IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ANALYTICAL METHOD DEVELOPMENT OF DIACERINE AND BULK FORMULATION

Roshani Uttamrao Suryavanshi [1], Research Scholar

Bhagwant University, Ajmer, Rajasthan, 305004, India.

Rajasekaran S.[2]

Department of Pharmacology, Bhagwant University, Ajmer, Rajasthan, 305004, India.

ABSTRACT:

The drug analysis is playing an important role in the development of drugs, their manufacture and therapeutic use. For the simultaneous estimation of drugs present in dosage forms, lot of suitable methods are adopted like uv –spectrophotometer, HPLC, HPTLC etc These methods are powerful and rugged method. They are also extremely precise, specific, accurate, linear and rapid. A pharmaceutical industry depends upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specification. The drugs will occur as a single component or multi component dosage forms. The later proves to be effective due to its combined mode of action on the body.

KEYWORDS: HPLC, HPTLC, spectrophotometer, Glucosamine Diacerein, Ramipril, Analytical method.

AIM AND OBJECTIVE

The number of drugs or drug formulations introduced into the market is increasing at a fast rate. These may be either new entries in the market or structural modification of the existing drugs or novel dosage forms or multi component dosage forms. The complexity in the dosage forms, including that of the multi component dosage forms creates considerable challenges to the analytical chemist during the development of assay procedure for its accurate estimation. The estimation of individual drugs in these multi component dosage forms becomes difficult due to tedious extraction or isolation procedure. The combination of Glucosamine Diacerein and Methyl sulfonyl methane, combination of Ramipril and hydrochlorothiazide was selected for the present study.

OBJECTIVES:

- Development of UV and RP-HPLC Spectrophotometric method
- Validation of all the developed methods using marketed formulation.

UV method development was obtained as

- Optimized UV conditions
  - Isobestic point of Glucosamine, Diacerein and Methyl sulfonyl methane
  - Chromatographic conditions

RP-HPLC method development was obtained as

- Selection and optimization of mobile phase and stationary phase.
- Selection of detector wavelength.
- Selection of extraction procedure.
- Optimization of chromatographic condition.
- Estimation of Glucosamine Diacerein and Methyl sulfonyl methane
- Estimation of Ramipril and hydrochlorothiazide
- Method validation.

1. MATERIALS AND METHODS

5.1 Drugs and Chemicals

- ✓ Diacerein and ramipril, hydrochlorthiazide standards were obtained as gift sample from Spectrum Pharma Research Solution, Hyderabad, Telangana.
- ✓ Tablet formulation (8mg of Diacerein, 200mg Ramcor H 5 of ramipril 5 mg and hydrochlor thiazide 12.5mg, Cipla) was purchased from local market.
- ✓ HPLC grade Acetonitrile, HPLC grade Water were purchased from Rankem. Mumbai, India.
- ✓ Potassium dihydrogen phosphate AR grade and Acetic acid was obtained from RANKEM, Mumbai, India.
- ✓ Orthophosphoric acid AR, Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine Chemicals, Hyderabad and all solvents used in this work are HPLC grade.

5.2 Instruments

Table 8: list of Instrument

Sl. No.	Instruments/Equipments/Apparatus
1.	SHIMADZU with class-10vp Software with Isocratic Gradient with UV-Visible Detector (SPD-IOA), PUMP (LC-IOAT) and (LC-IOATvp).
2.	UV-Visible Spectrophotometer (ELICO SL-159).
3.	Electronic Balance (AFCOSET)
4.	Ultra Sonicator(ENERTECH)
5.	Thermal Oven
6.	Hypersil Pack ODS (C_{18}) RP Column, 250 mm x 4.6 mm.
7.	P^H Analyzer (ELICO)
8.	Triple Quartz Distillation Unit (BOROSIL)
9.	HPLC Injecting Syringe (25 µl) (HAMILTON)

3 METHOD DEVELOPMENT OF GLUCOSAMINE, DIACEREIN AND METHYL SULFONYL METHANE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS.

5.3.1 Preparation of Standard and Sample Solutions

5.3.1 Preparation of Mobile Phase

The mobile phase was prepared by mixing 650 ml 0.1% KH_2PO_4 buffer and 350 ml of Acetonitrile. The mobile phase was degassed for 10 min by sonicating the solution before use.

Preparation of Diacerein and Standard Solution

Accurately Weighed and transferred 5mg of Diacerein and 25 mg working Standards into a 50ml clean dry volumetric flask, add $3/4^{th}$ volume of diluent, sonicated for 15 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipetted out in to a 10ml volumetric flask and then made up to the final volume with diluents. The concentrations of the Diacerein are 150µg/ml solution.

Preparation of sample solutions for method validation

10 tablets are weighed, powdered (equivalent to 75mg of 5mg of Diacerein and was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent. The concentrations of the Diacerein a 150µg/ml solution.

Preparation of sample solutions for method validation

5.4.1 Accuracy

Accurately weighed and transferred 5mg of Diacerein and 25mg of into 50 mL clean and dry volumetric flask, add 15ml of diluents (Water:ACN 50:50), then sonicated for 20min and make up the volume with diluent. From the above solution, 50%, 100% and 150% of analyte concentrations were prepared and 10µl of the solution was injected into HPLC system.

5.4.2 Linearity

Six different concentrations of Diacerein (2.5-15 µg/ml) was prepared separately and injected into HPLC system.

5.4.3 Precision

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

5.4.4 Intraday Precision

To study the intraday precision, a known concentration of six replicates of Diacerein (10µg/ml) standard solution was injected on the same and peak area was recorded.

Robustness

To validate the robustness of the developed method, a known concentration of freshly prepared Diacerein standard solution were injected and record the peak tailing, USP plate count and peak area.

Preparation of sample solutions for forced degradation studies

Preparation of Acid induced degradation product

In acid hydrolytic degradation, to 1 ml of stock ssolution Diacerein. 1 ml of 2 N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 150µg/ml,10 µg/ml, 50µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Preparation of Base induced degradation product

In acid hydrolytic degradation, to 1 ml of stock solution Diacerein and 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 150µg/ml, was injected into the system and the chromatograms were recorded to assess the stability of sample.

Preparation of Peroxide induced degradation product

In peroxide degradation, 1 ml of 20% hydrogen peroxide (H_2O_2) was added to 1 ml of stock solution of Diacerein and separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 150µg/ml was injected into the system and the chromatograms were recorded to assess the stability of sample.

Preparation of Dry heat degradation product

In thermal degradation studies, the standard drug solution was placed in oven at 105^0c for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Preparation of Photolytic degradation product

The photochemical stability of the drug was also studied by exposing the 1500µg/ml, 100 µg/ml, 500 µg/ml solutions to UV Light by keeping the beaker in UV Chamber for 1hrs or 200-Watt hours/m^2 in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 150µg/ml, 10 µg/ml, 50 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Preparation of Neutral Degradation product

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 150µg/ml, 10 µg/ml, 50 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

NEW UV METHOD FOR SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND RAMIPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

A basic UV spectrophotometric method was created for the assurance of Hydrochlorothiazide and Ramipril in pure and its formulations. Hydrochlorothiazide shows max absorbance at 212nm and Ramipril at 226nm in methanol separately and obeyed linearity in the concentration range of 1.25 -40µg/ml and 0.5-16 µg/ml.The proposed strategy was statistically validated.

Experimental

Instrumentation:

Otherworldly and absorbance estimations were made on an electron corporation double beam uv-vis spectrophotometerby by utilizing 1 cm quartz cells. Shimanzu electronic balance was utilized for gauging the samples. Commercially accessible tablets of Hydrochlorothiazide and Ramiprilwere procured from the neighborhood market and assessed.

Reagent used: Methanol (Merck).

Procedure:

Preparation of Stock Solutions:

Standard stock solution was set up by dissolving 25 mg of each medication in 25 ml of methanol to get convergence of 1mg/ml (1000 µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph:

The readied stock solution was additionally diluted with methanol to get working standard solutions of 10 µg/ml and 100 µg/ml of Ramipril and Hydrochlorothiazide. To build Beer's law plot for pure drug, diverse aliquots Hydrochlorothiazide (1.25-40µg/ml) and Ramipril (0.5-16µg/ml) (1:2.5) were taken and weakened to 10 ml with methanol. The absorbance was estimated most extreme at 212 and 226nm. against methanol as blank. The outcomes were appeared in table. The standard chart was plotted by taking grouping of concentration on x-side and absorbance on y-side and was appeared in Fig. the medication has complied with Beer's law in the concentration range of 1.25-40µg/ml (for Hydrochlorothiazide) and 0.5-16 µg/ml (for Ramipril).

NEW RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND RAMIPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Experimental:

Instrumentation:

Quantitative HPLC was performed on Shimadzu HPLC with LC 10AT VP series pumps besides SPD 10AVP UV-Visible detector. Shimadzu Class-VP version 6.12 SPI software is used along with C-18 (250 x 4.6 mm, packed with 5 µ) column for the separation.

Reagents used:

1. Methanol HPLC grade (MERCK).
2. Acetonitrile HPLC Grade.
3. Tetra Butyl Ammonium Hydrogen Sulphate. (T.B.H.S).

Preparation of mobile phase

Methanol HPLC grade, Tetra Butyl Ammonium Hydrogen Sulfate. (T.B.H.S) and Acetonitrile HPLC grade and were appropriately blended in the proportion of 35:35:30.

Preparation of standard drug solutions:

10 mg of Hydrochlorothiazide and 250mg Ramipril pure drug were gauged and broken down in 3.5ml of Methanol, 3.5ml of T.B.H.S and 3.0ml of Acetonitrile from these further dilutions were set up in concentration of 50ng to200µg/ml.

Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets containing Hydrochlorothiazide and Ramipril of marketed formulation were taken and a part of powder comparable to 10mg of the dynamic fixing was precisely weighed into a 10ml volumetric cup and versatile stage was added to make upto volume. The volumetric flasks were sonicated for 20minutes to impact total disintegration of medication and the arrangement was made up to volume with mobile phase. Aliquots arrangement were sifted through whatman channel comprised of cellulose nitrate (0.45micron) and 0.1, 0.2ml of the v was moved to a 10ml volumetric flasks and made up to volume with mobile phaseto yield concentrations of medication in range of linearity previously portrayed.

6 Procedure for calibration curve:

The substance of the mobile phase was filtered before use through Whatman channel paper comprised of cellulose nitrate (0.45micron), and pumped from the particular dissolvable reservoirs to the segment at a predefined flow rate. Before infusion of the drug solutions, the column was equilibrated for in any event 30 min with the mobile phase moving through the systems. At that point, 20µl of every one of standard and sample solutions were infused into the HPLC system to get the chromatograms. The retention time, peak areas of medication were recorded. Taking conc. plotted a chart on X-hub and region under bend on Y-hub. The linearity range was discovered to be in the between1.251-250µg/ml for Hydrochlorothiazide and 0.52-100µg/ml for Ramipril respectively.

RESULT AND DISCUSSION

Method development of glucosamine, diacerein and methyl sulfonyl methane in bulk and its pharmaceutical dosage forms.

Optimized UV conditions

UV – visible spectra from 400-200nm for Diacerein and standard solutions were taken. Diacerein shows λmax 285nm. By observing the UV spectra of standard solutions, a common wavelength was observed at 285nm and it was taken as isobestic point for trials to develop HPLC method.

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

6.1.3 Trial 1:

Chromatographic conditions:

Mobile phase	**:**	0.1% OPA:Acetonitrile(50:50)
Flow rate	**:**	1 ml/min
Column	**:**	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	**:**	285.0 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	10 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50.
Results	:	In this trial all peaks were eluted but peaks shapes are not good and less USP plate count so, further trial is carried out.

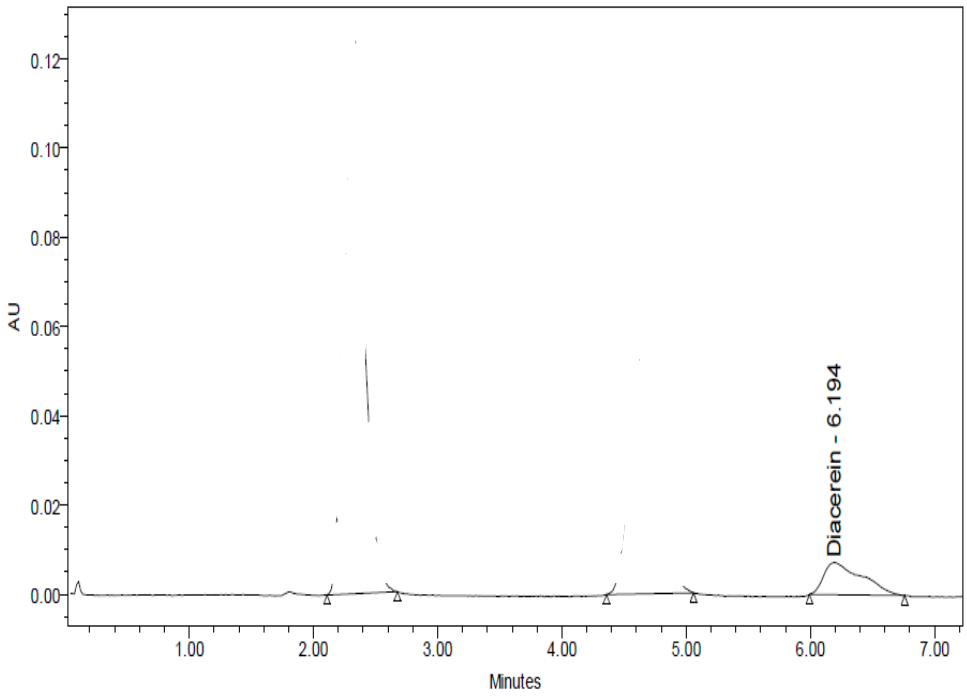
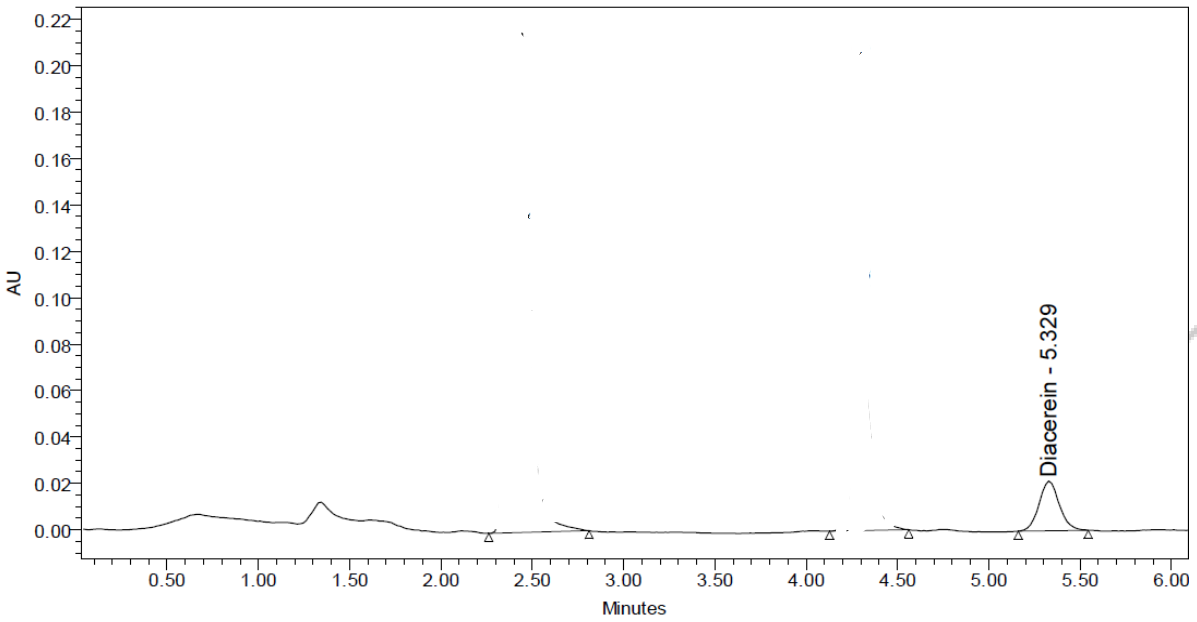


Fig 1: Trial chromatogram 1

Trial 2:

Chromatographic conditions:

Mobile phase	**:**	0.1% OPA: Acetonitrile (55:45)
Flow rate	**:**	1 ml/min
Column	**:**	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	**:**	285.0 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	6 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	All peaks were eluted but base line is not good and retention time is more so, further trials are conducted**.**



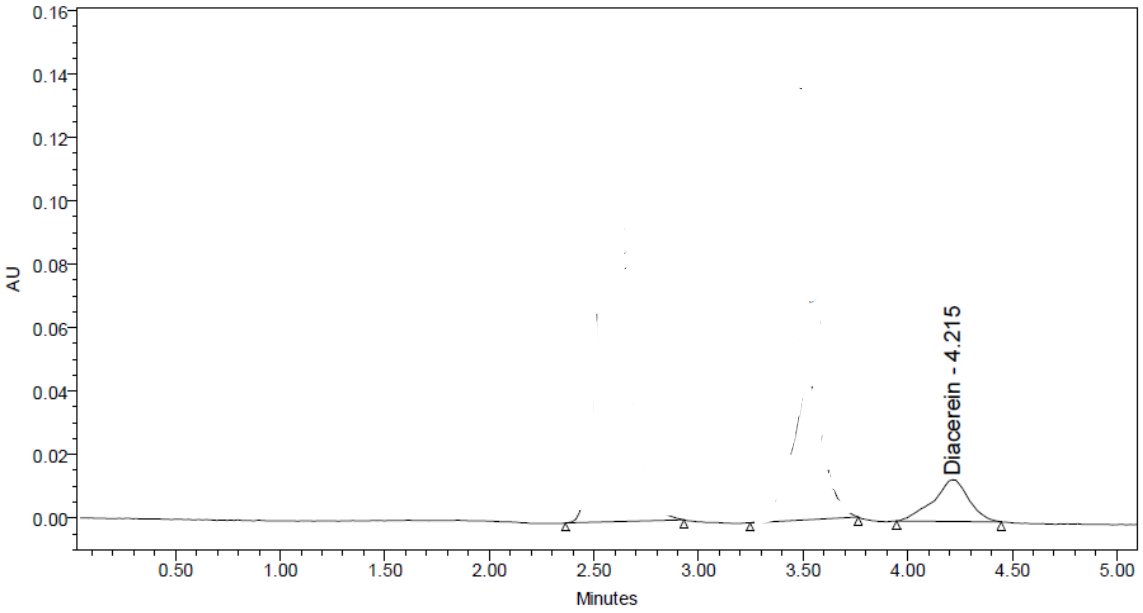
Trial chromatogram 2

Trial 3:

Chromatographic conditions:

Mobile phase	:	55% 0.1% OPA: 45% Acetonitrile (55:45)
Flow rate	**:**	1 ml/min
Column	**:**	Altima C18 (4.6 x 150mm, 5µm)
Detector wave length	**:**	285.0 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	10 min
Diluent	**:**	Water and Acetonitrile in the ratio 50:50
Results	**:**	In this trial also all peaks are eluted but diacerien peak

shape was not good so, further trials are carried out.



Trial chromatogram 3

Optimized method:

Chromatographic conditions:

Mobile phase : 65% 0.1N KH_2PO_4: 35% Acetonitrile

Flow rate : 1 ml/min

Column : Altima C18 (4.6 x 150mm, 5µm)

Detector wave length : 285.0 nm

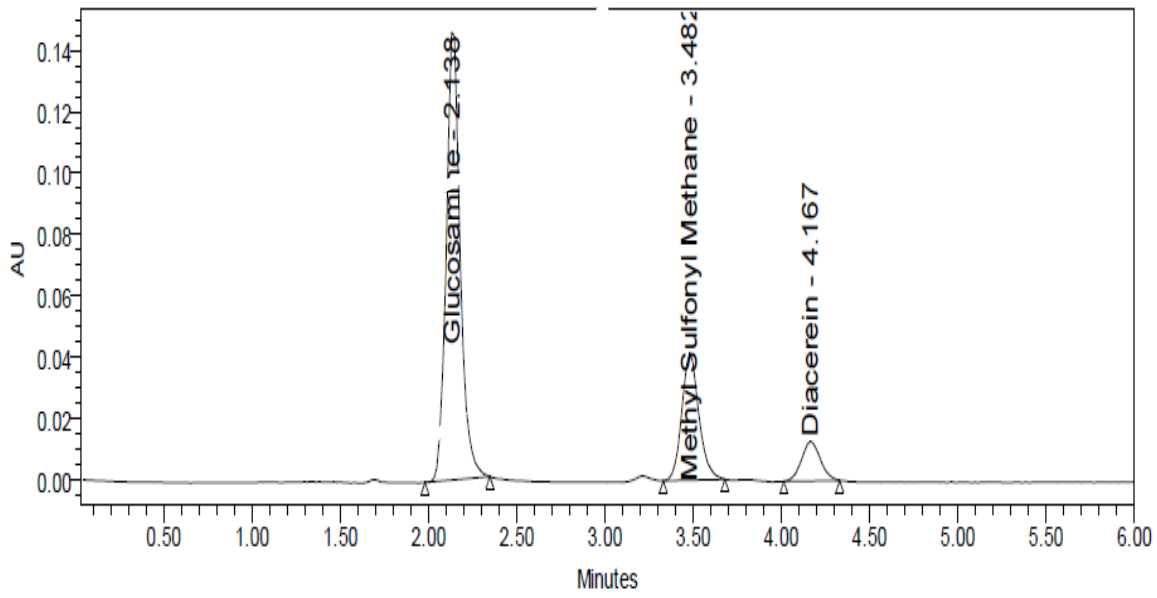
Column temperature : 30°C

Injection volume : 10µL

Run time : 8 min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : Both peaks have good resolution, tailing factor, theoretical plate count and resolution.



Optimized Chromatogram

Observation: Diacerein were eluted 4.167 respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Method Validation

The objective of validation of analytical method is to verify the characteristics of the proposed method suitability for its intended purpose. After developing a suitable method, it was validated for the following typical parameters such as accuracy, linearity, specificity, ruggedness, precision, robustness, LOD and LOQ according to ICH guidelines

Specificity

The specificity of an analytical method is ability to measure the analytes accurately and specifically in the presence of unexpected components in sample matrix. It was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The specificity of the method for the drug was also established by checking for interference with drug quantification from degradation products formed during the forced degradation study. The peak purity spectra of the Diacerein and was found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak.

Specificity

The specificity of an analytical method is ability to measure the analytes accurately and specifically in the presence of unexpected components in sample matrix. It was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The specificity of the method for the drug was also established by checking for interference with drug quantification from degradation products formed during the forced degradation study. The peak purity spectra of the Diacerein and was found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak.

System suitability

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of freshly prepared standard solution containing 150µg/ml Diacerein was injected. Parameters that were studied to evaluate the suitability of the system are retention time, peak area, number of theoretical plates and USP Peak tailing. The values of the system suitability parameters were given in table

System suitable parameters Diacerein

PARAMETERS	Diacerein
Retention Time (min)	4.167
USP Plate count	6500
USP Tailing	1.15

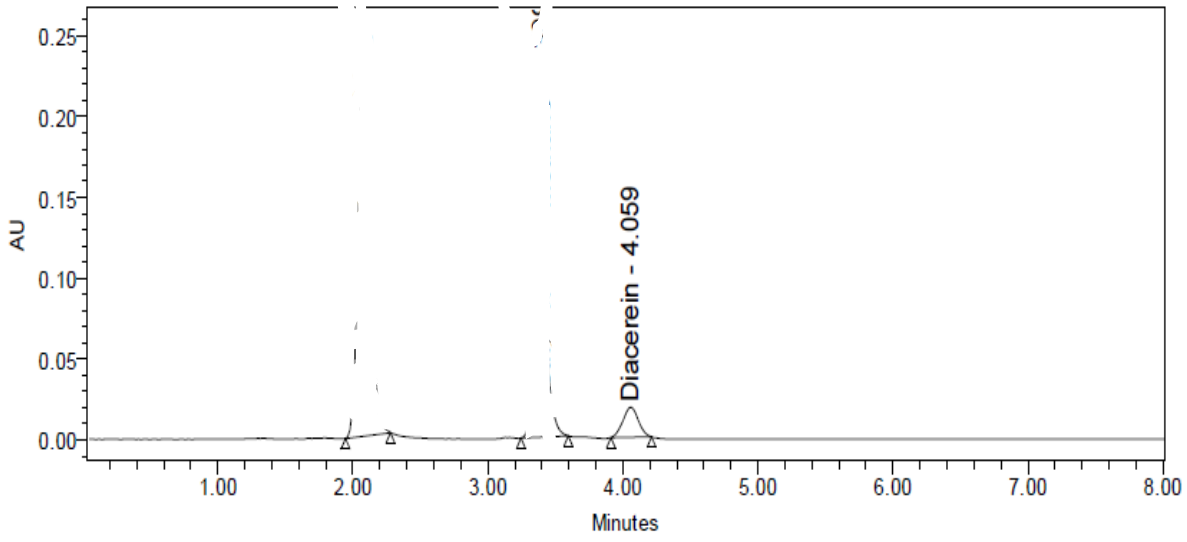
3 Accuracy

The percentage recoveries for Diacerein and was found to be 99.79%, 99.68% and 99.82% respectively. The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

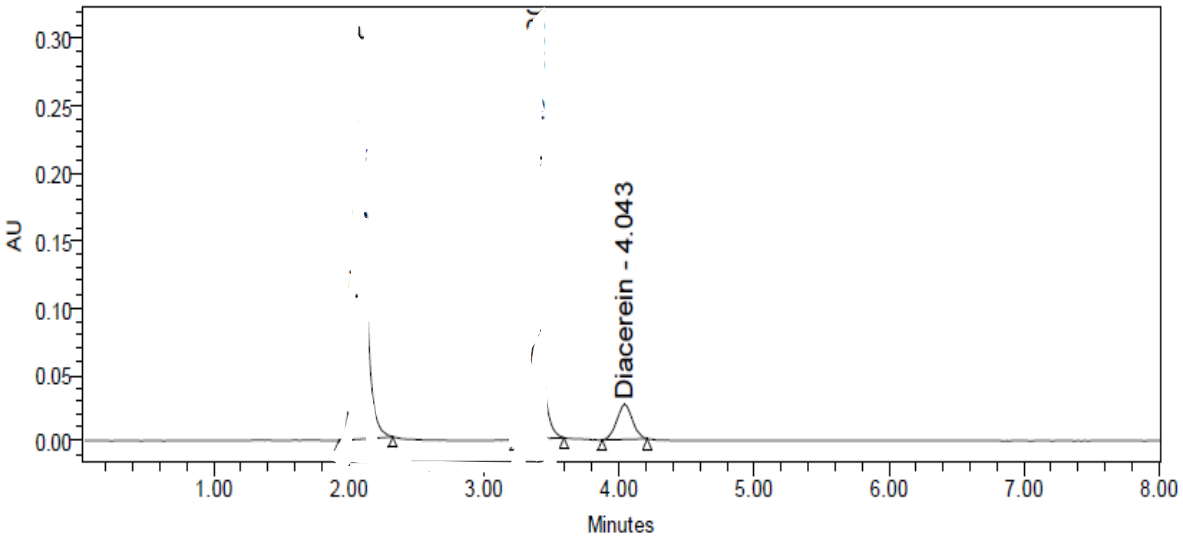
Recovery results of diacerein

% Level	Amount added (µg/ml)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	5	5.023735	100.47	99.68 %
	5	4.944618	98.89	
	5	4.970129	99.40	
100%	10	9.936868	99.37	
	10	10.0091	100.09	

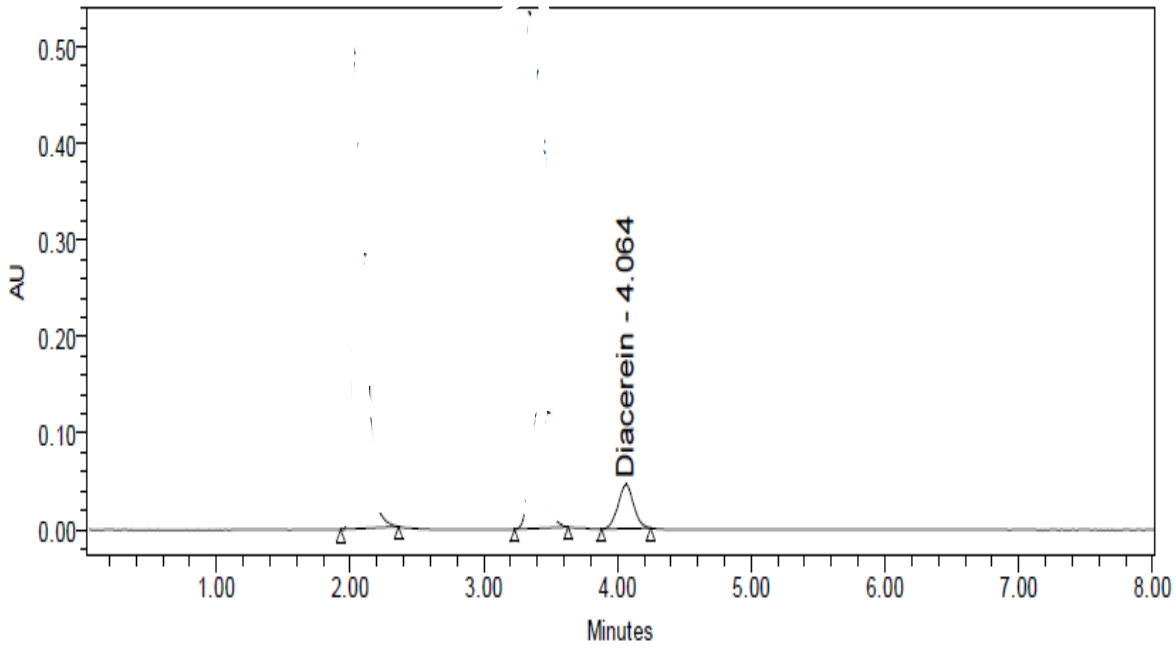
	10	9.964855	99.65	
150%	15	14.83708	98.91	
	15	15.049	100.33	
	15	14.996	99.97	



A typical standard chromatogram of Diacerein at Accuracy 50 %



A typical standard chromatogram of Diacerein at Accuracy 100 %



A typical standard chromatogram of Diacerein at Accuracy 150 %

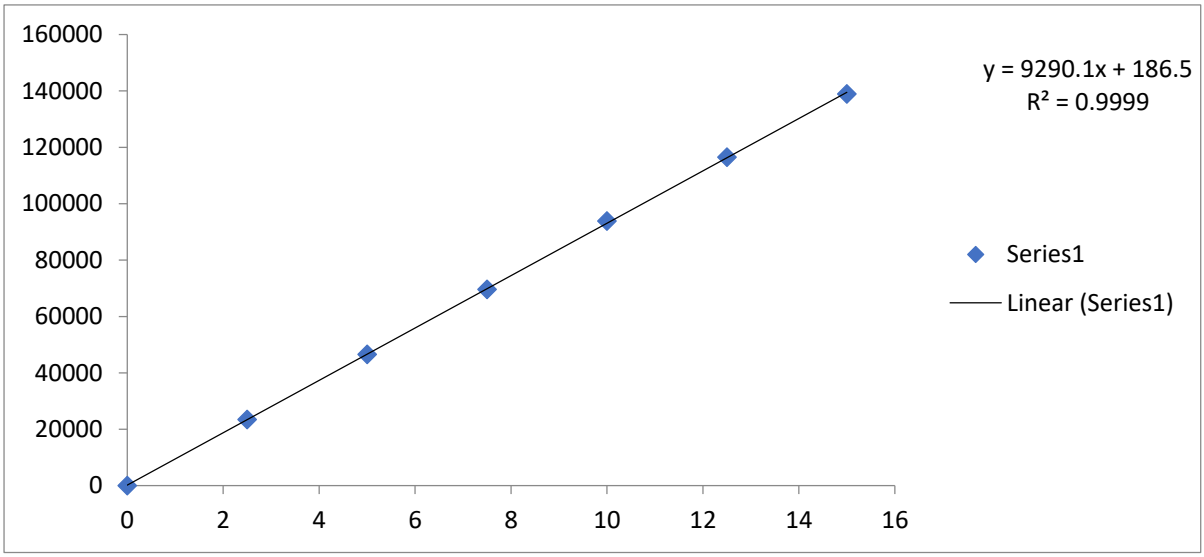
Linearity

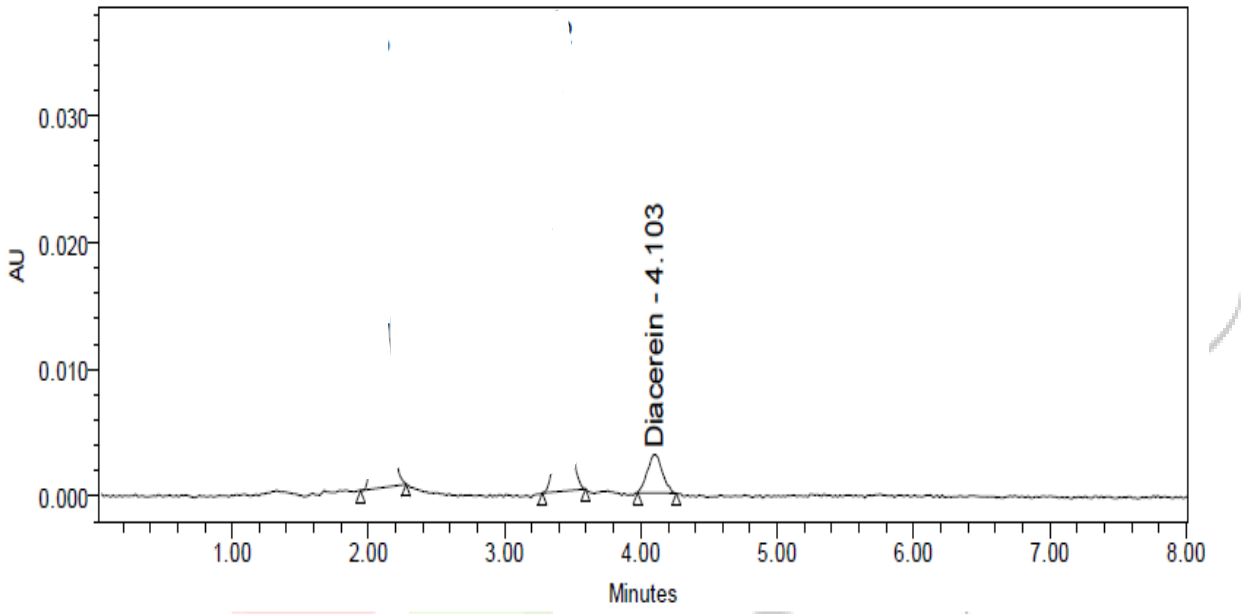
The linearity range was in the interval of Diacerein (2.5-15µg/ml). These were represented by a linear regression equation as follows: y (Diacerein) = 9290.x + 186.5 (r^2 =0.999). Regression line was established by least squares method and correlation coefficient (r^2) for Diacerein was found to be greater than 0.999. A calibration curve was plotted and the response of the drugs was found to be linear and the linearity results were given in table.

Linearity table for Diacerein

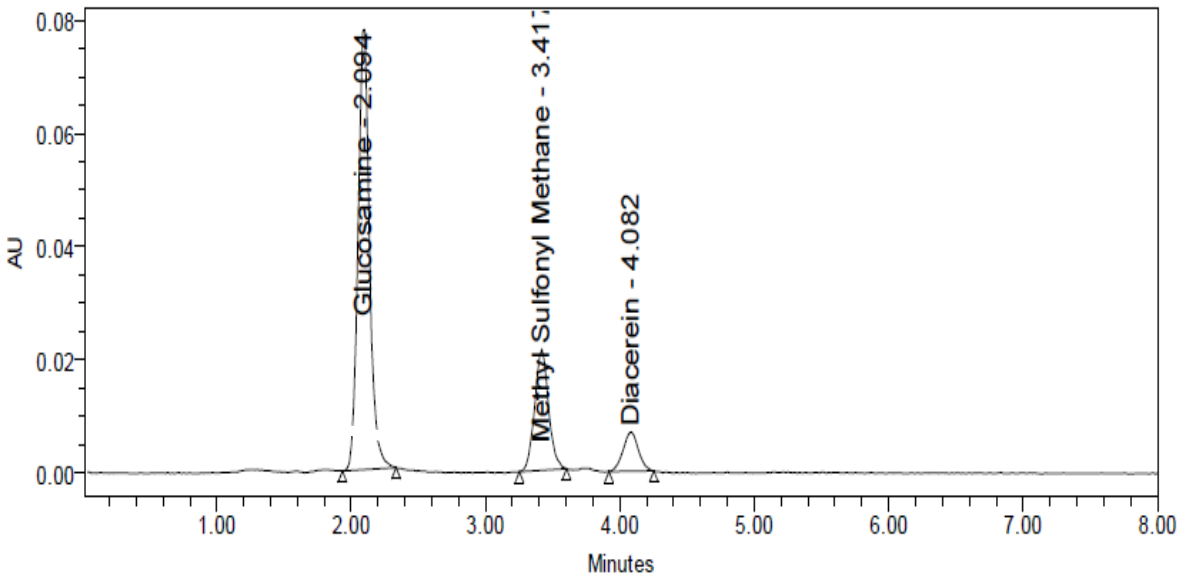
Diacerein	
Conc (µg/mL)	**Peak area**
0	0
2.5	23480
5	46560
7.5	69644
10	93844
12.5	116545
15	138965

Linearity curve for Diacerin

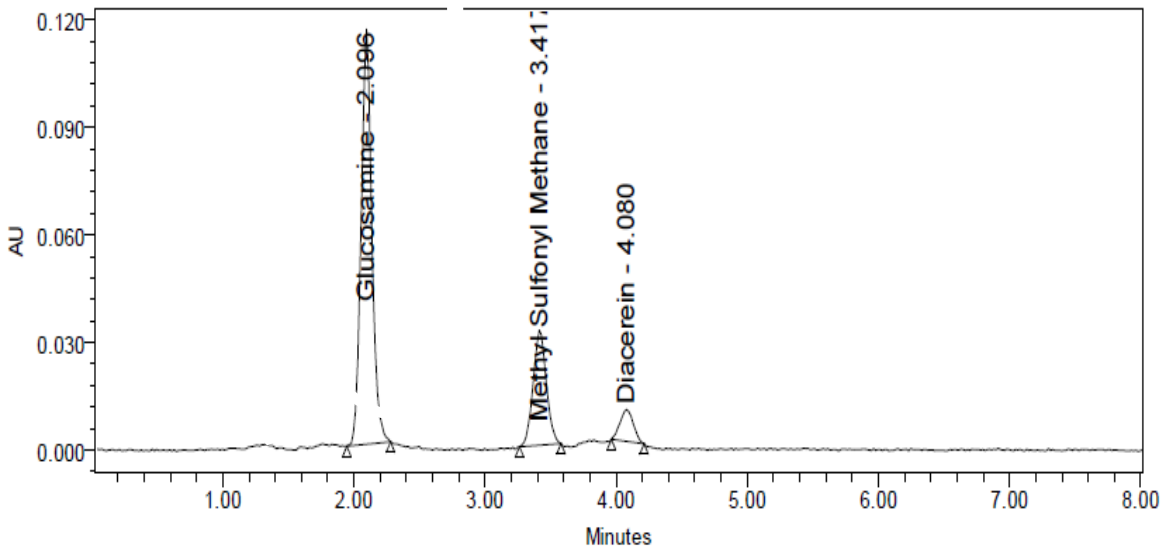




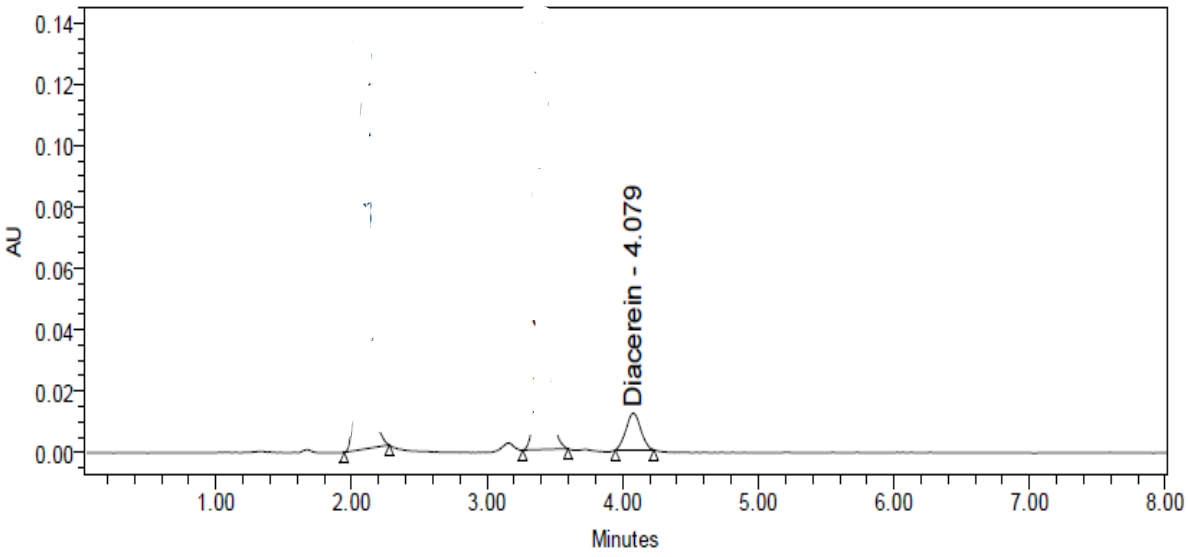
A typical standard chromatogram of Diacerein and at Linearity 25 %



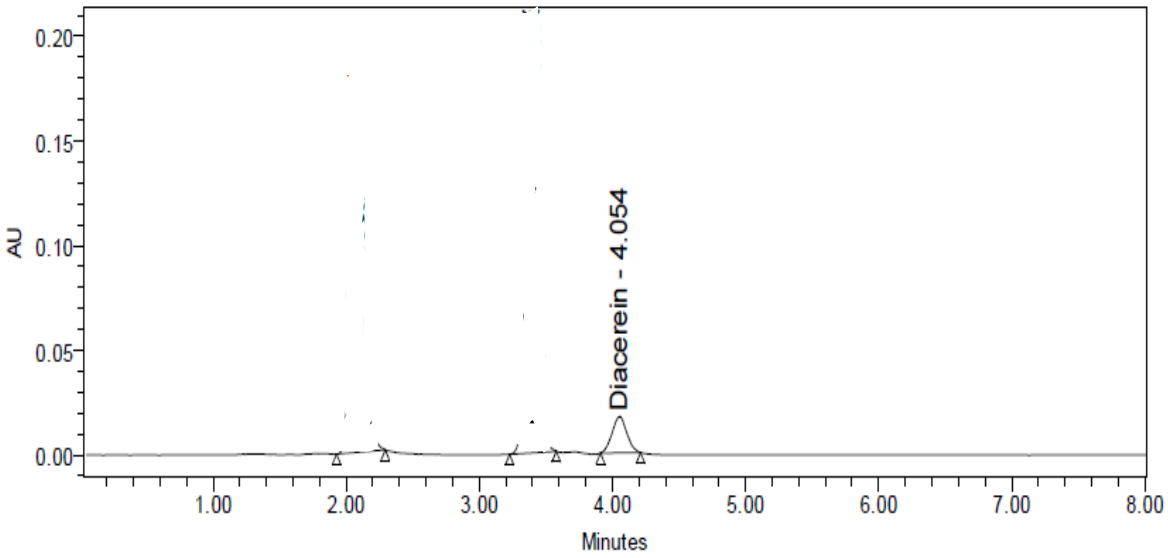
A typical standard chromatogram of Diacerein and at Linearity 50 %



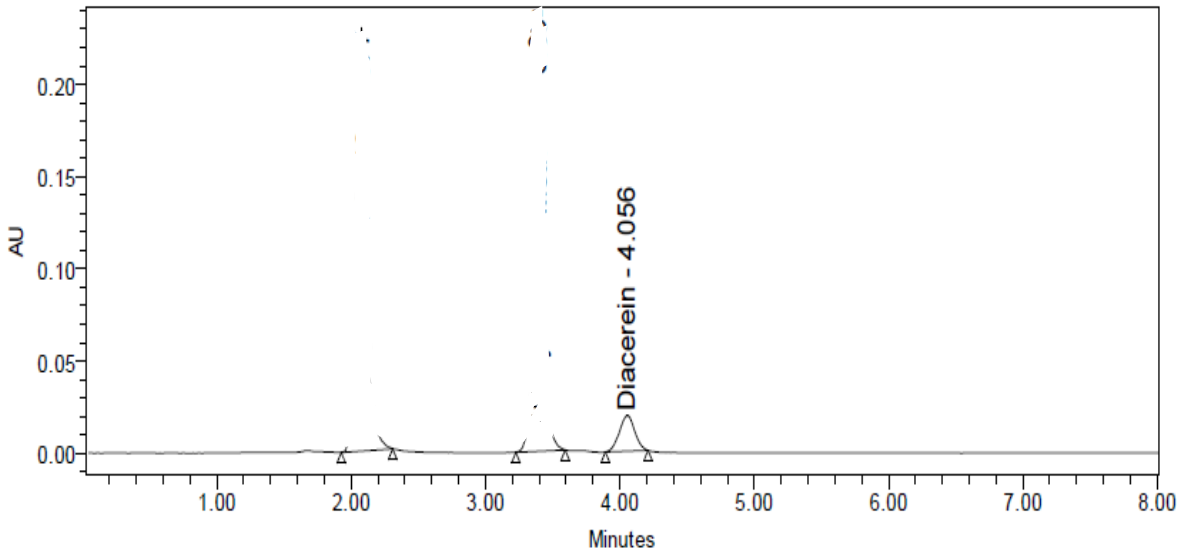
A typical standard chromatogram of Diacerein at Linearity 75 %



A typical standard chromatogram of Diacerein at Linearity 100 %



A typical standard chromatogram of Diacerein at Linearity 125 %



A typical standard chromatogram of Diacerein at Linearity 150 %

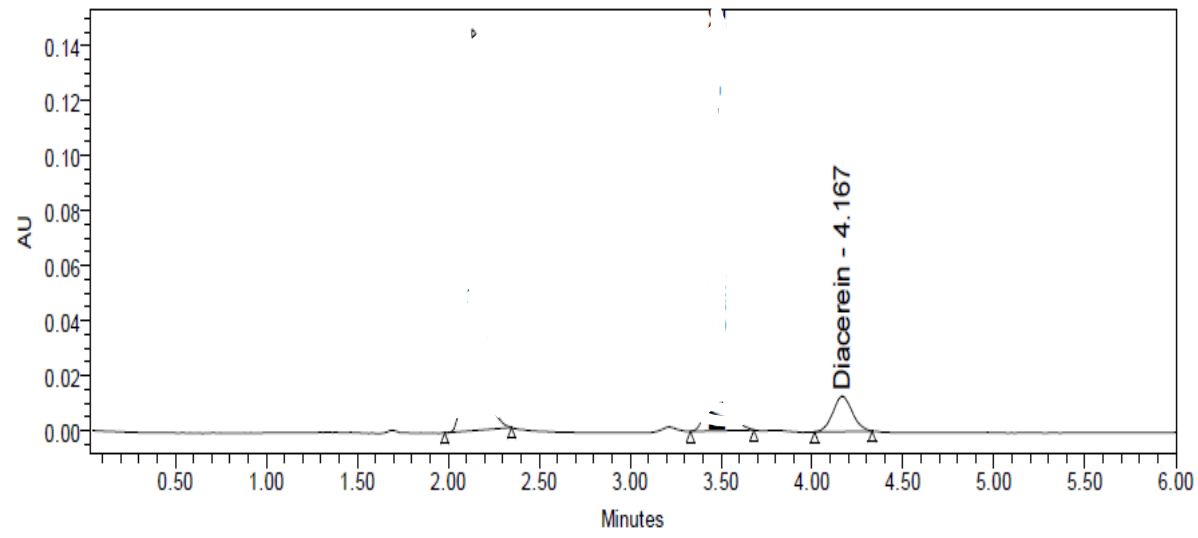
Precision

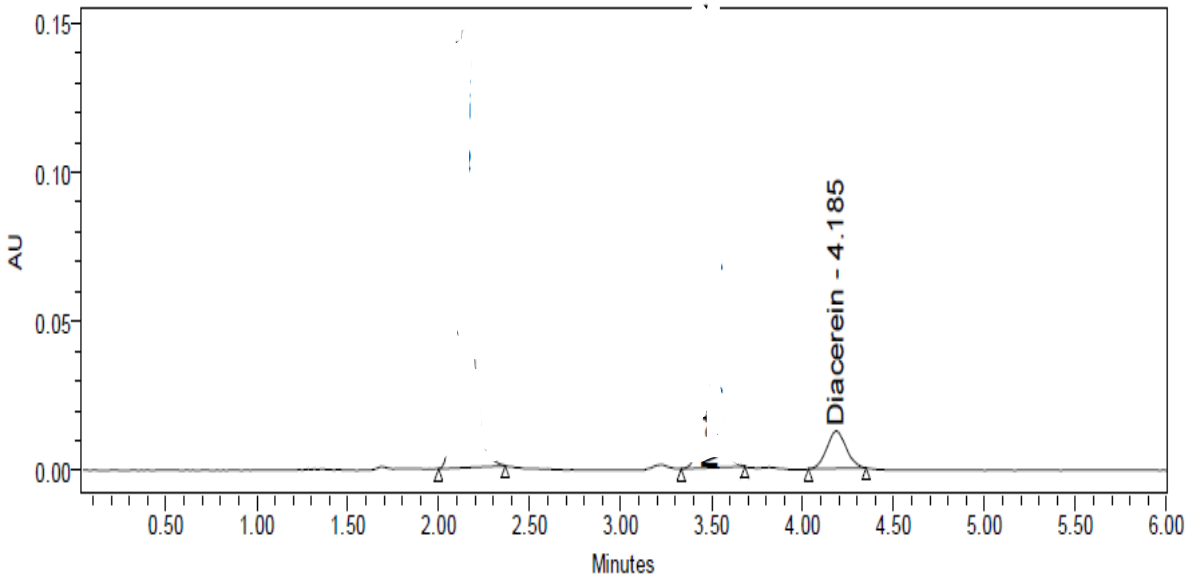
The precision of the proposed method was investigated in terms of intraday and inter day precision. Intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the % RSD and the interday precision (reproducibility) of the method was checked by performing same procedure on different days under the same experimental conditions. A known concentration of Diacerein (10µg/ml) solutions was prepared and analyzed. The % RSD was calculated for intraday and inter day precision and found to be below 2% which indicates the developed method was precise. The precision data of the drugs were shown in table.

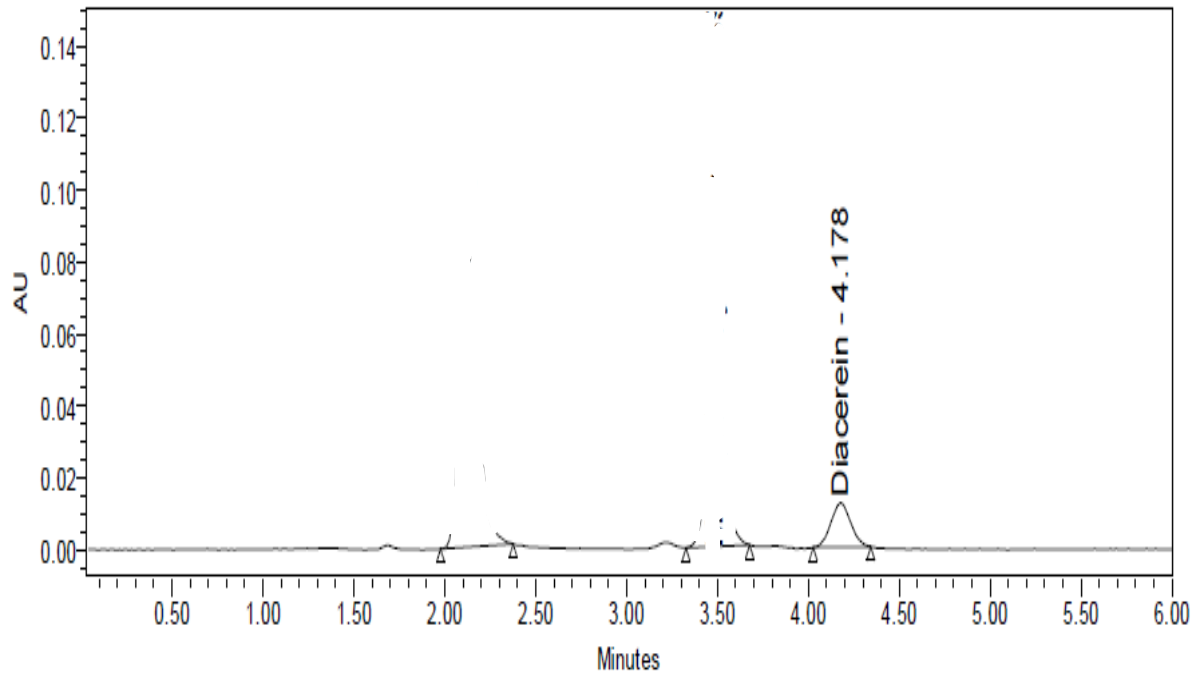
Intraday Precision data of Glucosamine, Diacerein and Methyl sulfonyl methane

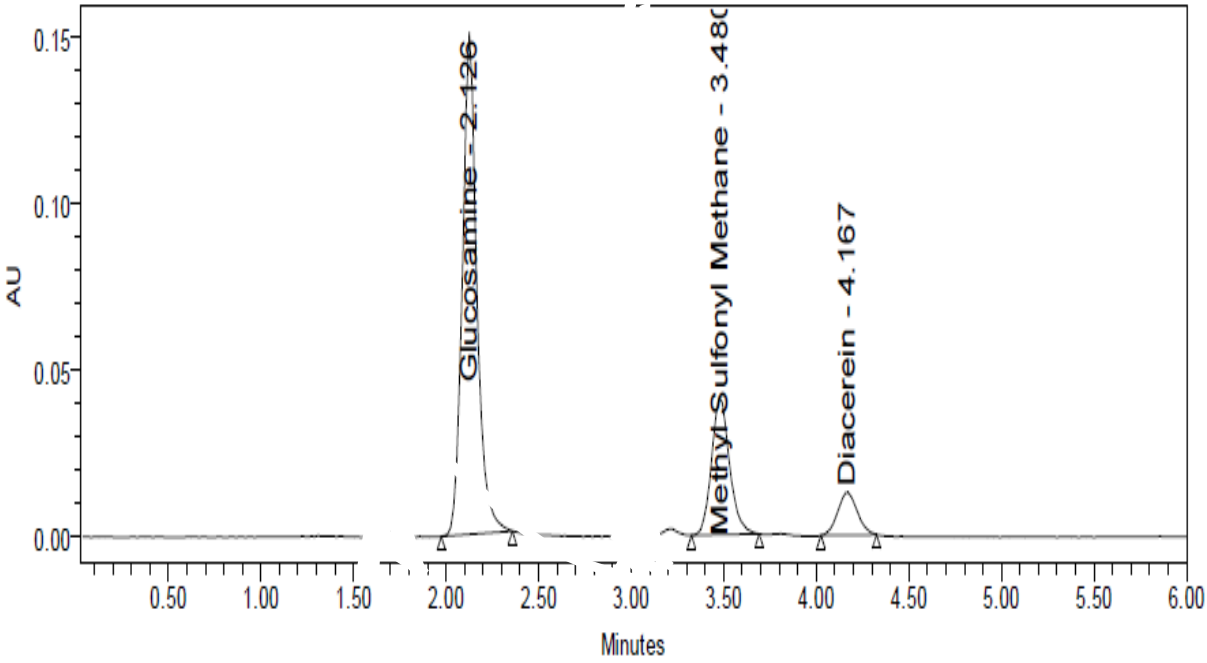
S.No	**Diacerein**
	Intraday Peak area
1	93875
2	93246
3	95381
4	93789
5	93336
6	93570
AVRG	93866
SD	781.6
%RSD	0.8

A typical intraday precision chromatogram of Diacerein





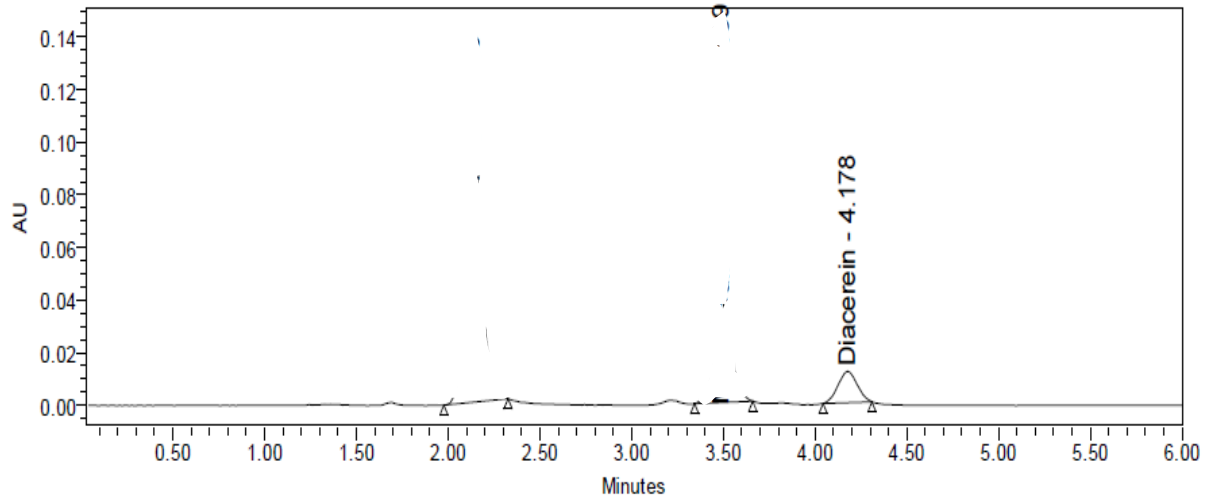


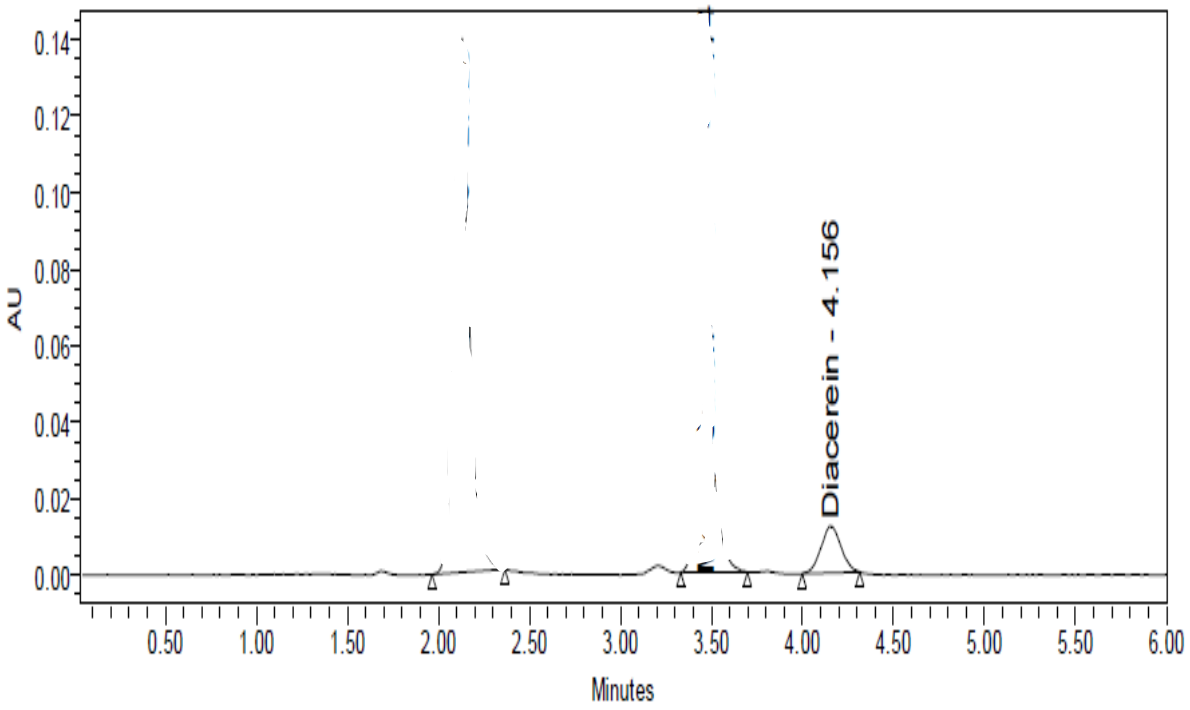


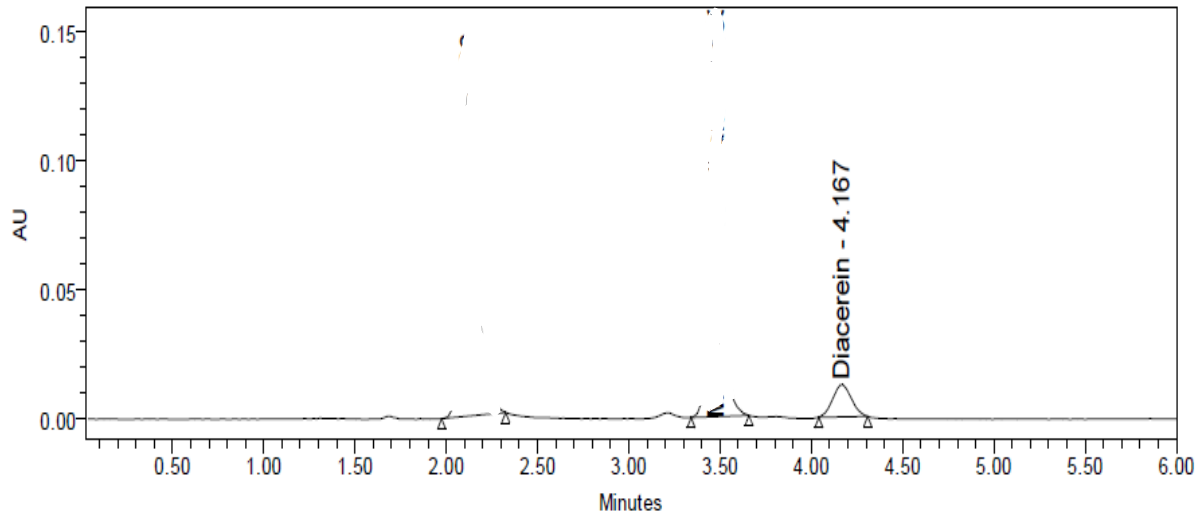
Interday Precision data of Diacerein

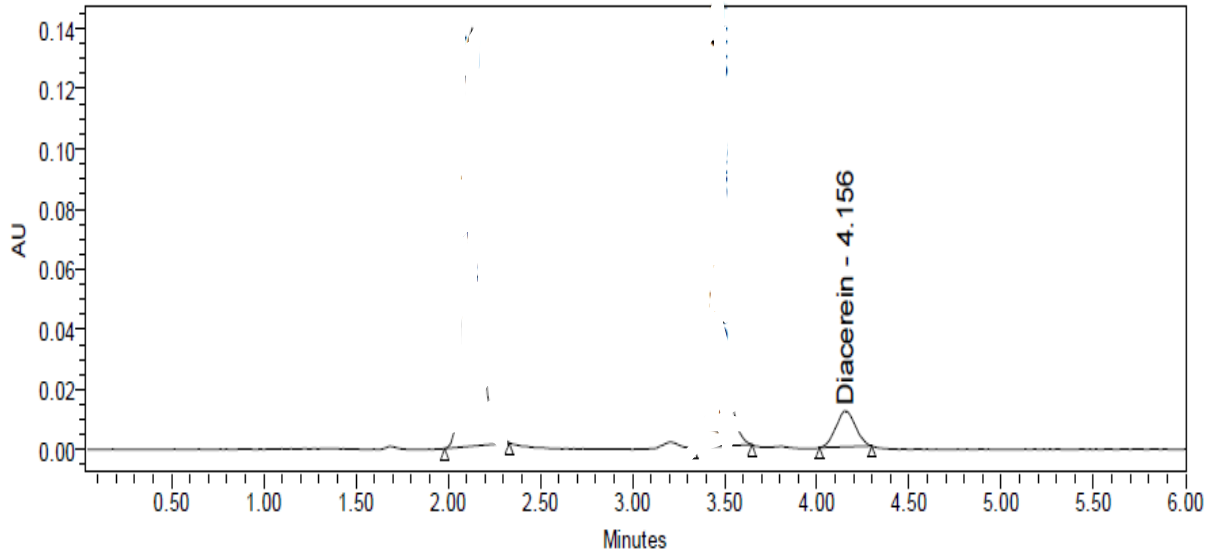
S.No	Diacerein
	Interday Peak area
1	93399
2	93876
3	93999
4	92949
5	93671
6	93989
AVRG	93647
SD	410.5
%RSD	0.4

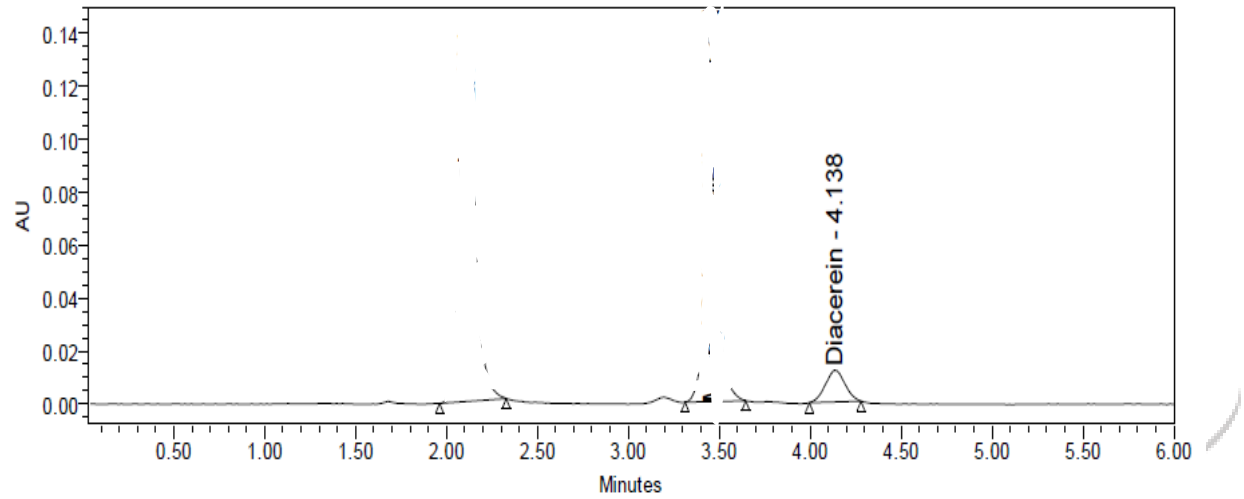
A typical interday precision chromatogram of Diacerein











Robustness

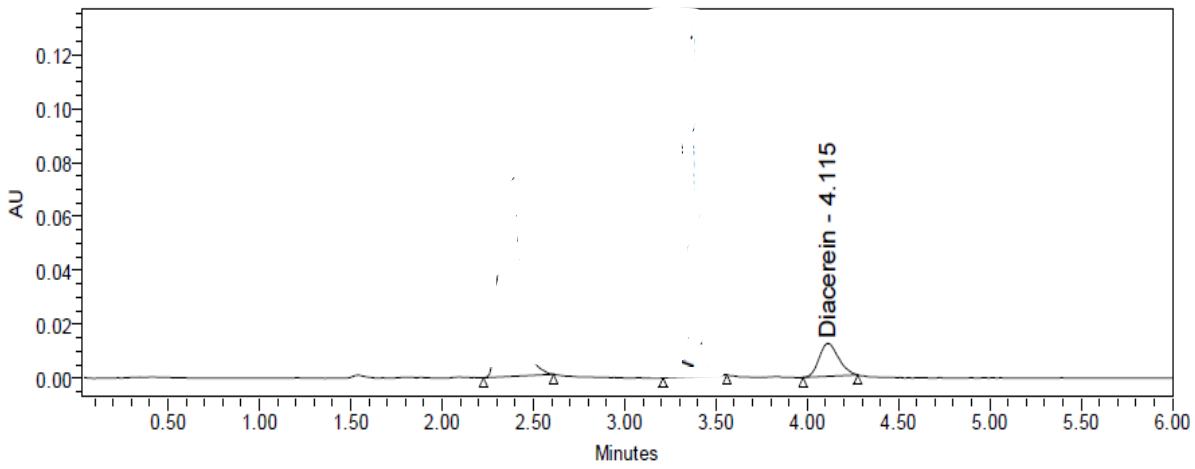
Robustness of the proposed method was studied by analyzing the samples of Diacerein for some relevant factors which may influence the reliability of the developed method. Deliberate change in some of the factors including, flow rate (±0.1ml), mobile phase (±10%) and temperature ($\pm 5^0$) was investigated for their degree of change with respect to proposed method by comparing the change in response with the original one. In all the conditions, good separation of the Glucosamine, Diacerein and Methyl sulfonyl methane were achieved which indicate the developed analytical method remained selective and robust. The % RSD was determined in all the conditions and found to be within the limits of acceptance.

Actual conditions and proposed conditions of the method

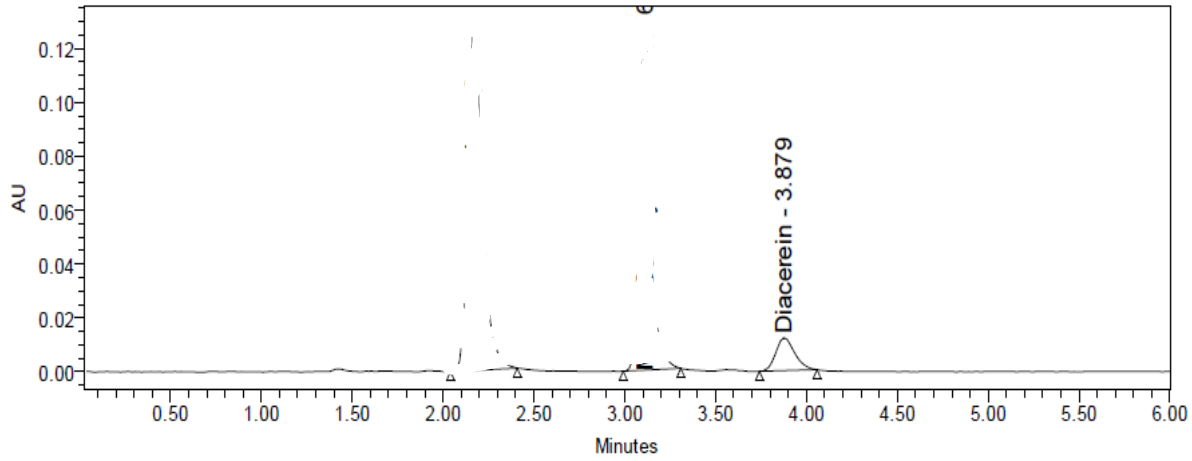
Parameters	Actual conditions	Proposed variations
Flow rate	1ml/min	0.9, and 1.1ml/min
Mobile phase ratio	66:35 % v/v	±10%
Temperature	30 ^{0}C	25 ^{0}C, 35 ^{0}C

Robustness data for Glucosamine, Diacerein and Methylsulfonyl methane

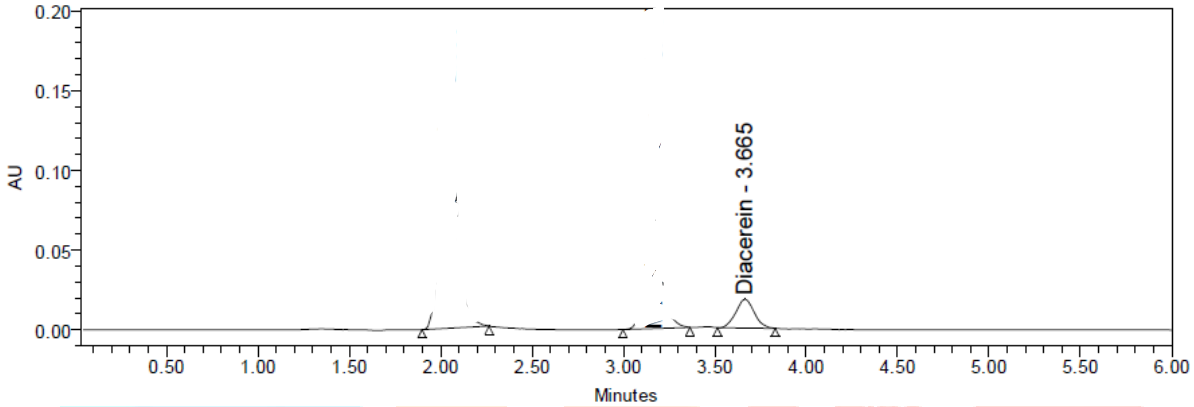
S.no	Condition	%RSD of Diacerein
1	Flow rate (-) 0.8ml/min	0.6
2	Flow rate (+) 1.0ml/min	0.2
3	Mobile phase (-) 70B:30A	0.4
4	Mobile phase (+) 60B:40A	0.3
5	Temperature (-) 25°C	0.5
6	Temperature (+) 35°C	0.2



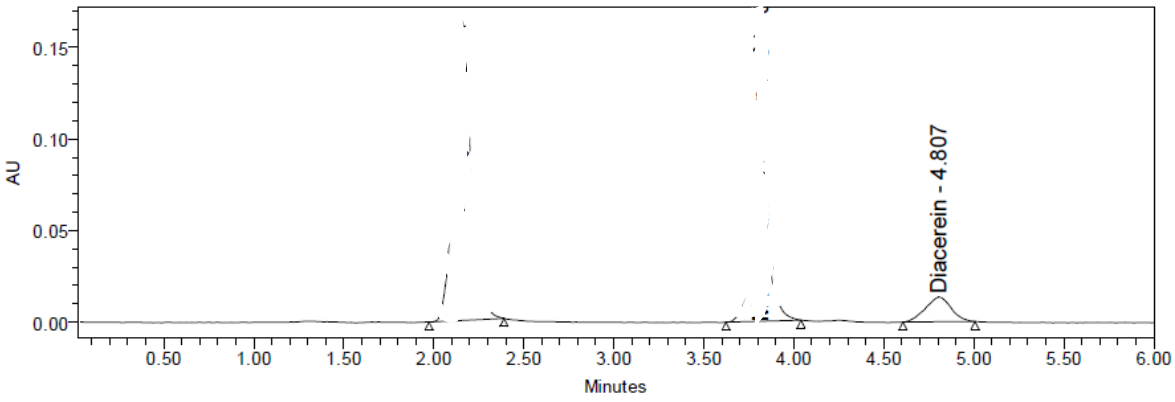
A typical robustness chromatogram of Diacerein at flow rate 0.9ml/min



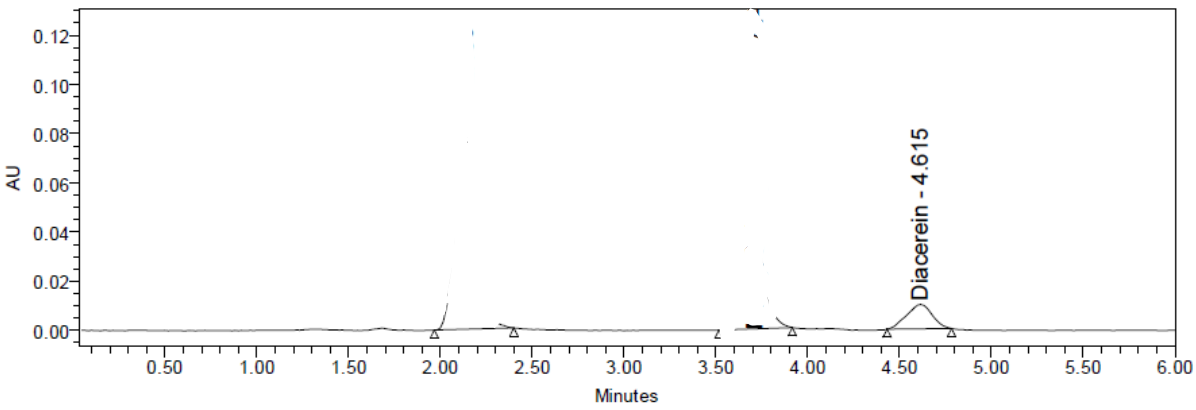
A typical robustness chromatogram of Diacerein at flow rate 1.1ml/min



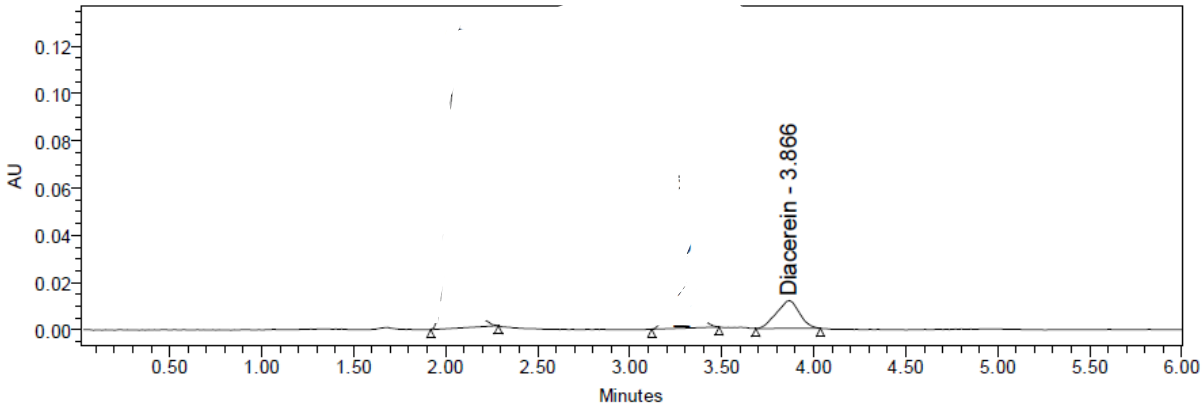
A typical robustness chromatogram of Diacerein at temperature 25 ^{0}C



A typical robustness chromatogram of Diacerein at temperature 35 ^{0}C



A typical robustness chromatogram of Diacerein at mobile phase 65:35% v/v



A typical robustness chromatogram of Diacerein at mobile phase 55:45% v/v

LOD and LOQ

Limit of Detection (LOD) of an individual analytical procedure is the lowest amount of analyte is a sample, which can be detected but not necessarily quantitated and limit of quantitation (LOQ) of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated from standard deviation of the response and the slope values of the three linearity curves using the formula 3.3 α/S for LOD and 10 α/S for LOQ, where α is standard deviation of response and S is mean of slope of three calibration curves. The LOD was calculated and found to be 0.14µg/ml for Diacerein and 0.53 µg/ml. The LOQ was calculated and found to be 0.41 µg/ml for Diacerein. The LOD and LOQ data was given in table

LOD and LOQ results of Glucosamine, Diacerein and Methyl sulfonyl methane

Sample	LOD	LOQ
Diacerein	0.53 µg/ml	1.60 µg/ml

REFERENCES:

1. Taverniers I, De Loose M, Van Bockstaele E. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Analyt Chem. 2004; 23(7):480-490.
2. Araujo P. Key aspects of analytical method validation and linearity evaluation. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877 (23):2224–2234.
3. United States Pharmacopeia and National Formulary (USP 35 NF30), The United States Pharmacopeial Convention, Inc., Rockville, MD, 2012.
4. Rozet E, Ceccato A, Hubert C, et al. Analysis of recent pharmaceutical regulatory documents on analytical method validation. J Chromatogr A. 2007; 1158(1- 2):111–125.
5. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," *Federal Register***62**(96), 27463–27467 (1997).

6. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," *Federal Register (Notices)***65**(169), 52776–52777 (2000).
7. www.fda.gov/cder/guidance/cmc3.pdf
8. USP 25–NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopeal Convention, Rockville, Maryland, USA, 2002) p 2256.
9. G.A. Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation Requirements of FDA, the US Pharmacopeia and the ICH," *J. Chromatogr. A.***987**(1-2), 57-66 (2003).
10. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 2nd. ed. B. Magnusson and U. Ornemark (eds.). 2014.