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Başlık: İlaç ürün imalat ekipmanının yüzeyindeki dipiridamol kalıntısının swab örneklemesi kullanılarak ve yüksek performanslı sıvı kromatografisi tekniği kullanılarak tahmin edilmesi için temizleme metodu validasyonu.

Title: Cleaning method validation for estimation of dipyridamole residue on surface of the drug product manufacturing equipment using the swab sampling and by using high performance liquid chromatographic technique.

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Özet

Amaç: Temizlik validasyonu, temizleme maddesinin, ilaç üretildikten sonra ekipman yüzeyinde etkin madde kalıntılarını uzaklaştırmak için uygulanan bir prosedürdür. Dipyridamolün modifiye salım yapan kapsülleri imal edildikten sonra ekipman yüzeyinden alınan örneklerde dipiridamolun kantitatif tahmini için basit, hassas, sağlam ve doğru bir HPLC yöntemi geliştirilmiştir.

Gereç ve Yöntem: Metot, Hypersil BDS C18 (150 mm x 4.6 mm, 5 µm) kolonu kullanılarak mobil faz içeren tampon (Potasyum dihidrojen fosfat tamponu, pH 7.0 ± 0.05) ve metanolün 30:70 (v/v) oranında kullanılmasıyla geliştirilmiştir. Akış hızı 1.5 mL/min, kolon sıcaklığı 45°C ve enjeksiyon hacmi 5 µL olarak elde edildi.

Sonuçlar: Yöntem onaylandı ve özgünlük çalışması, seyreltici çözeltinin ve swab seyrelticisinin tepe dipiridamol tutma süresi içinde mevcut olmadığını göstermek için yapıldı. Saptama limiti ve sınırlama limiti, lineerlik çözüm serileri kullanılarak belirlenmiş ve sırasıyla 0.041 µg/mL ve 0.124 µg/mL bulunmuştur. Niceleme seviyesi 8.6 % RSD, Yöntem hassasiyeti 0.2 % RSD ve sağlamlık çalışma sonuçlarında 0.3 % RSD bulundu. Yöntem 0.13 µg/mL ila 21.80 µg/mL konsantrasyonundan doğrudur,

geri kazanım sonuçları kabul kriterlerini karşılamaktadır. Yöntemin doğrusallığı 0.12 µg/mL ila 20.14 µg/mL arasında bulunmuştur ve bulunan (r2) değeri 0.997'dir. Akış hızı, dalga boyu, kolon sıcaklığı, tampon pH'sı ve mobil faz oranı değişimleri için sağlamlık çalışması yapıldı ve tüm sistem uygunluk parametreleri karşılandı. **Sonuç:** Yöntem validasyonu, yasal gereklilikler ve kurallara göre gerçekleştirilmiştir. Validasyon parametreleri kabul kriterlerini karşıladı ve önerilen swab rutin analizi için önerilen metot uygulanabilir.

Anahtar kelimeler:

Dipiridamol, swab, yöntem geliştirme, doğrulama ve temizleme.

Abstract

Objectives: The cleaning validation is the procedure use to ensure the cleaning process to eliminate the residues of the drug substance after drug product manufactured in the equipment surface. A simple, sensitive, robust, accurate HPLC method was developed for the quantitative estimation of dipyridamole in the swab samples from drug product manufacturing of the Dipyridamole modified release capsules equipment surface after manufacturing.

Materials and Methods: The method was developed by using Hypersil BDS C18 (150 mm x 4.6 mm, 5 µm) column with mobile phase containing mixture of buffer (Potassium dihydrogen phosphate buffer, pH 7.0 ± 0.05) and methanol in the ration of 30: 70 v/v, flow rate 1.5 mL/min, column temperature 45°C and injection volume as 5 µL.

Results: The method was validated, the specificity study was conducted to prove that, there was no interference from blank and swab blank at the retention time of dipyridamole. The limit of detection and limit of quantification limit was established by using the series of linearity solutions and found 0.041 µg/mL and 0.124 µg/mL respectively. The method precision at limit of quantification level 8.6 % RSD, Method precision 0.2 % RSD and ruggedness study results were found 0.3 % RSD. The method was accurate from the concentration of 0.13 µg/mL to 21.80 µg/mL, the recovery results were meeting the acceptance criteria. The linearity of the method was found from 0.12 µg/mL to 20.14 µg/mL and found (r2) value is 0.997. The robustness study for the flow rate, wave length, column temperature, buffer pH and mobile phase ratio variations were conducted, and all the system suitability parameters were meeting.

Conclusion: The method validation was performed as per the regulatory requirements and guidelines. The validation parameters were meeting the acceptance criteria and proposed method can be applied for the intended swab routine analysis.

Key words

Dipyridamole, swab, method development, validation and cleaning.

1. INTRODUCTION

The cleaning validation should be performed to confirm the efficiency of any cleaning procedure for the pharmaceutical product contact with the equipment. The cleaning validation in the pharmaceutical manufacturing industry is well known that, the manufacturing equipment and manufacturing area should be cleaned after every manufacturing process of the drug product and this process was strictly indorsed by regulatory authorities. The cleaning validation, is a perilous analytical responsibility of the quality management system in the pharmaceutical industry and this process is to ensure the cleaning procedure which effectively eliminate the residue from the manufacturing equipment and manufacturing area below a predetermined tolerable limit. The cleaning process is not only to ensure the product quality of the different products and this process will helpful tool to avoid the cross-contamination and this was also requirements of European union guidelines for Good Manufacturing Practice and United States Food and Drug Administration. The cleaning validation involves two different activities one is, development and validation of the cleaning process used to remove the drug from the manufacturing equipment surfaces and second one is, development and validation of the methods used to measure the residues on the surfaces of the manufacturing equipment's. The valuation of the sensitivity and specificity of the analytical method used to detect residue is critical. The residue analytical method should able to detect and quantify the drug substance at very lower level from the manufacturing equipment. The residue analytical procedure should be test in the mixture of sampling method used to show that residue can be recovered from the equipment surface with the specified levels in the accuracy study before

concluding the sampling procedure. In general, two types of sampling procedure were found acceptable by the regulatory and frequently practicing the pharmaceutical industries. The popular sampling method is the direct method of sampling on the surface of the manufacturing equipment and another method is to use of rinse solutions from the manufacturing equipment. The positives of direct sampling of the equipment surface areas hardest to clean and which are reasonably available can be projected, important to founding a level of residue per given surface area. In case of the rinse samples, the two benefits of using rinse samples are that a larger surface area may be sampled, and unreachable systems or ones that cannot be routinely disassembled can be sampled and estimated. The disadvantage of rinse samples is that the residue may not be soluble or may be physically occluded in the manufacturing equipment surface area.

The direct surface sampling, there is a possibility of getting the interference from the swab sticks as the swab sticks has some glue content and before finalizing the sampling procedure the specificity also should be evaluated. The selection of the extraction solvent is very important and critical step during the cleaning method development, the drug substance should be soluble and recoverable across the accuracy swab sample level and the results should be meet the acceptance criteria.

The drug product manufacture rationale for the residue limits established should be logical based on the drug product manufacturer's scientific knowledge of the materials involved. The important to describe the analytical method sensitivity of the residue method in order to fix the sensible acceptable limits. According to the USFDA, the limit should be based on logical criteria, involving the risk associated with residues of determined products. The calculation of an acceptable limit of residues and a maximum allowable carryover (MAC) for an active pharmaceutical ingredient (API) in the production equipment should be based on therapeutic doses, toxicity and a general limit (10 µg). Several mathematical formulas were proposed to establish the acceptable residual limit [1-17].

The drug substance Dipyridamole [Fig 1], chemical name: 2,2',2'',2'''-[[4,8-Di(piperidin-1-yl)pyrimido[5,4-d] pyrimidine-2,6-diyl]dinitrilo] tetraethanol, CAS Registry number: 58-32-2, molecular formula: $C_{24}H_{40}N_8O_4$, Molecular mass: 504.6, Appearance: Bright yellow, crystalline powder, Solubility: Practically insoluble in water, slightly soluble in acetone, soluble in anhydrous ethanol and It dissolves in dilute mineral acids.

The aim of this study is to develop the simple and fast analytical method for the, estimation of the dipyridamole content in the swab samples after manufacturing of the Dipyridamole modified release capsules in the surface of the manufacturing equipment and to meet the regulatory requirements. Hence, the developed method was subjected for the analytical validated with respect to specificity, linearity, precision, accuracy, robustness and ruggedness. The specificity studies were performed on the diluent, swab and placebo during the analytical method validation as per ICH guidelines [18]. The developed and validated method can be used for the routine swab samples analysis.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

The HPLC/Analytical grade water, Potassium dihydrogen phosphate, methanol, sodium hydroxide. The Dipyridamole drug substance and Dipyridamole working standard were supplied by Bluefish Pharmaceuticals Private Limited, India.

2.2. Equipment

The analytical method was developed and validated by using the HPLC from Agilent 1200 with VWD/PDA detector. The output signal was monitored and processed using specific software. Analytical balance from Mettler Toledo, Sartorius, pH meter and Refrigerator were used.

2.3. Chromatographic Conditions

The proposed method was developed by using Hypersil BDS C18 (150 mm x 4.6 mm) 5µm column with mobile phase containing mixture of mobile phase (Buffer: Potassium dihydrogen phosphate buffer, pH 7.0 ± 0.05) and methanol solution in the ration of 30:70 v/v. The flow rate was used as 1.5 mL/min with column temperature of 45°C, detection wavelength at 295 nm and the sample injection volume is 5 µL.

2.4. Preparation of Solutions

Diluent solution

Methanol was used as diluent.

Preparation of Dipyridamole Standard Solution

Weigh and transfer about 50 mg of dipyridamole working standard in to 50 mL volumetric flask. Add about 35 mL of diluent, sonicate for 2 to 3 minutes until material gets completely dissolved. Pipette out 1 mL of the above solution into a 100 mL volumetric flask, make up to volume with diluent and mix well. Pipette out 4 mL of the

above solution into a 10 mL volumetric flask, make up to volume with diluent and mix well.

2.5. Preparation of Test Tubes and Swabs

Take the clean and dry test tubes. Rinse the required number of swabs and test tubes with about 10 mL of swabbing solvent for two times. Squeeze out the swab against the side of the test tubes and discard swabbing solvent.

2.6. Preparation of Blank Solution

Transfer 10 mL of swabbing solvent to the above cleaned test tube. Place a cleaned swab into the test tube and sonicate for 10 minutes. Squeeze the swab and take it out and mix well.

2.7. Preparation of Test Solution

Transfer 10 mL of swabbing solvent to the above cleaned test tube. Place a cleaned swab into the test tube to wet the swab with swabbing solvent. Squeeze the swab by pressing it against wall of the test tube. Take the swabbing at the prescribed area of equipment. After swabbing, place the swab in the above test tube containing swabbing solvent and sonicate for 10 minutes.

Squeeze the swab by pressing it against the wall of the test tube and take it out and filtered through membrane filter and inject.

2.8. System Suitability Criteria

The present relative standard deviation of dipyridamole peak area for six replicate injections should not more than 5.0.

The tailing factor for dipyridamole peak in standard solution should be not more than 2.0.

The present relative standard deviation of dipyridamole peak retention time for six replicate injections should not more than 1.0.

The % recovery for dipyridamole check standard solution should be not less than 95.0 % and 105.0%.

3. RESULTS AND DISCUSSION

3.1 Method Development

During the method development stage, the standard solution was prepared with the known concentration, blank solution was scanned in UV spectrophotometer and collected the diluent blank [Fig 2] and dipyridamole working standard [Fig 3] spectrums to check the wave length maxima. The dipyridamole peak retention time was found about 2.8 minutes in the chromatogram, the relative standard deviation for the six

replicate injections were found 0.2 % and this was proven that, the method is reproducible. The accuracy study was conducted by spiking the know concentration of dipyridamole solution in the SS plate from about 0.4 µg/mL, 4 µg/mL and 6 µg/mL and the % recovery for all the levels, calculated the % recovery and results were found in the range of 99 % to 100% and this was proven that, the method is accurate. The linearity study was conducted by spiking the know concentration of dipyridamole solution from starting from about 0.4 µg/mL, 1 µg/mL, 2 µg/mL, 4 µg/mL, 5 µg/mL and 6 µg/mL, calculated the square of correlation coefficient, it was found 0.999 and this was proven that, the method is linear from the level 0.4 µg/mL to 6 µg/mL. Based on the above mentioned satisfactory results, the below mentioned chromatographic conditions were finalized for the quantitative estimation of dipyridamole in the swab samples from drug product manufacturing of the dipyridamole modified release capsules equipment surface after manufacturing. The chromatographic conditions are Hypersil BDS C18 (150 mm x 4.6 mm) 5µm column with mobile phase containing mixture of mobile phase (Buffer: Potassium dihydrogen phosphate buffer, pH 7.0 ± 0.05) and methanol in the ration of 30:70 v/v. The flow rate was 1.5 mL/min with column temperature of 45°C and detection wavelength at 295 nm. The injection volume was 5 µL with the isocratic flow. Hence, this method can be validated to introduced for the routine swab analysis.

3.1. Method Validation

The proposed analytical method for the quantitative estimation of dipyridamole in the swab samples from drug product manufacturing of the dipyridamole modified release capsules equipment surface area after manufacturing was validated as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [18]. The following validation characteristics specificity, precision, accuracy, linearity, range, ruggedness and robustness were conducted.

3.1.1. System Suitability

To check the system suitability criteria, the solutions were prepared and injected as per the test method of analysis. All the system suitability parameters were found well within the acceptance criteria. The summary of the system suitability was tabulated in the [Table 1].

3.1.2. Specificity

To study the specificity, the required solutions like diluent as blank, swab blank and standard solution were prepared and injected as per test method. It was observed that no peak interference at the retention time of dipyridamole from blank and swab blank solutions in the chromatogram. Find the specimen chromatograms of diluent as blank [Fig 4], swab blank [Fig 5], standard [Fig 6] and overlaid chromatograms of (diluent as blank, swab blank, standard) [Fig 7].

3.1.3. Estimation of Limit of Detection (LOD) and Limit of Quantification (LOQ)

To evaluate the concentration limits as limit of detection (LOD) and limit of quantification (LOQ), the series of linearity solutions were prepared ranging concentration starting from about 0.1 µg/mL to 2.0 µg/mL and calculated the square of correlation coefficient, slope of the curve and y-intercept. The limit of quantification was calculated based on the standard deviation of the response and the slope as mentioned in the below formula. The summary results of the LOD and LOQ estimation study were tabulated in the [Table 2] and find the graph in [Fig 8].

$$LOQ = \frac{10 \times \sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

3.1.4. Method Precision at LOQ Level

The method precision at LOQ concentration was performed by preparing the six replicate test preparations (n=6) of dipyridamole stock solution and samples were analysed as per test method. The % RSD for response of dipyridamole six replicate injections were calculated and found within the acceptance criteria. The summary of the method precision at LOQ level study results find the graph in [Fig 9].

3.1.5. Method Precision (Repeatability)

The method precision was performed by preparing the six replicate test preparations (n=6) of dipyridamole stock solution spiked on to the SS plate (4 x 4 inch) and samples were analysed as per test method. The % recovery for replicate injections and % RSD for response of dipyridamole six replicate injections were calculated and found within

the acceptance criteria. The summary of the method precision study results finds the graph in [Fig 10].

3.1.6. Accuracy

In the accuracy study, a series of sample solutions were prepared in triplicate (six preparations for lower level (LOQ) and higher level (500%) by spiking the dipyridamole drug substance stock on SS plate (4 x 4) at LOQ, 50 %, 100 %, 200 %, 300 % and 500 %.and analysed as per the test method. The spiked concentrations of dipyridamole are 0.12 µg/mL, 2.01 µg/mL, 4.03 µg/mL, 8.05 µg/mL, 12.08 µg/mL and 21.14 µg/mL. Individual % recovery, mean % recovery, % RSD and squared correlation coefficient for linearity of the test method were calculated and the results were found within the pre-defined acceptance criteria. The summary of the accuracy study results was tabulated in the [Table 3] and find the graph in [Fig 11].

3.1.7. Linearity

The linearity was studied by analysing the standard solutions. A series of solutions of dipyridamole standard solutions were prepared in the range of LOQ to about 500% and injected into the HPLC system. Linearity of detector response was established by plotting a graph between concentration Vs response of dipyridamole. The detector response was found to be linear from about LOQ to 500% and injected into HPLC system and analysed as per the test method. The concentrations of dipyridamole 0.1208 µg/mL, 2.0137 µg/mL, 4.0274 µg/mL, 8.0548 µg/mL, 12.0821 µg/mL and 20.1369 µg/mL.

The square of correlation coefficient, slope, and % y-intercept at 100% level, intercept and residual sum of squares were calculated and the results were found within the acceptance criteria. The summary of the linearity study results was tabulated in the [Table 4] and find the graph in [Fig 12].

3.1.8. Ruggedness

The intermediate precision was performed by preparing the six replicate test preparations (n=6) of dipyridamole stock solution spiked on to the SS plate (4 x 4 inch) and samples were analysed as per test method by using different HPLC system, different column of same make by different analyst on different day. The % recovery for replicate injections and % RSD for response of dipyridamole six replicate injections were calculated and found within the acceptance criteria. The summary of the ruggedness study results was tabulated in the [Table 5] graph in [Fig 13].

3.1.9. Solution Stability and Mobile Phase Stability

The solution stability of dipyridamole by keeping swab sample solution and standard solutions in tightly capped volumetric flasks at room temperature for 1day, 2day and measured against freshly prepared standard solution. The standard solution and swab sample solutions was found stable for 2 days at room temperature.

The stability of mobile phase was also determined by freshly prepared solutions of dipyridamole at 1day and 2 days. The mobile phase was found stable for 2 days at room temperature.

3.1.10. Robustness

Robustness of the proposed method was performed by keeping the chromatographic conditions constant with the following deliberate variations.

i) Change in flow rate
ii) Change in wave length
iii) Change in mobile phase buffer pH
iv) Change in HPLC column temperature
v) Change in mobile phase composition

The standard solution was injected in replicate for each above mentioned change. The system suitability parameters were recorded for dipyridamole peak and the system suitability found well within the acceptance criteria. The summary of the robustness study results was tabulated in the find the results in the [Table 6].

4. APPLICATION OF THE PROPOSED METHOD

The proposed developed analytical method can be applied for the analysis of swab samples from the dipyridamole drug product manufacturing unit. All the analytical validation parameters were meeting the pre-defined acceptance criteria and method was proven to be suitable for analysis the swab samples from drug product manufacturing of the dipyridamole modified release capsules equipment surface area after manufacturing.

5. CONCLUSIONS

The developed method was validated as per the ICH guidelines and can be used for the quantitative estimation of dipyridamole in the swab samples from drug product manufacturing of the dipyridamole modified release capsules equipment surface area after manufacturing The method was found precise, accurate, linear, robust, rugged, specific and there was no interference found during the specificity study at the retention time of dipyridamole peak. The validated method can be applied for the swab

samples from drug product manufacturing of the dipyridamole modified release capsules equipment surface area after manufacturing.

6. ACKNOWLEDGMENT

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TABLES

Table 1: System suitability criteria and results

Parameter	Acceptance criteria	Result
The present relative standard deviation of dipyridamole peak area for six replicate injections	Not more than 5.0	2.0 %
The tailing factor for dipyridamole peak in standard solution.	Not more than 2.0	1.2
The present relative standard deviation of dipyridamole peak retention time for six replicate injections.	Not more than 1.0	0.2 %
The % recovery for dipyridamole check standard solution.	Not less than 95.0 and not more than 105.0	96.7 %

Table 2: Estimation of limit of detection (LOD) and limit of quantification (LOQ)

Description	Dipyridamole
Square of correlation coefficient (r2)	0.999
Slope	11073.25
Y-Intercept	50.4388
Limit of Detection (µg/mL)	0.041
Limit of Quantification (µg/mL)	0.124

Table 3: Accuracy data of dipyridamole

Spike Level	% Mean recovery of dipyridamole	Average amount added (µg/mL)	Average amount found (µg/mL)
Level-1	101.5	0.12	0.13
Level-2	97.5	2.01	1.97
Level-3	98.7	4.03	3.98

Level-4	99.7	8.05	8.03
Level-5	105.1	12.08	12.70
Level-6	103.1	21.14	21.80

Table 4: Linearity data of Dipyridamole

Linearity Level	**% Linearity**	**Concentration (µg/mL)**	**Area Response**
Level-1	LOQ	0.1208	1514
Level-2	50 %	2.0137	24558
Level-3	100 %	4.0274	49035
Level-4	200 %	8.0548	97506
Level-5	300 %	12.0821	152086
Level-6	500 %	20.1369	231128
Square of correlation coefficient (r2)			0.997
Slope			11622.7
Y-Intercept			2686.68
Residual sum of squares			122556978.290215

Table 5: Ruggedness data

Sample No.	**Method Precision % Recovery**	**Intermediate Precision % Recovery**
1	99.0	102.6
2	98.7	103.0
3	98.6	102.5
4	98.9	102.3
5	99.2	102.8
6	98.7	102.3
Mean	**98.9**	**102.6**
% RSD	**0.2**	**0.3**
Over all % RSD	**0.3**	

Table 6: Robustness data

Parameter Variation	The present relative standard deviation of dipyridamole peak area for six replicate injections should not more than 5.0.	The tailing factor for dipyridamole peak in standard solution should be not more than 2.0.	The present relative standard deviation of dipyridamole peak retention time for six replicate injections should not more than 1.0.	The % recovery for dipyridamole check standard solution should be not less than 95.0 % and 105.0%.
Flow 1.30 mL/min	0.3	1.2	0.1	98.0
Flow 1.70 mL/min	0.2	1.2	0.2	98.0
Wavelength 293 nm	0.2	1.2	0.2	97.4
Wavelength 297 nm	0.3	1.2	0.2	97.1
Column Temp-40°C	0.2	1.2	0.0	99.5
Column Temp-50°C	0.2	1.2	0.0	99.4
Buffer pH 6.8	0.3	1.2	0.0	97.1
Buffer pH 7.2	0.7	1.2	0.3	96.5
Mobile phase ratio 35:65 % v/v	0.2	1.2	0.0	99.9
Mobile phase ratio 20:80 % v/v	0.2	1.2	0.0	99.7

FIGURES

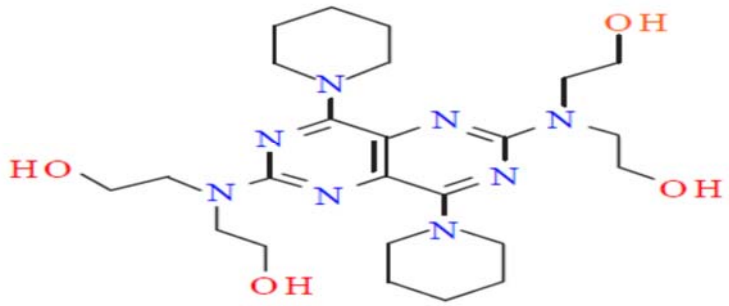

Fig 1 Structure of dipyridamole

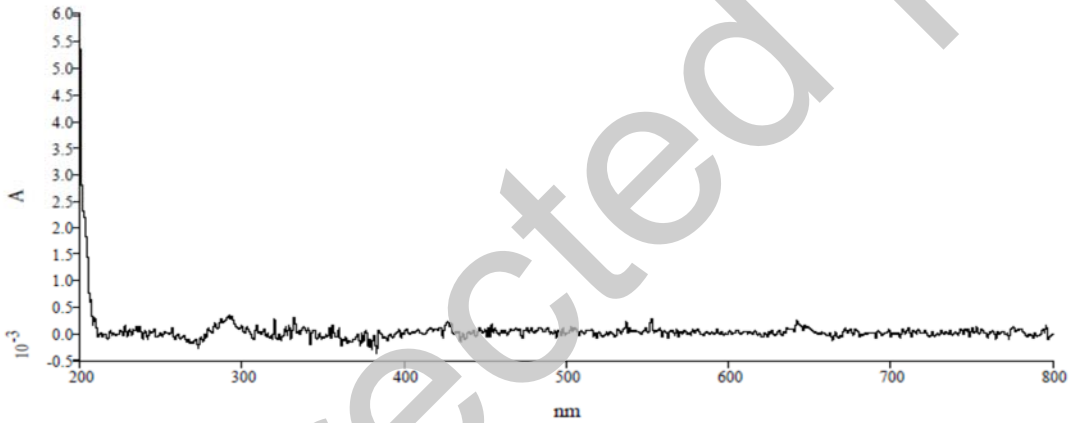

Fig 2 Spectrum of diluent blank

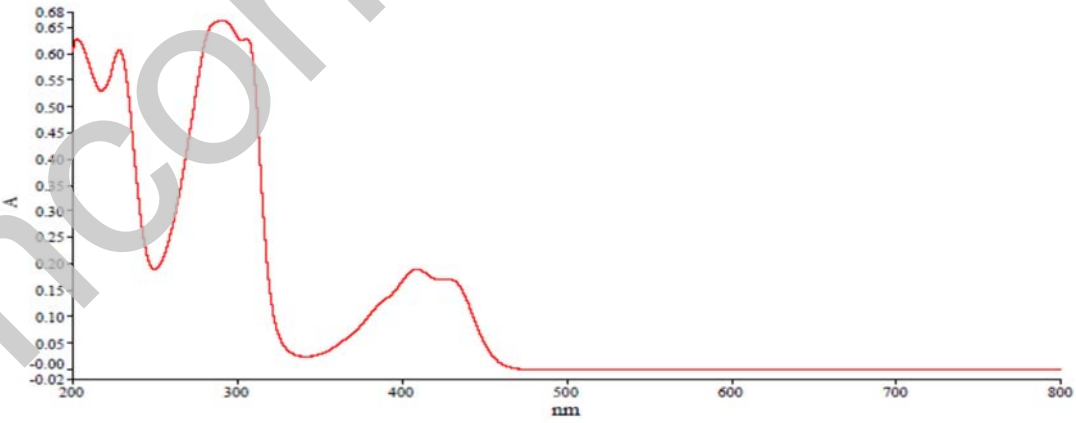

Fig 3 Spectrum of standard

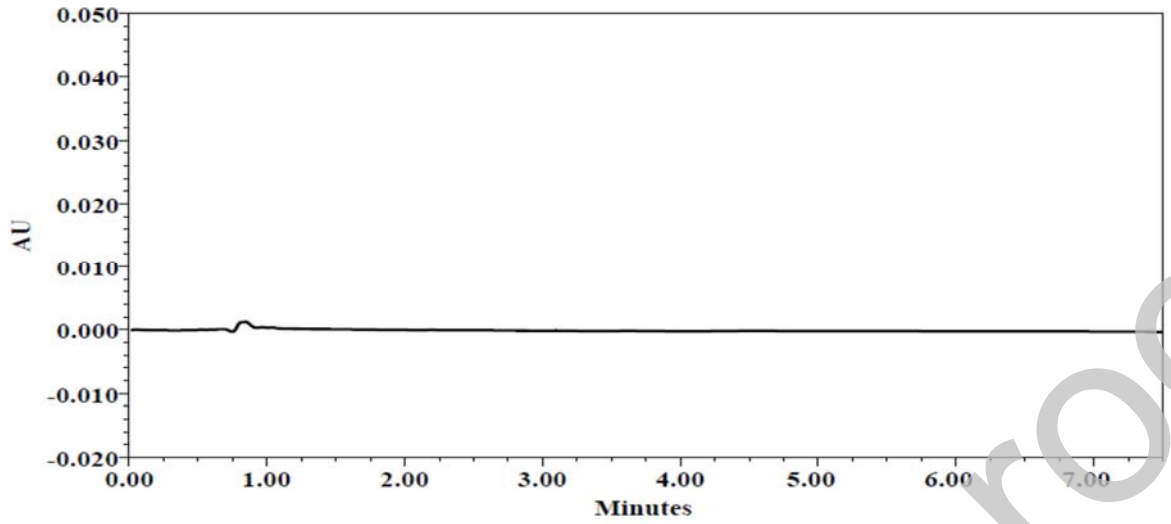

Fig 4 Specimen diluent blank chromatograms

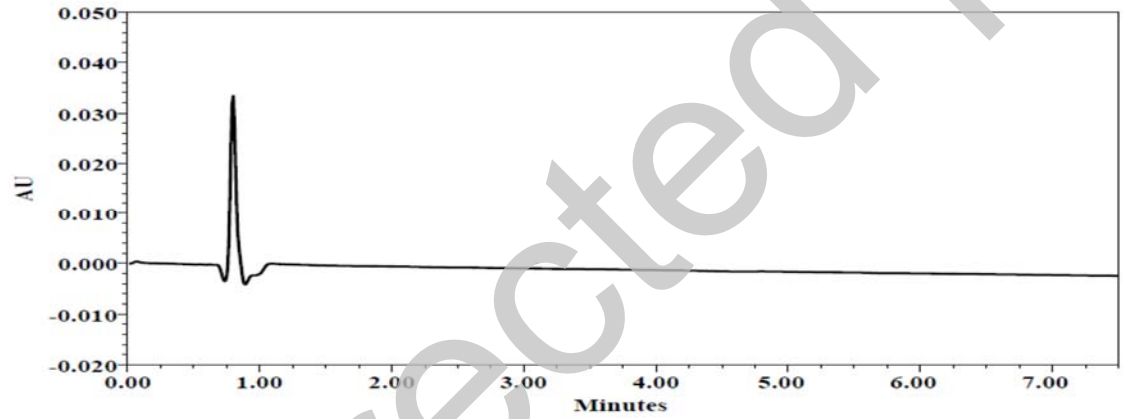

Fig 5 Specimen swab blank chromatograms

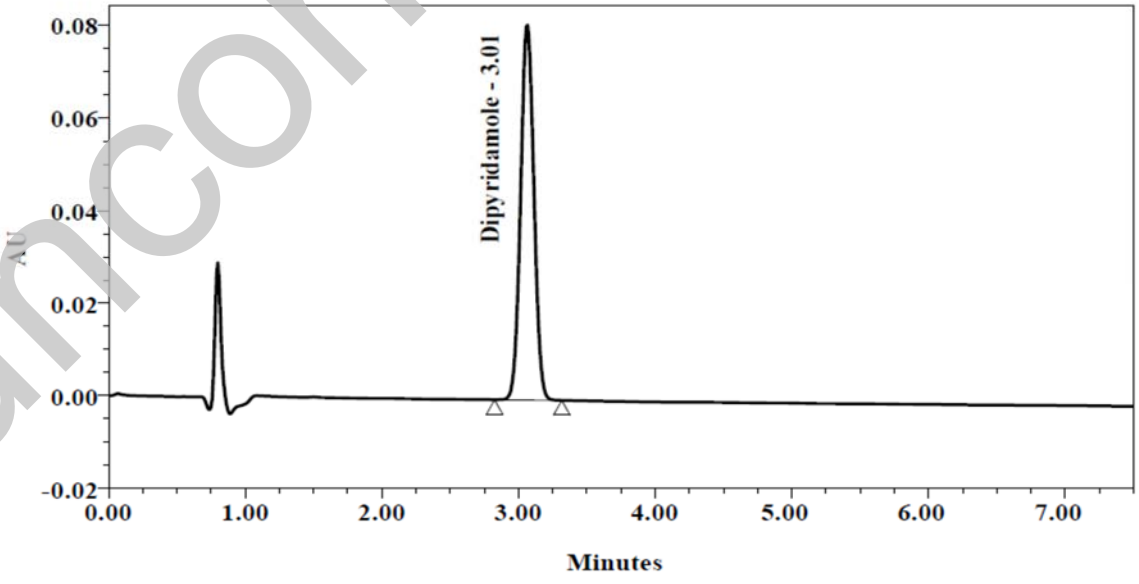

Fig 6 Specimen standard chromatograms

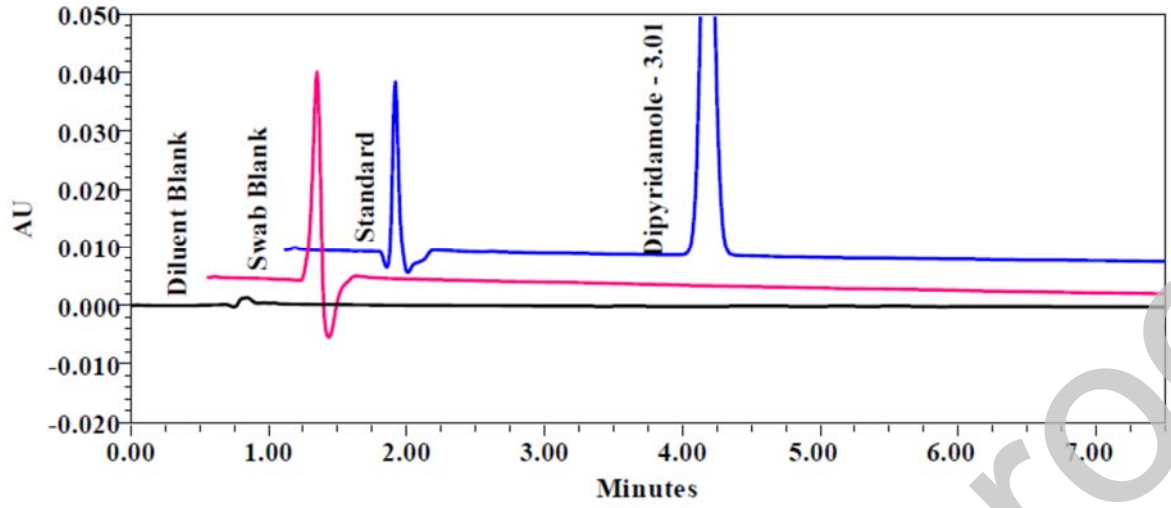

Fig 7 Specimen overlaid chromatograms

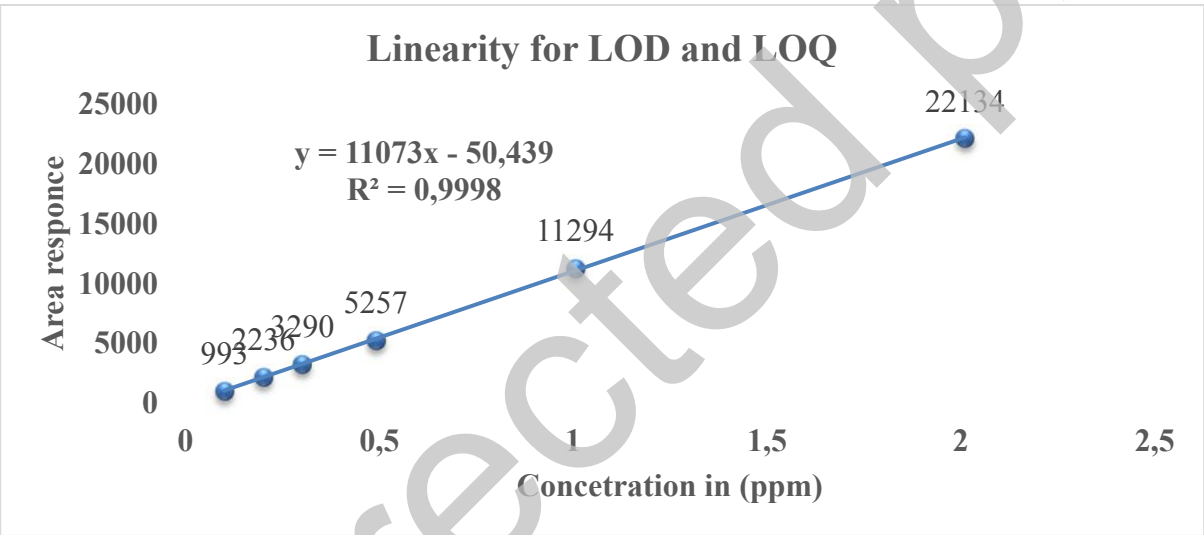

Fig 8 Linearity for LOD and LOQ

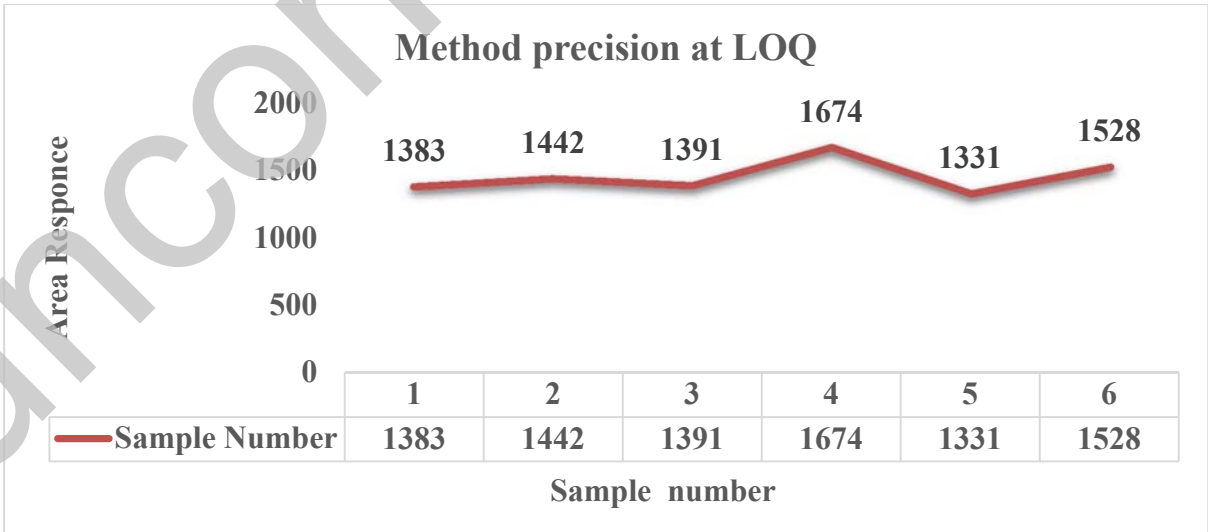

Fig 9 Method precision at LOQ level

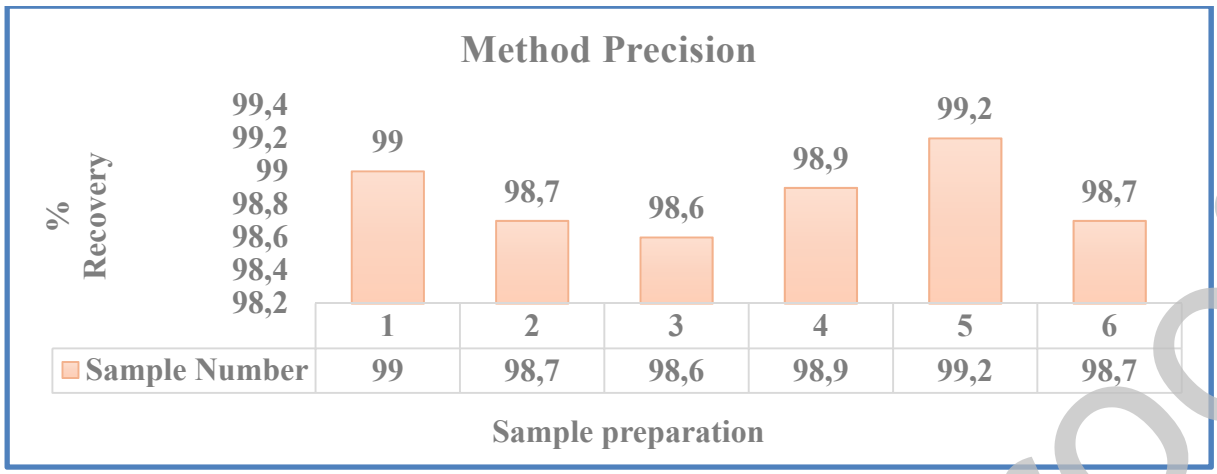

Fig 10 Method precision

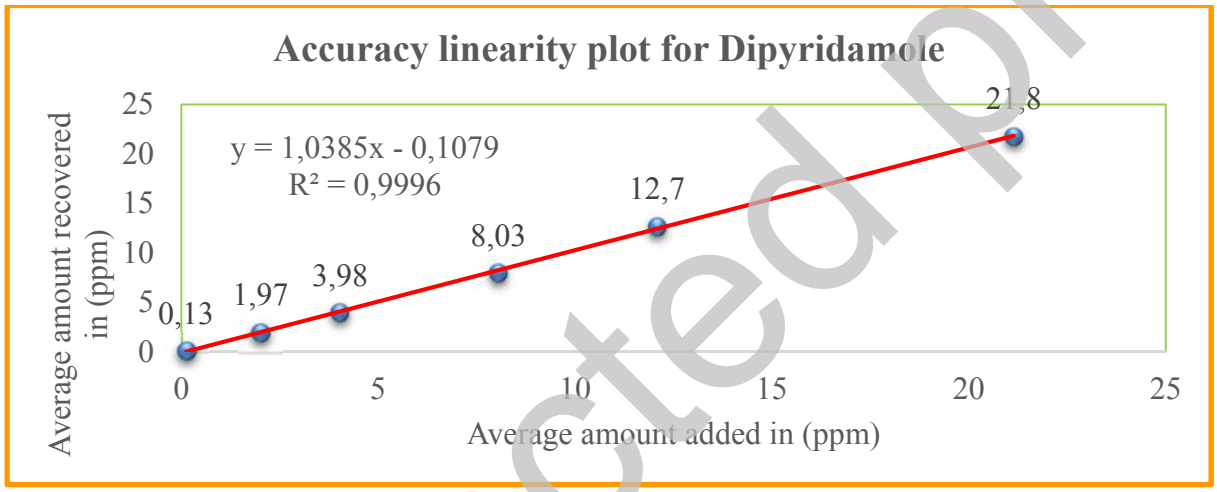

Fig 11 Accuracy linearity plot for dipyridamole

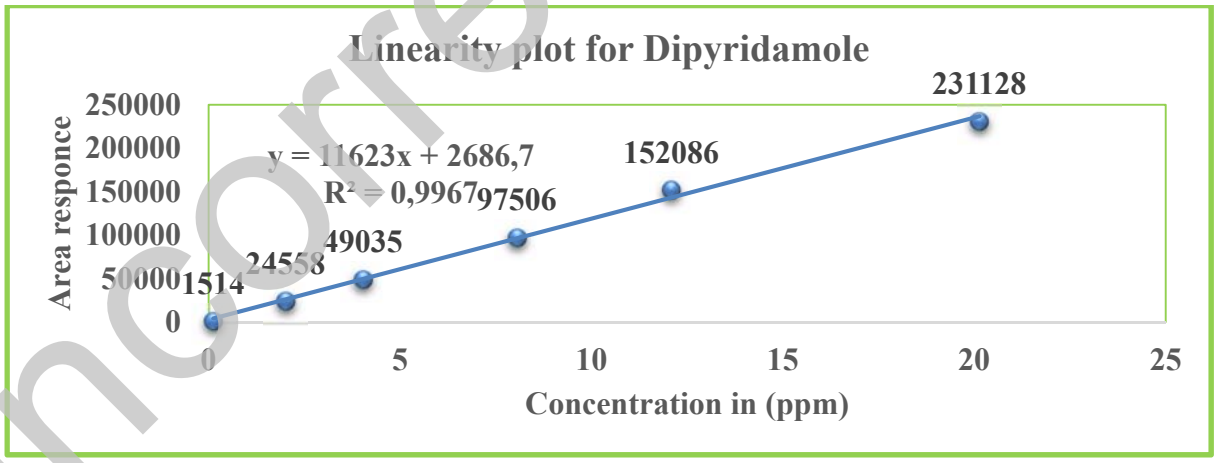

Fig 12 Linearity plot for dipyridamole

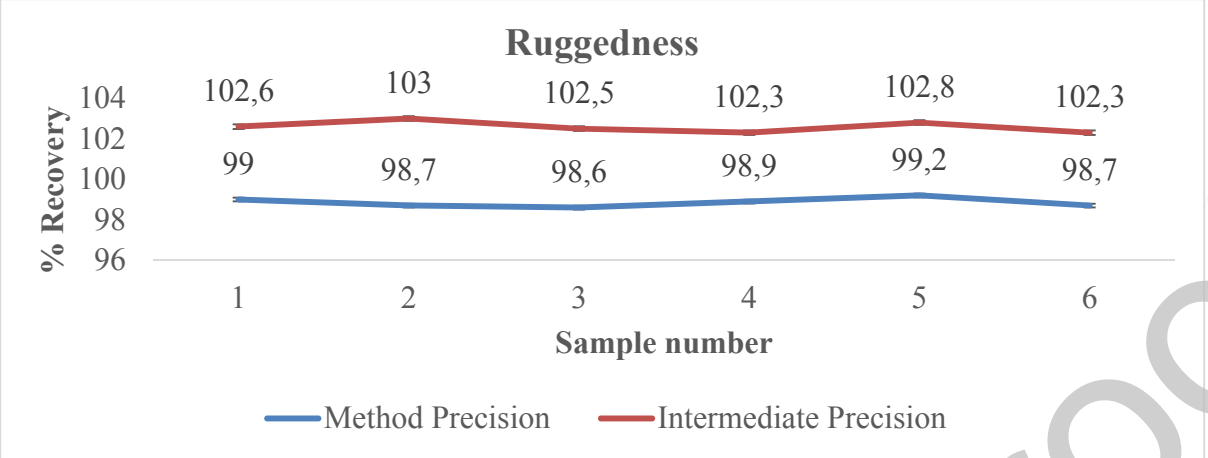

Fig 13 Ruggedness