SIMULTANEOUS ESTIMATION OF ISOMERIC *(R-S)* CEFPODOXIME PROXETIL AND DICLOXACILLIN SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP- HPLC AND HPTLC METHODS

KashyapN.Thummar
Assistant Professor
Graduate School of Pharmacy
Gujarat Technological University
ap_kashyap@gtu.edu.in

Sakariya Vijay
M.Pharm Student
Department of Pharmaceutical Science
Saurashtra University
vijaysakariya@gmail.com

Lalwani Krishna
M.Pharm Student
Department of Pharmaceutical Science
Saurashtra University
lalvanikrina772@gmail.com

DudhatraBhumika
M.S.Student
Gujarat Forensic Science University
bhumikadudhatra@yahoo.com

Prof.(Dr.) NavinSheth
Vice Chancellor
Gujarat Technological University
navin_sheth@yahoo.com

ABSTRACT
The pressurized liquid chromatography (RP- HPLC) and planner chromatography (HPTLC) were developed for the simultaneous estimation of dicloxacillin sodium and isomeric *(R-S)* cefpodoximeproxetil in bulk and pharmaceutical dosage form. In RP-HPLC, the chromatographic separation was achieved on HIBER C18 (250 x 4.6, 5 µm) column for dicloxacillin (DCX) and cefpodoximeproxetil (CPD), consisted a moving phase of methanol: 10 mM potassium dihydrogen phosphate: triethylamine (55:45: 0.13, v/v/v) having pH-6 using ortho phosphoric acid with 1.0 mL/min of analytical flow rate. The quantification of analytes were done at 225 nm using photo diode array detector. The elution times of DCX and CPD were 5.8 min and 10.6 min, respectively. In HPTLC, chromatograms were developed using a mobile phase containing toluene: methanol: ethyl acetate: glacial acetic acid (7.0:2.5:0.5:0.1, v/v/v/v) on precoated silica gel $60F_{254}$ as the stationary phase. Saturation time was 15 min. and densitometric detection at 235 nm. The R_f value of DCX and CPD were 0.46 and 0.69, respectively. The proposed methods were fully validated in terms of ICH (Q2) R1 guideline. These methods were successfully assessed for the simultaneous quantification of DCX and CPD in combined marketed tablet dosage form.

Keywords: Dicloxacillin sodium, Cefpodoximeproxetil, RP-HPLC, HPTLC, Validation

1. INTRODUCTION

Dicloxacillin (DCX), (Figure: 1(a)) a narrow-spectrum beta-lactam antibiotic of the penicillin class [1]. It is commonly used in pneumonia, septic arthritis and throat infections [2]. It is official in IP [1], BP [3] and USP [4]. Cefpodoximeproxetil(CPD), (Figure : 1(b)), is third generation cephalosporin antibiotic to cure spreadable disease triggered by bacteria such as bronchitis, urinary tract infections, pneumonia; ear, gonorrhea, throat, and skin infection [5]. It is official in Indian Pharmacopoeia[6],and United States Pharmacopoeia [7] which recommends liquid chromatography method for its analysis.
The combination therapy is available for DCX and CPD and used in the treatment of infection causes by bacteria. Review of Literature revealed that few spectrophotometric [8-9], and HPLC [10-11], methods were reported for the quantification of individual DCX or with its combined other drug. On the other hand various spectrophotometric [12-14], HPLC [15-16], and HPTLC [17] methods were available for estimation of CPD individually or in combination with other drugs. Hithero, First orderDerivative spectroscopy [18],

method was reported for analysis of DCX and CPD in combination. Therefore, the present work is focused on to develop a rapid, accurate and precise RP-HPLC and HPTLC method and, fully validated as per international council on harmonization (ICH) guidelines [19].

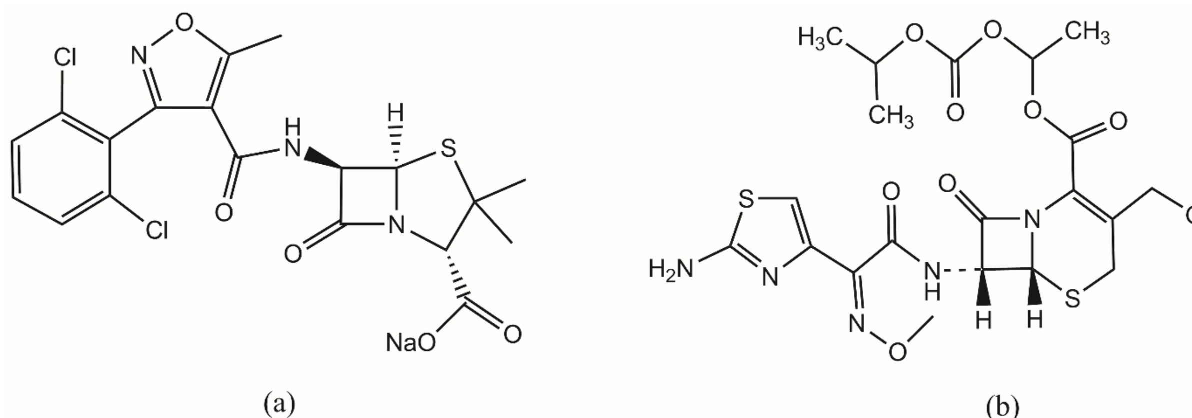

Figure 1: Chemical structure of (a) Dicloxacillin sodium, (b) Cefpodoximeproxetil

2. EXPERIMENTALS:

2.1 Instrumentation:

The chromatographic system consisted of HPLC (Shimadzu-Kyoto, Japan) containing LC-20AD pump, variable wavelength programmable SPD -M20A-PDA detector and having 20 µL fixed loop of manual rheodyne injector. The DCX and CPD were quantified at 225 nm. The chromatographic separation was employed on column having dimension was HIBER® C18 (250 × 4.6 mm, 5 µm). In HPTLC Sample applicator- Linomat – V and TLC scanner, Twin trough chamber, Hamilton Syringe (100 µL). The densitometric detection were set at 235 nm. The system was controlled with WinCATS software V 4.0.

2.2 Chemicals and Reagents

The standrard of DCX and CPD were collected form Department of pharmaceutical sciences, Saurashtra University, Rajkot. All HPLC and analytical grade chemicals were purchased from Merck Ltd., India. Methanol, Triethylamine both (HPLC grade) and potassium dihydrogen phosphate, benzene, anhydrous acetic acid, ethyl acetate, toluene of analytical grade. HPLC grade water was prepared from milliQ synergy UV apparatus (Merck Millipore, India). Tablet formulation Zedocef® DXL 200 (Macleod's Pharmaceutical Ltd. Mumbai, India.) having labeled claim of 500 mg of DCX and 200 mg of CPD, were procured from market.

2.3 Preparation of standard stock solutions

The equivalent amount of 50 mg of DCX and 20 mg CPD were weighed and transferred in two different 10 mL volumetric flasks. Both analytes were dissolved in 5 mL of methanol by ultra-sonication and then dilute up to 10 mL with same solvent to obtain final concentration 5000 µg/mL for DCX and 2000 µg/mL for CPD. The appropriate volume (1 mL) was taken and dilute to 10 mL in volumetric flask to achieve 500 µg/mL and 200µg/mL considered it as standard stock solution and use for both methods.

2.4 Chromatographic conditions

The various combination of mobile phase were tried for RP-HPLC and HPTLC. In RP-HPLC, the satisfactory results shown to the mobile phase containing water: 10 mM potassium dihydrogen phosphate: triethylamine (55:45:0.13, v/v/v) with flow rate 1 mL/min using a HIBER C18 (250 × 4.6, 5 µm) column

and UV detection at 225 nm by PDA detector.In HPTLC, TLC aluminum plates precoated with silica gel $60F_{254}$ used as the stationary phase with a moving phase containing toluene: methanol: ethyl acetate: glacial acetic acid (7.0:2.5:0.5:0.1, v/v/v/v). Saturation time was set for 15 min and detection was performed at 235 nm. The application of the samples were done by a 100 µLhamilton syringe.

2.5 Method Validation

The RP-HPLC and HPTLC methods were validated according to ICH Q2 R1 guideline.

2.5.1 Linearity and Range

In HPLC, the aliquots of DCX and CPD were prepared in separate 10 mL volumetric flask with methanol having concentration range 62.5-162.5 µg/mL for DCX and 25-65 µg/mL for CPD respectively. The standard solutions were inserted using a 20 µL of injection volume and chromatograms were recorded.
In HPTLC, the calibration curve was plotted by analyzing five individual levels in the linear range of 1-5 µg/band for DCX and 0.4-2 µg/band for CPD, the calibration curve was qualify by its correlation co-efficient value for both drugs.

2.5.2 Precision

The intra-day precision were performed by analyzing the corresponding responses 3 times within the day and 3 times on the 3 different days for the inter-day precision. For three same concentrations of DCX (100 µg/mL), CPD (40 µg/mL) in HPLC and DCX (3 µg/band), CPD (1.2 µg/band) in HPTLC and the results were expressed in relative standard deviation. The instrumental precision were performed by evaluating response six times of same concentrations of DCX (100 µg/mL) and CPD (40 µg/mL) in HPLC and DCX (3 µg/band) and CPD (1.2 µg/band) in HPTLC. The precision were measured by relative standard deviation.

2.5.3 Accuracy

The accuracy of the methods were evaluated by percentage recoveries of DCX and CPD by standard method of additions at 3 levels i.e. 80%, 100% and 120%. Known amount of DCX (80, 100, 120 µg/mL) and CPD (32, 40, 48 µg/mL) were added to a pre-quantified sample solution (having DCX and CPD in 100:40 µg/mL proportion, respectively), in HPLC, for HPTLC, known amount of DCX (1.6, 2, 2.4 µg/band) and CPD (0.64, 0.8, 0.96 µg/band) were added to a pre-quantified sample solution (having DCX and CPD in 2:0.8 µg/band proportion, respectively) and the final amount of DCX and CPD were calculated by measuring the peak areas and by fitting these values to the linear regression equation of calibration curve to measure the recovered amount and thereby % recoveries.

2.5.4 Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated using the following equation as per ICH guidelines. Both LOD and LOQ were analyzed by equation methods.
$LOD = 3.3 \times \sigma / S$;
$LOQ = 10 \times \sigma / S$;
Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

2.5.5 Robustness

Robustness of the method was studied by minor changing the experimental conditions such as pH of mobile phase, flow rate and % of organic phase in HPLC and while saturation time, mobile phase ratio in HPTLC. The methods were prove against the value of relative standard deviation obtained.

2.5.6 Analysis of marketed formulations

A marketed formulation having 500 mg of DCX and 200 mg of CPD was taken in sufficient quantity, the test solutions were properly sonicate for 10 min to dissolve the analytes and further the test solutions were prepared in manner to made clear solution. The test solution was diluted with methanol to get thefinal solution containing DCX and CPD in 50:20 µg/ mL proportion, respectively. The final test solution was assessed as per above discuss chromatographic conditioned and peak areas were measured. The estimation of DCX and CPD were done by keeping these values to the linear regression equation of calibration curve.

3. RESULTS AND DISCUSSION:

3.1 Mobile phase optimization

Several trials were taken containing methanol, water, ACN and aqueous buffer in various mixture as mobile phases in the development stage of method. At last, the mixture of 10 mM KH_2PO_4: Methanol: Triethylamine (45:55:0.13, v/v), pH adjusted to 6 with o-phosphoric acid was found to appropriate and gave well-resolved peaks for DCX and CPD with isomeric separation in HPLC. The retention time for DCX was 5.8 min and for isomer of CPD (R-S) was found to be 12.8 and 10.6 min, respectively (Figure 2). The separation between DCX and CPD was found to 6.52. The mobile phase flow rate was maintained at 1 mL/min. Detection was performed at 225 nm.

In HPTLC Finally, the system containing mixture of toluene: methanol: ethyl acetate: glacial acetic acid (7.0:2.5:0.5:0.1, v/v/v/v) was found to satisfactory and gave two well-resolved peaks for DCX and CPD. The R_f value for DCX and CPD were 4.6 and 6.9, respectively. Saturation time of mobile phase was 15 min after several optimization. Detection wavelength was finalize at isosbestic point at which both drug spectra was crossed constantly that was at 235 nm. The data indicates, method represent good separation of both compounds (Figure 3).

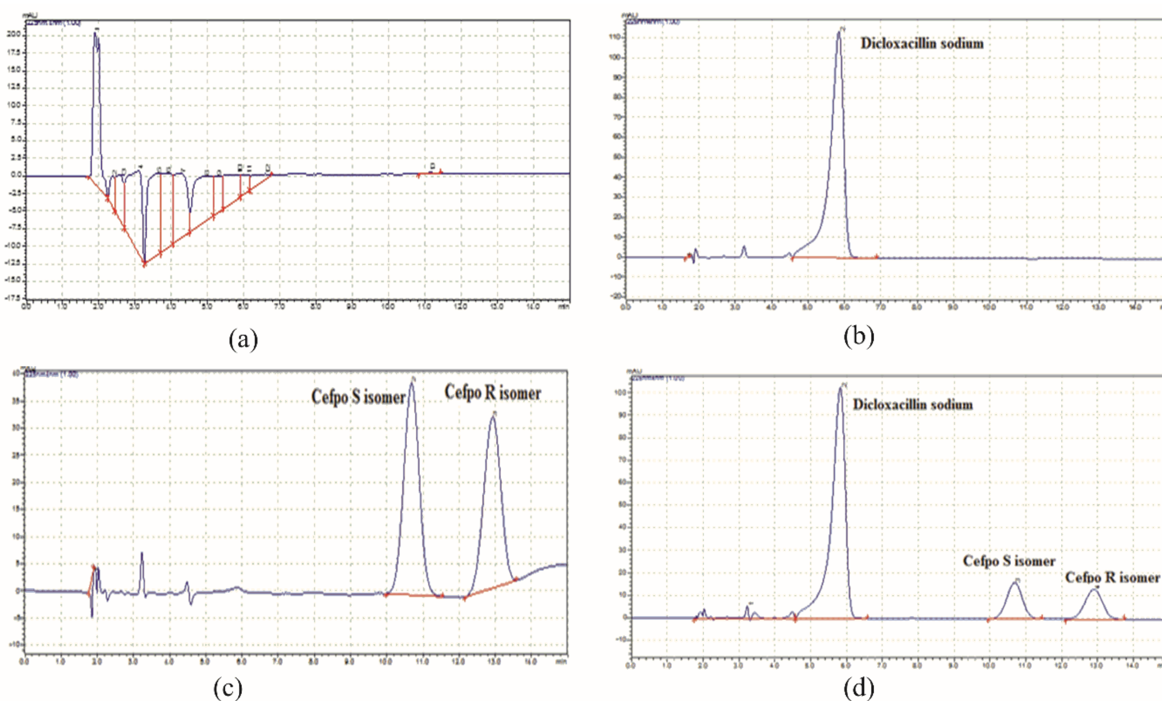

Figure 2: (a) HPLC chromatogram of blank, (b) HPLC Chromatogram of DCX (R_t 5.8 min), (C) HPLC chromatogram of CPD *S-isomer* (R_t 10.6 min) and CPD R-*isomer* (Rt 12.8) and (d) standard chromatogram of Mixture solution of both drugs,

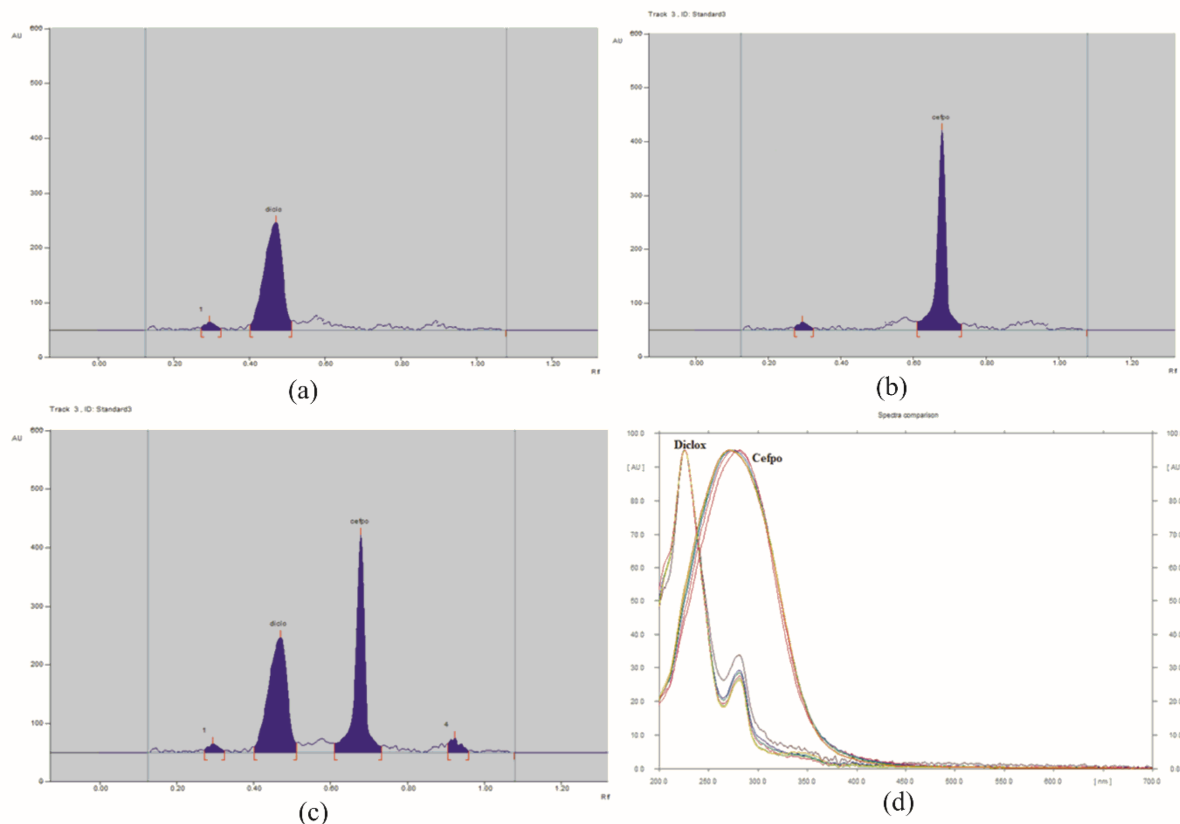

Figure 3: (a) HPTLC chromatogram of DCX (R_f 0.46), (b) HPTLC chromatogram of CPD (R_f 0.69), (C) HPTLC chromatogram of standard mixture solution of both drugs, (d) Peak purity spectra of both drugs.

3.2 Validation of the Proposed Method:

3.2.1 Linearity and Range

The linear range DCX was found for the calibration range of 62.5–112.5 µg/mL and 1–5 µg/band within a correlation coefficient of 0.992 and 0.998 in HPLC and HPTLC respectively. And linear range for CPD, it was 25–45 µg/mL and 0.2–2 µg/bands within a correlation coefficient of 0.996 and 0.999 in HPLC and HPTLC respectively. The linearity data were reported in table 1.

Table 1: The measured data from calibration curvesf

Parameters	HPLC		HPTLC	
	DCX	**CPD**	**DCX**	**CPD**
Conc.Range	62.5-112.5 (µg/mL)	25-45(µg/mL)	1–5(µg/band)	0.4-2 (µg/band)
Slope (m)	27198	11361	2001	4066
SD of slope	101.25	739.78	110	650
Intercept(c)	76348	45148	945.2	1484
Correlation coefficient (R^2)	0.992	0.996	0.998	0.999

3.2.2 Precision

The precision values (RSD) for DCX and CPD were found to 0.80–0.99% and 0.55–0.58% in HPLC while 5–2% and 0.5–1.14% in HPTLC respectively. The RSD values for intra-day were within the 1.94 % for both drugs in HPLC and HPTLC methods and inter-day precision were within the 2 % for both drugs in HPLC and HPTLC methods. The all RSD values fall in acceptable limit hence it indicate that the methods were precise.

3.2.3 Accuracy

The trueness of the methods were done by calculating recoveries of DCX and CPD by standard method of addition. The percentage recoveries were found to 99.86–100.09% and 99.54–100.15% for DCX and CPD, respectively in HPLC. While for HPTLC recoveries were 99.40–99.84% and 98.21–99.57% for DCX and CPD respectively (table 3). The high values indicate that the method was accurate.

Table 3: The accuracy data for theproposed method

Drug	Level(n=3)	Amount found (µg/band)		% recovery		RSD	
		HPLC	HPTLC	HPLC	HPTLC	HPLC	HPTLC
DCX	80 %	180.12	3.55	100.06	99.56	0.51	0.42
	100%	199.72	3.95	99.86	99.84	0.35	0.27
	120%	220.21	4.37	100.06	99.40	0.23	0.56
CPD	80%	71.67	1.43	99.54	98.21	0.11	1.01
	100%	80.12	1.58	100.15	99.26	0.47	1.0
	120%	87.52	1.75	99.57	99.57	0.19	0.55

3.2.4 LOD and LOQ

The LOD for DCX and CPD were 1.44 µg/mL and 0.59 µg/mL, respectively while LOQ were 4.36 µg/mL and 1.80 µg/mL, respectively in HPLC while in HPTLC, LOQ limit for DCX and CPD were 0.24 µg/mL and 0.08 µg/mL, respectively, while LOQ were 0.53 µg/mL and 0.24 µg/mL, respectively The determined value represented the methods were sensitive, accurate and precise.

3.2.5 Robustness

Table 4 shows that the methods were found robust and have no any detrimental effect on the response of analytes. The RSD values indicated that the methods were robust.

Table 4: Results (RSD values) of robustness parameters of HPLC and HPTLC methods

HPLC Method			
Sr. no.	Method parameter/Condition	RSD of Peak area (n = 6)	
		Condition 1*	Condition 2#
1	Flow rate (± 0.1 mL/min)	0.73	0.56
		0.64	0.31
2	Mobile phase ratio (± 1 v/v)	0.89	0.56
		0.53	0.27
HPTLC Method			
Sr. no.	Method parameter/Condition	RSD of Peak area (n = 6)	
		Condition 1*	Condition 2#

1	Saturation time 15min.(± 1 min)	2.0	1.50
		1.58	1.55
2	Mobile phase ratio (± 0.5 v/v)	1.75	1.82
		1.97	1.45

[*]Positive deviation (+) from the original condition

[#] Negative deviation (-) from the original condition

3.2.6 Analysis of marketed formulation

The test solution were prepared in methanol and clean solution having DCX and CPD in 50:20 µg/ mL proportion, respectively, were injected and peak areas were measured. The % assay of DCX and CPD in tablet dosage form were calculated and reported in (Table 5).

Table 5 Analysis of marketed formulation by HPLC and HPTLC

Brand (Tablet)	Amount taken		% assay ± SD (n=5)	
Zedocef® DXL	DCX	CPD	DCX	CPD
HPLC (µg/mL)	50	20	100.02 ± 1.65	90.48 ± 1.21
HPTLC (µg/band)	3	1.2	99.65 ± 1.70	99.08 ± 1.58

3.3 Statistical Analysis

The statistical analysis was also evaluated to compare both methods by paired t-test using accuracy data of both HPLC and HPTLC method. It was concluded that there was no significant difference between the HPLC and HPTLC methods (Figure 4)

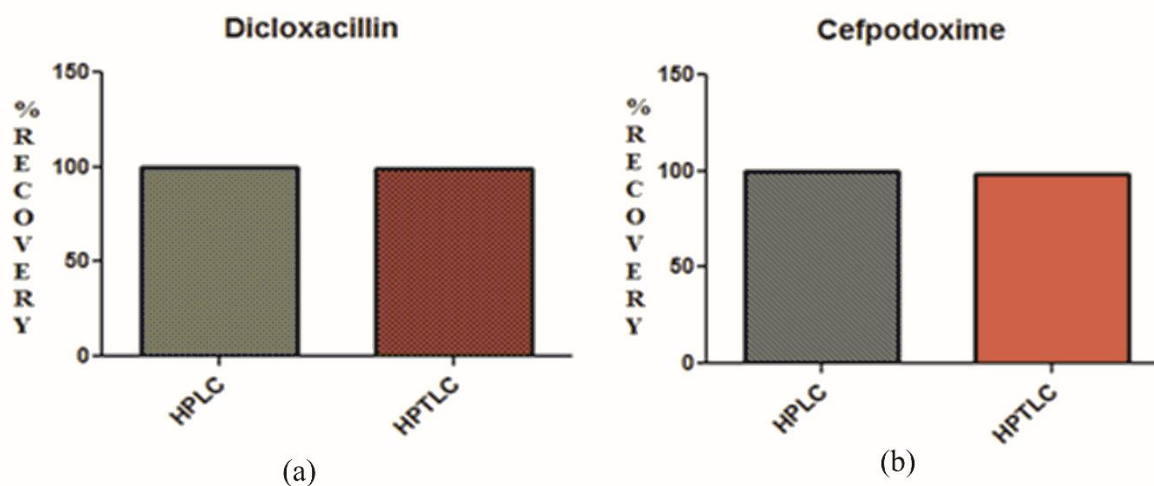

Figure 4 : T-test comparison graph of (a) DCX and (b) CPD

CONCLUSION

The RP-HPLC and HPTLC method have been successfully developed for simultaneous estimation of DCX and CPD. This method could be used for pure drug analysis, assay of drug formulation and stability study. The purposed method did not use any extraction step in recovering of drug from the formulation excipients and matrixes and their by decrease degree of error, and overall cost of drug analysis. This both method RP-HPLC and HPTLC were fully validated as per ICH guideline and found to simple, accurate, simple, precise, and economical. This method could be applied in routine quality control laboratories.

ACKNOWLEDGEMENT

We kindly acknowledge the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for providing the instrument facility for this research work.

CONFLICT OF INTEREST

The authors does not have any conflict of Interest.

REFERENCES

1. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Indian Pharmacopoeia Commission, Ghaziabad, 6th edition ,2010, 2,1201-1202.
2. Tripathi KD, Essentials of medical pharmacology, 7thedition, Jaypeebrothers medical publishers 2008, 902.
3. British Pharmacopoeia, Vol. I. London, The British Pharmacopoeia Commission, 2007,606-607.
4. United States Pharmacopoeia 32 NF 27. The United States Pharmacopoeial Convention, Rockville, 2009 , 378
5. Tripathi KD, Essentials of medical pharmacology, 7th edition, Jaypeebrothers medical publishers 2008, 726-728.
6. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Indian Pharmacopoeia Commission, Ghaziabad, 7th edition, 2010, 1018.
7. United States Pharmacopoeia 32 NF 27. The United States Pharmacopoeial Convention, Rockville , 2009 , 1860
8. Nour F, Mohamed G and Farag E, "Spectrophotometric Determination of Flucloxacillin and Dicloxacillin in Pure and Dosage Forms." SpectrochimicaActa, 2006, part A 64, 210–215.
9. Morelli B, "Second-derivative spectrophotometric assay of mixtures of Dicloxacillin sodium and Ampicillin sodium in pharmaceuticals." Journal of Pharmaceutical Sciences. 77, 1988, 1042–1046.
10. Lauriault G, Awang D and Kindack D, "The High performance liquid chromatographic determination of Dicloxacillin in presence of its degradation products" Journal of Chromatography. 283, 1984, 449-452.
11. Barot T, Patidar K, Kshartri N and Vyas N, "Development and Validation of LC Method for the Determination of Ampicillin and Dicloxacillin in Pharmaceutical Formulation Using an Experimental Design." Journal of Chemistry. 6, 2009, 955-964.
12. Kavar R, Lakkad A, Savaliya B, Kapuriya K and Faldu S, "Development and validation of spectrophotometric method for simultaneous estimation of CefpodoximeProxetil and Levofloxacin Hemihydrate in bulk drug and pharmaceutical dosage form by simultaneous equation method" Inventi Rapid: Pharm Analysis & Quality Assurance, 2013, 1-4.
13. Kavar R, Savaliya B, Lakkad A, Soriya S, Kapuriya K and Faldu S, "Q-absorbance ratio spectrophotometric method for the simultaneous estimation of CefpodoximeProxetil and Levofloxacin Hemihydrate in their combined dosage form" IBDR, 2012, 2, 22-30.
14. Mohamed N, Abdel W and Ahmed S, "An efficient one-potreaction for selective fluorimetric determination of Cefpodoxime and its prodrug." Talanta. 85, 2011, 2121- 2127.
15. Kakumanu V, Arora V and Bansal A, "Development and Validation of isomer specific RP-HPLC method for quantification of CefpodoximeProxetil." Journal of Chromatography B. 835, 2006, 16–20.
16. Jing X, Qin H and Hong J, "Development and validation of an HPLC method for the determination of CefpodoximeProxetil and its related substances in dry syrups." Chinese Journal of Antibiotics. 10, 2003, 476-484.
17. Darji B, Shah N, Patel A and Patel N, "Development and validation of a HPTLC method for the estimation of CefpodoximeProxetil." Indian Journal of Pharmaceutical Science. 69, 2007, 331-333.
18. Acharya DR, Patel DB, Patel VV. Development and validation of first order derivative spectrophotometric method for simultaneous estimation dicloxacillin and cefpodoximeproxetil in tablet dosage form. J.D.D.T, 2(1), 2012.
19. Validation of Analytical Procedures: Methodology (Q2R1), ICH Harmonized Tripartite Guidelines,1996 , 1-8.