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Research Article

NOVEL VALIDATED REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF GLUCOSAMINE, DIACEREIN, AND METHYL SULFONYL METHANE IN MICRO SAMPLE RAT PLASMA AND ITS APPLICATION TO PHARMACOKINETIC AND DISSOLUTION STUDIES

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ABSTRACT

Objective: The main objective of this research is to develop and validate a simple, specific, precise, sensitive, cost-effective, and rapid reversed-phase high-performance liquid chromatography method for simultaneous quantification of glucosamine (GLU), diacerein (DIA) and methyl sulfonyl methane in bulk and pharmaceutical dosage forms, and micro-sample of rat plasma using ultraviolet (UV) detection, to perform the studies of drug dissolution from tablets.

Methods: Sprague-Dawley rats were used for pharmacokinetic study after intravenous administration of the drug samples at dose 5 mg/kg. The drug samples were extracted by liquid-liquid extraction technique using acetonitrile, which also acted as a deproteinization agent. The separation of the analyte was carried out on a phenomena C_{18} column with a mobile phase composed of 0.1 % orthophosphoric acid:acetonitrile (80:20 v/v) delivered at a flow rate of 1.0 ml/min, and separation has been monitored by a UV detector, at detection of the wavelength of 285 nm.

Results: This method was proven to be linear over a concentration range of 30–450 µg/ml for GLU, 2–30 µg/ml for DIA, and 10–150 µg/ml for methyl sulfonyl methane with a correlation coefficient of 0.999. The retention time of GLU, DIA, and methyl sulfonyl methane were 2.89, 6.32, and 9.87 min, respectively. Recovery of the drugs was found to be in the range of 98.0–102.0%. Validation results were found to be satisfactory and the method applicable for bulk and formulation analysis. Hence, it was evident that the proposed method was said to be a suitable one for the regular analysis and quality control of pharmaceutical preparations which contain these active drugs either individually or in combination.

Conclusion: The validation results were in good agreement with acceptable limits. Relative standard deviation values which are less than 2.0% are indicating the accuracy and precision of this method. The usefulness of the method is that the common chromatographic conditions have been adopted for assay, dissolution, and pharmacokinetic studies. This developed method showed reliable, precise, and accurate results under optimized conditions.

Keywords: Glucosamine, Diacerein, Methyl sulfonyl methane, Pharmacokinetics, Dissolution profile.

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INTRODUCTION

Glucosamine (GLU) (Fig. 1) is chemically called (3R, 4R, 5S)-3-amino-6-(hydroxymethyl) oxane-2, 4, 5-triol. It can act as an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids [1, 2]. It is found in the hard covering of shellfish [3]. It is one of the most common non-vitamin, non-mineral, dietary supplements, and natural products. It is used for osteoarthritis, back pain, and joint pain.

Diacerein (DIA) (Fig. 2) chemically called 4, 5-bis (acetyloxy)-9,10-dioxo-2-anthracenecarboxylic acid [4,5]. It is slow-acting drug and is used in the treatment of osteoarthritis, analgesic, and nonsteroidal anti-inflammatory drugs [6-8]. It may be safe on the stomach [9-11]. It can inhibit interleukin-1 and retards all pathological processes initiating in osteoarthritis [12] and also inhibits superoxide production, chemo taxis, and phagocytic activity of neutrophils.

Methylsulfonylmethane (MET) (Fig. 3) can acts as a precursor for the synthesis of methionine, cysteine, and sulfur-containing amino acids [13]. As it is suggested anti-inflammatory and analgesic effects, it has been promoted as a possible supplement for osteoarthritis [14] and is used to protect muscles from damage by reducing the amount of oxidative stress.

A dissolution test is an important tool for characterizing drug product performance and is a vital component of the overall quality control program [15,16]. It is used to determine whether a drug the product can release its active pharmaceutical ingredients in a timely manner [17]. The study of the dissolution rate is observed to be sensitive, reliable, and rationale for predicting *in vivo* drug bioavailability behavior among all tests that can be performed on different combinations of drugs.

Literature survey reveals that several methods have been present for the estimation of each drug [18-20] and two drugs at a time [21]. However, one of the analytical methods for assay has been reported for the estimation of GLU, DIA, and MET in pharmaceutical dosage forms [22]. However, it is of interest to refer that the studies of dissolution rate and relevant kinetic parameters of these active drugs have not yet been reported previously so that present research work was undertaken.

The present paper explains the development of method that meets the suggested aim: The simple, specific, precise, sensitive, cost-effective, and rapid reversed-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantification of GLU, DIA, and MET in bulk and pharmaceutical dosage forms, and micro-sample of rat plasma using ultraviolet (UV) detection, to carry out drug dissolution studies from tablets. The proposed method showed reliable, precise, and accurate results under optimized conditions. Hence, this method appears to be appropriate for the quality control technique in the pharmaceutical industry.

EXPERIMENTAL

Reagents and solutions

Pure samples of GLU, DIA, and MET used were obtained from Glenmark Pharmaceutical Private Ltd., Andheri (E), Mumbai, India (99.7–99.9% purity). All other chemicals such as acetonitrile, orthophosphoric acid, and water were of HPLC grade, purchased from Merck (India) Ltd. Worli, Mumbai, India.

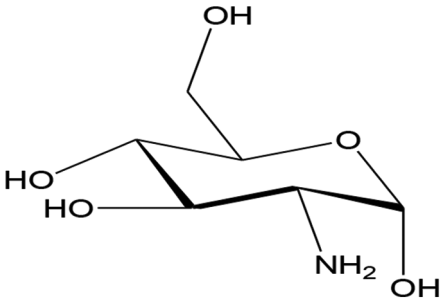

Fig. 1: Chemical structure of glucosamine

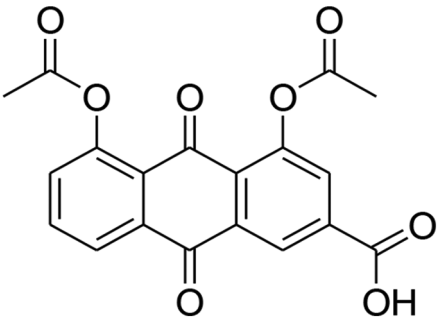

Fig. 2: Chemical structure of diacerein

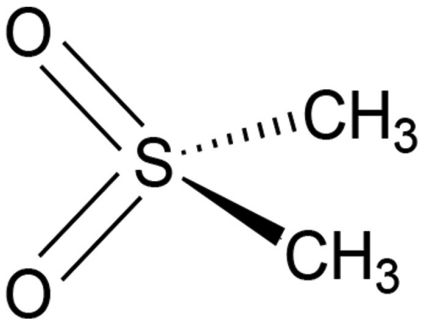

Fig. 3: Chemical structure of methylsulfonylmethane

Table 1: System suitability data

System suitability parameter	Acceptance criteria	Drug name		
		DIA	GLU	MET
% RSD	NMT 2.0	0.26	0.32	0.18
USP tailing	NMT 2.0	0.84	0.75	0.65
USP plate count	NLT 3000	5628	7458	4514

DIA: Diacereinm GLU: Glucosamine, MET: Methylsulfonylmethane, NMT: Not less than, NMT: Not more than

Table 2: Recovery data for GLU

???	Recovery solution (area) (mAU)	% Drug recovery
150	911,741	100.3
300	1,813,188	100.5
450	2,705,571	100.1

GLU: Glucosamine

Instrumentation

Water alliance-2695 chromatographic system equipped with a quaternary pump, variable UV, and photodiode-array detection detectors were used. For data collection and processing chromatographic software, Empower-2.0 has been used.

Selection of buffer

Simple, economical, and proper acidic buffer were selected like 0.1% orthophosphoric acid.

Mobile phase

The mobile phase selected was 0.1% orthophosphoric acid buffer:acetonitrile in the ratio of 80:20 (v/v), and the mobile phase was degassed before analysis. The selected mobile phase has given sharp peaks with low tailing factor (2.0) and also plate count was more than in 2000.

Diluent preparation

The diluent was optimized as a mixture of water and acetonitrile (50:50 v/v).

Selection of wavelength

The maximum absorption of the solution of three drugs was scanned in the UV region against acetonitrile as blank using a photodiode

Table 3: Recovery data for DIA

Amount of DIA drug (mg/ml)	Recovery solution (area) (mAU)	% Drug recovery
10	231,904	100.6
20	473,832	99.8
30	690,546	100.5

DIA: Diacerein

Table 4: Recovery data for MET

Amount of MET drug (mg/ml)	Recovery solution (area) (mAU)	% Drug recovery
50	508,305	100.3
100	1,082,508	100.5
150	1,516,126	100.1

MET: Methylsulfonylmethane

Table 5: Precision data of GLU

Concentration of GLU drug (mg/ml)	Area mAU	RSD
300	1,794,877 1,846,300 1,829,898 1,783,160 1,770,378 1,827,609	1.66

GLU: Glucosamine

Table 6: Precision data of DIA

Concentration of DIA drug (mg/ml)	Area mAU	RSD
20	477,064 473,060 460,715 470,723 474,850 474,079	0.67

DIA: Diacerein

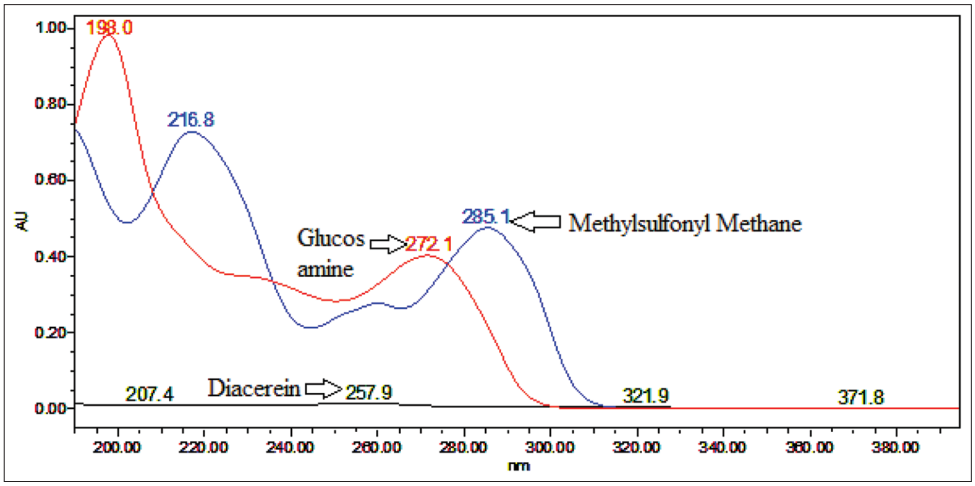

Fig. 4: Photodiode-array detection spectrum for glucosamine, diacerein, and methylsulfonylmethane

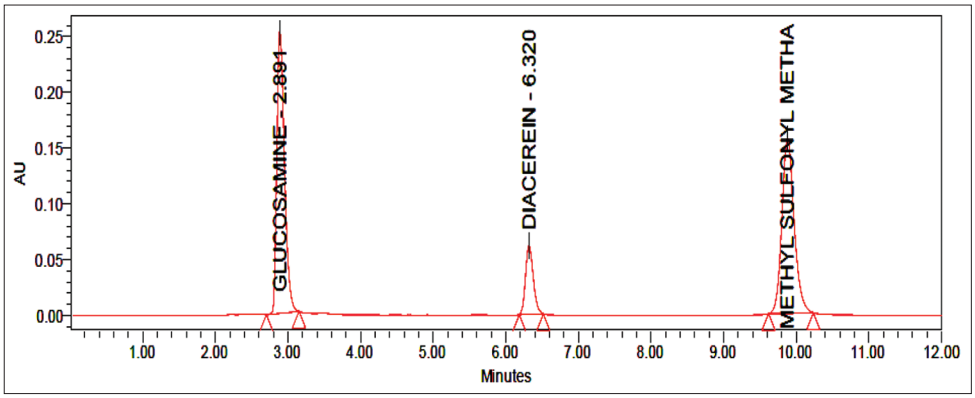

Fig. 5: Method developed chromatogram

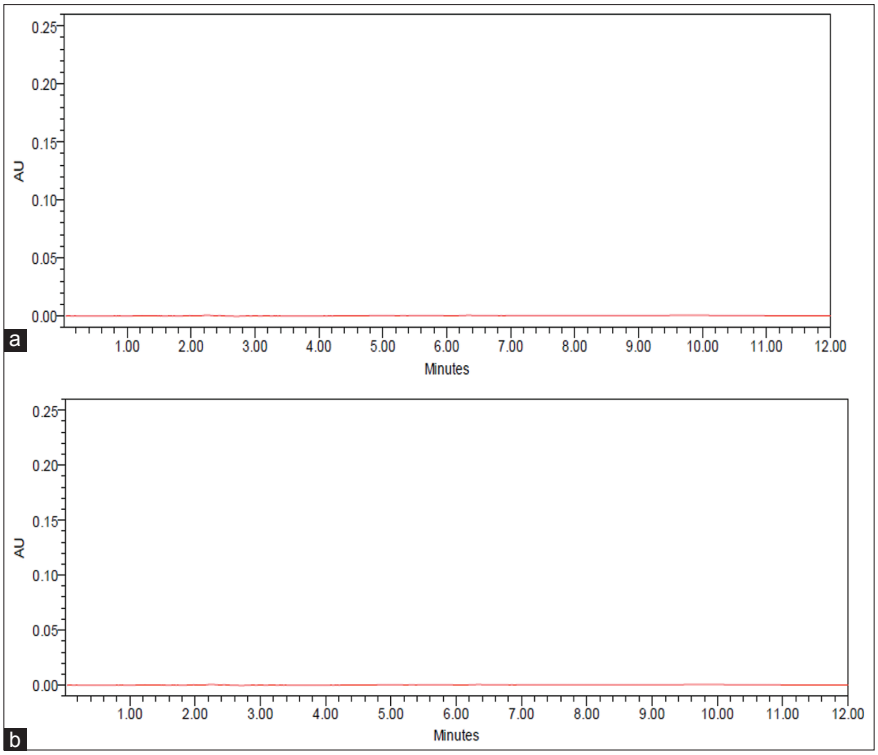

Fig. 6: Chromatograms for (a) blank, and (b) placebo

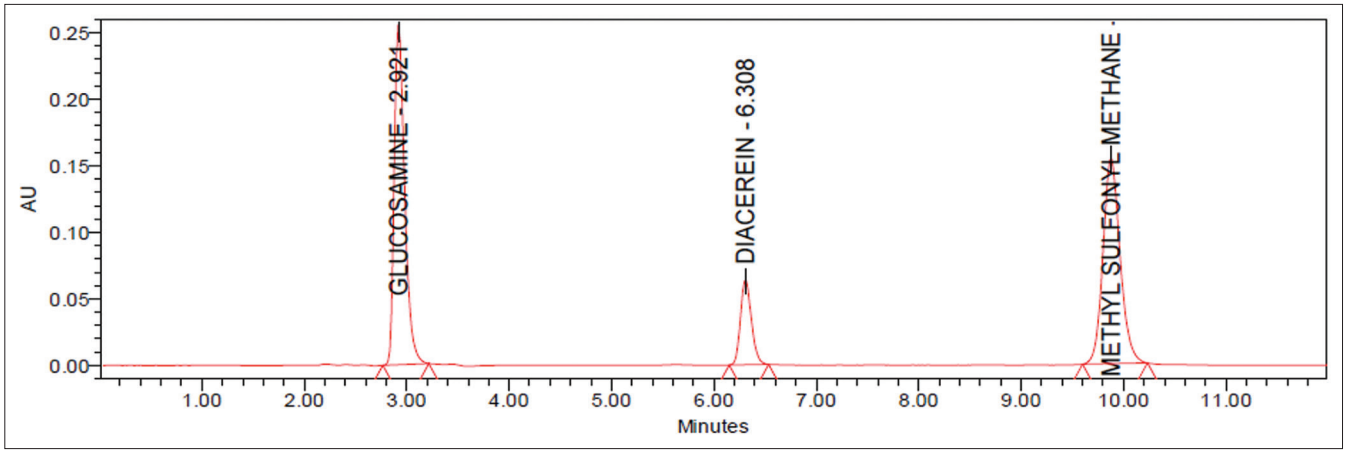

Fig. 7: Chromatogram for system suitability

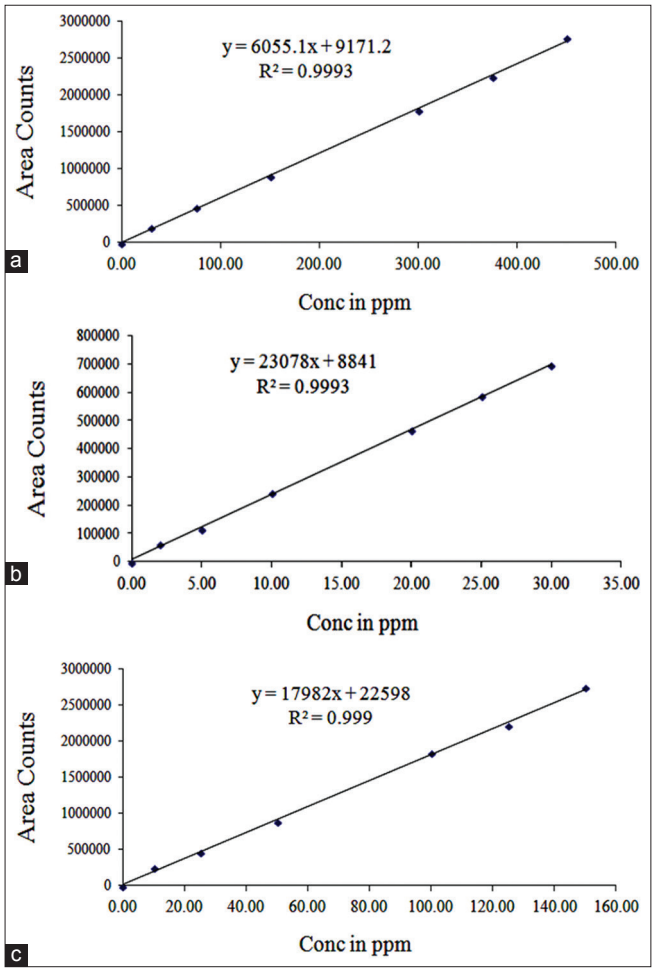

Fig. 8: Linearity plots for (a) Glucosamine, (b) Diacerein, and (c) Methylsulfonylmethane

spectrophotometer. The spectra of GLU, DIA, and MET (Fig. 4) show different λmax, namely, 272.1, 285.1, and 257.9, respectively. 285 nm was selected as detector wavelength for the proposed method by considering the highest response for three drugs.

Chromatographic conditions

HPLC studies have been done on Phenomenex C18 (250 × 4.6 mm, 5 µm) column. The separations were attained by isocratic elution and acetonitrile: H_3PO_4 (0.1%) (20:80 by volume) as mobile phase delivered

Table 7: Precision data of MET

Concentration of MET drug (mg/ml)	Area (mAU)	RSD
100	1,071,675 1,085,374 1,073,846 1,015,706 1,036,260 1,017,498	1.27

MET: Methylsulfonylmethane

Table 8: Results of stress degradation studies

Stress condition/duration/solution	Degradation (%)
Acid degradation (0.5 N HCl, 1 h)	25
Alkaline degradation (0.5 N NaOH, 1 h)	22
Oxidative degradation (30% H2O2, 80°C for 15 min)	28
Reduction degradation (10% NaHSO4, 80°C for 15 min)	27
Thermal degradation (Solid sample, 80°C, 3h)	23
Photolytic degradation (sample expose sun light 6 h)	22

at a flow rate of 1.0 ml/min. The injection volume was 10 µl with 12 min run time and the column the temperature was maintained at 60°C and absorbance measured at 285 nm.

Standard solution preparation

300 mg of GLU, 20 mg of DIA, and 100 mg of MET (working standard) were accurately measured and transferred into a 100 ml clean and dry volumetric flask, about 70 ml of mobile phase was added, sonicated for complete dissolution and it was made up to the mark with the diluent. 5.0 ml of this solution was diluted to 50 ml with the mobile phase and mixed well.

Sample preparation

Ten tablets were weighed and triturated in a mortar. The tablet powder equivalent to 480 mg of active ingredient (sample) presents in ten tablets has been transferred into a 100 ml clean and dry volumetric flask, 70 ml of diluent was added, sonicated to about 30 min with occasional stirring and made up the volume with diluent. From this solution, 5.0 ml was diluted to 50 ml with the mobile phase. These solutions were filtered through 0.45 µ nylon syringe filter.

RESULTS AND DISCUSSION

The purpose of this study is to develop accurate, specific, cost-effective, and a single isocratic HPLC method for the simultaneous quantification

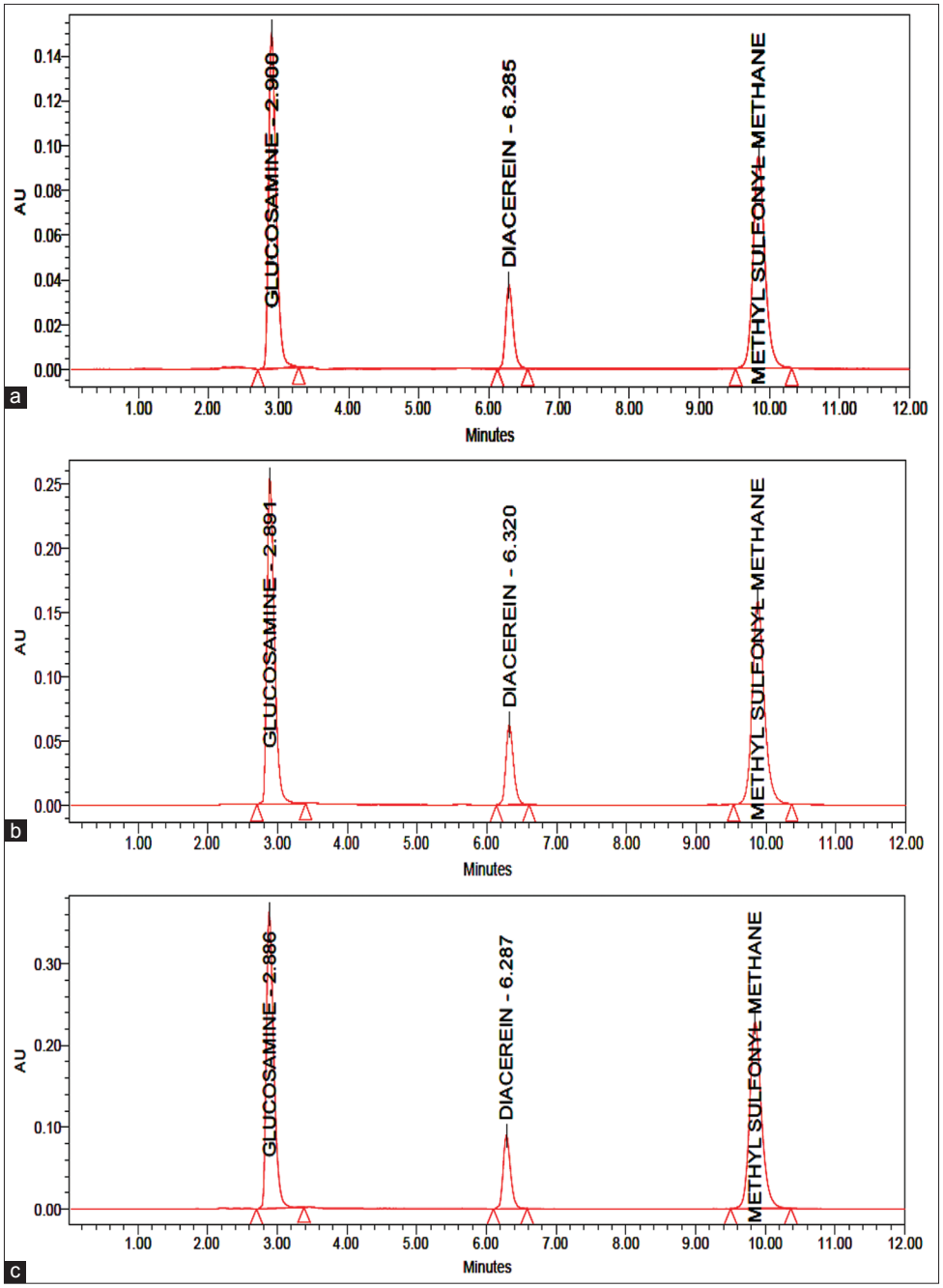

Fig. 9: Chromatograms for (a) accuracy 50%, (b) accuracy 100%, and (c) accuracy 150%

Table 9: Results of robustness studies

Change in parameter	% RSD
Flow (0.8 ml/min)	0.65
Flow (1.2 ml/min)	0.92
Organic phase composition (+5 %)	0.24
Organic phase composition (-5 %)	0.22
Wavelength (290 nm)	0.74
Wavelength (280 nm)	0.56

RSD: Relative standard deviation

Table 10: Results of stability studies

Stability	% Label claim	% Deviation
Initial	100.6	0.00
6 h	100.4	0.02
12 h	100.0	0.04
18 h	99.8	0.08
24 h	99.6	1.00

of GLU, DIA, and MET in bulk and pharmaceutical dosage forms, and micro-sample of rat plasma using UV detection, and to carry out the studies of drug dissolution from tablets. Appropriate wavelengths for simultaneous estimation of three drugs were selected according to the UV spectra of these compounds.

Method optimization

Development trials were carried out with acidic buffers, methanol, and acetonitrile using isocratic and gradient mode. Different stationary phases such as phenyl, biphenyl, amino, C4, and C8 were used to analyze the method. In addition, the mobile phase composition was modified at each trail to enhance the resolution and to achieve good retention

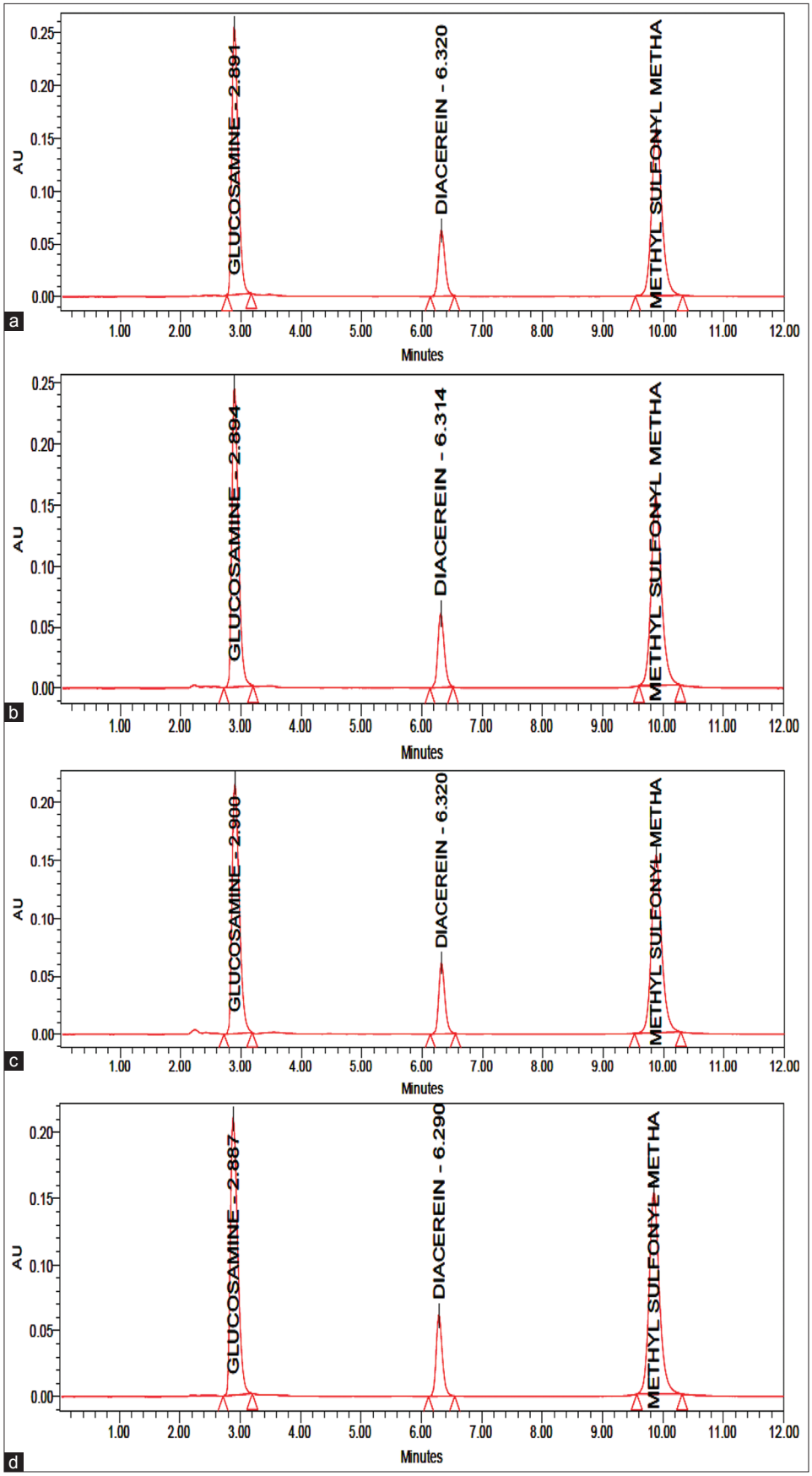

Fig. 10: Chromatograms for method (a) Precision-1, (b) Precision-2, (c) Precision-3, (d) Precision-4, (e) Precision-5, and (f) Precision-6

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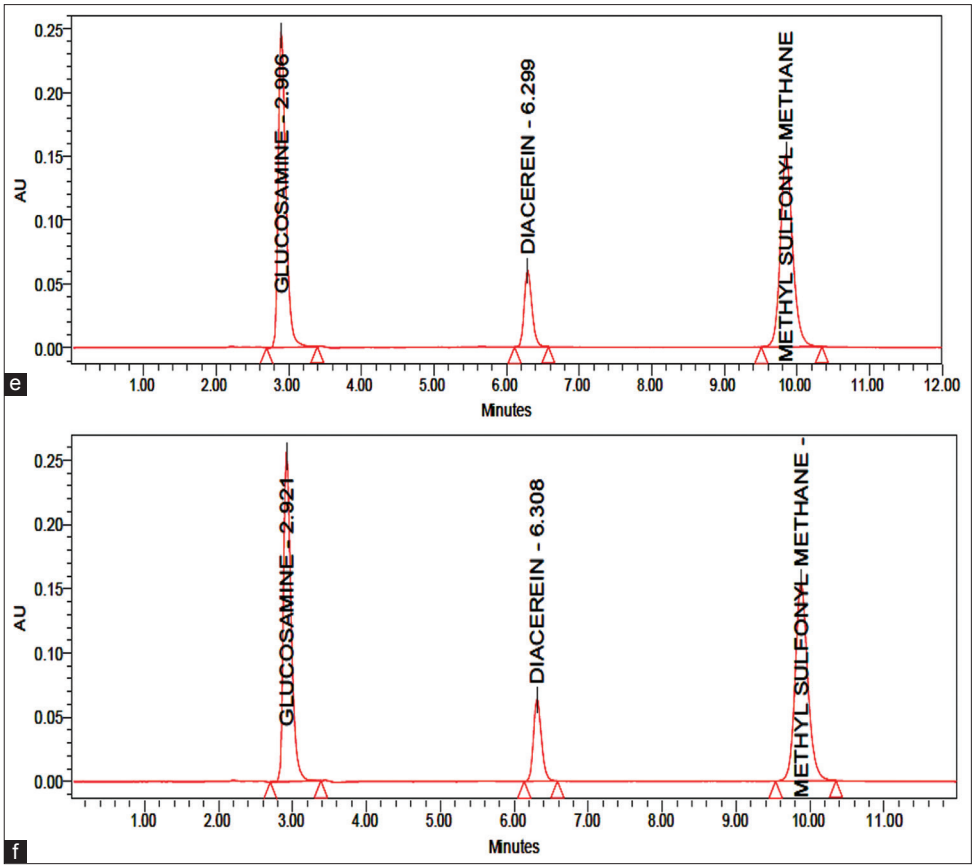

Fig. 10: (*Continued*)

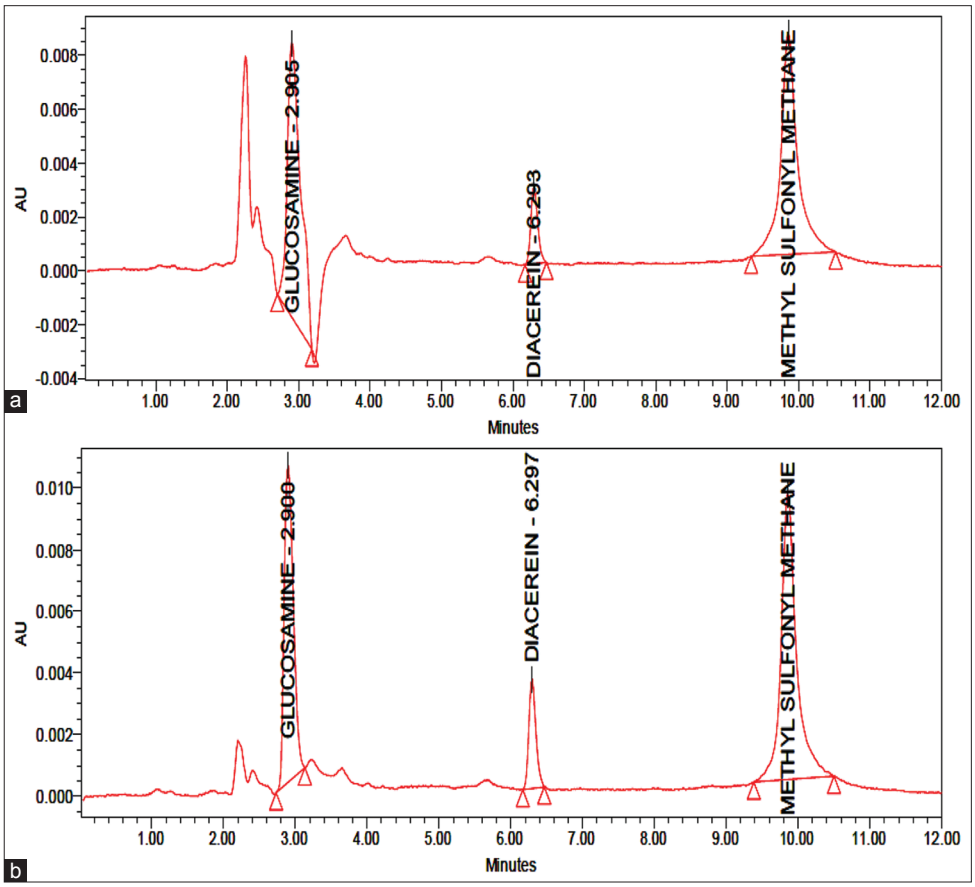

Fig. 11: Chromatograms for (a) limit of detection and (b) limit of quantification

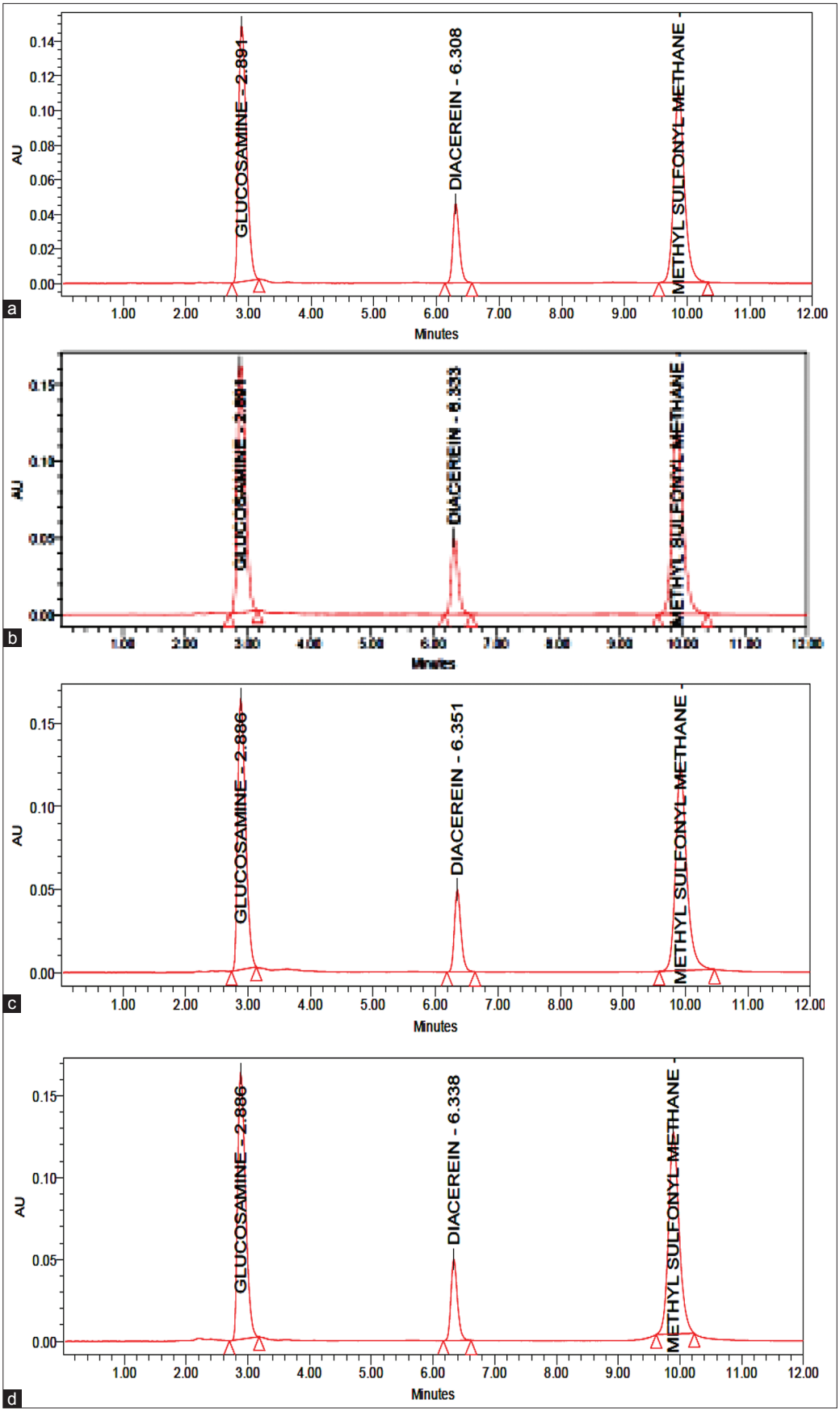

Fig. 12: Chromatograms for (a) acid degradation, (b) alkali degradation, (c) peroxide degradation, (d) reduction degradation, (e) thermal degradation, and (f) photolytic degradation

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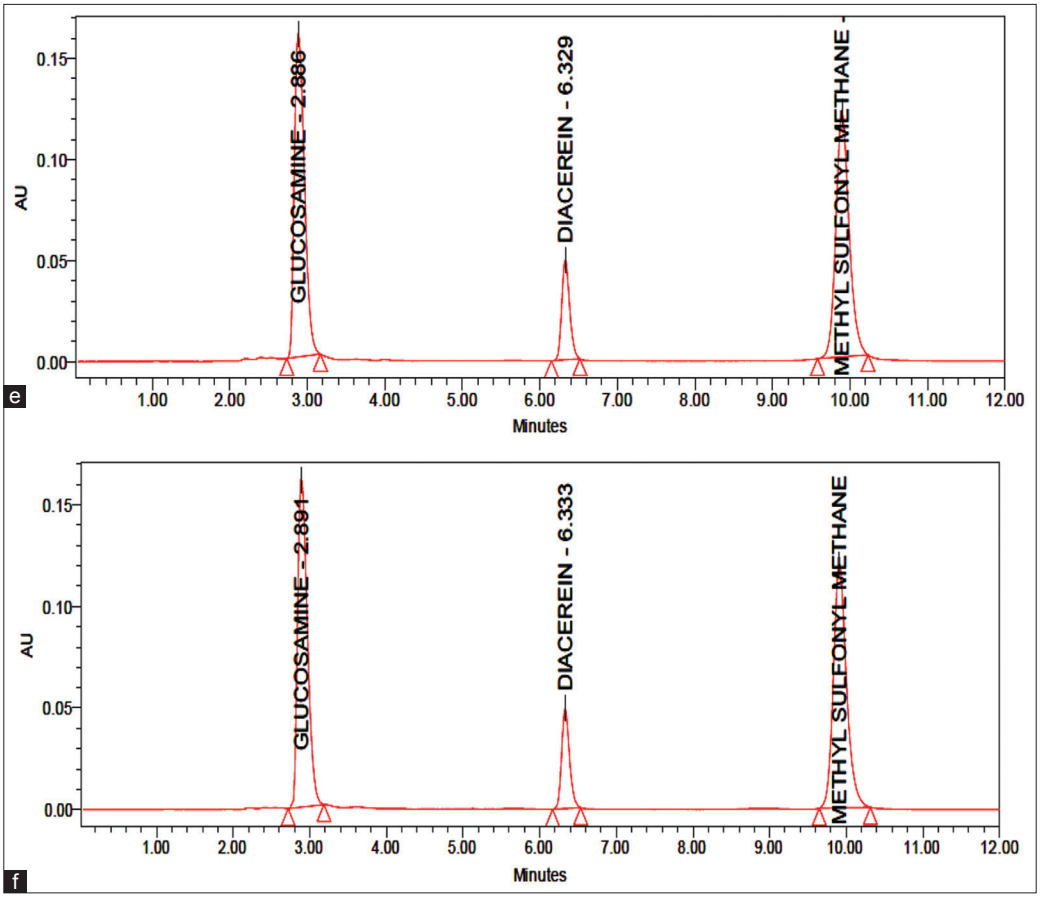

Fig. 12: (*Continued*)

Table 11: Results for pharmacokinetic studies

Time intervals	Glu (ng/ml)	Dia (ng/ml)	MET (ng/ml)
0 h	0.00	0.00	0.00
0.5 h	30.36	38.25	35.47
1.0 h	42.68	45.14	46.32
1.5 h	55.38	58.26	60.32
2.0 h	95.87	93.32	96.21
2.5 h	75.31	70.68	72.47
3.0 h	68.36	60.55	65.32

DIA: Diacerein, GLU: Glucosamine, MET: Methylsulfonylmethane

Table 12: Results for dissolution studies

Time intervals	GLU % Drug release	DIA % Drug release	MET % Drug RELEASE
15 min	32	35	38
30 min	58	60	57
60 min	95	94	96
Recovery	99	98	98

DIA: Diacerein, GLU: Glucosamine, MET: Methylsulfonylmethane

times. Finally, the chromatographic separation was achieved on a phenomena C_{18} column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase composed of 0.1 % orthophosphoric acid:acetonitrile (80:20 v/v) delivered at a flow rate of 1.0 ml/min, and separation was monitored by a UV detector, at a detection wavelength of 285 nm. The total run time was less than 12 min (Fig. 5).

Method validation

The developed method was validated as per the International Council for Harmonization (ICH) guidelines [23-24] with the aspect of system suitability, linearity, and range, precision in terms of % relative standard deviation (RSD), accuracy in terms of % recovery, and robustness study.

Specificity

It is the capability of the method for the accurate and specific measurement of the analyte of interest in the presence of matrix and other components.

Procedure

The solutions of standard, sample, and blank were injected into the system and chromatograms were recorded. There was no interference of the placebo with the principal peak, which indicates that the proposed method was specific. These results are furnished in Fig. 6.

System suitability

System suitability tests were conducted out to check the system functioning before or during the analysis. The parameters such as retention time, theoretical plate number, tailing factor, peak area, and the resolution were monitored for the suitability of the system (Table 1).

Procedure

Six replicate injections of standard solutions were injected and system suitability parameters such as theoretical plate number, time, peak area, tailing factor, and the resolution were calculated. The results are furnished in Fig. 7.

Linearity and range

Linearity is its ability to obtain the test results which are directly proportional to the concentration of the analyte in the sample. This

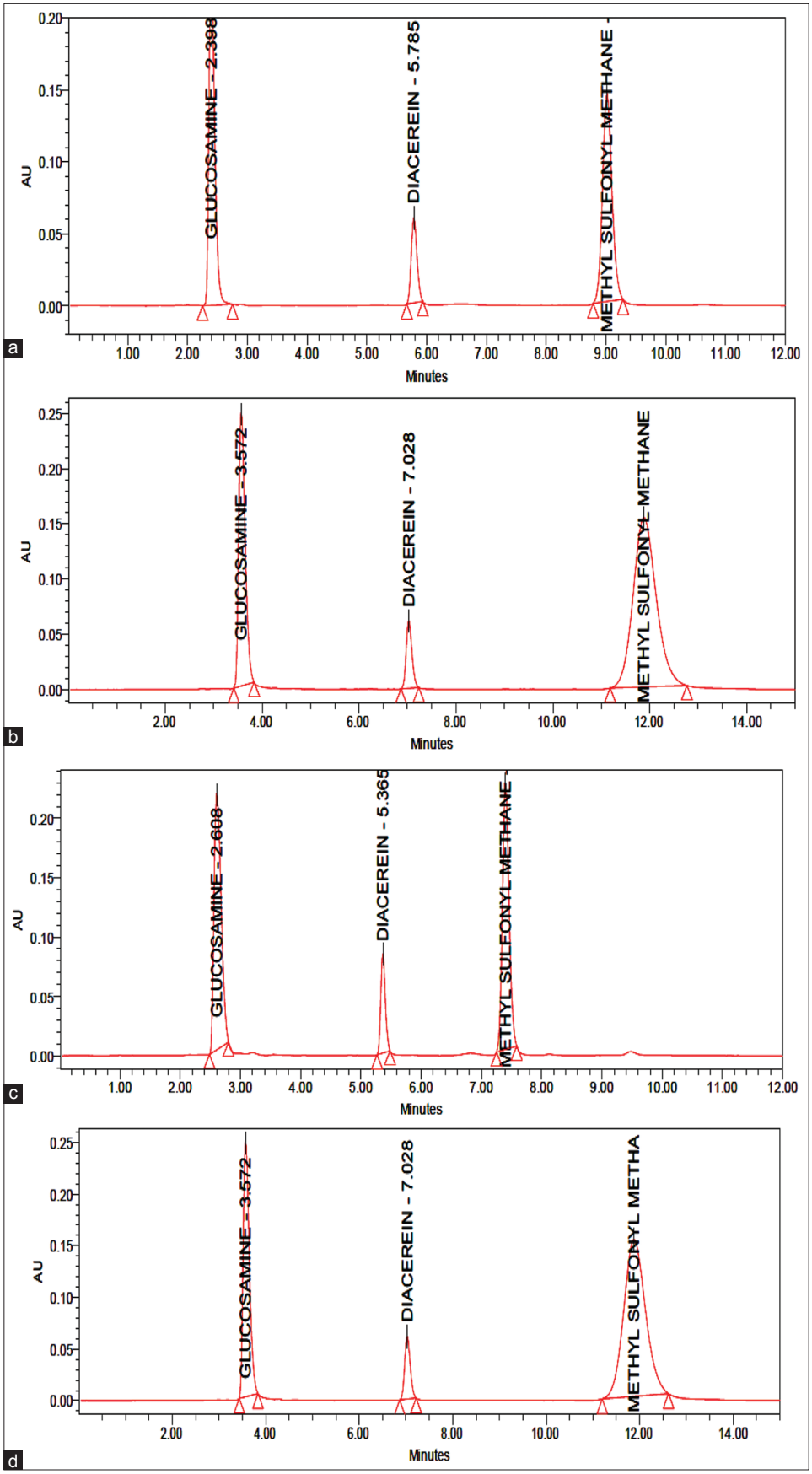

Fig. 13: Chromatogram for (a) flow plus, (b) flow minus, (c) organic plus, (d) organic minus, (e) Wavelength plus, and (f) wavelength minus

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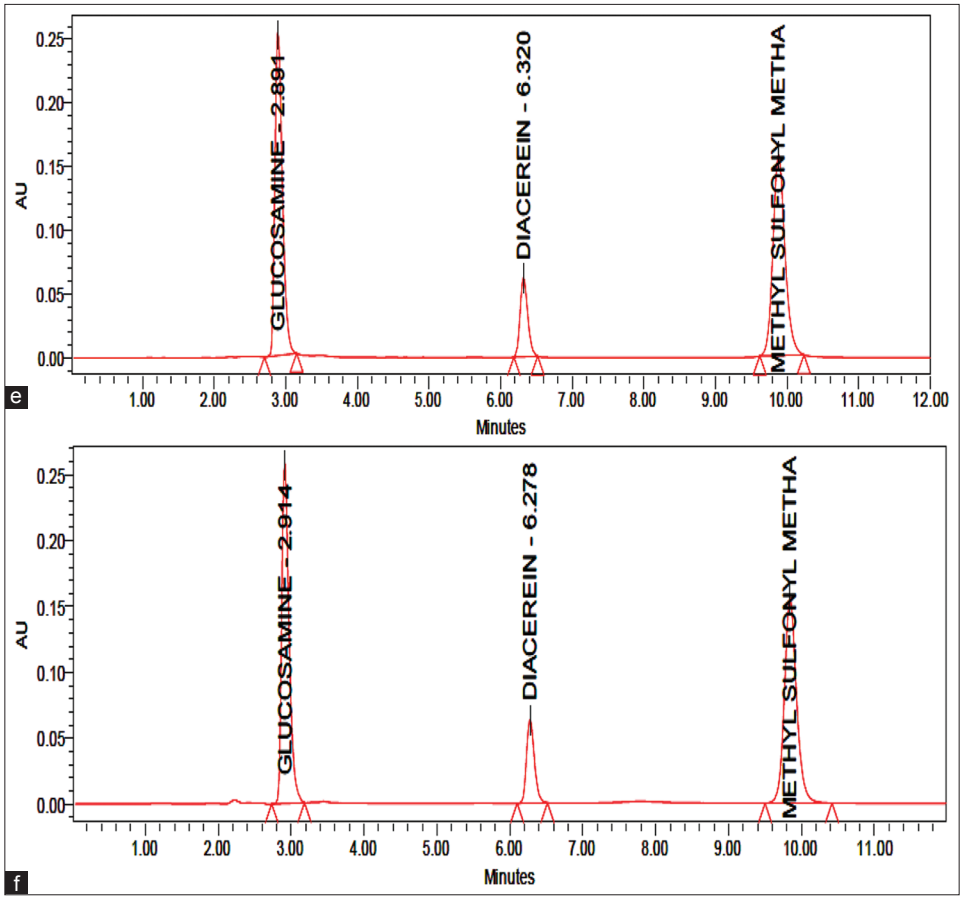

Fig. 13: (*Continued*)

linearity was measured by plotting a calibration curve of peak area against their respective concentration.

Procedure

Six different concentrations of the mixture of DIA, GLU, and MET prepared for this study. The solutions were injected and calibration curves were constructed, showed linear relationship. Peak areas were observed. The SD slope, intercept and the coefficient of variation of the curves were determined. The regression equations for the calibration curve were y=23078x+8841(0.9993) for DIA, y=6055.1x+9171.2 (0.9993) for GLU and y=17982x+22598 (R2=0.999) for MET. The results are furnished in Fig. 8.

Accuracy

Accuracy is in agreement with acceptable true value and actual result. It was determined by calculating the recovery of the drugs at three kinds of concentration levels.

Procedure

The accuracy was measured at three different concentration levels such as 150, 300, and 450 µg/ml of GLU, 10, 20, and 30 µg/ml of DIA and 50, 100, and 150 µg/ml of MET. As per the test method, the test solution was injected 3 times for every spike level and the assay was performed. The recovery the percentage was calculated and results were furnished in Tables 2-4, (Fig. 9).

Precision

The precision of the analytical procedure demonstrates the closeness of an agreement between a series of measurements obtained from multiple sampling of homogeneous samples. It has been demonstrated by repeatability, reproducibility, and intermediate precision.

Procedure

Repeatability was calculated by injecting six replicates of standard solution into the HPLC system. The mean, SD and % RSD of the peak areas were calculated. As per the test method for reproducibility, six samples of the single batch were analyzed. The proposed method has been found to be precise as the percentage of RSD values was found to be less than 2%. Results are furnished in Tables 5-7, (Fig. 10).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD is the smallest the concentration of the analyte which gives the measurable response (3.3 σ/S) and LOQ is the smallest concentration of the analyte which gives accurately quantified response (10 σ/S), where σ is the SD of the response and S is the slope of the calibration plot (Fig. 11).

Procedure

The LOD and LOQ were measured by injecting progressively low concentrations of the standard solutions. LOD and LOQ values of GLU, DIA, and MET were found to be 0.30 and 3.00 µg/ml, 0.002 and 0.20 µg/ml, and 0.10 and 1.00 µg/ml, respectively.

Stress degradation

Active drugs were subjected to different stress conditions according to ICH guidelines Q1A (R2). Stress degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Procedure

As per the ICH guidelines, QA1 (R2) stress degradation conditions such as thermal, acidic, oxidative, hydrolysis, reduction, and photolytic stresses were attempted. There is no interference between the peak and were well separated with the resolution at least 1.0 and the purity of the principal peaks should pass (Fig. 12). The results are furnished in Table 8.

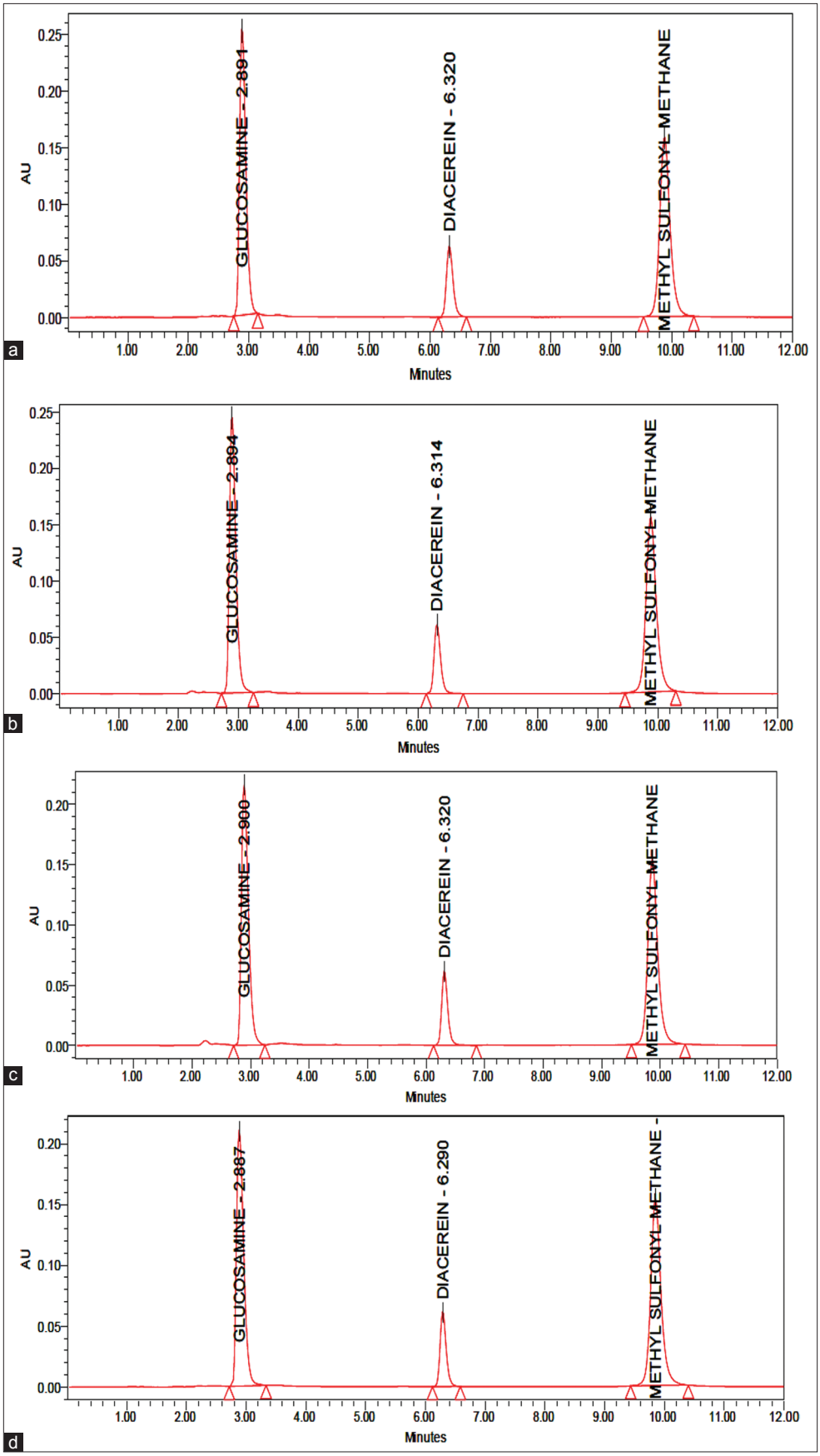

Fig. 14: (a) Chromatogram for stability initial, (b) chromatogram for stability 6 h, (c) chromatogram for stability 12 h, (d) chromatogram for stability 18 h, (e) chromatogram for stability 24 h

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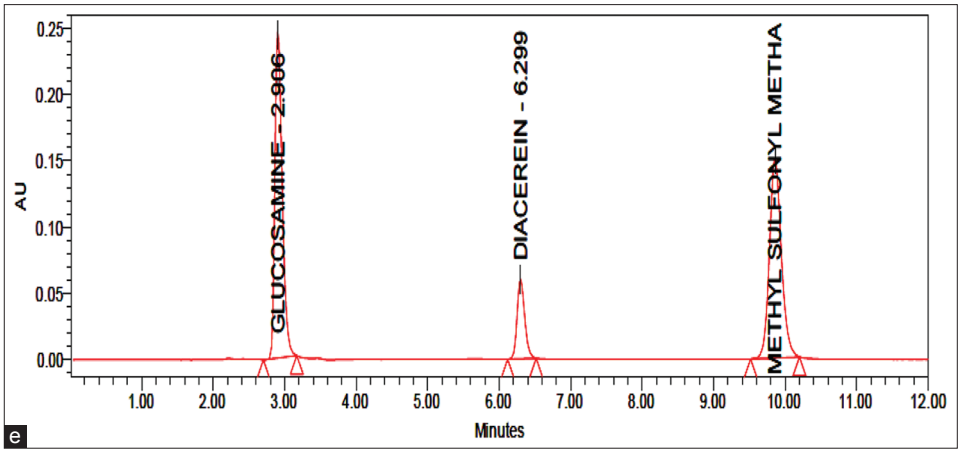

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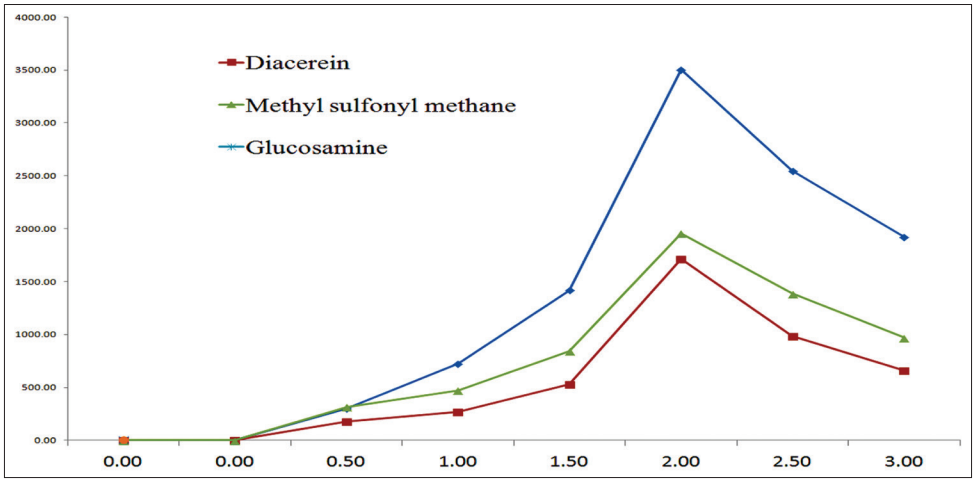

Fig. 15: Diagram for plasma study

Robustness

Deliberate small modifications in the method parameters were done and the results were not influenced by various changes in the method parameters.

Procedure

The small changes in optimized parameters, such as ±0.2 change in flow rate, ±5 change in the mobile phase, and a change of ±5 in wavelength, were done to analyze the robustness of the method. There was no remarkable impact on retention time, plate count, and tailing factor (Fig. 13). The results are furnished in Table 9.

Stability

The standard and the sample solutions were subjected to 24 h stability studies. The stability of these solutions was studied and changes in area and retention time of the peaks were observed. The results were compared with the pattern of the chromatogram of the freshly prepared solution.

Procedure

The sample solutions were analyzed initially to 24 h at laboratory temperature. Retention time and pea area of the drugs were unchanged. No significant degradation was observed within the period sufficient for performing the analytical process and the percentage deviation is not more than 5% (Fig. 14). The results are furnished in Table 10.

Recovery studies in spiked rat plasma samples

The method of liquid-liquid extraction was utilized to isolate GLU, DIA, and MET in rat plasma. Active drug sample was injected into rat body and samples have collected at different time periods such as 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h. Before processing, the plasma samples were kept at –20°C and allowed to thaw at normal temperature. After gentle thawing 100 µl, aliquot plasma sample (respective concentration) was added in polypropylene centrifuge tubes, vortexed briefly and 2.5 ml of acetonitrile was added. The tubes were vortexed for 10 min and then it was centrifuged at 4000 rpm. The supernatant liquid was taken carefully transferred into another conical glass tube and completely evaporated the organic layer at 40°C. After completion of evaporation, these samples were reconstituted with 500 µl of acetonitrile and vortexed for 5 min and then the sample was transferred into autosampler vials for injection. These samples have been injected in developed chromatographic conditions and values were recorded. A 2^{nd} h the sample reaches the maximum result, suddenly down to 3.0 h (Fig. 15). The results are furnished in Table 11.

Dissolution study

The medium of dissolution phosphate buffer (pH 6.8).

Preparation of dissolution media 0.235 M dibasic sodium phosphate was taken and pH adjusted to 6.8 with 0.1 N HCl.

For the dissolution study of GLU, DIA, and MET analysis was performed using the above chromatographic conditions with the aid of a paddle stirrer type of apparatus in 900 ml of pH 6.8 phosphate buffers at a stirring rate of 100 rpm and the temperature was maintained at 37±5°C. Accurately weighed and placed one tablet in each of the six dissolution vessel containing dissolution media. The samples were collected at 30, 60 min, and recovery (150 RPM extra 30 min). The samples were prepared as per assay test concentration and equal volumes (10 µl) of these test solutions were injected into the system with autosampler and peaks areas were measured (Table 12).

CONCLUSION

The suggested method of RP-HPLC is simple, specific, precise, sensitive, cost-effective, and rapid for the simultaneous quantification of GLU, DIA, and MET in bulk and the pharmaceutical dosage forms, and micro-sample of rat plasma using UV detection, to carry out drug dissolution studies from tablets. The validation results were in good agreement with acceptable limits. RSD values which are less than 2.0% indicating the accuracy and precision of this method. The usefulness of the method is that the common chromatographic conditions have been adopted for assay, dissolution, and pharmacokinetic studies. This developed method showed reliable, precise, and accurate results under optimized conditions. Hence, it can be concluded that the proposed method can be used for regular analysis of GLU, DIA, and MET in rat plasma samples.

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AUTHORS' CONTRIBUTIONS

All authors have contributed equally in developing the concept of the study, data collection, data analysis, and drafting the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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