ORIGINAL ARTICLE

DEVELOPMENT OF CONTINUOUS ANTI-SOLVENT RECRYSTALLIZATION METHOD TO PRODUCE CEFTRIAXONE SODIUM NANO CRYSTALS INJECTION USING CERAMIC FILTRATION

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ABSTRACT

The aim: In this work we developed a method of continuous recrystallization to meet industrial requirements.

Materials and methods: Continuous recrystallization method was investigated using porous ceramic filter for water purification with pour size less than 1 μm, that ensures high mixing rate of ethanol and water.

Results and conclusions: The results of experiments using crystallization through ceramic filter, gives superior products in particle size, and produced needle shaped ceftriaxone crystals form, that showed significant improvement in dissolution time and obtained ceftriaxone sodium powder to be reconstituted in injectable formula that give clear solution without insoluble microparticles.

KEY WORDS: Ceftriaxone, Recrystallization, Anti-solvent crystallization, Supercritical fluid processes, Continuous Anti-solvent Recrystallization

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INTRODUCTION

Ceftriaxone is a hemisynthetic third generation cephalosporin antibiotic, that has some unique criteria such as very long half-life and wide spectrum of activity against both gram positive and gram negative bacteria [1]. Ceftriaxone chemical formula is represented in Figure1. Ceftriaxone also could cross blood brain barrier and reach cerebrospinal fluid [2]. Ceftriaxone is administered only via parenteral route of administration [3] and is available in the market as Ceftriaxone Sodium for Injection, that contains sterile powder equivalent to 0.25 g, 1 g, 2 g and 10 g of ceftriaxone (as sodium salt). The pH of freshly prepared solutions usually ranges from 6 to 8. [4]. Although, Ceftriaxone sodium is highly water-soluble, investigations show that Ceftriaxone preparations take a while to fully dissolve in water for injection, and it is expected that this property highly affects using it in a clinical practice. Tange, et al. investigated the dissolution time for eight marketed Ceftriaxone products. They found that difference in dissolution time between the eight products was related to differences in powder surface characteristics, such as water interaction and crystal shape [5] .

In the pharmaceutical industry practice, crystallization is frequently used to obtain crystal products with controlled purity, crystal shape, crystal size and polymorphic form in high producibility. Usually, for the final pharmaceutical product, the control of crystal (size, shape, and crystal form) is fundamental, as these properties can influence the

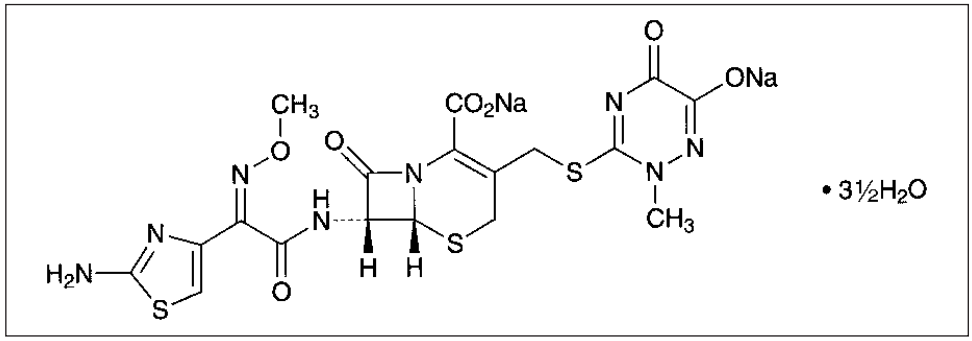


Fig. 1. Structure formula of Ceftriaxone sodium, Adapted from (United States Pharmacopeia) [17]

physicochemical properties such as solubility and dissolution rate[6]mixed-product removal (MSMPR).

Thus, assertiveness has been engaged on predicting appropriate design and operation of industrial crystallization processes to convene the increasing requirement of reliable crystal products. Commonly, controlled size crystals with uniform size distribution are desired to improve downstream pharmaceutical processes (such as centrifugal separation and drying after crystallization), bioavailability and product stability [7].

Recrystallization method, sometimes called "bottom up method", means that crystal grows from solution at molecule level to well defined particle size crystal [8]. The principle of crystallization method is how to reach supersaturation and the size of crystals could be maintained by balancing rate of crystal growth and rate of nucleation which is determined by the extent of supersaturation situation [9].

Anti-solvent crystallization is a straightforwardly scalable technique. It enables to prepare crystalline products with particle size ranged from micro to nano size as far as; it could be used to control produced crystals morphology. It is one of the clean and efficient, purification and crystallization techniques that can replace thermal (cooling and heating) crystallization. The main benefit of this process is the requirement of cold temperature which is favorable for thermo-sensitive materials to avoid degradation by eliminating the thermal energy such as pharmaceutical products [10].

Ceftriaxone sodium is commonly purified by crystallization using anti-solvent techniques by means of ethanol or acetone as anti-solvent, since it is heat-sensitive and has weak solubility temperature dependence [11]. The Japanese pharmacopeia [12] maintained that powder for injection obtained by this method, dissolving active ingredient(s) then re-crystallization from the solution or mixing additionally the powders with sterilized excipients.

Currently, the anti-solvent crystallization technique has been developed in batch, semi-continuous, and newly in continuous mode. The continuous method that is known as automatization anti-solvent process or automatization of supercritical anti-solvent induced suspension process [13]"ISSN":"19994923","abstract":"Solid multicomponent systems (SMS. The supercritical fluid technology has experienced an intensive development in the past decade, being widely represented as a complementary to many conventional solvent-based processes. Several authors have used supercritical fluid methods in the production of micro to nano size particles of a wide range of materials including medicine, pharmaceutics. Many supercritical fluid processes have been established with the main objective of controlling the phenomena of nucleation and growth of crystals. The list of processes is based on three fundamental properties of supercritical fluids: its solvent power, which strongly depends on pressure; its miscibility with organic liquids that causes an antisolvent effect to the materials dissolved; and its atomization enhancement when depressurized together with a liquid in a coaxial nozzle [14].

THE AIM

In this work we developed a method of continuous recrystallization to meet industrial requirements. Continuous recrystallization method was investigated using porous ceramic filter for water purification with pour size less than 1 μm, that ensures high mixing rate of ethanol and water.

MATERIALS AND METHODS

MATERIALS

Ceftriaxone Sodium was purchased from Shanghai pharmaceutical LTD., China. Absolute ethanol (99.5%) was supplied from Hayman Ltd., U.K. Activated Charcoal was purchased from Micropore Inc, Taiwan. Ultra-purified double distilled water was used during all experiments.

For chromatographic HPLC Method, Ceftriaxone Sodium E-Isomer and Ceftriaxone Sodium, Pharmacopoeia Reference Standard (RS) were supplied from Merck, Germany. Acetonitrile, HPLC Grade and Methanol, HPLC Grade were obtained from Chem-Lab, Belgium. Citric acid anhydrous was supplied by Alpha chemika, India. Phosphoric acid 85% (ultra-pure) was obtained from Chem-Lab, Belgium. HPLC grade water was obtained from a Milli-Q.

All apparatus and laboratory devises were used, listed in the Table I.

METHODS

CHARACTERIZATION OF CEFTRIAXONE SODIUM

Melting point

Melting point of ceftriaxone was determined according to British pharmacopeia technique [15]. using Stuart (SMP10) melting point apparatus and soda glass melting tubes closed from one end. The tube dipped inside drug powder, allowing little quantity of drug to be packed inside tube (about 3 mm). Then this tube is located inside melting point apparatus with the temperature raised gradually. The melting point will be obtained as temperature at which the drug powder particles all converted to liquid.

Identification of ceftriaxone sodium pH

For identification of ceftriaxone sodium pH, 0.6 g of pure drug was dissolved in 5mL double distilled water, the temperature of solution fixed at 25 C°, pH meter (WTW, inolab pH720) calibrated with (pH 7.00, pH 4.01and pH 9.21 buffers) and probe washed by deionized water before and after testing the sample for the pH value of the solution. The pH value was measured triplicate [12].

Chromatographic assay of ceftriaxone sodium

Chromatographic assay for ceftriaxone sodium was performed according to united state pharmacopeia [16].

The following steps and calculation procedures were carried out:

Preparation of Mobile phase

Mobile phase was prepared by dissolving of 3.2 g tetraheptylammonium bromide in 400 mL acetonitrile. Then 44 mL of (pH 7) buffer and 4 mL of (pH 5) buffer were

Table I. List of apparatus used in experimental work and manufacturer, model and origin.

Apparatus	Manufactures, Model and Origin
AC Fluid Pump	HAIKE,HK-200, China
Automatic thermal analyzer (DSC)	Shimadzu, DSC-60, Japan
Ceramic Nano cartridge	SWS, Hitech cartridge, China
DC Fluid Pump	DENSO, ZZE133, Japan
Digital Timer Delay Relay	Omron, DH48S-S, Japan
Dynamic light scattering Nano-laser particle size analyzer	Angstrom Advanced Ltd., ABT-9000, USA
FTIR Spectroscopy	Shimadzu, IR Prestige 21, Japan
High performance liquid chromatography (HPLC)	Shimadzu, Prominence UFLC, Japan
Hot plate stirrer	Stuart, CB 162, U.K Labinco, L81, Netherland
Lyophilizer (Freeze Dryer)	Martin Christ, alpha 2-4 LD plus, Germany
Melting point apparatus	Stuart, SMP10, U. K
pH-meter	Crison, Spain WTW, inolab pH720, Germany
Scanning Electron Microscope (SEM)	FEI, USA
Sensitive Electrical Balance	Kern and Sohn GMBH, Germany
Ultra-Sonic Cleanser bath	Kudos, Shanghai Co. Ltd, China.
Ultrasonic-Bath	Hwashin, power sonic 410, Korea
Water Deionizer	BIBBY, Aquatron de-ionizer, U.K
Water Distillator	GFL, 2001/4, Germany
X-ray diffractometers (XRD)	Shimadzu, XRD 6000, Japan

added and diluted with ultra-pure water to 1L solution.

This solution was filtered through membrane filter with pore size of 0.25 µm and degassed by vacuum and sonication.

Preparation of Buffer Solutions

- (pH 7) Buffer, was prepared by dissolving of 13.6 g dibasic potassium phosphate(K_2HPO_4) and 4 g monobasic potassium phosphate (KH_2PO_4) in water to obtain 1L solution. After sonication for 10 min, using power sonic 410 ultrasonic-bath, temperature maintained to 25 C°. Then the solution pH was adjusted to value of (7±0.1) by phosphoric acid or 10N potassium hydroxide.
- (pH 5) Buffer, was prepared by dissolving of 25.8 g sodium citrate ($Na_3C_6H_5O_7$) in 500 mL of water, after 10 min sonication the solution pH was adjusted to value of (5±0.1) by citric acid 20% solution, and diluted with water to 1L solution.

Preparation of samples

Solution for indicating suitability of the system was prepared by dissolving 50 mg of USP Ceftriaxone Sodium E-Isomer reference standard in 100 mL of mobile phase then also 50 mg of ceftriaxone reference standard (RS) were also added.

Standard solution was prepared by dissolving 30 mg of ceftriaxone reference standard RS in 100mL mobile phase to get concertation of 0.3mg/mL.

Sample solution was prepared by dissolving 30 mg of ceftriaxone sodium in 100mL mobile phase to get concertation of 0.3mg/mL.

Chromatographic system

The HPLC system was tuned according to USP chromatography <621> [17].

Shimadzu Prominence UFLC devise was used, Mode LC.

Detector uses UV 254 nm.

Column 4.6 mm X 25 cm; 5-µm packing L1.

The volume injected 20 µl.

Flow rate was 1.5mL/min.

The quantity of ceftriaxone sodium in the sample was calculated by following below equation 1:

$$Quantity = \left(\frac{ru}{rs}\right) \times \left(\frac{Cs}{Cu}\right) \times P \quad (1)$$

ru = Peak response from sample solution.

rs = Peak response from standard solution.

Cs = Concentration of USP ceftriaxone reference standard in standard solution (mg/mL).

Cu = Concentration of ceftriaxone in sample solution (mg/mL), ceftriaxone taken for assay in defined volume.

P = Potency of ceftriaxone in USP ceftriaxone reference standard (µg/mg)

HPLC – System suitability

The ability of method is to detect the analyte in the presence of another components that may cause interference [18].

Specificity and suitability were determined by injection of ceftriaxone sample solution and comparing the retention time with ceftriaxone reference standard RS and USP Ceftriaxone Sodium E-Isomer reference standard in solution for indicating suitability.

HPLC – System linearity

The linearity was determined by plotting calibration curve with selected serial five concentrations of ceftriaxone in mobile phase ranged from 0.1 – 0.5 mg/mL, these samples were injected in separate run and in single run [19].

PREPARATION CEFTRIAXONE SODIUM CRYSTALS BY CONTINUOUS METHOD

In order to develop the method of crystallization to meet industrial requirement continuous crystallization method was investigated, using porous ceramic filter for water purification with pour size less than (1 μm) that ensures high mixing rate of ethanol and water.

The ceftriaxone solution was prepared as in above mention crystallization technique by dissolving (2g) in (40mL) de-ionized water, decolorized by charcoal, after filtration the solution and rinsing with extra (5mL) deionized water, the solution is left to reach room temperature. Then the procedure modified from [20]low energy input, and ease of scale-up for a variety of industrial processes. Porous hollow fiber membranes have also been shown to produce more efficient mixing than conventional mixing equipment mostly because in mixing binary fluids, they provide sufficient mixing time, retention time, and a large contact interface for the drug solution and the antisolvent, allowing for the precise control of nucleation and crystal growth necessary to form nano-size particles. This study reports an experimental and numerical approach to obtain a further understanding of the fundamental principles of antisolvent crystallization using a porous hollow fiber membrane. This includes producing a particle size-controlled drug nanosuspension experimentally using a commercial microfiltration (MF for continuous synthesis drug crystals by antisolvent crystallization using a porous hollow-fiber membrane was applied in this experiment.

The simple mixing method (Figure 2)

Ceftriaxone solution was pumped by flow rate 15ml/min (flow rate was calibrated by using volumetric cylinder and electrical timer) through filter syringe with pore size (0.45μm) to simulate sterilization filtration in industrial process. At same time ethanol was pumped by flow rate 17mL/min into beaker that contains magnetic bar fitted on magnetic stirrer with (600 rpm) for 5 min. This experiment was conducted for three times, the product was treated as in following table II.

The resulted mixtures were kept in an air tight vials to prevent ethanol evaporation and covered with aluminum foil to prevent light influence, at temperature 5-10 C° until further experiment.

CRYSTALLIZATION WITH CERAMIC FILTER

Continuous crystallization by ceramic filter was conducted by pumping ethanol through ceramic filter against ceftriaxone aqua solution (5%) Figure 3. Ceftriaxone solution was prepared

Table II. Components of Ceftriaxone Sodium recrystallization by simple mixing method

	F1Smix	F2Smix	F3Smix
Ceftriaxone Sodium (g)	2	2	2
Distilled-deionized water (mL)	45	45	45
Ethanol (mL)	51	51	51
Ceftriaxone solution flow rate (mL/min)	15	15	15
Ethanol flow rate (mL/min)	17	17	17
Operation time (min.)	3	3	3
Initiation crystallization temp (°C)	10	10	10
Final crystallization temp (°C)	-10	-10	-10
Stirring time (min.)	0	10	360

Table III. Crystallization with ceramic filter

	F1Filt	F2 Filt	F3 Filt
Ceftriaxone Sodium (g)	2	2	2
Distilled-deionized water (mL)	45	45	45
Ethanol (mL)	51	51	51
Ceftriaxone solution flow rate (mL/min)	10	15	17
Ethanol flow rate (mL/min)	20	20	20
Operation time (min.)	3	3	2.5
Initiation crystallization temp (°C)	10	10	10
Final crystallization temp (°C)	-10	-10	-10
Stirring time (min.)	180	180	180

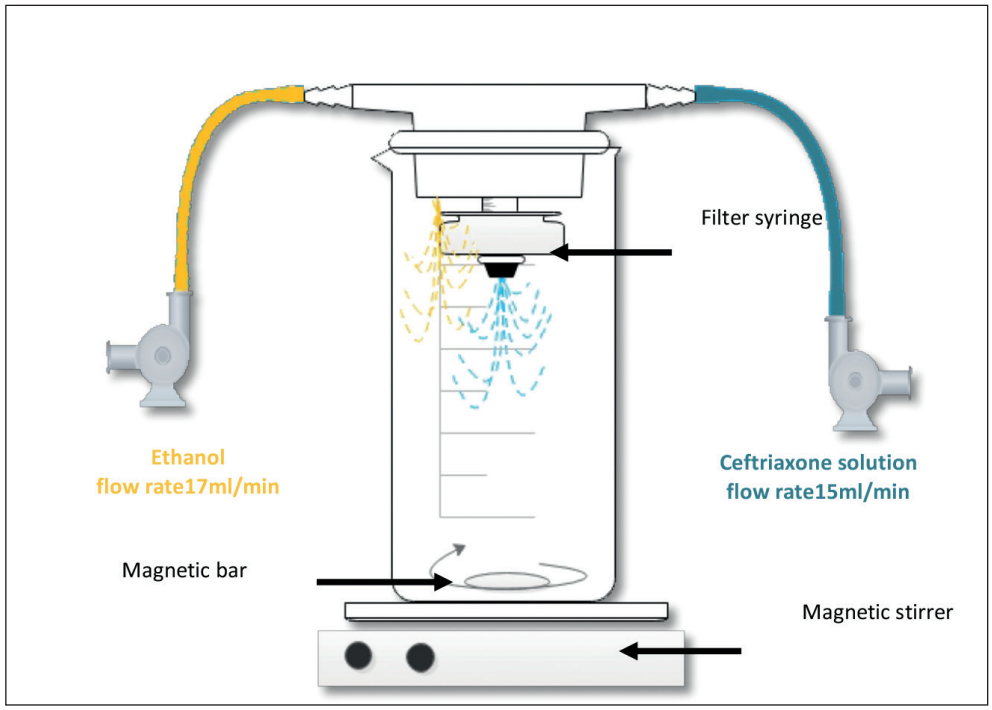


Fig. 2. Schematic illustrate the simple mixing method

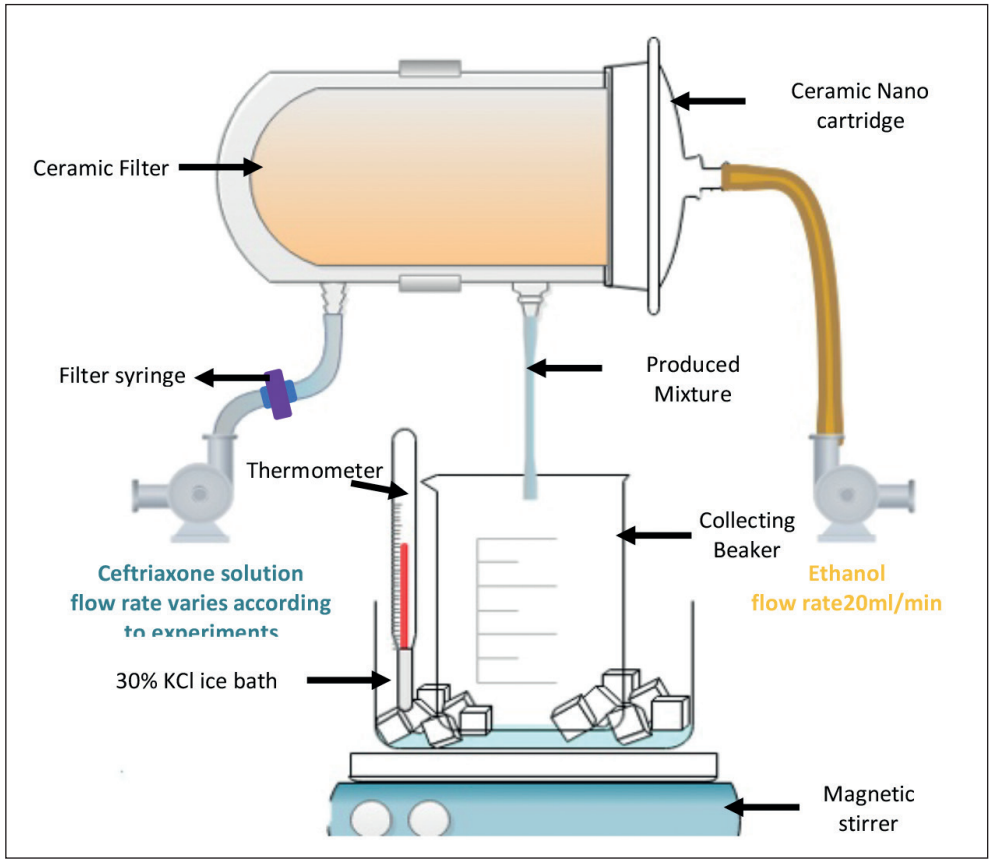


Fig. 3. Continuous crystallization by ceramic filter

in the same method of above experiments, after decolorization with charcoal and additional filtration (5mL) of water added to wash filtrate. The solution was left to cool down to reach room temperature (25 C°), then pumped through filter syringe with pore size (0.45μm) to simulate sterilization filtration in industrial process. The flow rate of both aqua-solution and ethanol was calibrated before the experiment using Omron delay timer and by sitting the pump intake gate. The flow rate of ethanol through ceramic filter kept constant (20 mL/min) and ceftriaxone solution flow rate vary (10 mL/min, 15 mL/min, 17 mL/min), the flow of ethanol designed to be higher to prevent crystallization of ceftriaxone inside filter pores of the system. The produced mixture was collected in beaker fitted inside (30% KCl ice bath) and contained magnetic stirrer that stirring the mixture with

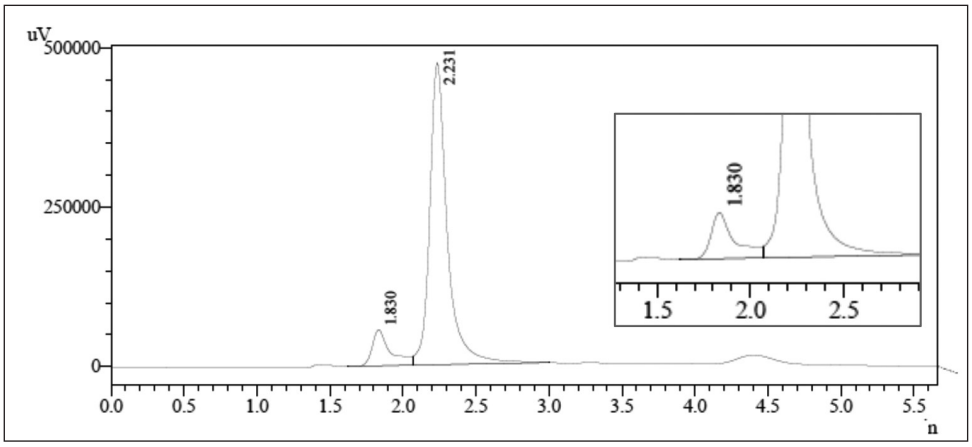


Fig. 4. Chromatograph of system suitability solution

(400 r.p.m), until reaching initial crystallization temperature (10C°). Then the produced mixture was kept in control final crystallization temperature (-10C°) for 180 min. The products were kept in an air tight vials to prevent ethanol evaporation and covered with aluminum foil to prevent light influence, at temperature (5-10 C°) until further experiment. [20]low energy input, and ease of scale-up for a variety of industrial processes. Porous hollow fiber membranes have also been shown to produce more efficient mixing than conventional mixing equipment mostly because in mixing binary fluids, they provide sufficient mixing time, retention time, and a large contact interface for the drug solution and the antisolvent, allowing for the precise control of nucleation and crystal growth necessary to form nano-size particles. This study reports an experimental and numerical approach to obtain a further understanding of the fundamental principles of antisolvent crystallization using a porous hollow fiber membrane. This includes producing a particle size-controlled drug nanosuspension experimentally using a commercial microfiltration (MF.

EVALUATION OF CEFTRIAXONE SODIUM CRYSTALLIZATION PRODUCTS

Particle size analyzing

Produced mixture was measured after 6hr crystallization using ABT- 9000 Beta sizer. This light scattering particle size analyzer measures the particle size as function of light scattering at angle 90º. Mean particle size was calculated based on triplicate recorded results.

Powder X-ray Diffraction

The degree of crystallinity was investigated by X-ray diffraction pattern for ceftriaxone row powder, formulas (F11CE- F13CE) for detection best crystallization time and selected formula from continuous method of crystallization and simple mixing method of ceftriaxone also. The X-ray powder pattern was obtained, using Shimadzu, XRD6000, Japan; with Cu radiation at 40 (kV) and 30 (mA) current, the sample was put in a zero-background holder and scanned by scan speed of 5 (degree / min) through range of (2θ) 5°-80° continuous scan [21], [22].

Scan electron microscope

Surface morphology and crystals shape of ceftriaxone row powder and selected formulas were studied, using scan electron microscope, samples placed in carbon tape and coated with thin layer of gold [5], [22].

Infrared spectroscopy (FTIR)

Shimadzu, IR Prestige 21, was used to determine Fourier transform infrared spectroscopy (FTIR) spectra. A sample was prepared by diluting 100mg of ceftriaxone sodium in potassium bromide, at weight ratio of 1:100 (ceftriaxone sodium sample: potassium bromide). A 150mg of homogeneous powder mixture were compressed for 10 minutes to obtain translucent pellets.

Scanning and the obtained spectra were between the wave number of 4000 – 400 [2], [23] .

Differential scanning calorimetry (DSC)

DSC thermograms were obtained by automatic thermal analyzer system (Shimadzu, DSC–60, Japan), this thermograms used to evaluate the crystalline state of drug.

Samples accurately weighing 5 mg were heated at a scan rate of 5°C/min in non-hermetically sealed DSC sample pan placed on the sample furnace. Against an empty DSC pan that was placed on the reference furnace. Covering temperature range of (50˚ C to 300˚ C). [5], [24].

RESULTS AND DISCUSSION

CHARACTERIZATION OF CEFTRIAXONE SODIUM

Identification of ceftriaxone sodium melting point

The melting point optioned for ceftriaxone sodium was found to be 162 C°. This value totally concurs the value mentioned in reference that indicates drug purity[4].

Identification of ceftriaxone sodium pH

The pH of 0.6 g of ceftriaxone dissolved in 5mL double distilled water, was measured in triplicate using pH meter at temperature kept at 25 C°, the mean pH value was equal to 6.81 and RSD (a relative standard deviation) of 0.30%.

The results obtained conform the references that stated pH will be excepted between 6-8 [12], [16].

CHROMATOGRAPHIC ASSAY OF CEFTRIAXONE SODIUM

HPLC – System suitability

Specificity and suitability of HPLC determined by measuring ability of method to detect the analyte in the

Table IV. Standard solution retention time and peak area

Sample	Retention time	Mean Retention time	Peak area	Mean peak area	RSD %
1	1.999min	2.001min	8478372	8488155	0.528%
2	2.006min		8448997		
3	1.997min		8537095		

Table V. Particle Size Analyzing for Continuous Crystallization Samples

Formula	D [50] particle size (nm)	PDI
F1Smix	Larger than 9500 (visible)	-
F2Smix	4102	0.008
F3Smix	2850	1.34
F1Filt	2694	0.006
F2Filt	236	0.080
F3Filt	66.8	0.209

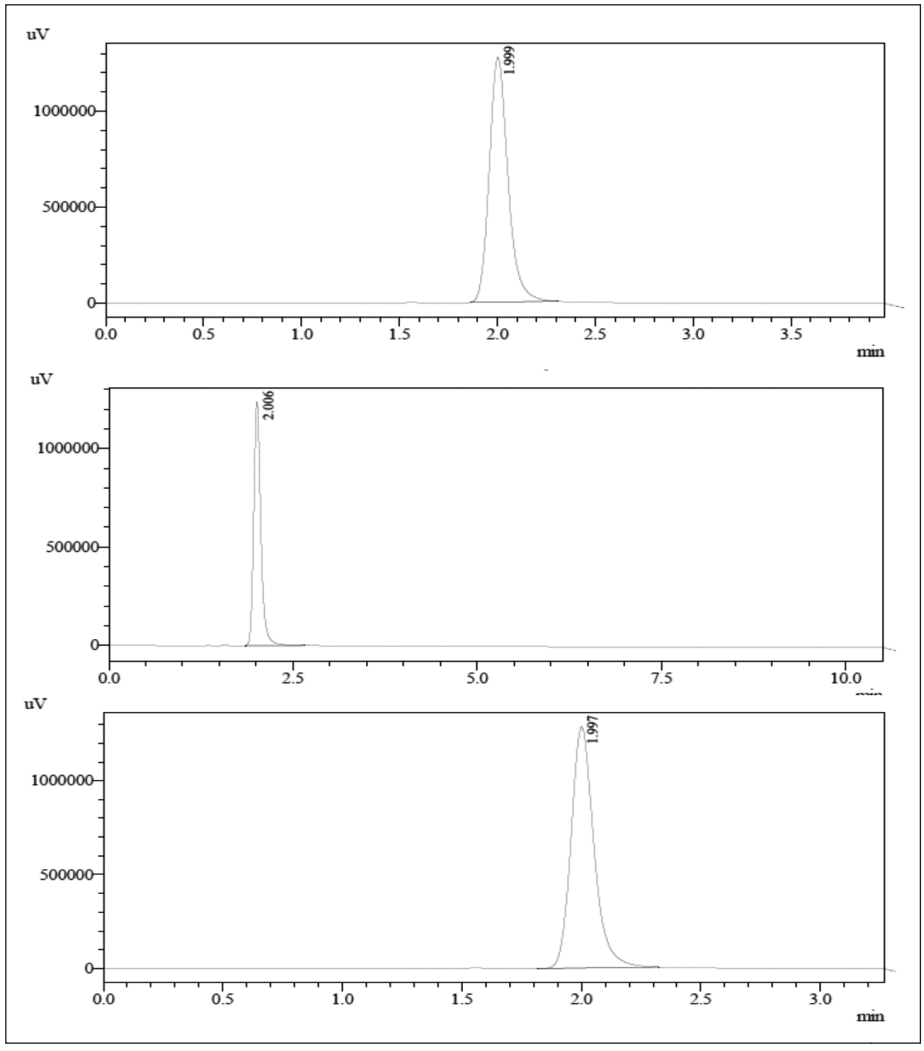


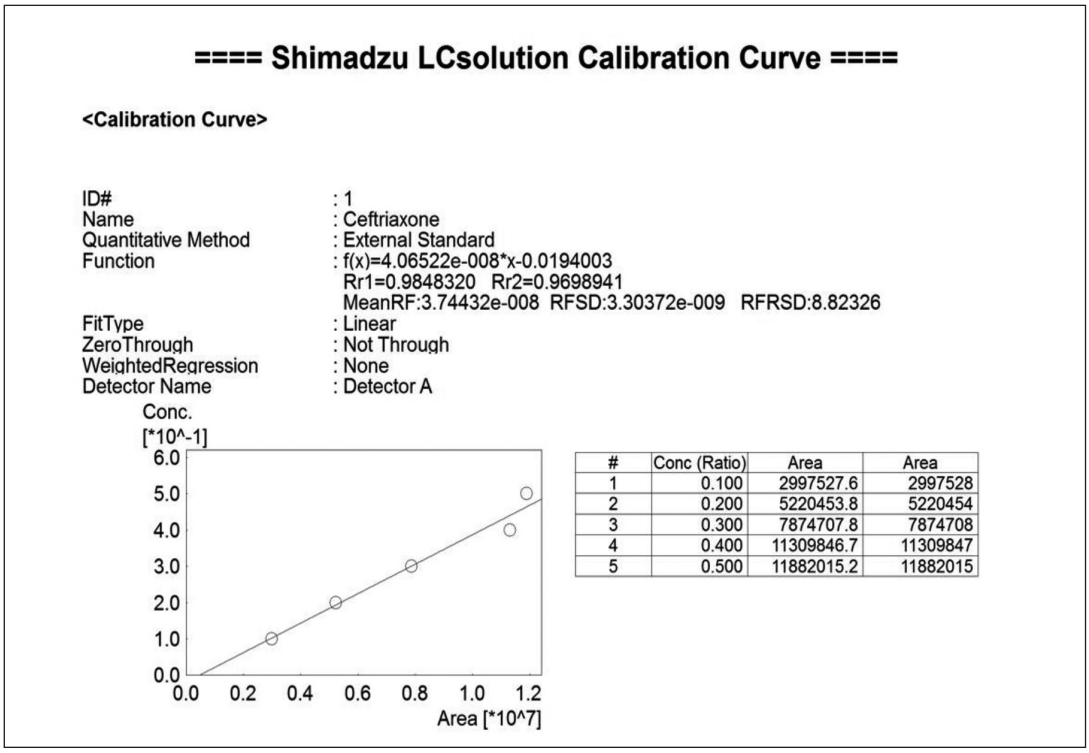
Fig. 5. Chromatographs of standard solution

presence of other components that may cause interference, for this test following samples were prepared:

• System suitability solution: 50 µg/mL of USP Ceftriaxone Sodium RS and 50 µg/mL of USP Ceftriaxone Sodium E-Isomer RS in Mobile phase" [16].

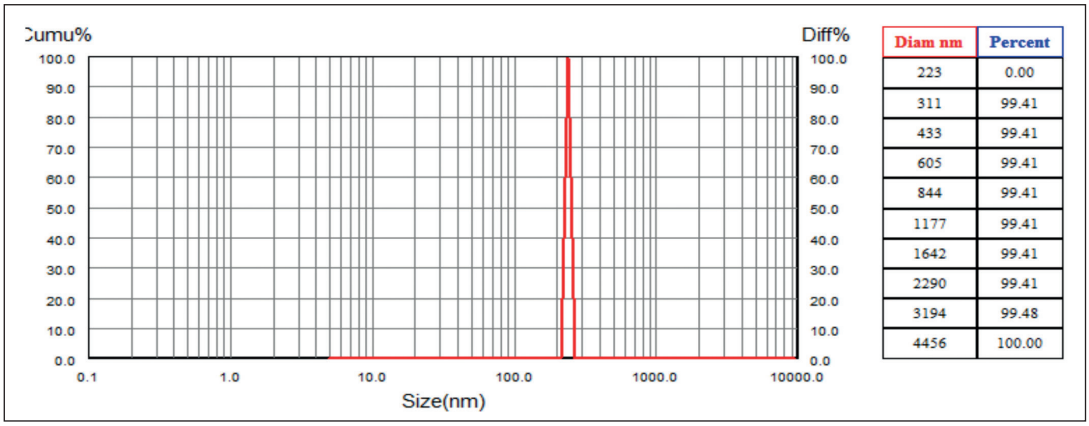
• Standard solution: prepared by dissolving 30 mg of ceftriaxone reference standard RS in 100mL mobile phase to get concertation of 0.3mg/mL

• Sample solution: prepared by dissolving 30 mg of ceftriaxone sodium in 100mL mobile phase to get concertation of 0.3mg/mL.



#	Conc (Ratio)	Area	Area
1	0.100	2997527.6	2997528
2	0.200	5220453.8	5220454
3	0.300	7874707.8	7874708
4	0.400	11309846.7	11309847
5	0.500	11882015.2	11882015

Fig. 6. HPLC Calibration curve



Diam nm	Percent
223	0.00
311	99.41
433	99.41
605	99.41
844	99.41
1177	99.41
1642	99.41
2290	99.41
3194	99.48
4456	100.00

Fig. 7. Particle Size Distribution for F2Filt Formula

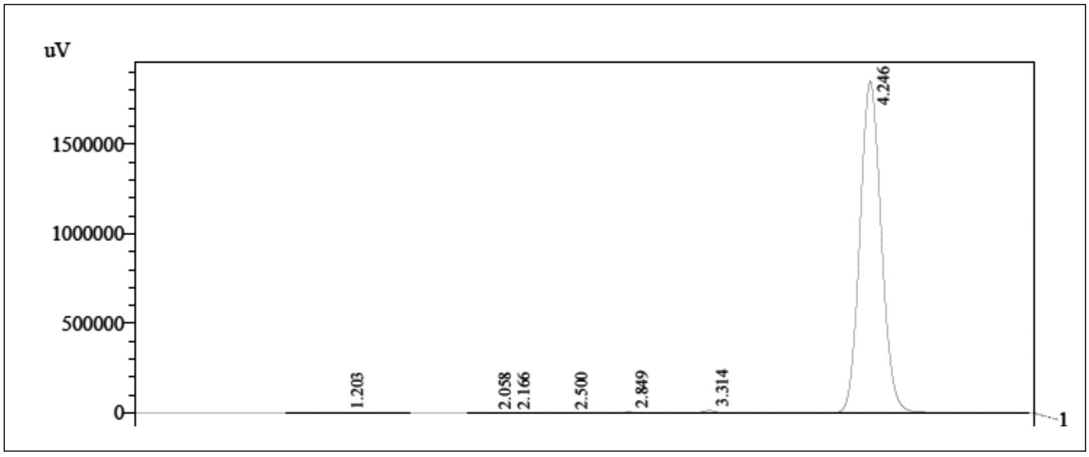


Fig. 8. Chromatogram of F2Filt Formula

System suitability

Samples injected in this test (system suitability solution and standard solution).

The chromatograph of system suitability solution illustrated in the following (Figure 4).

The mean retention time for ceftriaxone and cef-

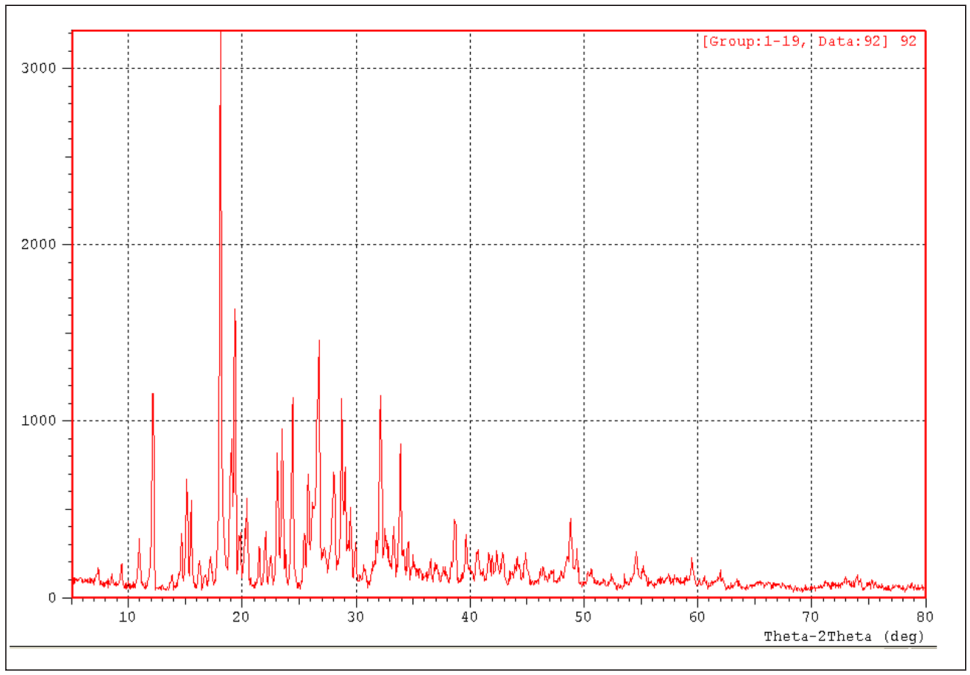


Fig. 9. X-ray Diffraction Pattern for Ceftriaxone Raw Powder

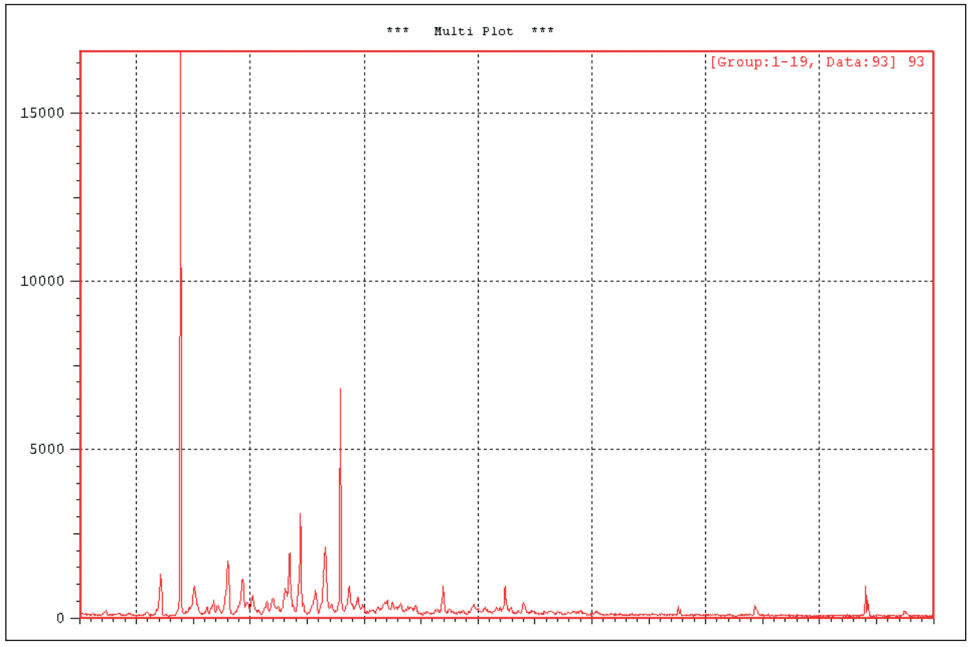


Fig. 10. XRD Pattern of Selected Formula F2Filt

triaxone sodium *E*-Isomer RS triplicate measures are (1.828 ± 0.003 S. D) min and (2.23 ± 0.0017 S. D) min respectively. The relative retention time equal what stated in references, and the mean calculated resolution are 3 that is identical to what listed in references (not less than 3.00), [16], [25].

The chromatographs of standard solution illustrated in the following (Figure 5), triplicate consequences sample runs of standard solution preformed in the same day, the results listed in following Table (4). The calculated mean standard solution peaks tailing factor is 1.23.

Both tailing factor and relative standard deviation RSD% are within acceptance criteria of the references for system selectivity [16], [19].

HPLC – System linearity

Shimadzu software has been used to determine linearity, by injection of serial five concentrations of ceftriaxone in mobile phase ranged from 0.1 – 0.5 mg/mL. These samples injected in separate run and in single run, (Figure 6) illustrated the calibration report.

Assay for ceftriaxone sodium

By injection of Standard solution and Sample solution the amount of in µg/mg of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) in the portion of Ceftriaxone Sodium taken can be calculated by Equation 1. The mean quantity obtained for triplicate sample readings : 409.64 µg/mg which mean that our row ceftriaxone powder contains half of required ceftriaxone ($C_{18}H_{18}N_8O_7S_3$)in each milligram (not less than 795 µg/mg), [16].

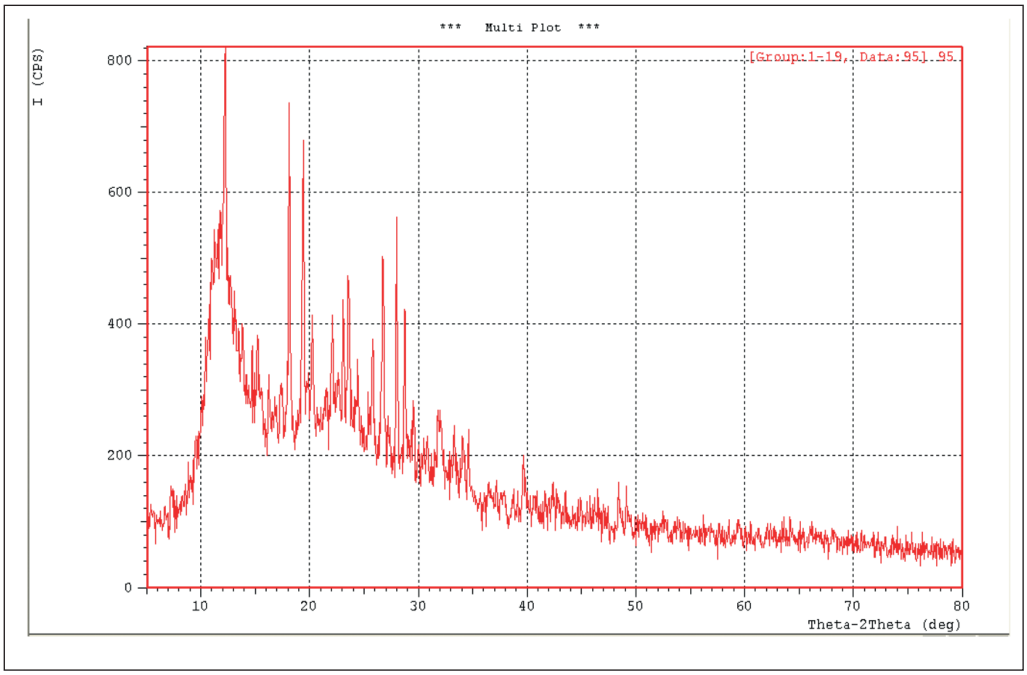


Fig. 11. F3Filt X-Ray Diffraction

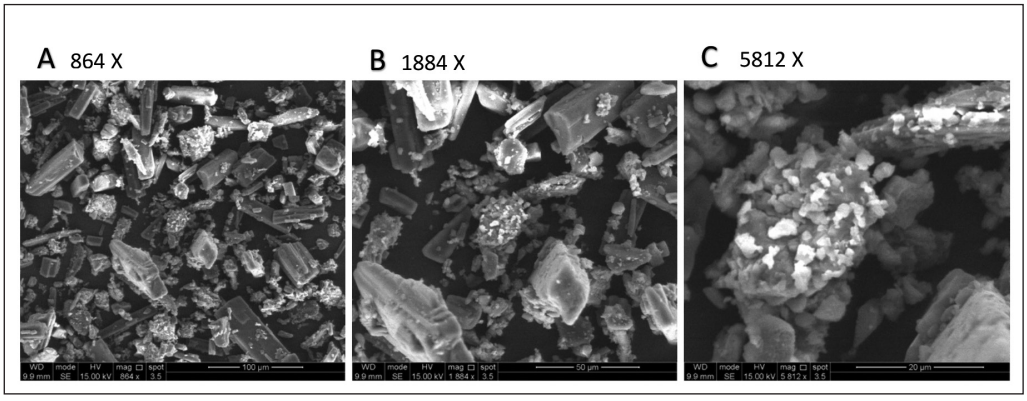


Fig. 12. Microscopic Photographs (SEM) for Raw Ceftriaxone Sodium

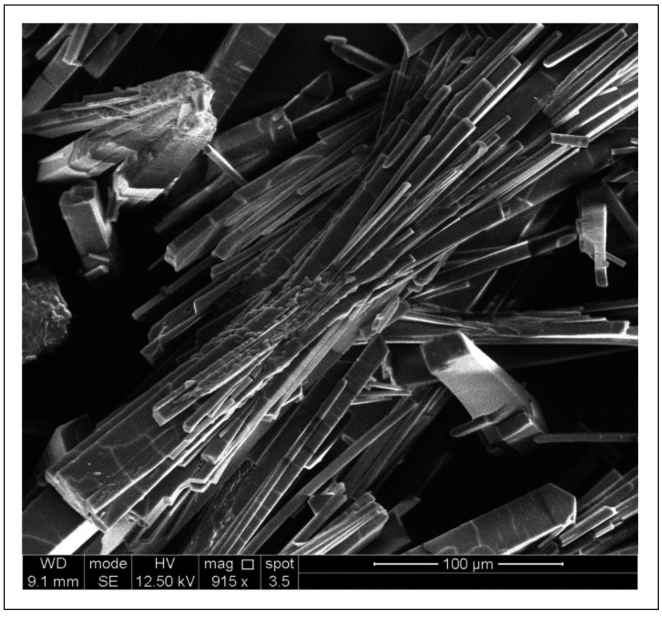


Fig. 13. Microscopic Photographs (SEM) for Formula (F1Smix).

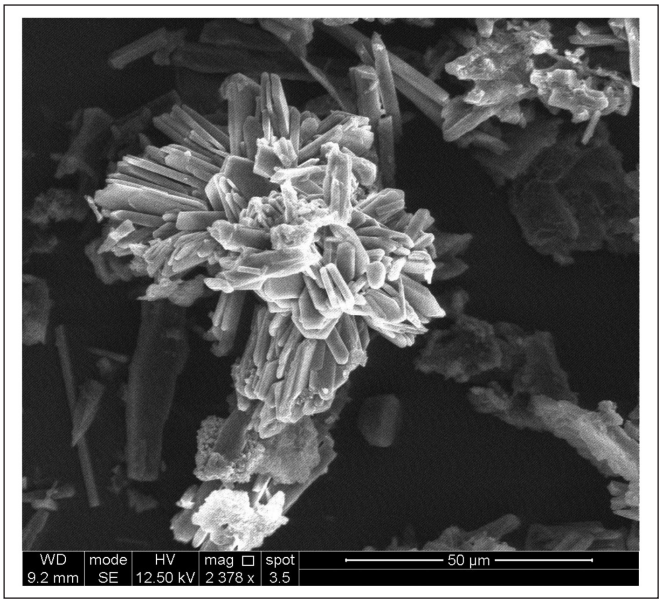


Fig. 14. SEM Microscopic Photograph for (F1Filt) Formula

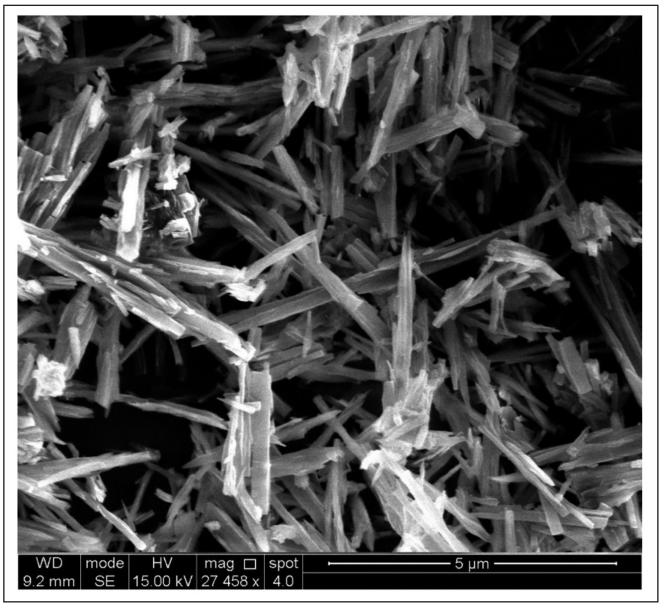


Fig. 15. The SEM Microscopic Photograph for (F2Filt) Formula

Ceftriaxone Sodium Crystals by Continuous Method

The particle size produced from F1Smix, F2Smix and F3Smix has been evaluated and compared with these produced from ceramic filter continuous flow F1Filt, F2Filt and F3Filt that have drug solution flow rate of (10 mL/min, 15 mL/min, 17 mL/min) respectively.

EVALUATION OF CEFTRIAXONE SODIUM CRYSTALLIZATION PRODUCTS

Particle Size Analyzing

The produced mixture was measured after 6hr crystallization, using ABT- 9000 Beta sizer. This light scattering particle size analyzer measure the particle size as function of light scattering at angle 90°. Mean particle size was calculated based on triplicate recorded results.

Following Table V illustrates particle size analyzing for continuous crystallization method.

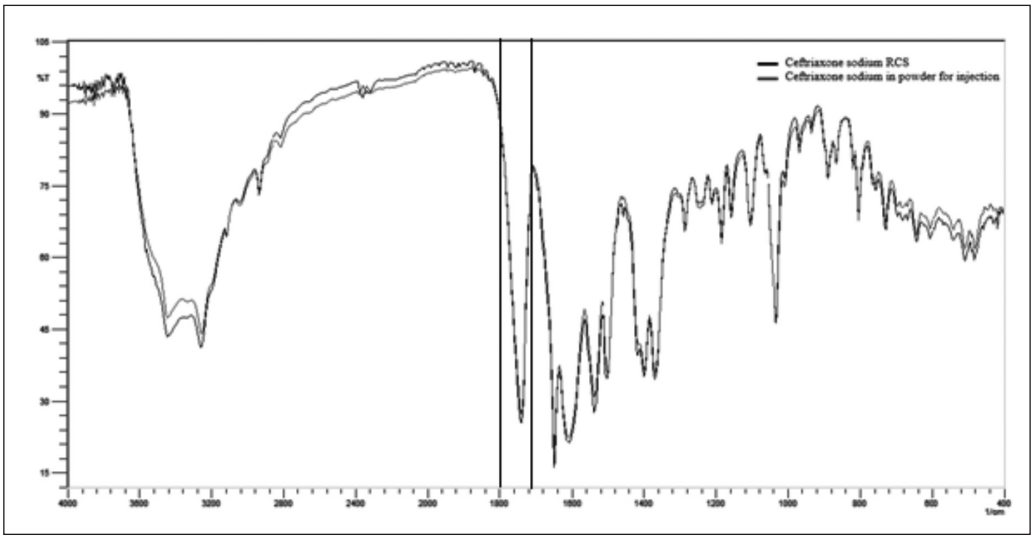


Fig. 16. Overlap of Ceftriaxone and Ceftriaxone Reference Standard, Adopted From (Teixeira, Regina and Salgado, 2017)

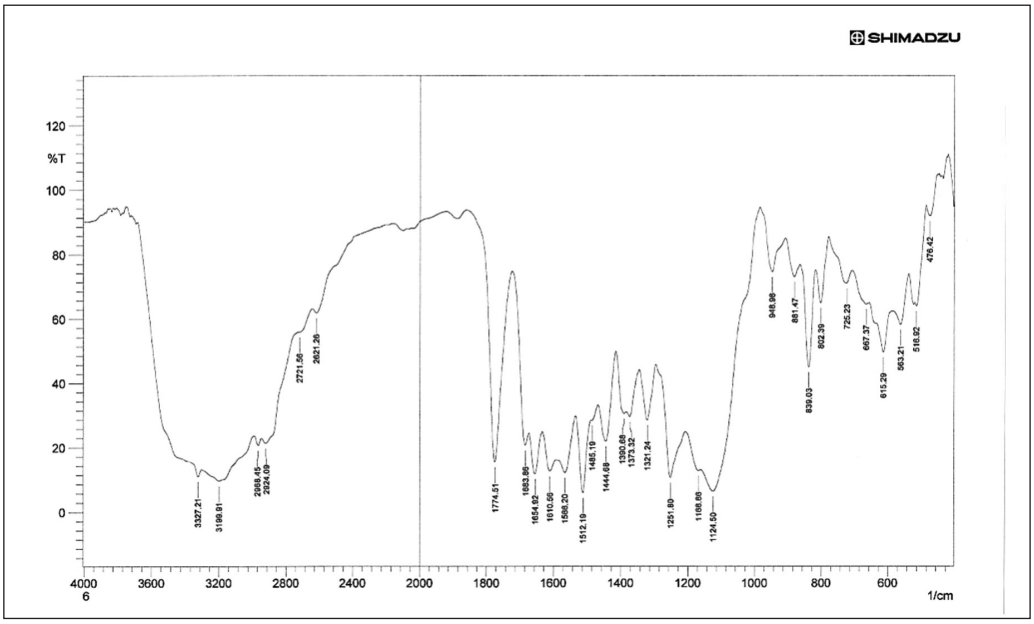


Fig. 17. FTIR Spectra for Ceftriaxone Selected Formula Crystallization

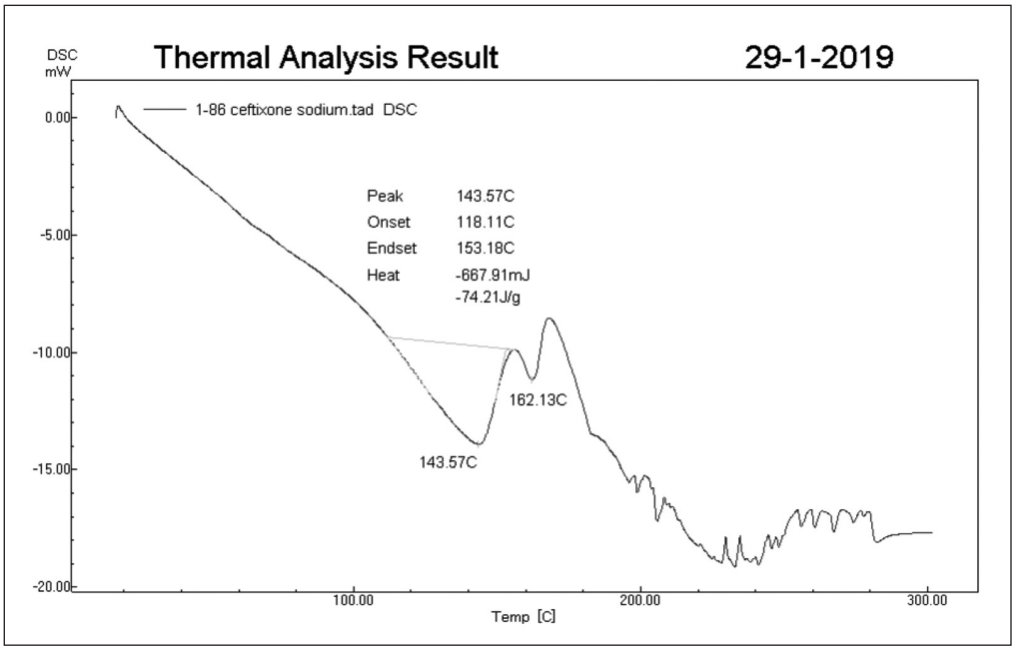


Fig. 18. Thermogram of Ceftriaxone Raw Powder

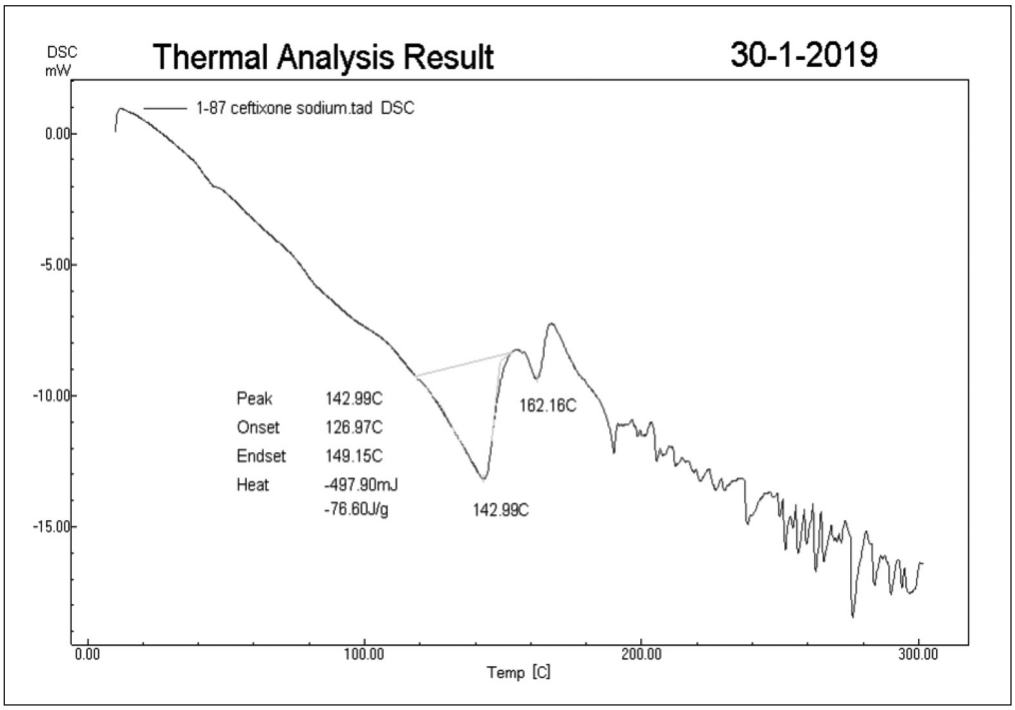


Fig. 19. DSC Thermogram F2Filt

The poly-dispersible index obtained by this devise, gave an idea about the distribution of particle size the lower value refer to homogeneous distribution of particle size inside the sample [26].

The obtained PDI values gives an idea about how crystal growing for each technique F2smix shows large particle size with lower PDI that's my indicate distribution of ceftriaxone during nucleation make uniform particle growing, F3Smix that give smaller particle size with higher PDI value indicate that continuous stirring disturbs nucleation of crystals and not sufficient to produce uniform crystal size. In the other hand, continuous mixing through ceramic pores giving lower PDI that conform high mixing efficiency of solute and anti-solvent that produce uniform nucleus for crystal growth the F1Filt but quantity of ethanol not sufficient to induce nucleation therefore, larger particle size obtained. F2Filt have smaller particle size with low PDI that conform the efficient mixing criteria that produce larger number of nucleolus growing to relatively smaller particle size (D50 236 nm) (Figure 7). The F3Filt show lower particle size in reference to X-ray diffraction and DSC result the larger number of ethanol produced both amorphous and crystalline state because of sudden sedimentation of ceftriaxone not enable the normal crystallization kinetics of ceftriaxone. This amorphous could affect both product stability and increase the risk of insoluble microparticle in the product.

Chromatographic Assay of F2Filt Formulas Using HPLC

The amount of ceftriaxone sodium (C18H18N8O7S3) in each milligram sample has been calculated using equation.1, by injecting (60 mg/mL) of ceftriaxone formula F2Filt, the peak response and concentration of this formula applied with peak response and concentration of ceftriaxone RS. Figure (8) illustrated the chromatogram of F2Filt formula. The calculated amount was 1.024 ± 0.152 S. D (μg/mg).

Powder X-Ray Diffraction

The degree of crystallinity was investigated by X-ray diffraction pattern for ceftriaxone sodium raw powder, Figure (9) illustrated the P-XRD diffraction pattern.

The pattern confirmed crystalline nature of ceftriaxone sodium and comply with this in reference [27].

Formula F2Filt P-XRD defection pattern shows higher intensity asymmetric sharp peaks that suggest different crystalline shape (needle shape) (Figure 10).

For F3Filt X-Ray Diffraction Figure (11). The intensity of 2theta pattern in 14,18,19,28 and 29 with 14 and strongest peak, this result confirms the lattice for amorphous and crystal properties that are produced because of antisolvent over pumping

Scan Electron Microscope

The raw ceftriaxone sodium powder has been visualized using scan electron microscope to the magnification power of 864 X,1884 X and 5812 X, as shown in the Figure (12).

For the formulas obtained from lyophilized continuous crystallization method, the SEM microscopic photograph shows F1Smix formula with 915X magnification power in Figure (13).

Figure (14) shows SEM microscopic photograph for (F1Filt) formula with 2378X magnification power. This photograph shows crystallization of ceftriaxone sodium but tendency to cumulate and form type of clusters.

The SEM microscopic photograph for (F2Filt) formula is shown in Figure (15) in which we may confirm low particle size with needle shape crystals, that comply with the reference [5], that found small needle particle ceftriaxone sodium product dissolute quickly.

Infrared Spectroscopy (FTIR)

Infrared spectroscopy (FTIR) studies were performed to characterized raw ceftriaxone powder. Furthermore, it has benefitable capabilities to detect any chemical interaction between drug and excipients, added in this study during mixing or processing(crystallization). This interaction would result in band shift compared in spectra for the drug and additives [28]. Ceftriaxone raw powder was investigated and FTIR spectra obtained between the wave number of 4000 – 400 . This absorption spectra show high degree of similarity with reference [2] that compares ceftriaxone vial with reference standard this spectra showed in Figure (16).

The FTIR result suggested no chemical reaction happened and confirmed that our selected formula contained ceftriaxone sodium (Figure 17).

Differential Scanning Calorimetry (DSC)

DSC thermograms were obtained by automatic thermal analyzer system. Following Figure (18) illustrates DSC thermogram of ceftriaxone raw powder, that gives endothermic peak in 148 C° and confirms hydration properties of the drug. Exothermic peak attend refers to melting decomposition transition of ceftriaxone sodium second endo thermic curve at 162.13 C°.

The DSC thermogram for F2Filt illustrated in following Figure (19). Endothermic curve until 142.99 C° reflects the loss of water, another exothermic from melting decomposition transition, another peak at 162.16 C°. The thermogram shows similarity with raw ceftriaxone sodium. Both thermograms show identical similarity with references [27].

CONCLUSIONS

Crystallization is purification and size reduction technique. It has good impact in final product behavior but it takes time and need more solvent (ethanol) to produce crystals with acceptable characteristic. This time needed for crystallization increasing of particle size that could affect dissolution rate and is associated with insoluble micro particle problem.

Continuous method was investigated and compared with ordinary method of stirring. It gives high mixing rate and nano product with superior properties of dissolution and purity. This method could be further investigated and developed to identify various variables and their effects on end products. Ceramic filter shows very good surface aid for continuous crystallization process.

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