Research Paper

Development and Validation of Favipiravir Severe Acute Respiratory Syndrome Coronavirus 2 Drug by Dissolution Method with Filter Compatibility Study

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Favipiravir drug is fitted into antiviral medication criteria and mainly used in the treatment of influenza. The mechanism is associated with choosy inhibition of viral ribonucleic acid-dependent ribonucleic acid polymerase. Development and validation of dissolution method and filter compatibility studies are conducted with reverse phase ultra-performance liquid chromatography method for the quantitative analysis. The validation of this method was performed as per International Council for Harmonisation Q2 (R1) guidelines with the optimized experimental conditions. The proposed method was achieved on Acquity ultra-performance liquid chromatography HSS C18 (100 mm×1.8 µ) column and temperature maintained at 30° and run time was 8 min. The mobile phase consists of A-Methanol, B-0.1 % Trifluoroacetic acid (v/v) in water (pH=4.8). The injection volume of samples was 1 µl and ultraviolet detection was carried out at 210 nm. Linearity ranges were covered from 1 % to 300 % of the sample concentration level. The newly developed dissolution profile will show good repeatability and reproducibility in solid dosage forms and proved the filter compatibility studies. The projected method has capable to produce swift retention time and maintained well percentage recoveries throughout the dissolution profile. Hence this method can be used in customary quantitative analysis in quality control department for solid dosage forms and active pharmaceutical ingredients.

Key words: Favipiravir, ultra-performance liquid chromatography method, dissolution, filter compatibility studies

The chemical name of favipiravir is 6-Fluoro-3,4-dihydro-3-oxo-2-pyrazinecarboxamide. Favipiravir mechanism is mainly to control the entry and exit of antivirals. As per the studies, purine will reduce the drug activity and there is a competition between favipiravir ribofuranosyl-5'-triphosphate and purine ribonucleic acid (RNA)-dependent RNA polymerase. Favipiravir is broadly absorbed in the urine. Oral dosage of favipiravir is 600 mg is recommended daily twice a day. That is also 2-5 d only preferable. In recent days, favipiravir drug has been used in the treatment of Ebola virus, Lassa fever and Coronavirus Disease (COVID-19). Favipiravir, advertising with the trade names of FluGuard, Avigan, Favulous, and FabiFlu[1,2]. It has a molecular formula of $C_5H_4FN_3O_2$ with molecular weight 157.104 (fig. 1). Its nature is white to light yellow powder and sparingly soluble in acetonitrile, methanol and also in water.

Favipiravir will come under small molecule category and the drug is approved in 2014 in Japan for treatment of influenza cases[3]. More recently, it is exhibited its effectiveness for aiming different strains of influenza. It is also having 54 % protein bound in plasma protein. Favipiravir is one of the repurposed

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leading drugs used for the treatment of Ebola and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)[4]. In the list of COVID-19 drugs, favipiravir having the chance to cure 80 % as per the studies[5]. Primarily, Indian Council of Medical Research (ICMR) approved favipiravir drug for only emergency usage[6,7].

They can be fast-tracked conventional into the final phase of clinical development, Phase III and can be effortlessly estimated for their safety and effectiveness as COVID-19 treatment[8,9]. The convenience of this approach has apprehended the mind of pharma companies and researchers committed to quickly resolving the COVID-19 pandemic[10].

MATERIALS AND METHODS

Drug substance:

Working standards favipiravir (99.9 %) was procured from Spectrum Pharma Labs, Hyderabad, India.

Instrumentation:

An Agilent-1100, Ultra Performance Liquid Chromatography (UPLC) consisting of ACQUITY UPLC Binary Solvent Manager (part numbers: 186015002), ACQUITY UPLC Sample Manager (part numbers: 186015005) and Waters Empower 2 workstation, Waters Acquity column manager (Part number: 186015007), Waters Photodiode Array Detector (PDA) detector (Part numbers: 186015032), supplied by M/s. Waters, USA. Dissolution apparatus (708 DS, M/s. Biocompare, USA), Mettler-Toledo analytical balance, model AG-245 capable of weighing 0.01 mg, supplied by M/s. Mettler AG, Switzerland. Sonicator supplied by M/s. Serwell instrument, India. Digital pH meter supplied by M/s. Serwell instruments, India.

Chemicals and reagents:

High-Performance Liquid Chromatography (HPLC) grade Methanol and HPLC water were purchased from Merck, India. AR grade Sodium acetate trihydrate, acetic acid, trifluoroacetic acid supplied by M/s. Rankem, Avantor performance materials, India used for present study, 0.45 µm pore size Nylon filter from Merck, India.

Preparation of mobile phase:

Mobile phase: A-0.1 % Trifluoroacetic acid (v/v), B-Methanol.

Preparation of standard solution:

Weigh accurately and transfer 20.0 mg of favipiravir standard into 100 ml volumetric flask, then add 25 ml of dissolution medium and allow dissolving completely by sonication and making up to volume with dissolution medium, shake well and transfer to vial.

Preparation of sample solution:

Set the parameters of dissolution apparatus as mentioned in dissolution parameters. Place previously weighed tablet in each of the six dissolution vessels and start the dissolution apparatus. At the end of specified time intervals, withdraw 10 ml of the sample solution from each of the dissolution vessel and filter the sample solution through 0.45 µm pore size Nylon filter (Make: mdi) and discard first 3 ml of the filtrate. Collect the filtrate and use as sample solution.

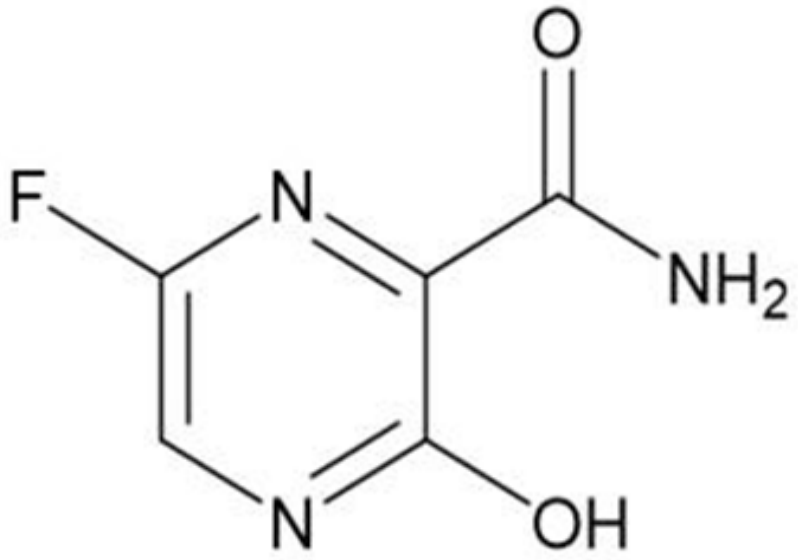

Fig. 1: Structure of favipiravir

Chromatographic conditions:

The mobile phase was a combination of mobile phase A-0.1% trifluoroacetic acid (v/v) in Water, B-methanol. The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed for 10 min and pumped from the respective solvent reservoirs to the column at a flow rate of 0.2 ml/min, Acquity UPLC HSS C18 (100 mm×1.8 µ). The column temperature was preserved at 30° and run time 8 min. The injection volume was maintained as 1 µl. Ultra-Violet (UV) detection was carried out using a UV-PDA detector at 210 nm.

Method development:

After several trials, optimal chromatographic conditions were fixed for better separations. The separate standard calibration lines were constructed to drug for dissolution profile. A series of aliquots are prepared from the above stock solutions using mobile phase to get the concentrations 1 % - 300 %). Each concentration was injected 3 times into chromatographic system. Each time peak area and retention time documented separately for the drug and impurities. Calibration curves were constructed by taking average peak area on Y-axis and concentration on X-axis separately for drug and impurities. From the calibration curves, regression equations were calculated as shown in the fig. 2.

Method validation:

In the validation, repeatability and reproducibility demonstrated for analytical method and was consistently produces the results for intended analytical applications[11,12]. The method was accomplished as per International Council for Harmonization (ICH) guidelines[13,14]. The developed method was supported by performing system suitability, linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, accuracy, selectivity and robustness.

System suitability parameters:

The system suitability, six replicates of favipiravir samples and favipiravir standard were injected and studied the parameters like tailing factor, and percentage of Relative Standard Deviation (% RSD). The results were revealed in Table 1.

Accuracy:

Accuracy was covered in range of 1, 50, 100 and 300 % with triplicate injections by adding a known amount of Active Pharmaceutical Ingredient (API) stock solution into Placebo and calculated the percentage of recoveries. The results were shown in Table 2.

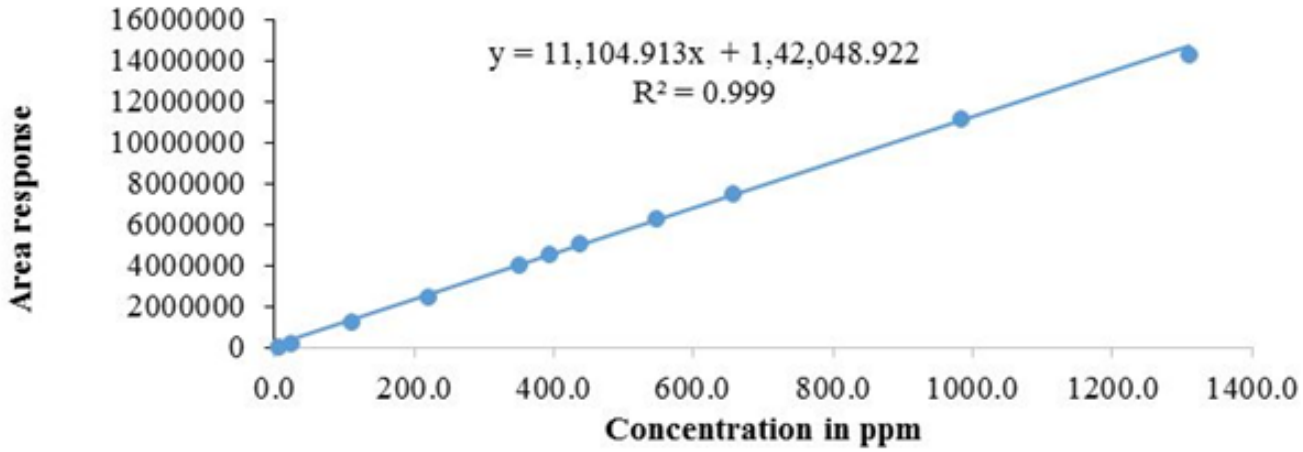

Fig. 2: Calibration curve for favipiravir

TABLE 1: SYSTEM SUITABILITY

S No	Acceptance criteria	Result
1	Tailing factor for favipiravir peak from standard solution should be not more than 2.0	1.02
2	The % RSD for favipiravir peak obtained from six replicate injections of standard solution should be not more than 2.0 %	0.51

TABLE 2: RESULTS OF THE RECOVERY STUDIES FOR FAVIPIRAVIR

Level	Area response	Amount added in(mg) (actual)	Amount of recovered(mg)	% Recovery	% Mean	% RSD
Level-1	28442.29	1.9789	2.23455	112.9	105.2	6.3
(1 %)	25660.28	1.9819	2.016	101.7		
	25458.24	1.9809	2.0001	101		
Level-2	1267876	99.0299	99.6101	100.6	100.6	0.1
(50 %)	1268528	99.1735	99.66135	100.5		
	1268978	99.08435	99.6967	100.6		
Level-3	2548125	198.2628	200.19225	101	101.1	0.1
(100 %)	2549591	198.139	200.30745	101.1		
	2551184	198.0994	200.4326	101.2		
Level-4	7266246	594.2141	570.86935	96.1	96	0.1
(200 %)	7261917	594.1794	570.5292	96		
	7258355	594.12	570.24945	96		

Precision:

Precision of the analytical method was demonstrated by analyzing six sets of sample solution. Favipiravir tablets (200 mg) of all six replicates sample solutions and calculated the mean percentage. Average relative peak area and % RSD were calculated and the obtained results were displayed in Table 3 and Table 4. Precision is the level of repeatability of results as reported for analyzed method. The precision test of the method, specifically, the method variation in the peak area of the drug formulation was calculated by using the below formula in terms of % RSD and the results were reported in the Table 3 and Table 4. Statistical results revealed that RSD of each drug for 6 times was less than 2.0.

% drug release calculation:

$A_{Test}/A_{Std}\times W_{std}/DS\times D_{Test}/1\times P/100\times 100/LC$

Where, A_{Test}=Average area response of favipiravir peak from sample solution, A_{Std}=Average area response of favipiravir peak from standard solution, W_{St}d=Weight of standard solution; DS=Dilution of standard solution, D_{Tes}t=Dilution of sample solution; P=Potency of standard in % w/w on as is basis and LC=Label claim in mg/tablets

Dissolution (mg)=% Release×Label claim/100

Linearity:

The linearity of the method was determined in the concentration range of 1 % to 300 % for favipiravir solid dosage form. Linearity graph was plotted peak area against solution concentration and calibration curve was presented in fig. 2. The results were presented in Table 5.

Robustness:

The robustness of the dissolution method was established by introducing trivial changes in the chromatographic condition which included the temperature (27° and 33°), flow rate (0.1 ml/min and 0.3 ml/min) and mobile phase (60 Buffer:40 Methanol and 50 Buffer:50 Acetonitile). The results were given in Table 6.

Specificity and selectivity:

Specificity is the degree to which the procedure applies to a drug formulation and is checked in each analysis by probing samples and impurities for any interfering peaks. The specificity of the method was assessed with regards to interference. The reverse Phase-Ultra Performance Liquid Chromatography (RP-UPLC) chromatograms recorded for the drug substance showed no interfering peaks within retention time range. The respective chromatogram for blank, placebo and favipiravir standard and sample which shows the selected drug was effectively separated (fig. 3). Thus, the projected RP-UPLC method in this study was selective.

Filter compatibility study:

Filter Compatibility studies was ensured for dissolution testing in favipiravir tablets (200 mg) sample and placebo[15,16]. 0.45 µm Nylon filter is used for filtration and sub fractions are collected for filter (after discarding 3 ml and 6 ml). Collected the filtrate and used the same sample solution for analysis and results are tabulated in Table 7-Table 9.

TABLE 3: SYSTEM PRECISION

S. No	Retention time	Peak area
1	3.961	2581812
2	3.921	2583062
3	3.933	2583338
4	3.941	2581117
5	3.923	2584478
6	3.922	2584096
Mean	3.934	2582984
% RSD	0.40	0.05

TABLE 4: METHOD PRECISION

S No.	Favipiravir
1	94.8
2	95.1
3	95.8
4	96.1
5	95.4
6	95.8
Mean	95.5
% RSD	0.51

TABLE 5: LINEARITY OF FAVIPIRAVIR

Level (%)	Concentration in ppm	Peak area
1	2.1816	23691.077
5	10.90805	129671.26
25	54.5402	642975.69
50	109.08045	1242671
80	174.5287	1976072.5
90	196.3448	2313945.7
100	218.16085	2554475.5
120	272.7011	3200799.1
150	327.2413	3761177.2
200	490.86195	5117290.6
300	654.4826	7179481.3
Correlation coefficient (r)	0.999	
Regression coefficient (r2)	0.999	
Slope	11104.913	
y-intercept	142048.922	
% intercept	2.8	

TABLE 6: ROBUSTNESS

Method parameters	Conditions	Retention time (R_T) favipiravir
Flow rate	0.1 ml/min	4.112
Flow rate	0.3 ml/min	3.809
Temperature	27°	4.211
Temperature	33°	3.795
Mobile phase	60Buffer:40Methanol	4.226
Mobile phase	50Buffer:50Methanol	3.802

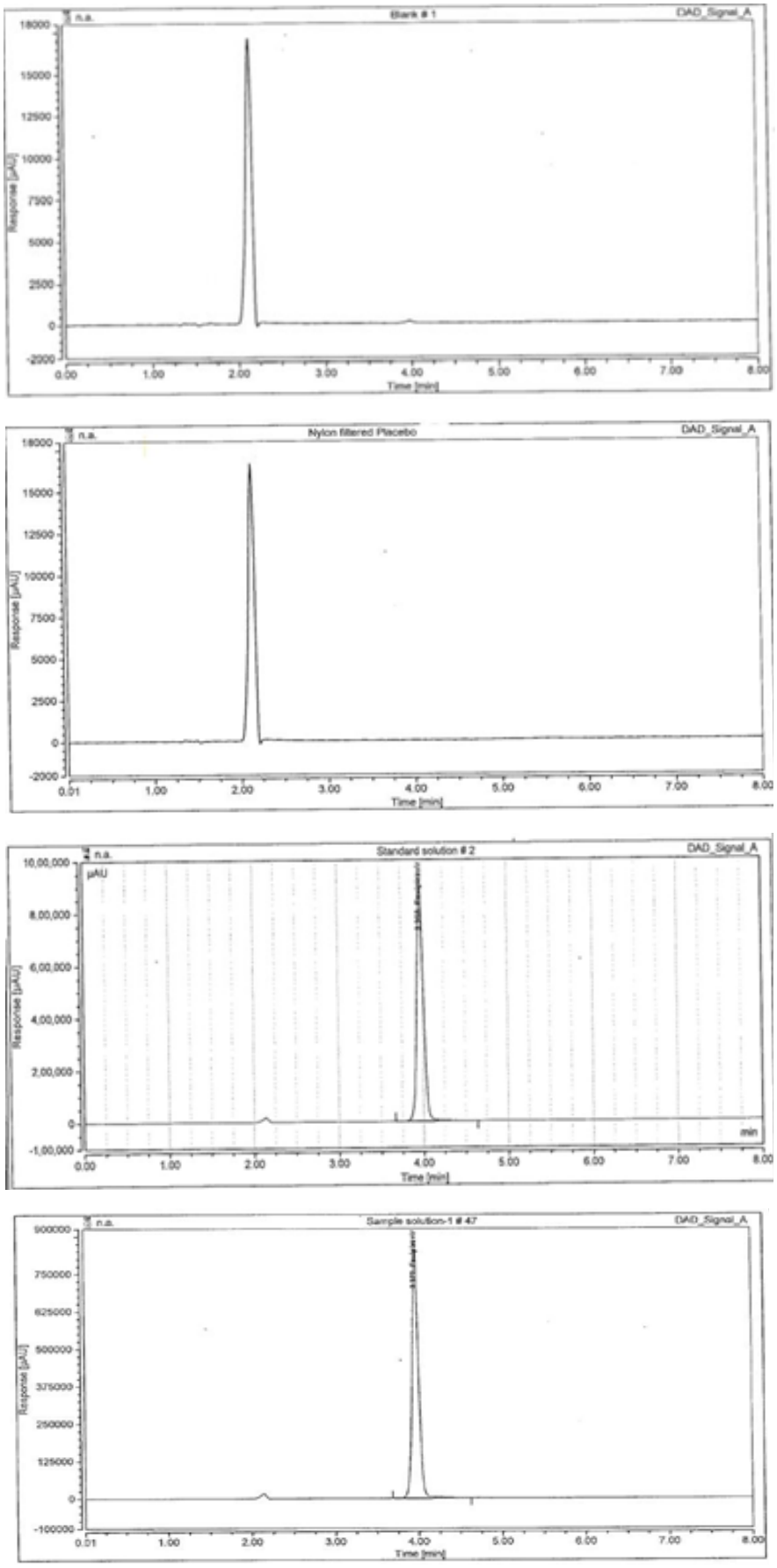

Fig. 3: Typical RP-UPLC chromatogram for blank, placebo and favipiravir standard and sample
Chromatogram derived from the RP-UPLC after collection of samples from the dissolution test. The chromatogram indicates peak elution at 4 min and 8 min run time.

RESULTS AND DISCUSSION

The optimized dissolution profile and chromatographic conditions are stated above. Based on the dissolution profile, the best peak shape and extreme separation was achieved with mobile phase composition of A-0.1 % Trifluoroacetic Acid (v/v) in Water, B-Methanol. The best peak separation, peak symmetry and reproducibility were obtained on Acquity UPLC HSS C18 (100 mm×1.8 µ). The optimum wavelength for detecting the analyte was found to be 210 nm with a flow rate of 0.1 ml/min. Most of the developed methods are reported High performance Liquid Chromatography (HPLC) till date use C8 column or C18 columns without dissolution profile. Also, most of them carried out with complex mobile phase compositions. Hence challenges were directed towards the development of a simple and better method on a commonly used C18 column with dissolution profile. Different logical amendments were tried to get good dissolution profile in solid dosage formulation. These changes included a change in mobile phase composition, column temperature.

The percentage recovery of favipiravir was attained in the range 1, 50, 100 and 300 % with triplicate injections by adding a known amount of API stock solution into placebo respectively. Related standard deviation value of replicated sets was less than 2.0 % which indicates that this method is highly accurate. The results are revealed in Table 2.

The precision of the method was determined by repeatability and method precision of favipiravir solid dosage formulation. The obtained results of repeatability and method precision were less than 2. Percentage of RSD value of replicated sets was less than 2.0 which indicates that this method is highly precise. The results are exposed in Table 3 and Table 4.

The calibration curve was plotted in the concentration range of 1 % to 300 % for favipiravir solid dosage form. The linearity graph is plotted for peak area against solution concentration and the correlation coefficient (r^2) was attained (0.99). The standardization curve was constructed on regression equation. The statistical data exposed that the projected method was linear and the results were as shown in Table 5.

The robustness of the related substance method was established by introducing minor changes in the chromatographic condition which included the percentage of flow rate (0.1 and 0.3 ml/min), Mobile Phase (60Buffer:40 Methanol and 50 Buffer:30 Methanol) and temperature (27° and 33°). The developed method was unaffected by minor deliberated changes, which represents that the proposed method was robust. The results were shown in Table 6.

Filter compatibility study was established for dissolution testing in favipiravir tablets 200 mg sample and placebo and proved that 0.45 µm nylon filter is suitable for dissolution sample testing and the percentage drug release and percentage calculation was calculated for different test solutions and results are tabulated in Table 7-Table 9.

Dissolution profile with method development is an important part of drug formulation in development process and the pharmaceutical industries always show much interest in this area to effective release of drug in human body. A rapid, accurate and precise stability-indicating RP-UPLC analytical method with dissolution profile, filter compatibility study have been developed and validated for the quantitative analysis of favipiravir dosage formulation. The presented RP-UPLC method with dissolution profile for separation of drug in placebo and found to be capable of giving swift retention times with accurate drug release in dissolution and with filter compatibility. Validation is followed as per International Council for Harmonization guidelines divulged that the method is more specific and stability-indicating. Henceforth, this method can be functional in the quality control samples and stability sample analysis. As per the current COVID pandemic situation, this method is valuable in pharmaceutical industries for fast release of batches in the market for the purpose of patient safety.

TABLE 7: ROBUSTNESS

Sample details	Filter details	% Drug release	% Difference
Centrifuged sample	NA	92.6	-
3 ml discarded sample	Nylon 0.45 µm	92.8	-0.2
6 ml discarded sample	Nylon 0.45 µm	92.7	-0.3

TABLE 8: RESULTS OF FILTER COMPATIBILITY STUDIES TEST SOLUTION-2

Sample details	Filter details	% Drug release	% Difference
Centrifuged sample	NA	95.8	-
3 ml discarded sample	Nylon 0.45µm	94.9	-0.1
6 ml discarded sample	Nylon 0.45µm	95.4	-0.2

TABLE 9: RESULTS OF FILTER COMPATIBILITY STUDIES TEST SOLUTION-3

Sample details	Filter details	% Drug release	% Difference
Centrifuged sample	NA	95.8	-
3 ml discarded sample	Nylon 0.45 µm	94.9	-0.1
6 ml discarded sample	Nylon 0.45 µm	95.4	-0.2

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Conflict of interest:

All authors declare that they have no conflict of interest.

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