RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CEFIXIME TRIHYDRATE IN BULK AND DOSAGE FORM

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Abstract

For the detection of Cefixime trihydrate, a straight forward, accurate, precise, and reliable reverse phase high performance liquid chromatography (RP-HPLC) approach has been devised and tested. Drug was resolved on a C18 column, utilizing mobile phase of Triethylamine: Methanol: Acetonitrile: Water (2:10:20:68 V/V%). The mobile phase was degassed by Sonication (10 min) and filtered under vacuum just before HPLC analysis. The flow rate used to provide mobile phase was 1.0 mL/min. Ultra violet detection was carried out at 291 nm. The drug exhibited a peak at retention time 1.547 min. The calibration curve was linear in the range of 2-12 µg/mL and coefficient of regression was found to be 0.9995. The mean percentage recoveries (S.D) for lower intermediate and higher concentrations were found to be 100.0093±0.02837, 100.00±0.00 and 100.0458±0.02178 respectively. With a relative standard deviation (RSD) for intra and inter-day precision of 1.0 over the specified concentration range, the method was determined to be reproducible. The method was successfully applied to the determination of quantity of medicine in SMEDDS formulation of Cefixime trihydrate, it can be very useful and an alternate to perform the stability studies.

Keywords: Cefixime Trihydrate, RP HPLC, SMEDDS.

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Introduction

Cefixime is a semi synthetic, aminothiozolyl, broad spectrum third generation cephalosporin antibiotic, active against gram positive and gram negative aerobic bacteria. Its pharmacokinetics profile has been extensively studied in healthy volunteers as well as patients[1].

Besides, its use in urinary tract infection, respiratory tract infection it has been documented efficacious in the treatment of gonorrhea1. It is used also to treat many different types of bacterial infections such as bronchitis, tonsillitis, ear infection and skin infection. Cefixime is available in oral formulation[2].

Like ceftriaxone and cefotaxime, cefixime has enhanced antibacterial activity and increased stability against many of the β-lactamase. Very few methods for analysis of cefixime trihydrate are reported in the literature. Those reported include high performance liquid chromatography[3]. The aim of the present work was to develop a relatively simple, sensitive, validated reliable, and inexpensive HPLC method for the determination of Cefixime trihydrate and its product[4].

The cephalosporins are the largest and most diverse family of beta-lactam antibiotics. They are linked to penicillin, both structurally and pharmaceutically[5]. Cephalosporins have a beta-lactam ring structure infused to a 6-membered dihydrothiazine ring thus forming the cephem nucleus.

MATERIALS AND METHODS

Cefixime trihydrate was obtained as a gift sample from FDC Mumbai (Mumbai, India). Acetonitrile and Methanol, Ethanol, Hydrochloric acid (HCL) used in the present study were of high performance liquid chromatography (HPLC) grade. All other chemicals were reagents grade.

Method Development

Preparation of Buffer Solution

Cefixime trihydrate(100 mg) was accurately weighed and transferred to the 100 mL volumetric flask. It was dissolved properly in methanol (30 mL) and diluted up to the mark with phosphate buffer pH 6.8 to obtain final concentration of 1000 µg/mL and used as a stock solution.

Preparation of Stock Solution

Cefixime trihydrate (100 mg) was accurately weighed and transferred to the 100 mL volumetric flask and diluted up to the mark with methanol to obtain final concentration of 1000 µg/mL and used as a stock solution. From the stock solution working standard solutions ranging from 2-12 µg/mL was prepared by appropriate dilution with phosphate buffer pH 6.8.

Determination of λ_{max}

Following an appropriate dilution of the standard solution with methanol , solutions containing 10 µg/ml of cefixime trihydrate were scanned in the 400–200 nm range to identify the wavelength of maximum drug absorption. Cefixime trihydrate had the highest absorption at 291 nm. The spectrum was obtained and the maximum absorbance was found out for detection of λ_{max} of Cefixime trihydrate in Methanol.

Preparation of Standard Solution

A wide range of solutions were prepared by calculating the amount of the stock solution and the amount of buffer solution. 2-12 µg/mL concentration range solution were prepared for the determination of validation parameters and others studies. 2,4,6,8,10,12 mL stock solution was taken in 100 mL volumetric flask and diluted with buffer up to the mark.

Preparation of Assay Solution

A 400 mg of drug from the tablet was weighed, then taken in a 100 mL volumetric flask and volume was adjusted up to 100 mL. Later, dilution was used to create the desired concentrations from this solution.

Quantitative Analysis of the Drug

A regression equation was obtained after constructing a calibration curve by graphing absorbance versus concentration. By taking the solution's absorbance at 291 nm and multiplying it by the dilution factor and total volume of solution, the standard curve equation was used to determine the quantitative analysis of the drug in a sample.

Method Validation

The proposed method was tested for linearity, precision, accuracy, specificity, robustness, LOD, LOQ and Assay accuracy. Validation of the proposed spectrophotometric method was carried out as per ICH guidelines by means of the following parameters.

Linearity

As per ICH guidelines the linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[6]. Least square linear

regression analysis was used to establish the method's linearity investigation. Linearity of the proposed method was determined by taking six separate series of solutions of Cefixime trihydrate (2-12 µg/mL) in methanol prepared from the pure drug stock solution and analyzed. For the gathered data, least squares regression analysis was done.

Recovery Study (Accuracy Study)

The degree of agreement between the value acknowledged as either a conventional true value or a recognised reference value and the value discovered is expressed as the analytical procedure's accuracy[7]. The accuracy of the proposed methods was checked by recovery studies (standard addition method), by addition of standard drug solution to pre-analysed sample solution at three different concentration levels within the range of linearity. Accuracy was measured as the percentage relative error and mean % recovery. An additional support to accuracy of the developed assay method, standard addition method was performed. In this study, different concentrations of pure drug (2.5, 5 and 7.5 µg/mL) were added to a known concentration of formulation sample and the total concentration was determined using the proposed method. The percent recovery of the added pure drug was estimated as, % recovery.

Precision

The degree of scatter between a set of measurements obtained from multiple sampling of the homogeneous sample under the specified conditions expresses the precision of an analytical process.

Repeatability: Repeatability describes the accuracy over a brief period of time while using the same operating conditions. Repeatability is also termed intra-assay precision[8]. Repeatability was calculated by taking different levels of concentrations, prepared from pure drug stock solution and analyzed.

Reproducibility: Reproducibility expresses the precision between laboratories. Intermediate precision was calculated by taking the variations of inter-day and intra-day response[9]. Three times throughout the day, triplicates of the respective concentrations from the stock solution were made and their intra-day and inter-day variability was examined. The relative standard deviation (% R.S.D.) of the estimated concentrations from the regression equation was taken as precision.

Specificity

The capacity to clearly evaluate the analyte in the presence of components that could be anticipated to be present is known as specificity[10]. These frequently include degradants, matrix, and contaminants. Comparison of the UV spectrum obtained from the solvent (blank), and Cefixime trihydrate smedds shown in figure sample revealed no significant interference, using same analytical conditions for samples. Cefixime trihydrate solution (10µg/mL) was prepared in selected medium. The solutions were scanned from 400 nm to 200 nm at a speed of 1 nm/sec and checked for any change in absorbance at specific wavelength.

Limit of Detection

The smallest quantity of analyte in a sample that can be identified, but not necessarily measured as an exact value, is the detection limit of a certain analytical process, according to ICH guidelines. According to ICH recommendations, the limit of detection can be computed using the following calculation.

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

Where, N= Standard deviation of the response
S= Slope of the calibration curve

Limit of Quantitation

The lowest amount of analyte in a sample that can be quantitatively measured with enough precision and accuracy is the quantitation limit of a specific analytical process. In quantitative assays for low concentrations of chemicals in sample matrices, the quantitation limit is a parameter that is particularly useful for identifying contaminants and/or degradation products. According to ICH recommendations, the limit of quantification can be computed using the following calculation.

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

Where, N= Standard deviation of the response
S= Slope of the calibration curve

Ruggedness

Variations within laboratories, such as various days, analysts, equipment, etc., are expressed as intermediate precision. The goal of intermediate precision validation is to confirm that after the development phase is complete, the method will provide the same findings in the same laboratory. The objective is also extent to verify that the method will provide the same results in different laboratories.

Robustness

A method's robustness is its ability to withstand minor, purposeful changes to its input parameters.

Robustness of the proposed method was performed by:

a) Changing pH of the media by ±1units
b) Stability of Cefixime trihydrate in the selected medium at room temperature ±1^{0}c for 48 hrs.

Results and Discussion

The absorption spectra in the range (200-400nm) were obtained for Cefixime trihydrate in methanol. The drug exhibited an absorption maximum at 291nm. A linear relationship between the concentration and absorbance of Cefixime trihydrate were established over the examined concentration range (2-12µg/mL).

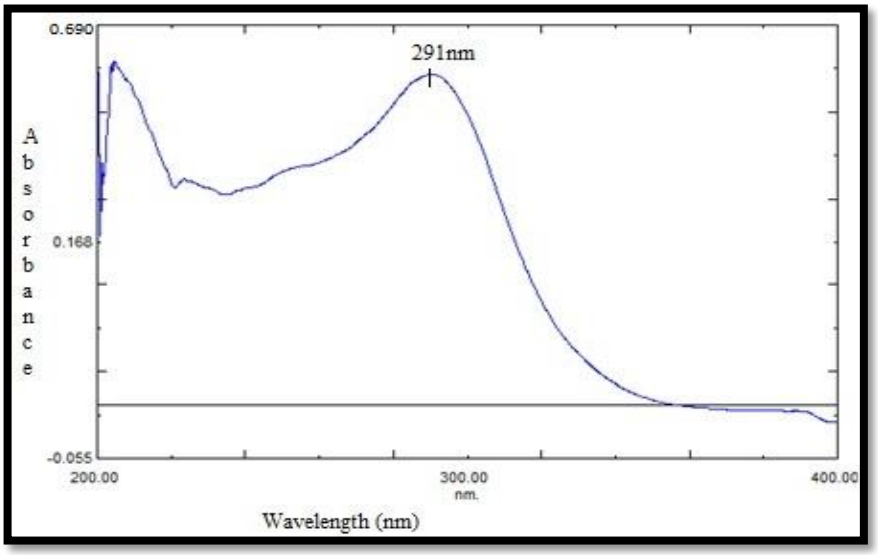

Figure 1: UV Spectrum Graph of Cefixime trihydrate Reference Standard

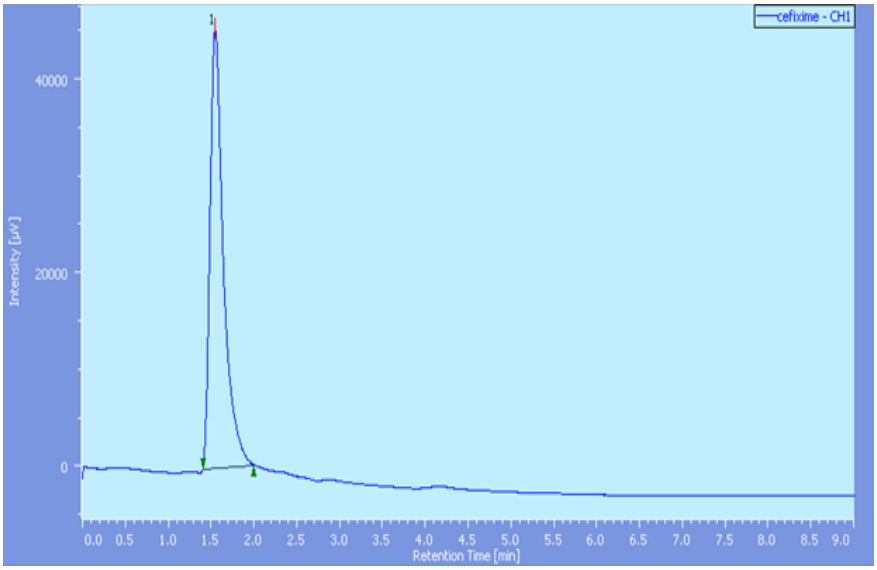

Figure 2: Test HPLC Chromatogram for the Analysis of Cefixime trihydrate Reference Standard by Developed Validated Method

Table 1: Calibration of Cefixime trihydrate by RP-HPLC

Sr. No.	Retention Time	Peak Area (µV/sec)	% Area	Symmetric Factor
1	1.547	493366	100	1.717

The HPLC chromatograms were obtained for Cefixime trihydrate in Triethylamine: Methanol: Acetonitrile: Water (2:10:20:68 v/v%). The drug exhibited a peak at retention time 1.547 min. C18 is common stationary phase suitable for many pharmaceutical and was found to be suitable in this study.

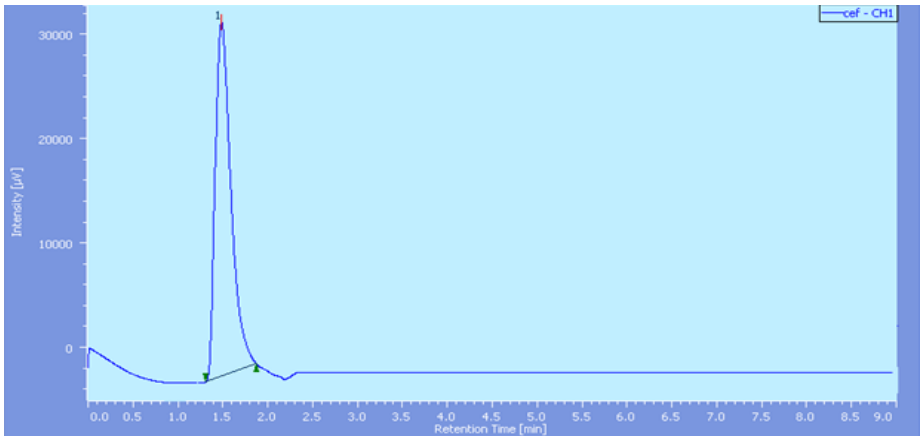

Figure 3: HPLC Chromatogram of Marketed Formulation

Table 2: HPLC Chromatogram Data of Marketed Formulation

Sr. No	Retention time	Peak area (µV/sec)	% Area	Symmetric factor	NTP
1	1.133	492191	100	1.500	4257

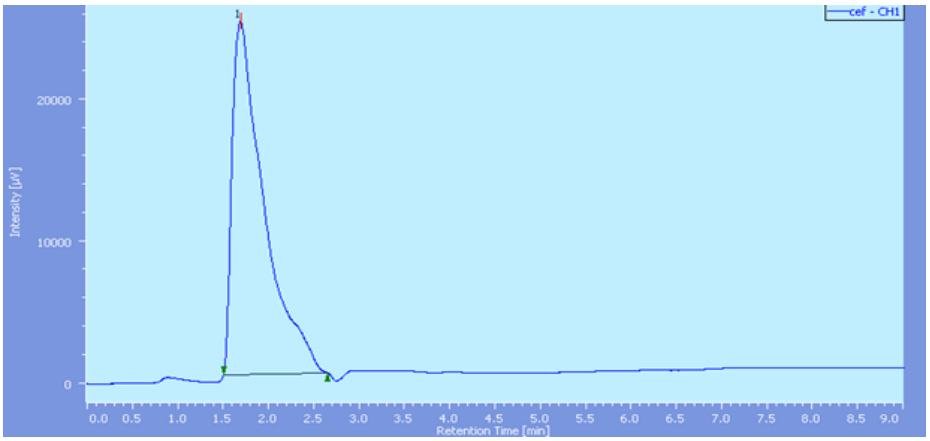

Figure 4: HPLC Chromatogram of SMEDDS Formulation

Table 3: HPLC Chromatogram Data of SMEDDS Formulation

Sr. No	Retention time	Peak area (µV/sec)	% Area	Symmetric factor	NTP
1	1.690	482233	100	1.918	5670

Linearity

Table 4: Linearity data of Cefixime trihydrate

Sr. No	Concentrations (µg/mL)	Absorbance
1	2	0.1256
2	4	0.2569
3	6	0.3978
4	8	0.5352
5	10	0.6785
6	12	0.7924
λ_{max}		291nm
Correlation Coefficient (R^2)		0.9995
Slope		0.1262
Intercept		0.0495

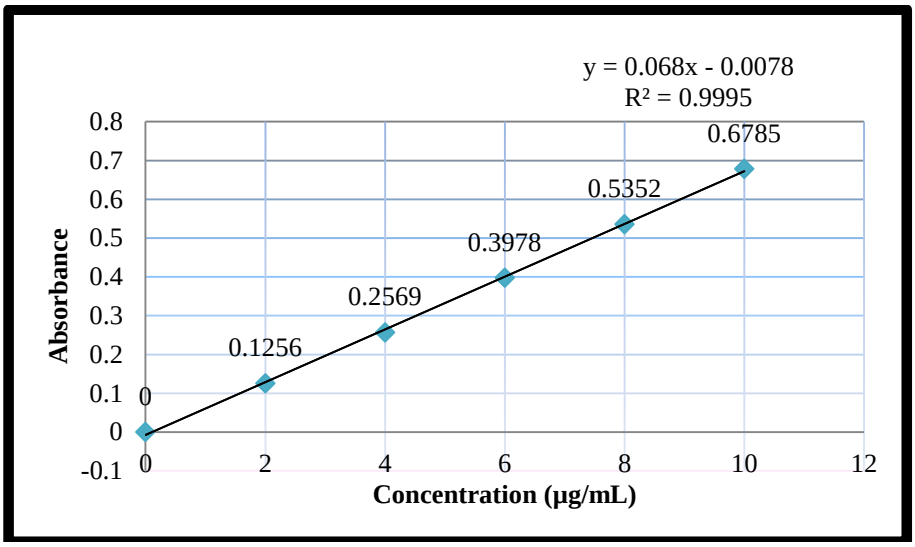

Figure 5: Calibration graph of Cefixime trihydrate in Methanol

The calibration curve of Cefixime trihydrate in methanol was found to be linear in the range of 2-12 µg/mL and coefficient of regression was found to be 0.9995.

Recovery Study (Accuracy Study)

Table 5: Recovery data of Cefixime trihydrate

Drug	% Level	Amount taken (µg/mL)	Amount added (µg/mL)	Total amount (µg/mL)	Amount recovered (µg/mL)	%Recovery ± SD*	RSD*
CT	50	5	2.5	7.5	7.5007	100.00 ±0.0283	0.0278
	100	5	5	10	10	100.00 ±0.0000	0.000
	150	5	7.5	12.5	12.5057	100.04 ±0.0217	0.0217

*Mean ±SD n=3

The excellent mean % recovery (close to100%) and low standard deviation (S.D. < 1.0) represent accuracy. The reliability and validity of the proposed method was determined by recovery studies of standard addition procedure (Table 5). The mean % recoveries (S.D) for lower, intermediate and higher concentrations were found to be 100.0093±0.02837, 100.00±0.00 and 100.0458±0.02178 respectively. These results have revealed that any small change in the Cefixime trihydrate concentration in the solution can be accurately determined by the proposed method.

Precision

Table 6: Precision data of Cefixime trihydrate

Conc. (µg/mL)	Intra-day	RSD*	Inter-day	RSD*
5	0.9905±0.0004	0.04391	0.9909±0.0001	0.01160
10	1.3214±0.0004	0.03057	1.3215±0.0004	0.03291
15	1.6922±0.0002	0.01483	1.6922±0.0005	0.03250

* Mean ±SD n=3

The method was found to be precise as the RSD values for the repeatability studies were <1 (Table 6).

Specificity and Selectivity

The UV-spectrum of Cefixime trihydrate was not changed in the presence of common pharmaceutical excipients in selected medium. Absorption spectrum of pure Cefixime trihydrate was matching with the prepared SMEDDS formulation in the selected medium (Figure 6). Therefore, proposed method is selective and specific for Cefixime trihydrate estimation.

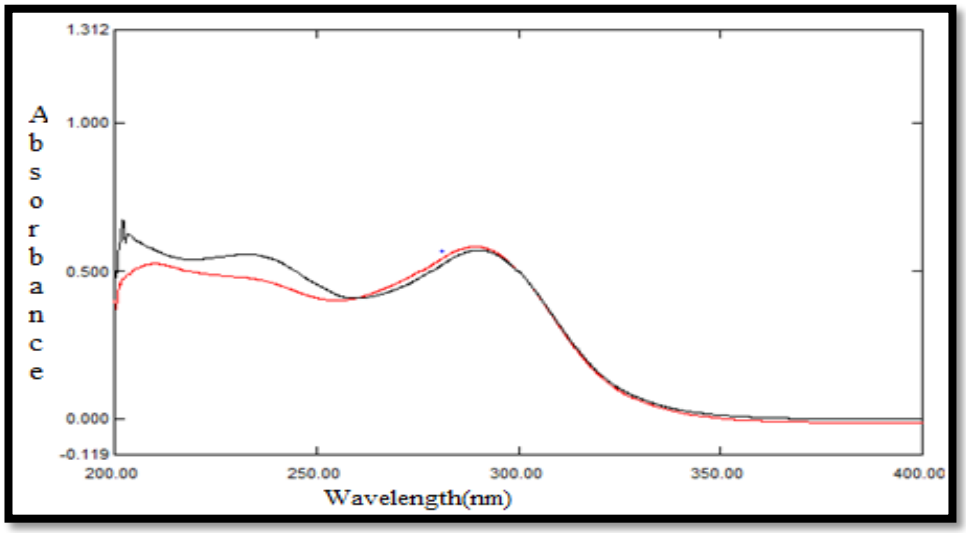

Figure 6: Specificity of the method determined by comparing the spectra of Cefixime trihydrate standard and SMEDDS formulation.

Limit of Detection

The detection limit for the proposed method for Cefixime trihydrate was found to be, LOD: 10.4468 µg/mL.

Limit of Quantitation

The quantitation limit for the proposed method for Cefixime trihydrate was found to be, LOQ: 30.6513 µg/mL.

Ruggedness

Table 7: Ruggedness data of Cefixime trihydrate

Parameter	**% Assay**	**SD***	**RSD***
Analyst -1st	99.58	±0.2173	0.2182
Analyst- 2nd	99.27	±0.2779	0.2799
Lab-1st	99.72	±0.4685	0.4698
Lab-2nd	99.82	±0.4400	0.4407
Reagent -1st	99.55	±0.3121	0.3135
Reagent-2nd	99.64	±0.4934	0.4951

* Mean ±SD n=3

The selected method verifies that in the same analyst, laboratory and reagent provides the same results once the development phase is over, hence the analytical method would pass the test for ruggedness.

Robustness

Table 8: Robustness data of Cefixime trihydrate

Sr. No	**Change in pH**			**Change in Temp.(^{0}C)**		
	6.0	**7.0**	**8.0**	**24**	**25**	**26**
1	99.17	99.18	99.12	99.19	99.10	99.25
2	99.50	99.49	99.44	99.24	99.49	99.40
3	99.35	99.45	99.28	99.47	99.35	99.29
Mean	99.34	99.37	99.28	99.3	99.31	99.31
SD	±0.1652	±0.1600	±0.1686	±0.1493	±0.1975	±0.0776
RSD	0.1662	0.1610	0.1698	0.1503	0.1988	0.0781

* Mean ±SD n=3

Variation of pH of the selected medium by 1% did not have any effect on absorbance (Table 8). The drug in selected media exhibited no spectrophotometric change for 48 hrs when kept at room temperature; hence the analytical method would be concluded as robust.

Results of Validation Parameters

Parameters	**Results**
Linearity (R^2)	0.9995
Y-Intercept	0.0495
Slope of regression line	0.1262
%RSD (Indicate precision)	000
Mean %Recovery	100%
Limit of Detection (LOD)	10.4468 µg/mL
Limit of Quantitation (LOQ)	30.6513 µg/mL
Range	2-12 µg/mL

Conclusion

In this study the method describe is a Simple and accurate approach for analyzing Cefixime trihydrate within the concentration ranges of 2 to 12 µg/mL (Table 4). The substances follows Beers law with an R^2 value of 0.9995 (Figure 5). According to ICH guideline the R^2 value should be greater or equal to 0.999 so the R^2 value of this research meets the required limits. Every concentration was prepared in triplicate in this study and absorbance of each one was measured and the average one was used for other concentration. The HPLC chromatography were obtained for Cefixime trihydrate in Triethylamine: Methanol: Acetonitrile: Water (2:10:20:68 v/v%). The drug exhibited a peak at retention time 1.547 min. and C18 is common stationary phase suitable for many pharmaceutical and was found to be suitable in this study.

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