Tropical Journal of Pharmaceutical Research November 2020; 19 (11): 2415-2424
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v19i11.24

Original Research Article

Validated RP-HPLC method for the simultaneous determination of glucosamine sulphate and curcumin in cream formulation: A novel stability-indicating study

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Sent for review: 12 April 2020 *Revised accepted: 15 October 2020*

Abstract

Purpose: *To develop and validate a stability-indicating reverse phase-high performance liquid chromatography (RP-HPLC) method for the simultaneous determination of glucosamine sulphate (GS) and curcumin (Cur) in drug solution and formulation.*

Methods: *The optimized chromatographic conditions were achieved by passing various compositions of mobile phases over different reverse phase chromatographic columns. Various validation parameters, including linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, specificity and system suitability were performed and evaluated. Stability studies under stressed conditions were done to evaluate the effects of acid, alkali, oxidation, heat and degradation by UV light.*

Results: *The validated method was linear over the concentration range of 0.094 to 1.5 mg/mL for GS and 0.125 to 1.5 mg/mL for Cur, with a correlation coefficient > 0.999. The Intra and inter-day precision were 1.9 % for GS and 0.5 % for Cur, while accuracy was 96 and 102 % for GS and Cur, respectively. Stability studies showed that GS was highly sensitive to acid, alkali and oxidation and less sensitive to heat and UV. Cur was stable against acid, heat and oxidation but sensitive to alkali and UV.*

Conclusion: *The developed and validated method was precise and accurate for both GS and Cur and can potentially be utilized for their identification and quantification at industrial, research and quality control laboratories.*

Keywords: *Curcumin, Glucosamine sulphate, Stability, System suitability*

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INTRODUCTION

Pharmaceuticals containing two or more drugs are said to be combination products and these products are proposed to meet patient's needs that were formerly unmet. However, the combination drug products can display overwhelming difficulties to the experts in

developing and validating their analytical methods. A validated method for the simultaneous determination of different ingredients in a drug product could be beneficial for industries and research facilities owing to its cost-effectiveness and to guarantee the identity, purity and potency of the pharmaceuticals [1].

The hexosamine biosynthetic pathway is used to synthesize the glucosamine (amino sugar) in the cell from glucose. Various types of glucosamine e.g. glucosamine hydrochloride, glucosamine sulfate and N-acetyl-glucosamine have beneficial effects in reducing the pain, slow down cartilage loss, and even restore articular cartilage in osteoarthritic patients. It is naturally found in the articular cartilage of mammals. But it is still controversial whether oral administration can repair hyaline cartilage defects [2].

Curcumin, a main ingredient in *Curcuma longa***,** is an important and well-known food spice which is a source of polyphenolic compound, named as curcuminoids [3]. It has been used for various pharmacological actions since ancient times including stopping hemorrhage, against biliary diseases, coryza, anorexia, diabetic wounds, hepatic disorders, sprains, rheumatic disorders and swellings caused by injury. Three important polyphenolic compounds namely curcumin (70 - 77%), desmethoxycurcumin (18 – 20%) and bis-desmethoxycurcumin (5 – 10%) are found in *C. longa*. Various pharmacological effects such as anti-inflammatory, anti-cancer, hypoglycemic and antimicrobial effects are observed in the herb (*C. longa*) [4]. In the past, separate analytical methods for the identification and quantification of GS and Cur have been published. High performance liquid chromatography has been used in different methods to quantify glucosamine sulphate [5,6], high performance thin layer chromatography (HPTLC) using silica gel plates [7], pre-column derivatization in liquid chromatography and detecting by UV Detector [8]. Rani and Devanna (2018) developed HPLC method for instantaneous analysis of diacerein, glucosamine and methyl sulfonyl methane in tablet [9]. In the case of Cur, various methods using thin layer chromatography, thin layer chromatography–densitometric [10], spectrophotometric and electrochemical detection [11], HPLC–UV and LC- Mass Spectrometry [12] and HPLC–UV [13-17] have been reported.

Presently, both GS and Cur have been used in different commercially available formulations like tablets and creams but there is not a single analytical method available for determination of GS and Cur simultaneously in a formulation. Therefore, our aim was to develop a novel, simple, cost effective, reproducible and stability indicating RP-HPLC-UV method for the simultaneous identification and quantification of both compounds in drug solution and cream formulation.

EXPERIMENTAL

Materials

The glucosamine sulphate reference standard (99.8 % purity) was purchased from Pharm herb Co. Limited, (China) and curcumin reference standard (98.0% purity) was purchased from Natural Remedies Pvt. Limited (Bangalore, India). The solvents and chemical reagents utilized throughout the study were of HPLC or analytical grade. Acetonitrile was bought from Fischer Scientific (UK), ortho-phosphoric acid from Merck (Germany), Sodium Hydroxide, Hydrochloric Acid and Potassium Hydroxide was purchased from Sigma-Aldrich (Germany). Hydrogen Peroxide (35 %) was purchased from VWR BDH Polarbo and ultrapure water was obtained from in-house Smart 2 Pure water purification system (Thermo Scientific USA). Other chemicals used during the study were purchased from Sigma-Aldrich, USA.

Instrumentation

The HPLC was Waters Alliance e2695 equipped with automatic sampler and column oven and PDA detector (2998). The Empower® 3 Software (RF-3) was used to acquire all the chromatographic data.

Chromatographic conditions

Reverse phase HPLC analysis was performed on Pursuit XRs-C_{18} (250 × 4.6 mm; 10 µm) column (Agilent, USA) by setting flow rate at 1.0 mL/min, injection volume of 20µL using single wavelength of 195 nm at ambient temperature.

Column optimization

Both compounds are of different nature, as glucosamine sulphate is hydrophilic, and curcumin is hydrophobic. It was a bit difficult to elute both compounds together using a single column. So, in this regard, different columns as Tracer Excel 120 CN (150 × 4.6 mm, 5 µm), Waters Spherisorb 5 µm NH2 (150 × 4.6 mm), Waters Spherisorb C8 (150 × 4.6 mm, 5 µm), Phenomenex Luna C18 (150 × 4.6 mm, 5 µm) and Agilent (Pursuit XRs-C18 (250 × 4.6 mm, 10 µm) having varying stationary phases were tried.

Mobile phase optimization

Different mobile phase compositions were passed over the selected column to observe the response of analytes and to achieve good resolution and appropriate elution. The filtered (through 0.45 µm Nylon Millipore Filters (HNWP04700) and degassed mobile phase was used prior to liquid chromatography (LC).

Preparation of stock and working standard solutions

The standard working solutions were separately prepared by pre-dissolving 15 mg GS and 25 mg Cur reference standards in 27.5 mL of buffer solution (pH 3.0) and 22.5 mL acetonitrile respectively in separate volumetric flasks of 50ml. The volumetric flasks were shaken using ultrasonic vibrator for 5 min and mixed together and made up to volume of 50ml with mobile phase. The standard stock solutions had concentrations of 0.3 mg/mL of GS and 0.5 mg/mL of Cur.

System suitability studies

System suitability parameters were performed by injecting six injections of a working standard. Parameters such as tailing factor (T), theoretical plate count (N), resolution (Rs) and coefficient of variation or relative standard deviation (%RSD) were determined [18].

Limit of detection (LOD)

The lowest concentration (amount) of the standard solution was determined from the linearity study by using the following formula:

$LOD = (\sigma/s) \times 3.3$

Where σ = Standard deviation of residuals or standard error and S= slope of the calibration curve

Lower limit of quantification (LLOQ)

The minimum amount of analyte which can be quantified with precision and accuracy in a given sample is called quantification limit. In the standard calibration curve, the LLOQ was the lowest point of concentration. The acceptance criteria were % bias of 2% for accuracy and RSD of 2% for precision.

Specificity

Specificity of a method is a measure of how efficient a method is in determining the analyte of interest in the presence of excipients and/or other analytes present in the sample. Diluent blank, which is actually the mobile phase was run over the column and compared it with GS and Cur standards as well as against the placebo (Cream base having no active ingredient).

Preparation of cream formulation

Paraffin oil and white soft paraffin were homogenized in a glass beaker using a hot plate magnetic stirrer. Cetosteryl alcohol and propyl paraben were added in oil phase. In oil phase Cur was added and thoroughly mixed until dissolved completely. Propylene glycol, citric acid, sodium citrate and methyl paraben were dissolved in distilled water with the aid of hot plate magnetic stirrer.

GS was added in water phase. Temperature of both phases were set to 55 °C. The two phases were homogenized for 15 min by adding water in oil phase through high speed ultra-turrax (IKA T 25 digital Blade-type Homogenizer). After homogenization, the beaker was removed from the mixing and stirring was continued manually with the help of spatula until a cream of smooth consistency was obtained.

Assay of glucosamine sulphate and curcumin in drug solution

Standard solutions of GS and Cur were prepared separately by pre-dissolving the 15 mg GS and 25 mg Cur reference standards in about 27.5 mL of buffer solution (pH 3.0) and 22.5 mL acetonitrile respectively in separate volumetric flasks. Both flasks were sonicated for five minutes and the solutions were transferred into 50 mL volumetric flask and made the volume up to the mark with the mobile phase if required. Final concentration of the standard solutions prepared were 0.3 mg/mL of GS and 0.5 mg/mL of Cur. Similarly, 15 mg of glucosamine sulphate powder and 25 mg of *C. longa* extract powder was weighed accurately and dissolved in the same manner as for standard solution. The % assay of GS and Cur is calculated by the following equation.

$$\% \text{ Assay} = (R_u/R_{std}) \times (C_{std}/C_u) \times P \quad \text{..... (1)}$$

Where, R_u = Peak response of GS (or Cur) in drug solution; R_{std}. = Peak response of GS (or Cur) in standard solution; C_{std} = Standard concentration of GS (or Cur) (mg/ml); C_u= Concentration of GS (or Cur) in Drug solution (mg/ml); P= Potency of GS (or Cur) Standard.

Assay of glucosamine sulphate and curcumin in cream

Similar procedure was adopted as mentioned above for the preparation of standard stock solutions of GS and Cur with same concentrations. One gram of cream equivalent to 15 mg of GS and 25 mg of Cur was taken in 50 mL screw capped glass centrifuge tube. After the addition of 10 mL acetonitrile, the tube was tightly capped and heated in circulating water bath. While heating, the intermittent shaking was done for proper dissipation of heat until the matrix was melted. The glass tube was then removed from the water bath and shaken vigorously until the specimen solidified. The whole process of melting and solidifying was repeated thrice before the final volume was made-up to 50 mL with mobile phase. The glass tube was placed in ice-methanol bath for about 10 minutes and the sample was filtered using 0.45-micron nylon syringe filter and transferred into HPLC-vials for analysis. The % assay of GS and Cur in cream formulation is calculated by the following equation:

$$\% \text{ Assay} = (R_{smp}/R_{std}) \times (C_{\cdot std}/C_{\cdot smp}) \times P \quad ..... (2)$$

Where, R_{smp} = Peak response of GS (or Cur) in sample solution; R_{std}. = Peak response of GS (or Cur) in standard solution; $C_{\cdot std}$ = Standard concentration of GS (or Cur) (mg/ml); $C_{\cdot smp.}$= Concentration of GS (or Cur) in sample solution (mg/ml); P= Potency of GS (or Cur) Standard.

Forced degradation studies

A perfect stability indicating technique is one that not only evaluate the standard drug but also has the capability to resolve its impurities or degraded products formed during reaction [19]. Stress degradation study of GS and Cur in drug solution was performed. The concentrations of GS and Cur in standard stock solutions were 0.3 mg/mL and 0.5 mg/mL respectively.

Alkali and acid degradation study

Standard stock solutions (5.0 mL) of GS and Cur were added into two 25 mL volumetric flasks. In each of the flasks 1 mL of 3 molar NaOH (sodium hydroxide) and 3 molar HCl (Hydrochloric acid) were added separately. The solutions in first set of flasks (zero-hour sample) were neutralized immediately and volume was adjusted with mobile phase. In second set of flasks, 1 mL of 3 molar HCl and 3 molar NaOH was added separately and put on the bench for 24 hours at room temperature (26 °C/65% RH) and marked as 24-hour sample. The solutions were then neutralized and made up to volume with mobile phase. The treated solutions passed through a 0.45-micron nylon syringe filter and injected in triplicate.

Oxidative degradation study

Standard stock solutions (5.0 mL) of GS and Cur were added into two 25 mL volumetric flasks. In each flask 0.344 mL of 35% H_2O_2 (hydrogen peroxide) was added. The volume of the first flask was adjusted immediately with mobile phase and marked the zero-hour sample, While the second flask (24 h sample) was left on the bench for 24 hours at room temperature and volume was adjusted with mobile phase. The final solution was passed through a 0.45-micron nylon syringe filter and injected in triplicate.

Heat degradation study

Stock solutions (5.0 mL) of GS and Cur were transferred into two 25 mL volumetric flasks. The volume of the first flask was adjusted with mobile phase and termed as the zero-hour sample. The second flask was placed in a water bath and heated to 80°C for 2 h and adjusted to volume with mobile phase after cooling to room temperature. The final solution was passed through a 0.45-micron syringe filter and injected in triplicates.

UV light degradation

Standard stock solutions (5.0 mL) of GS and Cur were transferred into two 25 mL volumetric flasks. The solution filled to the mark in first flask with mobile phase and termed as the zero-hour sample. The other flask was placed in a UV cabinet (366 nm) for 24 h and the solution adjusted to 25 mL with mobile phase. Both samples were injected in triplicate.

Evaluation of linearity

Concentrations of 0.125, 0.25, 0.5, 0.75 and 1.0 mg/mL of GS and Cur were prepared respectively in mobile phase. The calibration curve of standard was constructed using known concentrations of GS and Cur versus peak area. The linearity and concentration of sample was determined using regression line. The linearity correlation coefficient (r^2) should be equal to or near to 1.0 as an indication of linear response.

Assessment of precision and accuracy

Repeatability and intermediate precision were calculated. Repeatability was assessed by performing the analysis of six replicates at 100%

of the test concentration (0.5 mg/mL) of GS and Cur. Same procedure of analysis was adopted for determination of intermediate precision of developed analytical procedure for different days and on different instruments. For accuracy, one concentration (0.5 mg/mL each) of GS and Cur was used to determine the accuracy. Six fresh replicates were prepared and analyzed. After that, test concentration was spiked to 50%, 100% and 150% concentration of reference material to calculate the accuracy. For precision, the coefficient of variation (% RSD) was calculated while the accuracy was determined as %bias (relative percentage error).

Stock solution stability study

Stock solution of GS and Cur having concentration of 0.5 mg/mL each was kept at room temperature (26 ºC) for 24 h. The comparison of 24 h sample with fresh samples (zero hour) made through instrumental response.

Determination of recovery of glucosamine sulphate and curcumin

The developed validated method applied for the quantitative determination of both GS and Cur in a cream formulation. Standard solutions of GS and Cur at concentrations of 0.3 mg/mL and 0.5 mg/mL respectively were prepared as well as sample solution of cream that was prepared to match the concentrations for both APIs. Three replicates of the sample solution of cream and standard solutions were analyzed.

RESULTS

Selected chromatographic column

Initially, different trials were performed by selecting a mobile phase of potassium dihydrogen phosphate buffer (0.25 M KH_2 PO_4) and acetonitrile in a ratio of 70:30 % v/v respectively for the sequential analysis of GS and Cur. Mobile phase composition was kept constant because of the nature (polarity) of both analytes. As GS is hydrophilic in nature, it is eluted first because of the higher ratio of buffer with more affinity towards mobile phase, whereas elution of the Cur is delayed due to its hydrophobic nature. By using the same mobile phase over different chromatographic columns, it was observed that comparatively better elution was attained on Agilent (Pursuit XRs) C18 column.

Optimized mobile phase

Different mobile phase compositions were passed over the selected column -Agilent (Pursuit XRs-C18 (250 × 4.6 mm, 10µm) to observe the response of analytes. Relatively good separation with minimum peak splitting for both compounds were observed against the mobile phase (7.5mM phosphoric acid (H_3PO_4) and acetonitrile in a ratio of 55:45 % V/V, where the peak symmetry and resolution was relatively better. The retention time of the GS and Cur were 2.6 and 23.6 mins respectively. The response of both analytes in different mobile phases is summarized in Table 1 and chromatogram is shown in Figure 1.

System suitability studies

System suitability parameters like tailing factor (T), theoretical plates (N) and % RSD were determined by injecting five replicates of the standard preparation for the concentration of 0.5 mg/mL for both analytes. Tailing factor was found to be less than 2.0, theoretical plates was more than 2000 and % RSD of peak was less than 2.0% for both GS and Cur as shown in Table 2.

Table 1: Selection of mobile phase and observations

Mobile phase	Flow rate (ml/min)	pH	Observation
0.25 M KH_2PO_4: ACN (70:30)	1	3	Peak Splitting in GS, Chromatography of Cur improved.
0.25 M (NH_4) H_2PO_4: ACN (70:30)	1	3	Poor separation, No peak symmetry.
7.5mM phosphoric acid(H_3PO_4): ACN (70:30)	1	3	Peak splitting, Good separation of curcumin, High Retention Time.
7.5mM phosphoric acid (H_3PO_4): ACN (65:35)	1	3	High Retention time, Peak splitting
7.5mM phosphoric acid (H_3PO_4): ACN (60:40)	1	3	Peak splitting for GS, Good separation of Cur, High Retention Time.
7.5mM phosphoric acid (H_3PO_4): ACN (55:45)	1	3	Separation of GS and Cur improved.
7.5mM phosphoric acid (H_3PO_4): ACN (50:50)	1	3	Peak Splitting for GS, peak tailing observed for Cur.
7.5mM phosphoric acid (H_3PO_4): ACN (40:60)	1	3	Peak Splitting for GS, poor separation of Cur.

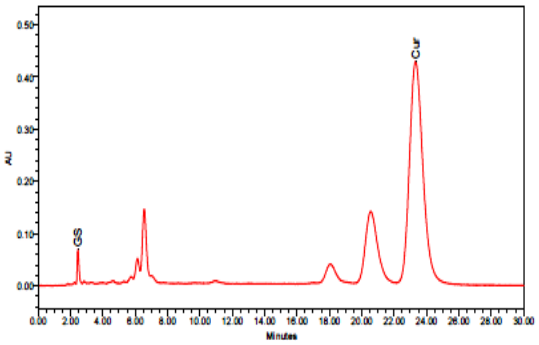

Figure 1: Chromatograms of GS and Cur obtained for mobile phase 7.5 mM phosphoric acid (H_3PO_4) and acetonitrile in a ratio of 55:45 at a flow rate 1.0 mL/min

System suitability values were within the limits mentioned in United States of Pharmacopeia and indicates the method is appropriate for the analysis of both analytes [18].

LOD of glucosamine sulphate and curcumin

The limit of detection was evaluated at different concentrations of GS and Cur. The entire data was reviewed statistically and the detection limit was determined to be 0.031 mg/mL for GS and 0.041 mg/mL for Cur.

LLOQ of glucosamine sulphate and curcumin

0.094 mg/mL and 0.125 mg/mL was the lowest amount of GS and Cur that can be quantified from this method. Variation in results and system suitability parameters were observed below this limit.

Specificity

Reference standards of GS and Cur were run by applying the method and it was observed that mobile phase did not interfere with the peaks of both standards in drug solution and in cream base (Placebo). Chromatograms of GS and Cur standard are shown in the Figures 2 and 3. There was no peak found at the retention times of the analytes in the blank (Figure 4). The placebo chromatogram is presented in Figure 5 and chromatogram of cream is shown in Figure 6.

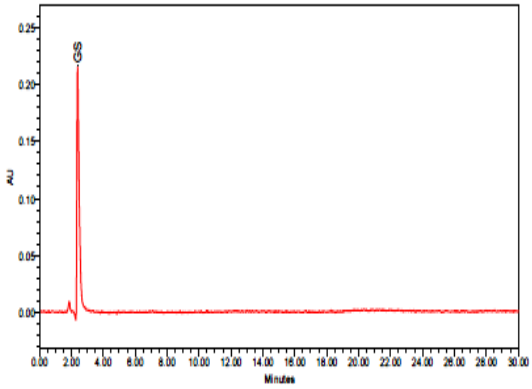

Figure 2: Chromatogram of GS standard at a flow rate of 1mL/min

Table 2: System suitability report

System suitability data							
S/no.	**Sample Name**	**Inj.**	**RT**	**Area**	**Tailing**	**K Prime**	**Plate Count**
1	Cur	1	23.18	21524274	1.06	10.627	4123.101
2	Cur	2	23.22	21815647	1.119	10.415	4265.414
3	Cur	3	23.11	21915974	1.074	10.657	4139.628
4	Cur	4	23.17	21933794	1.105	10.600	4134.346
5	Cur	5	23.2	22185568	1.095	10.587	4067.876
Mean			23.18	21875051.4	1.091		
Std. Dev.			0.042	238809.3268	0.024		
%RSD			0.179	1.091697214	2.172		
S/no.	**Sample Name**	**Inj.**	**RT**	**Area**	**Tailing**	**K Prime**	**Plate Count**
1	GS	1	2.659	101114	1.187	0.329	2659.96
2	GS	2	2.681	102756	1.098	0.516	2578.636
3	GS	3	2.597	103455	1.148	0.364	2668.791
4	GS	4	2.633	102146	1.145	0.412	2589.549
5	GS	5	2.645	103198	1.13	0.364	2681.563
Mean			2.643	102533.8	1.142		
Std. Dev.			0.031	936.1309737	0.032		
%RSD			1.184	0.912997444	2.821		

S/no. = serial number, Inj = injection, RT = retention time

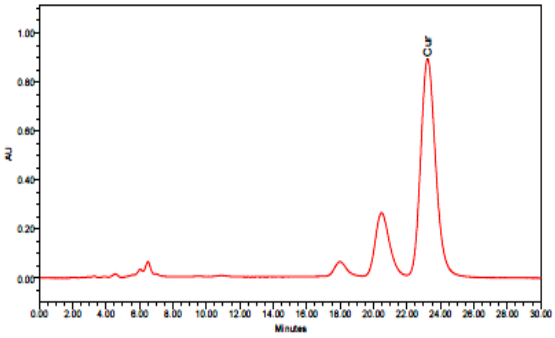

Figure 3: Chromatogram of Cur standard at a flow rate of 1 mL/min

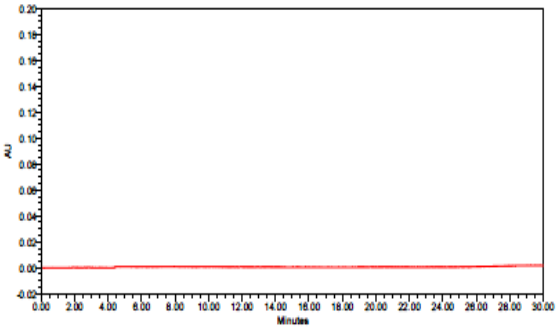

Figure 4: Chromatogram of diluent blank.

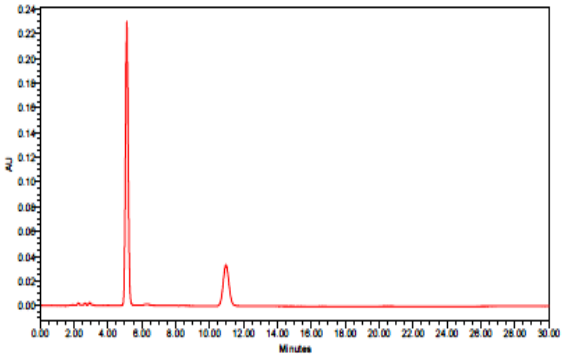

Figure 5: Chromatogram of placebo in cream formulation

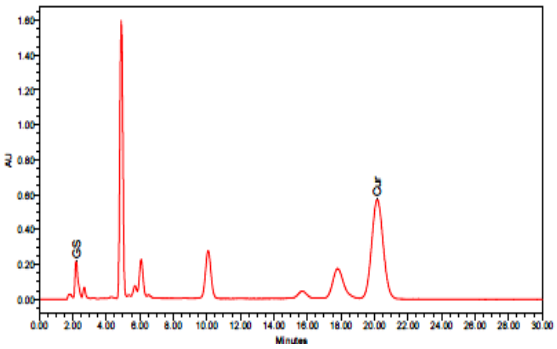

Figure 6: Chromatogram of cream sample

Forced degradation data

A perfect stability-indicating technique is one that not only evaluate the standard drug but also has the capability to resolve its impurities and degraded products [20]. The results of degradations caused by acid, alkali, oxidation, heat, oxidation and UV light are shown in Table 3.

GS degraded in the acid and alkaline medium as well as under oxidative stress. Under stressed conditions a new product is formed that merged with the peak of GS and resulted in the increase in the peak area. Cur has shown degradation in the acid and alkaline medium but to a very little extent. GS as well as Cur were stable against the heat for 2 h. Mild heat did not cause the structural modifications in the molecules. GS was stable against the UV radiation whereas the Cur showed a high rate of degradation in the UV radiations in 24 h exposure.

Linearity

The standard calibration curve exhibited an excellent linearity and a good correlation coefficient over a given range of 0.125 – 1.0 mg/mL with a correlation of 0.9997 and 0.9995 for GS and Cur respectively.

Table 3: Results of stress degradation studies of glucosamine sulphate and curcumin in cream formulation (mean ± SD, N = 3)

Parameter (%)	Glucosamine sulphate			Curcumin		
	0 h	24 h	2 h	0 h	24 h	2 h
Acid degradation (%)	98.96	Peak of degraded product merged with the peak of GS	-	96.74	96.81	-
Alkali degradation (%)	100.67	Peak of degraded product merged with the peak of GS	-	96.00	81.79	-
Oxidative degradation (%)	98.99	Peak of degraded product merged with the peak of GS	-	99.66	97.43	-
Heat degradation (%)	99.32	-	96.44	98.76	-	103.05
UV degradation (%)	100.19	104.56	-	99.74	61.25	-

Precision and accuracy

The repeatability results were expressed as % relative standard deviation (coefficient of variation) and it was observed as 0.6959 % for GS and 0.4249 % for Cur which were found less than the specified limit (the value of % RSD should be less than 2.0). In case of intermediate precision, the observed value for GS and Cur were 1.969 % and 0.5081 %, respectively. Both the statistical bias and practical bias of the stated method is within performance criteria, i.e. ± 2% (USP 39 [18]). The method indicates good precision and accuracy. Results of accuracy of GS and Cur are given in Tables 4 and 5.

Stock solution stability

The concentration of GS and Cur was 99.36 and 98.65 %, respectively, after keeping at room temperature for 24 h. Stock solution was found stable for 24 hours at room temperature after evaluating the results.

Recovery of glucosamine sulphate and curcumin in cream

The recovery determined by applying the developed method for GS and Cur was 94.12 and 100.33 %, respectively, from the cream formulation. Results of recovery for both compounds are presented in Table 6.

DISCUSSION

Different analytical techniques as UV Spectrophotometry, HPTLC, HPLC, LC-MS/MS for the identification and quantification of Glucosamine Sulphate and Curcumin in separate have been presented in literature. Present study develops and validate an innovative, specific, precise, suitable and stability indicating method of analysis for the simultaneous determination of both GS and Cur using RP-HPLC. Both ingredients (GS and Cur) were eluted on Waters Alliance HPLC system (e2695) by passing an isocratic mobile phase (mixture of 7.5mM phosphoric acid (H_3PO_4) and acetonitrile in a ratio of 45:55% v/v) over C18 column. The retention time (RT) of GS and Cur were 2.6 and 23.6 min respectively (fig. 1). RP-HPLC method was established by exploring the effects of equipment settings, different chromatographic columns and various mobile phase compositions. Mobile phase in a ratio of 55:45 (7.5 mM phosphoric acid and acetonitrile, respectively) exhibited better response regarding peak shape and peak area and is suitable for analysis as it has less organic component (acetonitrile) and more proportion of buffer.

Table 4: Accuracy of glucosamine sulphate

Replicate	Conc. Det. (mg/ml)	S - US (mg/ml)	Mean S-US (mg/ml)	Added amount (mg)	SD	%RSD (Precision bias)	Practical Bias	Statistical bias
D1-50%	0.74098	0.24098	0.2423	0.25mg	0.0019	0.7731	0.00769038	0.935
D2-50%	0.74363	0.24363						
D1-100%	0.98199	0.48199	0.4789	0.5mg	0.0044	0.9276	0.02114709	0.783
D2-100%	0.97571	0.47571						
D1-150%	1.227	0.72702	0.7262	0.75mg	0.0011	0.1542	0.02376681	0.783
D2-150%	1.22544	0.72544						

*D = Day; *Det. = Determined; *S-US = Spiked - Unspiked

Table 5: Accuracy of curcumin

Replicate	Conc. Det. (mg/ml)	S – US (mg/ml)	Mean S-US mg/ml)	Added amount (mg)	SD	%RSD (Precision bias)	Practical bias	Statistical bias
D1-50%	0.7562	0.2562	0.2551	0.25mg	0.0014	0.5626	-0.005	0.935
D2-50%	0.7541	0.2541						
D1-100%	1.0184	0.5184	0.5116	0.5mg	0.0097	1.8995	-0.0116	0.783
D2-100%	1.0047	0.5047						
D1-150%	1.2811	0.7811	0.7697	0.75mg	0.0162	2.0990	-0.0197	0.783
D2-150%	1.2583	0.7583						

*D= Day: *Det. = Determined: *S-US = Spiked – Unspiked

Table 6: Recovery results for glucosamine sulphate and curcumin

Analyte	Added amount (mg/g)	Recovery (%)	Uncertainty	Potency
Glucosamine sulphate	3.0	94.12%	2.4	2.8236 mg/g
Curcumin	5.0	100.33%	0.83	5.0165mg/g

System suitability studies indicated that the selected method is specific, precise, accurate and is suitable to use for the identification and quantification of both analytes simultaneously. Tailing factor was < 2.0, theoretical plates were more than 2000 and relative standard deviation of area were also < 2.0 % for both ingredients and meet the general guidelines of system suitability in USP 39-NF 34 [18].

Stress degradation results showed that GS was stable against UV light and heat but shows degradation when exposed to acid, alkali and oxidative stress. Whereas, Cur remains stable in acid, heat and oxidation but shown slight degradation in alkali and UV media. The excipients such as span 80 or tween 80 might react under stressed conditions with the APIs and form secondary products. These newly formed secondary products might interact with GS especially and to some extent with Cur altering their concentration in the sample. However, further studies are required for the characterization of the degradants formed especially of GS after exposure to acid, alkali and heat.

CONCLUSION

The method evaluated in this study is a simple, novel and cost-effective stability indicating RP-HPLC-UV method. It is precise and accurate for the concurrent determination of both GS and Cur at a single wavelength in drug solution and cream formulation.

DECLARATIONS

Acknowledgement

The authors thank Higher Education Commission (HEC), Pakistan for a grant (Project no. 5664/Punjab/NRPU/R&D/HEC/2016).

Conflict of interest

No conflict of interest is associated in this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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