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Efficient UPLC and spectrophotometric MC methods for simultaneous determination of cefixime and sodium benzoate in their dosage form and in its degradation product of cefixime-E Isomer. Application of lean six sigma and in-vitro dissolution studies

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Resumo

O objetivo principal deste estudo é desenvolver e validar um novo RP-UPLC e métodos espectrofotométricos de centralização média para a estimativa simultânea de cefixima e benzoato de sódio em sua forma farmacêutica e em seu produto de degradação do isômero cefixima-E e aplicação de ferramentas de qualidade e Lean Metodologias Seis Sigma para interpretar a integridade dos dados de atributos de qualidade que darão força, confiança e precisão para controlar e enfatizar que o Índice de Capacidade do Processo (Cpk) é> 1,33. A condição cromatográfica isocrática foi avaliada à temperatura ambiente usando coluna Waters CORTECS® C18 (50 mm × 4,6 mm, tamanho de partícula 2,7 µm), com uma fase móvel composta de acetonitrila: tampão fosfato 0,05 M (35:65 v / v) na taxa de fluxo de 0,3 mL / minuto e detecção de UV a 230 nm com volume de injeção de 0,5 µL e tempo total de execução de 7 min. O método de UV é a centralização média dos espectros de razão (MC), que se baseia na centralização média dos primeiros dados espectrais de razão de ambas as drogas em sua mistura binária a 225 nm para SDB, enquanto CFX foi detectado em espectros de ordem zero de 290 nm , já que nenhuma sobreposição da droga combinada foi encontrada. O método foi aplicado com sucesso a estudos comparativos de dissolução in vitro para cefixima (CFX) no produto Genérico; Rivaxime 200 mg Cap e Rivaxime 400 mg Cap portanto, havia sido considerado equivalente ao produto inovador; Suprax 200 mg Cap e Suprax 400 mg Cap usando meio de dissolução FDA.

Abstract

The main objective of this study is to develop and validate a novel RP-UPLC and spectrophotometric mean centering Methods for simultaneous estimation of cefixime and sodium benzoate in their dosage form and in Its Degradation Product of cefixime-E isomer and application of Quality tools and Lean Six Sigma methodologies to construe the data integrity of quality attributes which will give strength, confidence, and precision to control and emphasize that the Process Capability Index (Cpk) is >1.33. Isocratic chromatographic condition was evaluated at ambient temperature using Waters CORTECS® C18 column (50 mm × 4.6 mm, 2.7 µm particle size), with a mobile phase composing of acetonitrile: 0.05M Phosphate Buffer (35:65 v/v) at flow rate of 0.3 mL /minute and UV detection at 230 nm with injection volume of 0.5 µL and total run time of 7 min. The UV method is mean centering of the ratio spectra (MC), which is relied on mean centering of the first ratio spectral data of both drugs in their binary mixture at 225 nm for SDB, while CFX was detected at zero order spectra of 290 nm, as no overlapping from the combined drug has been found. The method was successfully applied to comparative in vitro dissolution studies for cefixime (CFX) in the Generic product; Rivaxime 200 mg Cap and Rivaxime 400 mg Cap therefore, it had been considered equivalent to the innovator product; Suprax 200 mg Cap and Suprax 400 mg Cap using FDA dissolution medium.

Keywords: Cefixime; Sodium Benzoate; Lean Six Sigma; In vitro dissolution; MC; RP- UPLC.

1. Introduction

A topic of Lean Six Sigma (LSS) has become more relevant due to the development of the medicines. During the last decades, more potent and complex drugs have been developed in which pharmaceutical companies show increasing to improve quality and productivity, reducing variation and decrease waste time. As well, it may use for growing the productivity or capacity of a manufacturing plant as many pharmaceutical factories which use Lean Six Sigma (LSS) to reduce time and eliminate defects in the process and increase the capability of the process. Cefixime (CFX), (Figure. 1a) as an antibiotic is a third-generation cephalosporin. It was chemically named as (6R,7R)- 7-[[(Z)-2-(2-Aminothiazol-4-yl)-2-[(carboxymethoxy) imino] acetyl] amino]-3- ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct2-ene-2-carboxylic acid trihydrate. It has a molecular formula of $C_{16}H_{15}N_5O_7S_2,3H_2O$ and a molecular weight of 507.5[1]. CFX for oral suspension is indicated in the treatment of adults and pediatric patients six months of age or older [2]. CFX E- Isomer, (Figure. 1b) was chemically named as 2-((((E)-1-(2-aminothiazol-4-yl)-2-((((2R)-5-methyl-7-oxo-1,2,5,7-tetrahydro-4H-furo[3,4-d] [1,3] thiazin-2-yl) methyl) amino)-2-oxoethylidene) amino) oxy) acetic acid. It has a molecular formula of $C_{16}H_{15}N_5O_7S_2$, and a molecular weight of 453.5. SDB is the chemical benzoate of soda ($C_7H_5NaO_2$) (Figure. 1c), produced by the neutralization of benzoic acid with sodium bicarbonate, sodium carbonate, or sodium hydroxide. The salt doesn't occur naturally. The excipient is used as antimicrobial preservative in pharmaceutical dosage forms [3]. HPLC method for determination of CFX and SDB were officially reported in both of British Pharmacopeia (BP) and United States Pharmacopeia (USP) [4]. Few analytical methods have been described for the determination of CFX and SDB individually or in combination with other drugs including (HPLC) [5-19], capillary electrophoresis and electrochemical methods [20-23], thin layer chromatography (TLC) [24-27], colorimetric and spectrophotometric [28-39], and comparative in vitro dissolution studies for cefixime [40,41]. In high analytical chemistry technique, the determination of a binary mixture or more analytes simultaneously using the same method of analysis lead to reduce time and cost of analysts. So, the current work introduces a new RP- UPLC and spectrophotometric MC methods for simultaneous estimation of cefixime and sodium benzoate in their dosage form, in the degradation product of cefixime-e isomer, and application of lean six sigma and in-vitro dissolution studies.

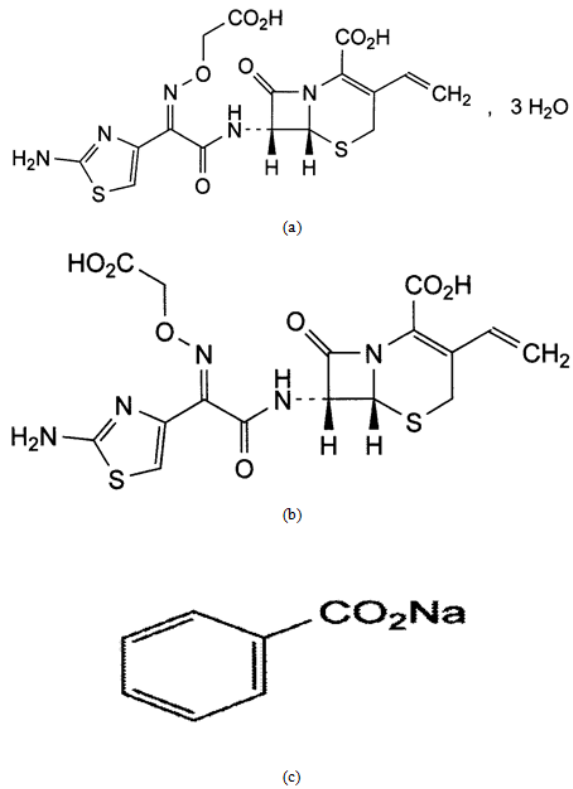

Figure 1. Chemical structures of (a) Cefixime, (b) Cefixime E- isomer and (c) Sodium Benzoate.

2. Experimental

2.1. Chemicals and reagents

CFX Astellas Pharma Inc. (Japan) and Orchid Chemicals & Pharmaceuticals Ltd (India) were kindly supplied by Hikma pharmaceutical industries company,

Beni-Suef, Egypt with potency (as anhydrous base) of 100.5% and 99.3%, respectively. SDB was supplied by Hikma pharmaceutical industries company, Beni-Suef, Egypt with claimed purity 100.1%. Innovator Product Suprax 200 and 400 mg Cap (Batch No. 2008& 2012) were supplied by Hikma pharmaceutical industries company. Generic product Rivaxime 200 mg and 400 mg Cap (Batch No. 2000&2001) were supplied by Riva Pharma company, Free zone Nasr City, Cairo, Egypt. Acetonitrile HPLC-grade, ultrapure water from an EMD Millipore Milli-Q Integral system, equipped with a Millipak 0.22 µm screen filter at the final point of use, potassium dihydrogen orthophosphate, potassium chloride, acetic acid, sodium acetate trihydrate, sodium hydroxide, hydrochloric acid, hydrogen peroxide and orthophosphoric acid analytical grade, were procured from (Scharlau, Spain).

2.2. Apparatus

UPLC Chromatographic condition was performed using Waters ACQUITY® Arc™ UHPLC System (Waters, USA) consisting of a quaternary liquid chromatography provides plug-and-play method compatibility for UHPLC separations with 2489 UV/Vis Detector (A17VTU408A), Sample Manager FTN-R (D17VSM260N), Quaternary Solvent Manager-R (C17VQS618G) Designed with unique Arc Multi-flow path™ technology to swap between HPLC and UPLC separations. Chromatographic data was monitored and processed using Empower™ 3 Software. Separation was achieved on a Waters CORTECS® C18 column (50 mm × 4.6 mm, 2.7 µm particle size, USA). Stepwise the mobile phase composing of acetonitrile: 0.05M Phosphate Buffer (35:65 v/v) at flow rate of 0.3 mL /minute and UV detection at 230 nm with injection volume of 0.5 µL and total run time of 7 min.

An 1800 double beam UV spectrophotometer (Shimadzu-Japan) with highest resolution was also utilized. Its spectral interval is 0.1 nm from (190-1100 nm range) was used for all absorbance measurements. Matched with 1 cm quartz cells. Perform data analysis by software (UV-Probe 2.5.2).

MATLAB R2015a ® 8.5using PLS toolbox software for MC method.

Statistical analysis was performed using Minitab® 17.1.0 and Origin® 2018.

2.3. Dissolution preparation (Buffer pH 7.2)

Dissolve 33.3 g of potassium dihydrogen phosphate anhydrous and 14.7 g of disodium hydrogen phosphate anhydrous in 7.0 liter with deionized water. If necessary, adjust the pH to 7.2 ± 0.05 with 1N phosphoric acid or 1N NaOH.

2.4. Solvent preparation (Buffer pH 7.0) for assay

Transfer 6.8 ml of concentrated phosphoric acid to a 1000 ml volumetric flask; add 300 ml of water. Adjust to pH 7.0 ± 0.1 with 10N NaOH. Then dilute to volume with water.

2.5. Sample preparation

Weigh 20 empty capsules and calculate the average content per capsule. Weigh accurately about the equivalent to 100 mg CFX. Then transfer completely to 500 ml volumetric flask with the aid of 400 ml solvent, sonicate for about 10 minutes and complete to volume with the same solvent and mix well. Filter through 0.45 µm PTFE membrane filter.

2.6. Preparation of standards

Stock solutions (1 mg/mL) of CFX and SDB were separately prepared and dissolved in assay solvent preparation. Separately transfer different dilutions from stock standard solutions into separate series of 10 mL

volumetric flasks covering the concentration ranges (5–250 µg/mL) of CFX and (3–75 µg/mL) of SDB. Mix well and filter through 0.45 µm PTFE membrane filter and inject into the chromatographic system (Figure. 2).

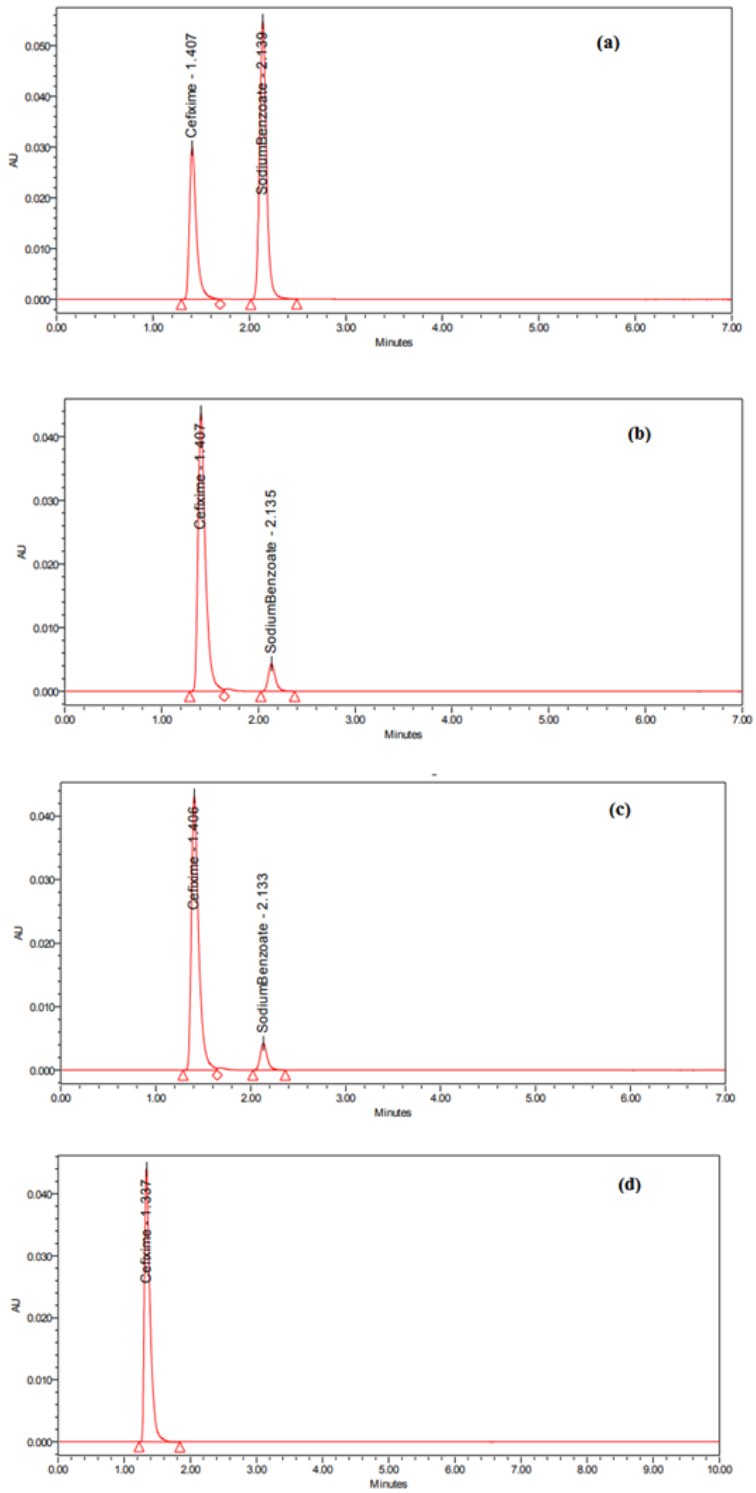

Figure 2. UPLC chromatograms of standard Solution of (a) 200 µg/mL of Authentic mixture of CFX and SDB, (b) 80 µg/mL of CFX and 2 µg/mL of SDB (c) Suprax 60 mL POS (d) Suprax 200 Cap using solvent as blank.

2.7. Comparative in vitro dissolution studies

In the early stages of development, in vitro dissolution testing guides were performed to test the optimal improvement of the drug release from the pharmaceutical dosage form. Over the past 50 years, it has been used as a measure of quality control (QC), in R&D to detect the impact of the critical manufacturing variables in Comparative Studies [42].

2.8. Dissolution Parameters

Apparatus: Type II (Paddle) with sinker

Dissolution medium volume: 900 mL of phosphate buffer pH 7.2

Speed: 100 rpm

Temperature: 37 ± 0.5 °C

Time: 10, 20, 30, 45 and 60 minutes

2.9. Test preparation for dissolution

Take 6 capsules of the product and insert each one in one sinker. Place each one from the generic product and the innovator in one dissolution apparatus vessel and immediately operate the apparatus. Discard the first 20 mL of the withdrawn sample and filter through 0.45 µm filter, Use the obtained filtrate solution.

2.10. Process Capability Sixpack

Quality tools and Lean six sigma were used for comparing and evaluating between different raw material suppliers and follow up the process of suppliers during the last two years ago and assure that the Process Capability Index (Cpk) is >1.33. moreover, the evaluation can also be useful in selecting or modifying the process at some stages in the product design and improvement the current process capability analysis.

2.11. Mean Centering of Ratio Spectra Spectrophotometric Method

In mean centering spectrophotometric method; both binary and ternary mixtures can determine without previous separation. The first ratio spectra were obtained after elimination the constant by mean centering of the ratio spectra, in addition to an extra step was performed for the ternary mixture where the mean centered ratio spectra were further divided by the mean centered vector of the other two drugs, then the second ratio spectra were mean centered [43].

3. Results and discussion

3.1. Methods development and optimization

To select buffer of mobile phase, different mobile phases were examined: methanol (100%); methanol: purified water (70:30, v/v); acetonitrile: purified water (70:30, v/v) and methanol: purified water (50:50, v/v). All solvents of the mobile phase were filtered through 0.45µ filter paper to remove particulate matter and degassed by sonication. Also, (0.2, -1.0 mL/min) flow rates are measured. Initial studies have been included trying C8, C18 reversed-phase columns. Wavelengths were scanned at (200 – 400 nm) for 5 µg/mL for both drugs, CFX and SDB in pure form (Figure. 3), showing maximum absorbances at 290 nm and 222 nm, respectively. However, the wavelength 230 nm was selected for the separation of both drugs because it has maximum intensity and resolution between the two peaks. The best developing mobile phase of the system was found to be a mixture of acetonitrile: 0.05M phosphate buffer (35:65 v/v) at flow rate of 0.3 mL /minute and UV detection at 230 nm with injection volume of 0.5 µL and total run time of 7 min. The retention times are quite short and sufficiently separated. The best system for dissolution was 900 mL of water, using dissolution media; phosphate buffer pH 7.2 using Type II (paddle) with sinker at speed rate of 100 rpm with time intervals; 10, 20, 30, 45 and 60 minutes.

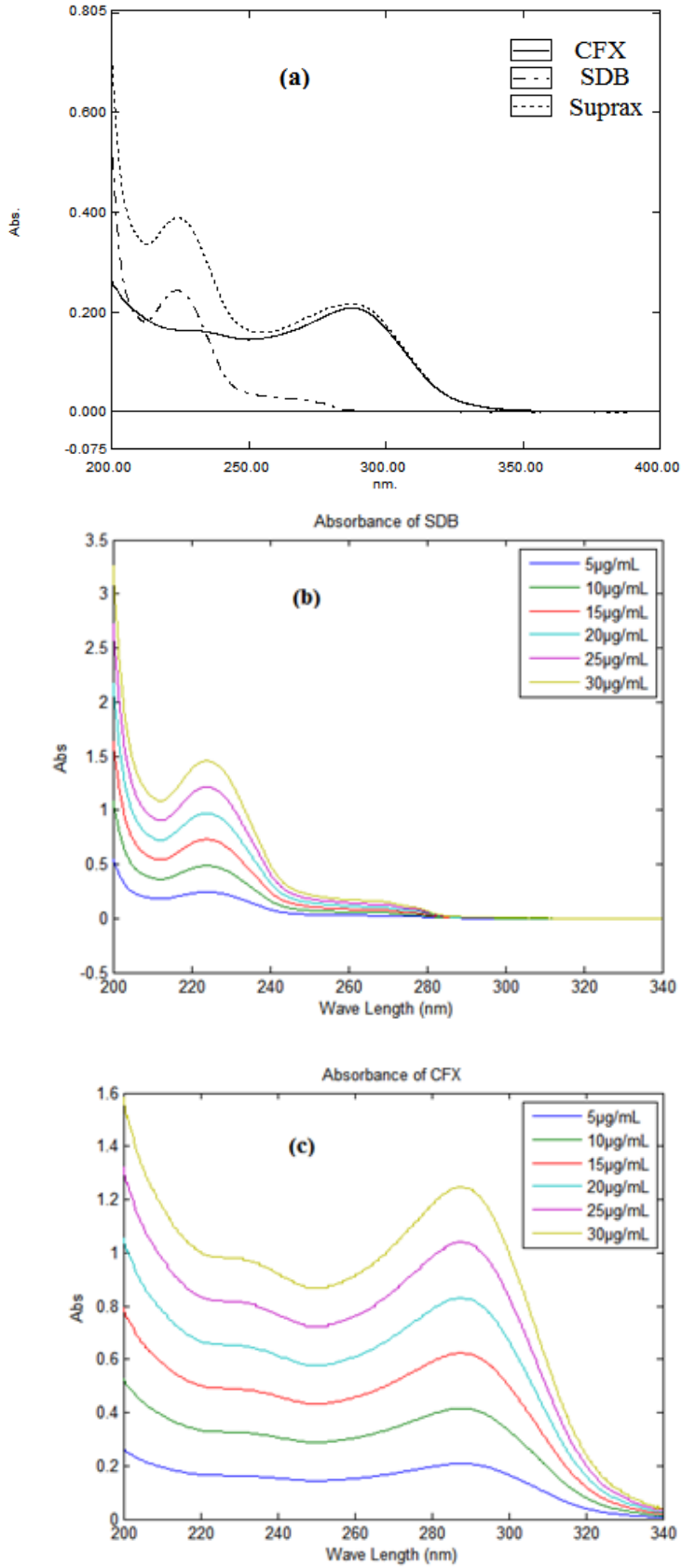

Figure 3. Zero order absorption spectra of each of (a) 5 µg/mL of CFX, SDB and dosage form, (5-30) µg/mL of (b) SDB, and (c) CFX using Solvent as blank.

3.2. Method validation

The proposed method was validated regarding linearity, detection and quantitation limits, accuracy, precision, stability of the analytical solution, system suitability and specificity, in accordance with ICH guidelines [44].

3.3. Linearity and range

The linearity of an analytical procedure is its ability to elicit test results that are directly, or by linear least squares regression of calibration curves in the concentration ranges (5–250 µg/mL) of CFX and (3–75 µg/mL) of SDB for UPLC method and (5–30 µg/mL) of both drug for MC method with coefficient of regression >0.999 for CFX and SDB, respectively. The linearity results were displayed in (Table 1).

Table 1. Regression and validation parameters of the proposed method for determination of CFX and SDB.

Parameter	UPLC		MC	
Drugs	CFX	SDB	CFX	SDB
Linearity Range (µg/mL)	5-250	3-75	5-30	5-30
Regression equation y = bx + a				
Slope	747.72	435.12	1.552	0.065
Intercept	947.45	117.87	0.82	0.03
Correlation coefficient(r2)	0.9996	0.9999	0.9997	0.9995
Accuracy (% R) a	99.53	100.94	99.72	100.02
Precision (% RSD)				
Repeatability	0.12	0.10	0.09	0.04
Intermediate precision	0.88	0.68	0.72	0.55
Robustness	0.95	0.83	1.12	1.59
Ruggedness	1.05	0.85	0.98	1.10
Stability of analytical solution	0.45	0.54	0.87	0.25
LOD b (µg/mL)	3.30	0.56	1.21	0.65
LOQ b (µg/mL)	10.0	1.76	3.68	2.11

[a] Average of three determinations

[b] Limit of detection (3.3× σ /Slope) and limit of quantitation (10× σ /Slope).

3.4. Detection and quantitation limits

LOD and LOQ are relied on the standard deviation of the response and the slope and can be calculated as LOD=3.3×σ /slope and LOQ=10×σ /slope, where σ = the standard deviation of the response as listed in (Table 1). Indicate that the method is sufficiently sensitive for the analysis of binary mixture in their dosage form.

3.5. Accuracy and recovery

The accuracy of an analytical procedure is the closeness of test results obtained by the procedure to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amounts of analyte. The recovery was calculated in triplicates of three concentrations (50, 100, 150 µg/mL) for CFX and (15, 30, 45 µg/mL) for SDB within linearity at all different media comparing the individual peak response with that of

the reference solution and (10, 15 and 20 µg/mL) for both drugs for the MC method. The mean % recoveries for CFX and SDB were between (98.0 – 102%). Results were shown in (Table 1).

3.6. Precision

Precision express of the degree of either reproducibility or repeatability of the analytical method. Reproducibility refers to the use of the analytical procedure in different laboratories. Repeatability refers to the use of the analytical procedure within a laboratory over a short period using the same analyst with the same equipment. Repeatability is approached using less than five replicates of the standard solution of the compound being studied (223 µg/mL) for CFX and (50 µg/mL) for SDB in all different media. and 20 µg/mL for both drugs for the MC method. The system was precise as the relative standard deviation RSD ≤ 2% as shown in (Table 1). Intermediate precision (Ruggedness): Intermediate precision expresses the within-laboratories variations parameter by RSD, evaluation has been tested for different days, different analysts and effect of use of different columns (different lots and/or suppliers). Good results were obtained as presented in (Table 1).

3.7. Robustness

The robustness of an analytical method is a degree of its capacity to remain unchanged by small but reflect variations in method's items such as: influence of variations of pH of the mobile phase (±0.2), flow rate change (±0.1 mL/min), wave length change (230 ± 2.0 nm) and column temperature change (30,25$_o$C). Good results were gained as presented in (Table 1).

3.8. Stability of the analytical solution

The standard solution was kept under specific conditions such as room temperature or fridge to ensure stability, then the solutions are measured against a freshly prepared standard; RSD < 2%. Results are showed in (Table 1).

3.9. Formulation assay

The proposed method was used to ascertain Analysis repeatability by evaluating six samples from the commercially dosage form (Suprax 60 ml POS, Suprax 200&400 mg ®Cap, Rivaxime 200 &400 mg ®Cap) to determine the assay of CFX. The obtained results were showed in (Table 2) designating that the method is sensitive

Table 2. Assay results for the determination of CFX and SDB in their dosage form by the proposed method.

Pharmaceutical formulation	Suprax 100 mg (60 ml) POS		Suprax 200 mg Cap	Rivaxime 200 mg Cap	Suprax 400 mg Cap	Rivaxime 400 mg Cap
	CFX	SDB	CFX	CFX	CFX	CFX
	101.76	99.87	101.75	99.93	101.29	100.42
	103.27	104.62	102.55	99.06	102.49	98.59
	102.87	107.72	103.09	101.76	103.60	100.20
	103.92	103.26	102.59	100.29	103.09	101.24
	102.94	104.87	101.09	98.84	101.75	102.94
	104.41	103.09	100.65	99.09	102.30	100.40
Mean ± RSD	103.20±0.89	103.91±2.49	101.95±0.94	99.83±1.10	102.42±0.83	100.63±1.42

for the samples without overlapping from the ingredient used to formulate.

3.10. System suitability

The system suitability is the established criteria, which must be met by the procedure in order to accept the results that are generated constitute an integral system that should be checked by calculating various parameters such as the number of theoretical plates (N), tailing factor (T), resolution (Rs), precision and selectivity (k') to ensure the performance of the system. All calculated parameters were found within the acceptable limits indicating good selectivity of the method as listed in (Table 3).

Table 3. System suitability testing parameters of the developed method.

Item	Obtained Value		Reference values
	CFX	SDB	
Tailing factor	0.99	1.10	T ≤ 2
Injection precision	0.12	0.22	RSD ≤1%
Number of theoretical plates (N)	5240	4500	N > 2000
Resolution	-	3.5	Rs>2
Retention time (R_t)	0.08	0.6	RSD ≤1%
Selectivity	-	4.2	k' > 2

3.11. Selectivity/Specificity

The purpose of this study is to determine the capability of the analytical procedure to measure accurately and specifically the analyte in the presence of the active ingredient, placebo and other ingredients. Selectivity may often be expressed as the degree of bias of test results obtained by analysis of samples containing added impurities, degradation products, related chemical

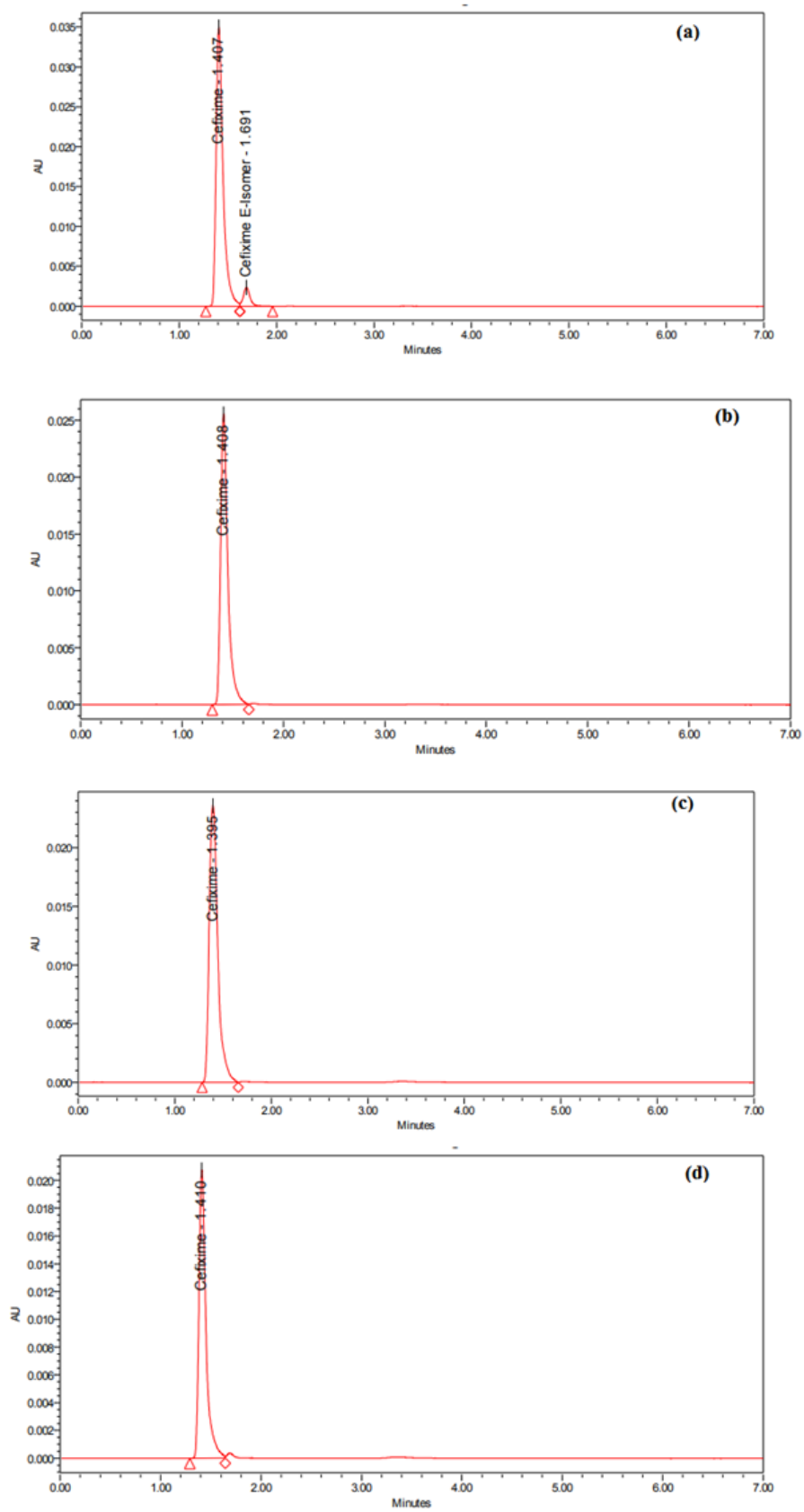

Figure 4. UPLC chromatograms of 80 µg/mL solution of CFX after exposure to (a) hydrolysis, (b) acid degradation, (c) base degradation, and (d) heat degradation.

compounds, or placebo ingredients when compared to test results from samples without added substances. Method specificity was performed for the dosage form being studied and placebos matrix containing all excipients of the finished product. The results indicated that no interference was detected at the retention times of CFX in placebo solution (Figure. 4).

3.12. Forced degradation

The forced degradation of API (CFX) was carried out as per ICH guidelines (ICH, Q2B) in hydrolysis, acid, base, and heat degradation.

A. Hydrolysis degradation

Dissolve 22.3 mg of CFX in 100 mL of water. Heat on a water-bath for 45 min and cool in situ preparation of impurity D (CFX E- Isomer). Filters through membrane filter 0.45- µm, reject the first portion then Inject by HPLC.

B. Acid degradation

Accurately weigh 22.3 mg of CFX and transfer into 100-mL volumetric flask. Add 70 mL of solvent and sonicate to dissolve, add 5 mL of 0.1 N HCl then keep the acidified solutions at room temperature for two hours, then complete to the mark with the same solvent and mix well. Filters through membrane filter 0.45- µm, reject the first portion then Inject by HPLC.

C. Base degradation

Accurately weigh 22.3 mg of CFX and transfer into 100-mL volumetric flask. Add 70 mL of solvent and sonicate to dissolve, add 5 mL of 0.1 N NaOH then keep the alkaline solutions at room temperature for two hours, then complete to the mark with the same solvent and mix well. Filters through membrane filter 0.45- µm, reject the first portion then Inject by HPLC.

D. Heat degradation

Keep the powder sample of CFX standard in a dry oven at 80ºC for 6 hours. Accurately weigh 22.3 mg of CFX and transfer into 100-mL volumetric flask. Add 70 mL of solvent and sonicate to dissolve, then complete to the mark with the same solvent and mix well. Filters through membrane filter 0.45- µm, reject the first portion then Inject by HPLC.

3.13. Mean Centering Method

For the estimation of SDB, the stored scanned spectra of laboratory prepared mixture of CFX and SDB were exported to MATLAB for posterior calculation. The spectra of SDB were divided by the spectrum of 5 µg/mL of CFX, the obtained First ratio spectra were mean centered (Figure. 5). The calibration curves for SDB was constructed by plotting the mean centered values at 225 nm versus the corresponding concentration and the regression equations were computed.

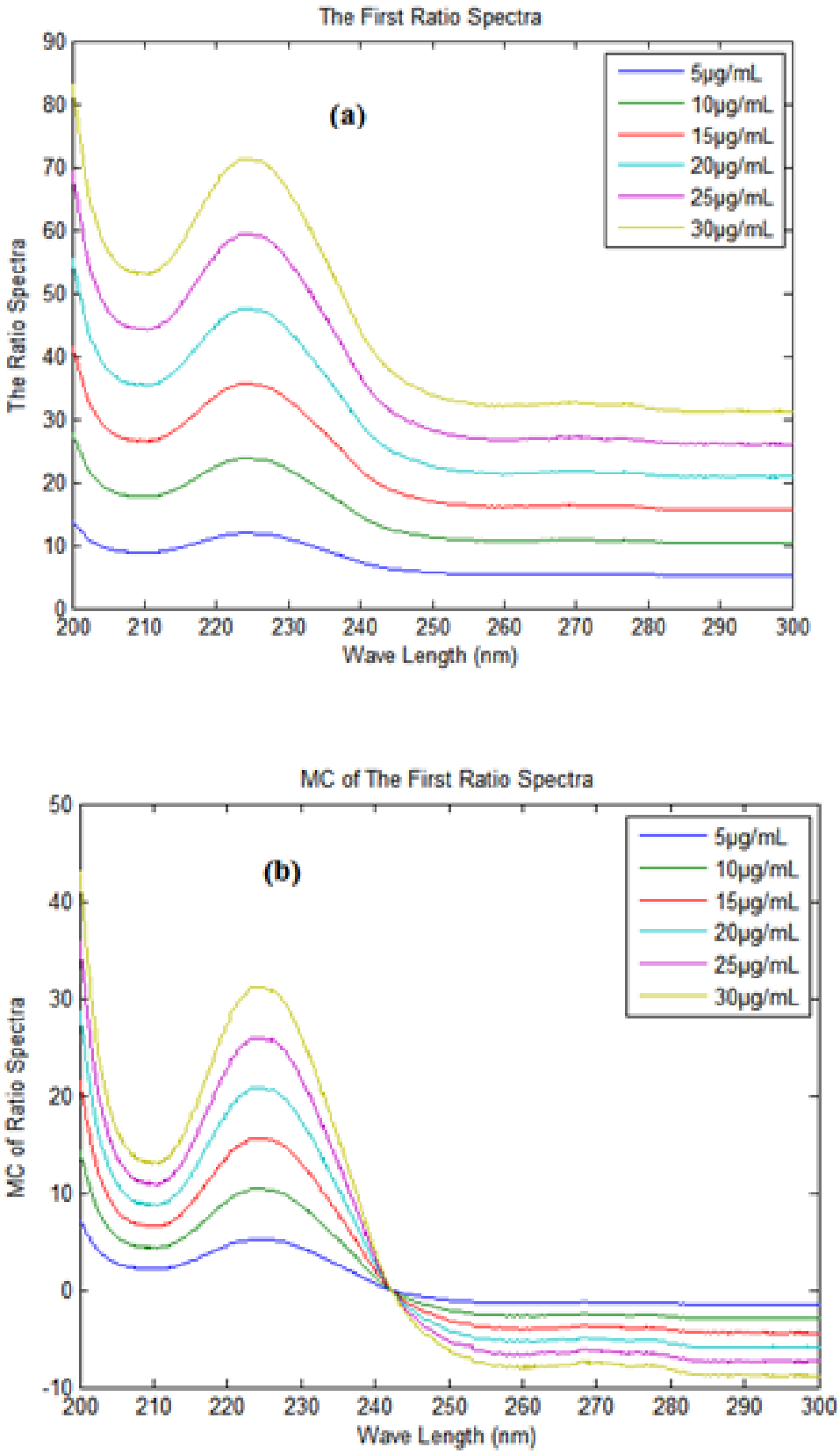

Figure 5. (a) First ratio spectra of SDB (5–30 µg/mL) using CFX (5 µg/mL) as a divisor, and (b) Mean centered ratio spectra of SDB (5–30 µg/mL) using CFX (5 µg/mL) as a divisor.

3.14. Application of Comparative in vitro dissolution study for the proposed method.

Comparative in vitro dissolution was performed on two generic products viz., Rivalexin 200mg Cap and Rivalexin 400mg Cap selecting dissolution parameters as described by FDA against innovator brand e.g., Suprax 200 mg Cap and Suprax 400 mg Cap and results were considered identical. The quantitative release of drug at specified intervals from the product was determined using the validated HPLC method. The dissolution results which were displayed in (Table 4) showed that the value of RSD % is less than 10 % at the initial point and less than 5 % for other intervals. Also, the in vitro dissolution profile and the cumulative percentage of generic and innovator products released were plotted against time (Figure. 6). The dissolution profile of generic brand showed similar behavior to the innovator one. There was an agreement that the *f*2 test is not necessary when the two products each provide at least 85% dissolution in 15 min. The percentage dissolved for all products was above 85% of the labeled claimed content from the first sampling time.

Table 4. Cumulative % Dissolution profile of CFX in Capsule dosage form at FDA media.

Name	Suprax 200 mg Cap	Rivaxime 200 mg Cap	Suprax 400 mg Cap	Rivaxime 400 mg Cap
Intervals		CFX		
10	87	85	88	86
20	89	87	90	88
30	95	92	94	92
45	100	97	98	95
60	103	100	102	99

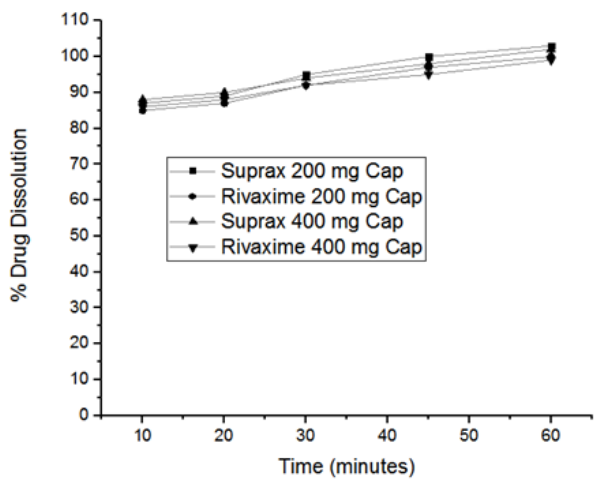

Figure 6. Dissolution Profile of CFX from Rivaxime 200 mg Cap, Rivaxime 400 mg Cap, the generic brand and Suprax 200 mg Cap, Suprax 400 mg Cap.

3.15. Application of Lean Six Sigma Methodology.

Trend data of CFX for the two suppliers were collected for the last two years concerning 100 batches of assay as summarized in (Table 5) by our quality control team for monitoring the manufacturing operation of the suppliers. Then data were exported to Minitab for subsequent calculation. Process capability six-pack analysis indicates that the data for the first supplier (Astellas Pharma Inc) was capable and more accurate than the second supplier (Orchid Chemicals & Pharmaceuticals Ltd) as Cpk is 1.96 (Figure 7), means that it meets 6 sigma level, while the second supplier was normally distributed, within the control specification and statistical control as the Cpk is 1.37 i.e., it meets 4 sigma levels.

Table 5. Application of quality control and statistical tools to monitor Manufacturing Operation.

Parameters Descriptive Statistic	Values Astellas Supplier	 Orchid Supplier
Mean	99.013	98.932
Standard Error	0.0615	0.110
Median	99.000	99.000
Mode	99.000	98.000
Standard Deviation	0.615	1.099
Variance	0.379	1.209
Kurtosis	-0.33	-0.58
Skewness	-0.36	0.21
Range	2.700	5.000
Minimum	97.300	96.000
Maximum	100.000	101.000
Sum	9901.300	9893.200
Count	100	100
Confidence Level (95.0%)	0.62	1.11

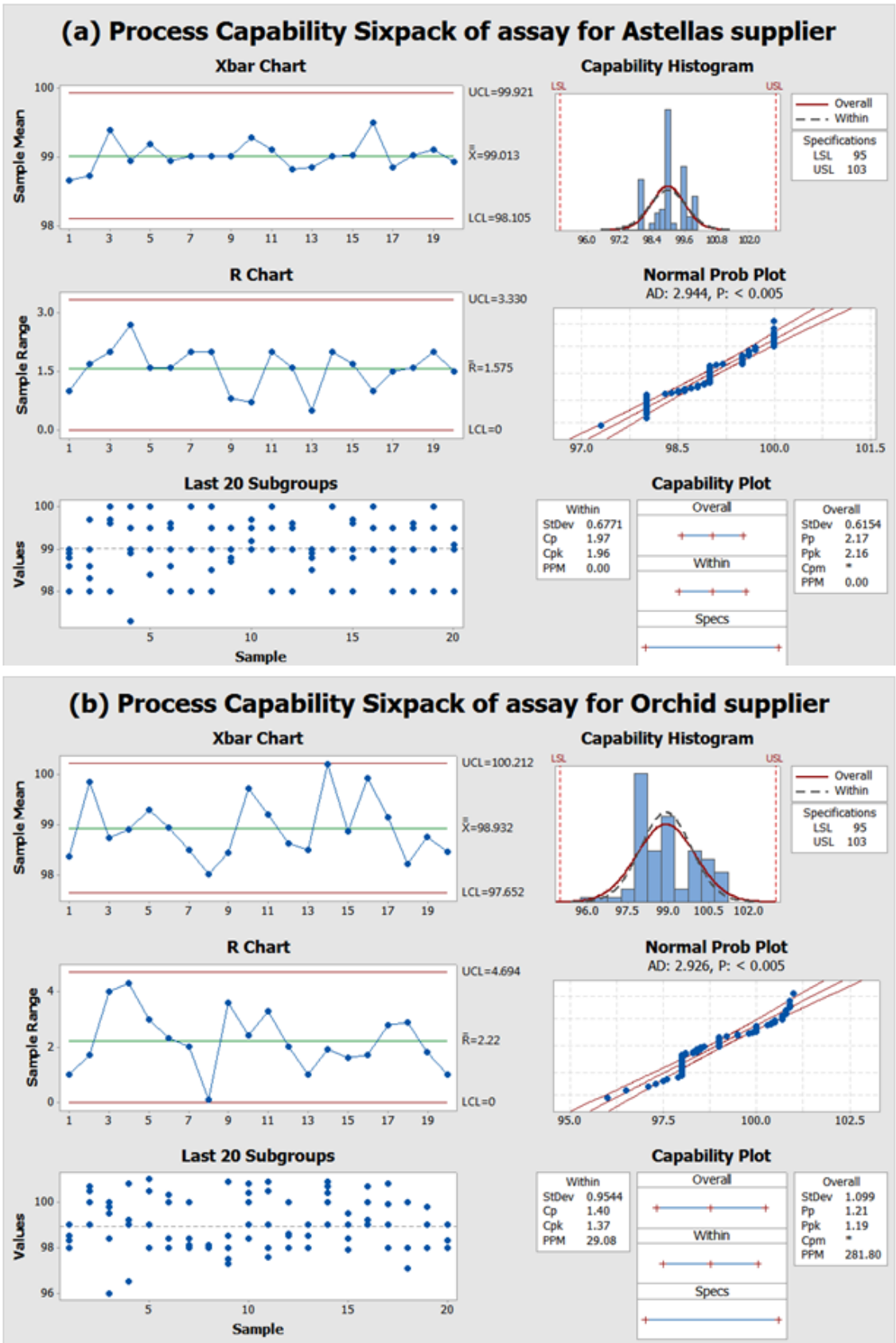

Figure 7. Process Capability Sixpack of quality tools for normally distributed assay results of 100 batches of CFX API for comparison between two suppliers (a) Astellas (Japan) supplier and (b) Orchid (India) supplier using Minitab® 17.1.0

4. Conclusion

Efficient and sensitive UPLC and spectrophotometric MC methods were validated and developed according to the requirements of ICH guidelines for simultaneous quantification of CFX and SDB in their pharmaceutical formulation and application of LSS methodology for reducing variation and increasing the productivity during the manufacturing operation, also Comparative in vitro dissolution studies have been achieved for two generic brands; Rivalexin 200&400 mg Cap and hence it was assumed equivalent to the innovator product of Suprax 200&400 mg Cap at FDA dissolution media and the two products were considered similar.

Conflict of interest

The authors declare no conflict of interest.

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