.17
Average	37386.77
SD	42.92
% RSD	0.115
Regression coefficient	1773.4x-595.63
Slope	1773.4
LOD	0.00159
LOQ	0.00481

Table 9: Assay Results of IMD.

Sample No.	Weight of standard (mg)	Sample weight (1ml contains 120 mg/ml)	Mean Area of standard at 243 nm	Area of sample at 243 nm	% Assay
1	10.09	1	37386.77	35280.96	101.33
2		1		35149.71	100.96
3		1		35279.66	101.33
4		1		34897.06	100.23
5		1		35194.56	101.04
6		1		35496.8	101.81
Mean					101.12
± SD					0.5269

DISCUSSIONS

A simple, isocratic and stability-indicating RP-HPLC method has been developed for

determination of IMD in injection dosage form. The obtimized chromatographic conditions are predicted by chemical structure of IMD shown in fig 1. Optimization of the method done on selection/choice of column, mobile phase, composition, injection volume and detection wavelength. The obtained chromatogram of IMD reference and sample are well resolved and sharp with retention time 6.01 min given in fig 3,4.

The system suitability parameters were applied to standard chromatograms and RSD of peak area, retension times were determined. The plates and tailing factors were also determined and found well within specified limits.

The concentration obtained was linear in range between 5-40 µg/ml. The linear calibration was constructed by analyzing the concentration over the selected range versus peak area of standard solution and regression coefficient was found to be 0.9999 from calibration curve.

The average of percent mean recovery at all three levels was found to be 101.60 % and mean percent RSD value found to be 0.030 %, which found to be within limits.

For method precision percent RSD result found to be 0.115 % for IMD, which are within the acceptable limit of 2.0 %. Intermediate precision was evaluated in terms of intraday precision by analyzing two different day intervals. Mean assay percentage are given for intermediate precision of IMD.

Robustness performed by keeping one parameter constant and other was kept altered. The concern variations on chromatographic method are compile and results obtained.

The obtimized method can determine and quantify the analyte at a lower concentration. LOD and LOQ of component IMD are determined by using mentioned formula.

The obtained assay results showed that percent assay of IMD in IMIX injection dosage form is 101.65 %, and are within defined limits. This results clearly state that developed method can be successfully applied for assay of pharmaceutical dosage forms. Results are shown in table 8.

Specificity was studies under various stressed conditions, it was reveled that there is no interference due to excipients. Chromatogram of degradation product are shown in fig 5-10. Significant fall in peak area of IMD was observed when it was subjected to basic hydrolysis.

The drug is highly sensitive forming four major degradation product at 3.24, 3.44, 3.31 and 4.88 and maximum degradation of drug were observed in basic hydrolysis compared to other stress condition. Less fall in peak area of IMD was observed when it subjected to water, thermal and photolytic hydrolysis. Maximum photolytic, oxidation, basic and acidic degradation of IMD observed at 223 nm, minimum degradation of IMD in all stress conditions were observed at 263 nm.

CONCLUSION

The present stability indicating method developed for quantitative and qualitative determination of IMD in injection dosage form was evaluated for specificity, linearity, range, accuracy, precision (repeatability and intermediate precision), and robustness. The method validate as per ICH Q2A guideline and results was within specification. Moderate regression values, percent RSD, standard deviation make it flexible and beneficial. As a result, present HPLC method could be adopted for quantitative quality control and routine analysis for injection dosage form in the laboratories.

ACKNOWLEDGMENT

The author is thankful to the Director of Central Drug Testing Laboratory, Mumbai and Gahlot Institute of Pharmacy, koparkhairane for the guidance and support. Special thanks to Dr. Vijay Kumar, Mrs. Smita Nayak and Dr. V.H. Bhaskar. The authors are extremely greatful to Mrs. S.U. Warde, Mrs. S. Parikh, Mrs. Ruchi Singh and others for their valuable guidance and support.

REFERENCE

1. Brayfield, A. Martindale: The complete drug reference (2011) 33th edition, pharmaceutical press, london, UK.
2. Donald, D. Treatment and drugg therapies of coccidiosis in carnivore, 2018; 445-463.
3. Antiprotozoal agents. Xpharma : The comprehensive pharmacology of reference, 2007; 1-2.
4. Trayonar M. Determination of Imidocarb residue in bovine and ovine liver and milk by immunobiosensor. Food additives and contaminants, 15 mar, 2013; 1108-1114.
5. Park J. Cho S. Determination of imidocarb in animal biosamples using simple high performance liquid chromatography with diode-array detection. Biomedical reports, 29 oct., 2013; 89-92.
6. Lehner A. Evaluation of mass spectrometric methods for detection of anti-protozoal drug

imdocarb. Journal of analytical taxicology, may, 2011; 35: 199-204.

7. Rei I. Analysis of imidocarb in livestock and sea food products using LC-MS/MS. Journal of food hygine and safety science, 2011; 52(1): 34-39.
8. Ghada S, Doha naguib. Validated stability indicating chromatographic methods for quantification of imidocarb dipropionate, application of determination of its residue in bovine meat and milk sample. Journal of AOAC internationals, 04 June 2020; 103: 980-998.
9. ICH, Q2B- validation of Analytical procedures; Methodology, International Conference on Harmonization, 1996.
10. Draft ICH Guidelines on validation of Analytical procedures definition and Terminologies. Federal Register, Vol 60. IFPMA, Switzerland, 1995; 1126.
11. ICH Stability Testing of New Drug Substances and Products Q1A[R2], in: Proceedings of International ConferenGhada ce on Harmonization, 2003.
12. G. Szepesi, Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. III. Method validation J. Chromatogr., 1989; 464: 265-278.
13. G.P. Carr, J.C. Wahlich. A practical approach to method validation in pharmaceutical analysis J. Pharm. Biomed. Anal., 1990; 86: 613-618.
14. D.R. Jenke. Chromatographic method validation: a review of common practices and procedures II J. Liq. Chromatogram., 1996; 19: 737-757.