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Research Article

QUANTIFICATION OF HYALURONIC ACID AND METHYLSULFONYLMETHANE IN DIETARY SUPPLEMENTS

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ABSTRACT

Objective: Osteoarthritis can be treated by taking oral supplements containing compounds that can nourish bones and joints such as hyaluronic acid, methylsulfonylmethane (MSM), chondroitin, glucosamine, and collagen. This study aimed to develop and validate tests for analyzing two compounds, namely, hyaluronic acid and MSM, simultaneously and to determine both their levels in a mixed sample.

Methods: Hyaluronic acid derivatization was carried out using fluorenylmethyloxycarbonyl chloride and then analyzed by liquid chromatography with fluorescence detection, while MSM was analyzed using gas chromatography. After the development of optimal conditions for each separation, system suitability tests were developed and calibration curves used for tests of accuracy and precision as well as for level determination. Hyaluronic was detected at an excitation wavelength of 255 nm and emission wavelength of 330 nm. The mobile phase used was acetonitrile-acetate pH 4.2 (1: 4) with a flow rate of 1.0 mL/min.

Results: The developed method was linear (r=0.9983) in the range of 5–50 ppm and the limits of detection (LOD) and quantitation (LOQ) were 3.55 and 11.84 ppm, respectively. The initial column temperature for MSM analysis was 110°C and the mobile phase used was nitrogen gas at a flow rate of 0.8 mL/min. The method was linear (r=0.9998) in the range of 4000–15,000 ppm and the LOD and LOQ were 332.90 and 1109.67 ppm, respectively.

Conclusion: A simulated sample containing both compounds was assessed to contained 98.63% hyaluronic acid and 99.35% MSM.

Keywords: Hyaluronic acid, Methylsulfonylmethane, High-performance liquid chromatography, Gas chromatography, Fluorenylmethyloxycarbonyl chloride, Component, Optimization, Validation.

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INTRODUCTION

Food supplements are products that contain nutrients and excipients and are available as capsules, tablets, powders, or liquids; they are used in cases where dietary insufficiency of their ingredients can lead to ill health [1]. Food supplements are only complementary, and not intended to be used as substitutes for a normal diet. These supplements usually contain chemical compounds that function as nutrients in the body and they can aid in supplying the specific nutritional needs of those with certain health conditions. For example, protein supplementation is used to prevent joint pain and help tighten the skin [2].

Two compounds that are often used in supplements to prevent these conditions are hyaluronic acid and methylsulfonylmethane (MSM), both of which can nourish bones and joints [1]. There are a number such supplement products sold to treat the symptoms of osteoarthritis and these usually contain glucosamine sulfate, chondroitin sulfate, MSM, Celadrin, nattokinase, hyaluronic acid, black catechu, skullcap, *Boswellia serrata,* and curcumin [3]. This study developed analytical methods for only hyaluronic acid and MSM because separation all these components require high-performance gradient liquid chromatography, while only isocratic high-performance liquid chromatography (HPLC) was available.

Hyaluronic acid exists in various molecular sizes in biological tissues and fluids and can be degraded by both normal physiological or pathological conditions [4]. It consists of linear macromolecular chains of repeated glucuronic acid and N-acetyl-D-glucosamine subunits that form glucosamine when hydrolyzed [5]. Hyaluronic acid molecules of different weights have different rheological and biological properties, and this, coupled to its intrinsic biocompatibility and viscoelastic physical character, means that it has been used as a treatment for osteoarthritis [6]. Thus, hyaluronic acid is commonly used as a supplement to maintain joint health and to control tissue hydration [7].

Methylsulfonylmethane is an organosulfur compound that is commonly used as a food supplement for the prevention of metabolic diseases since the amount of MSM and sulfur in the body decreases with age [8].

Hyaluronic acid does not contain any chromophores but can be derivatized with fluorescent compounds. Reagents that are often used for such derivatization before analysis include orthophtalaldehyde, phenyl isothiocyanate (PITC), and 9-fluorenylmetoxycarbonyl chloride [3].

Hyaluronic acid has previously been analyzed are using high-performance size-exclusion liquid chromatography [9], while a study using HPLC conducted by Harmita (2015) used a silica phase with phosphoric and potassium hydroxide in conjunction with UV detectors. Methylsulfonylmethane can be analyzed by gas chromatography (GC), with the 39th edition of the United States Pharmacopeia Edition 39 providing a method using dimethylpolysiloxane gum columns with a mobile phase of helium gas and flame ionization detection.

Here, we conducted an analysis of a mixture of hyaluronic acid and methylsulfonylmethane using HPLC in dimethyl sulfoxide with fluorescence detection for hyaluronic acid, and GC with flame ionization detection for methylsulfonylmethane analysis. Hyaluronic acid was derivatized with 9-fluoroenylmetoxycarbonil chloride, which was chosen because it can react with primary and secondary amine groups.

MATERIALS AND METHODS

Materials

Chemicals were purchased from the following suppliers: Hyaluronic acid standard (Sigma-Aldrich); methylsulfonylmethane standard (Merck); magnesium stearate (Merck); Amylum Oryzae (Merck); 9-fluoroenilmetoxicarbonyl chloride (Merck); glacial acetic acid (Merck); chloride acid (Merck); methanol pro HPLC (Merck); aqua pro

injection (Ikapharmindo Putramas); aquadest (Brataco); acetonitrile pro HPLC (Merck); NaOH (Merck); boric acid (Merck); anhydrous sodium acetate (Merck); nitrogen (Merck); and hydrogen (Merck).

Equipment

The HPLC (Shimadzu®) consisted of pumps, Shimadzu® C18 columns, RF 20A fluorescence detectors, manual injectors, a computer data processor, and HPLC syringes (SGE®, Australia). A Shimadzu® GC model GC-17A equipped with flame ionization detector, a capillary column with a length of 30 m, inner diameter of 0.53 mm, and film thickness of 5 µm with stationary phase G2 was used with a 10 µl micro-syringe (Hamilton Co.Nevada®).

Procedures

Standard hyaluronic acid solution

Two hundred milligrams of hyaluronic acid standard was dissolved in 100 mL of 0.1 N HCl and diluted to a concentration of 10 µg/mL.

Standard methylsulfonylmethane solution

Four milligrams of methylsulfonylmethane was dissolved in 1 mL of methanol, sonicated at 50°C for 1 min, and cooled to room temperature.

Wavelength optimization

The hyaluronic acid standard (300 µL) was pipetted into a test tube and 300 µl of borate buffer and 300 µL of 1.5 mM fluorenylmethyloxycarbonyl chloride (FMOC-Cl) reagent added. The mixture was vortexed for 20 s and allowed to stand for 2 min before injection of 20 µL into the HPLC to determine the excitation and emission wavelengths. This was achieved by varying excitation wavelengths at 255 nm and using three emission wavelengths (320, 325, and 330 nm). The selected wavelengths were those that produced the largest peak area.

Determination of optimal analytic conditions

A standard solution of hyaluronic acid with a concentration of 50 µL/mL as much as 100 µL each into the test tube add 100 µl of borate buffer pH 9.3, the solution is then added FMOC-Cl reagent to the selected volume. The mixture was vortexed for 20 s and allowed to react before HPLC analysis. Twenty microliter aliquots were analyzed using various combinations of the mobile phase of acetate (pH 4.2)-acetonitrile at 1:2, 2:3, and 1:4 and with different flow rates of 0.8, 1.0, or 1.2 mL/min.

One microliter of a standard solution of methylsulfonylmethane at a concentration of 4000 ppm was injected into the GC, and determination of optimal analysis conditions was performed by programing various initial column temperatures of the 100°C, 110°C, or 120°C and various flow rates of 0.8, 1.0, or 1.2 mL/min. The initial temperature was increased by 1°C/min to 250°C and the injector and detector temperatures were set to 250°C. The retention time, area, follow-up factor, number of theoretical plates, column efficiency (HETP), and resolution were determined for each condition, and the one that had the shortest retention time, the largest number of theoretical plates (N), the smallest HETP, the smallest follow-up factor (Tf), and the best separation with a resolution of 1.5 or more was selected.

Precision of the hyaluronic acid system

One hundred microliters of standard solution of hyaluronic acid with a concentration of 20 µL/mL was added to 100 µL borate buffer pH 9.3, the derivatization was carried out under the selected conditions. Twenty microliters of this solution were then injected into the HPLC 6 times and analyzed using the optimized method. The results were used to determine the coefficient of variance (% KV), which was found to be below 2%. The parameters seen were based on the separation between two adjacent peaks (R), the follow-up factor (Tf), peak discharge retention time, the column efficiency (HETP), and the number of theoretical plates (N).

A standard solution of methylsulfonylmethane at a concentration of 4000 ppm was vortexed to homogeneity and 1 µL injected into the GC and analyzed using the optimized method.

Validation of analytical methods

One microliter of 1000 mL/mL of hyaluronic acid was placed into a 10 mL flask and used to produce a 100-ppm solution, which was then used to produce solution of 10, 20, 30, 40, and 50 µg/mL. Samples were then derivatized and 20.0 µL of each was analyzed. A plot of peak area (*y*) versus concentration (x) produced a straight line with a correlation coefficient (r) of ≥0.999 (Fig. 1).

Different volumes (200 µL, 400 µL, 800 µL, 1000 µL, 1200 µL, and 1500 µL) of the 50,000 ppm methylsulfonylmethane standard solution were each added to 5 mL volumetric flasks, which were brought to volume using mobile phase solvent and then mixed to produce concentrations of 2000, 4000, 8000, 10,000, 12,000, and 15,000 ppm. One microliter of each solution was then injected into the GC and analyzed using the optimized conditions. The data were then used to produce a curve of peak area versus concentration. Result shown in Fig. 2.

Accuracy and precision tests for hyaluronic acid were carried out by analyzing simulated samples, which were produced by adding pure analytes added to the pharmaceutical vehicle. The concentrations used were low, medium, and high, namely, concentrations of 80%, 100%, and 120% of the target concentration. The 100% sample was made by dissolving 4 mg of the matrix of capsule fillers in a mixture of 20 mg of hyaluronic acid and 32 mg of methylsulfonylmethane. The 80% concentration was prepared by dissolving 3.2 mg of matrix in a mixture of 16 mg hyaluronic acid and 25.6 mg methylsulfonylmethane. The 120% concentration was produced by dissolving 4.8 mg of matrix in a mixture of 24 mg hyaluronic acid and 38.4 mg methylsulfonylmethane. All samples were dissolved in 100 mL 0.1 N HCl then pipette 1 ml and put into a 10 mL flask. Samples were filtered through a 0.45 µm membrane and 300 µL of each solution was added to 300 µL borate buffer pH 9.3 and 300 µL FMOC-Cl 1.5 mM (in acetonitrile) then the sample was filtered using a filter. Twenty microliters of each sample were injected into the HPLC in triplicate and the percent recovery (% recovery) and % KV were calculated using calibration curves to determine concentrations. Accuracy was considered acceptable if the percentage of recovery (% UPK) lays between 98% and 102% of target. Precision was considered acceptable if % KV was 2% or less.

Accuracy and precision tests for methylsulfonylmethane were conducted as for hyaluronic acid. The 100% concentration was produced by dissolving 5 mg of capsule matrix with 5 mg hyaluronic acid and 20 mg methylsulfonylmethane. The 80% concentration was

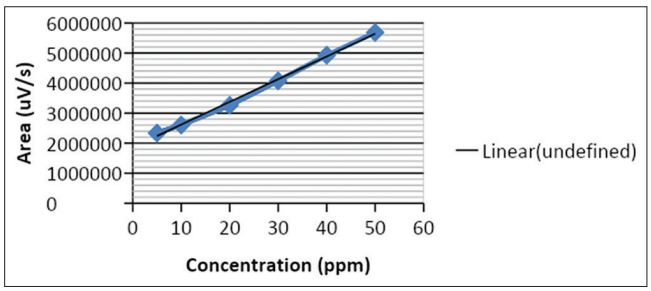

Fig. 1: Calibration Curve for hyaluronic acid

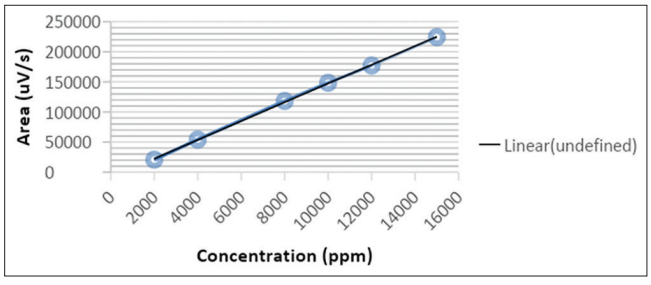

Fig. 2: Calibration curve for methylsulfonylmethane

produced by dissolving 5 mg of matrix with a mixture of 4 mg hyaluronic acid and 16 mg methylsulfonylmethane. The 120% concentration was produced by dissolving 5 mg of matrix with 6 mg hyaluronic acid and 24 mg methylsulfonylmethane, methanol was used as a solvent and samples were shaken until dissolved. Each sample was passed through a 0.45 µm filter and 1 mL placed into a 10 mL volumetric flask, which was filled to volume with n-hexane. One microliter of each sample was analyzed in triplicate, and the recover (% recovery) and % KV calculated using calibration curves to determine concentrations. Accuracy was considered acceptable if the percentage of recovery (% UPK) lays between 98% and 102% of target. Precision was considered acceptable if % KV was 2% or less.

Selectivity

The standard hyaluronic acid solution was derivatized and then 20 µL was injected into HPLC and the chromatograms examined for any differences in retention time between the sample and the standard and to determine whether or not another peak appeared at the hyaluronic acid retention time in the sample solution.

The 40,000 ppm methylsulfonylmethane standard solution of was vortexed to homogeneity and 1 µL injected into the GC. The same volume of a matrix solution was next injected and the chromatograms examined to determine whether any matrix components eluted at the same time as methylsulfonylmethane.

RESULTS AND DISCUSSION

The results of method optimization for hyaluronic acid are summarized in Tables 1-3. Based on the data, a wavelength of 330 nm with a mobile phase with a ratio 1:4 and a flow rate of 1.0 mL/min were selected as the optimal conditions because these produced relatively fast retention times, a number of theoretical plates with a relatively small HETP, and a tailing factor close to one.

Results for methylsulfonylmethane analysis optimization are shown in Tables 4-6. This showed that higher initial column temperatures and faster flow rates produced smaller retention times. The initial temperature of the column used was chosen to be 110°C with a flow rate of 0.8 mL/min because this produced relatively faster retention times, quite a high number of theoretical plates, a relatively small HETP, and a tailing factor of close to one. The 120°C temperature was not chosen because it resulted in the peak of the chromatogram at the retention time with the peak of the solvent chromatogram used so that it was feared that the proximity of the two chromatographic peaks could disrupt the chromatogram peak of methylsulfonylmethane compound. The 1.0 mL/min and 1.2 mL/min flow rates were not chosen because they resulted in an unfavorable separation between the substance and solvent peaks.

The conformity tests were carried out to qualify the optimized method by running six consecutive tests under the same conditions. This needs

Table 1: Effect of emission wavelength variations on retention time, peak area, theoretical plate, tailing factor, and HETP of hyaluronic acid derivatives

Excitation wavelength (nm)	Emission wavelength (nm)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
255	320	5.990	2909568	892	1.219	168.14
	325	5.981	4819277	869	1.207	172.60
	330	5.999	6855595	889	1.193	168.73

Table 2: Effect of variations in the composition on the mobile phase with respect to retention time, peak area, theoretical plate, tailing factor, and HETP of hyaluronic acid derivatives

Mobile phase	Mobile phase ratio	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
Acetonitrile-acetic buffer pH 4.2	1:2	41.312	2346676	2049	0.769	7368.27
	1:4	5.999	5688541	1019	1.241	147.22
	2:3	20.637	8123089	1626	1.563	851.63

Table 3: Relationship of flow rate to retention time, peak area, theoretical plate, tailing factor, and HETP of hyaluronic acid derivatives

Flow rate (mL/min)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
0.8	7.476	2955712	1305	1.221	114.99
1.0	5.999	3245385	1289	1.210	112.99
1.2	5.026	3157540	694	1.217	216.29

HETP: Height equivalent to a theoretical plate

Table 4: The relationship between retention time, peak area, number of theoretical plates, column efficiency, resolution, and tailing factor for methylsulfonylmethane at various flow rates and an initial column temperature of column 100°C

Column temperature	Flow rate (mL/min)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
100°C	0.8	3.837	47392	14810.85	0.996	2.03
		3.845	34775	25638.96	0.979	1.17
		3.826	33991	13886.27	0.982	2.16
	1.0	3.057	50043	19742.45	1.101	1.52
		3.056	50109	16555.80	1.069	1.81
		3.076	50178	11936.14	0.825	2.51
	1.2	2.594	50164	10021.11	1.358	2.99
		2.599	42272	8338.191	1.363	3.60
		2.621	62094	9347.068	0.924	3.21

HETP: Height equivalent to a theoretical plate

to be done because there will be variations on the results to be obtained so that it can be calculated and proven whether the results obtained are still in accordance with the objectives of the analysis and the applicable provisions or not. The data obtained for the two compounds passed the requirements for repeatability, with the coefficients of variation for both being below 2% (0.1% for methylsulfonylmethane and 1.11% for hyaluronic acid). The data shown in Tables 7 and 8.

Linear calibration curves are used to calculate the concentrations of compounds in a sample. A calibration curve for methylsulfonylmethane was produced that contained six points between 2000 and 15,000 µg/mL. The linear equation was y=15.596x–8673.5, where x is the concentration and y is the chromatogram peak area, and the correlation coefficient was 0.9998 (Table 9).

For hyaluronic acid compounds, a 6-point curve between 5 and 50 µg/mL produced a line with the equation y = 75714x–1859406 and a correlation coefficient of 0.9983 Table 10.

Accuracy is a value that describes the closeness of test results to the actual level of the analyte in the sample. Accuracy can be defined as the % recovery, i.e., the percentage of the test result relative to actual content

Table 5: The relationship between retention time, peak area, number of theoretical plates, column efficiency, resolution, and tailing factor for methylsulfonylmethane at various flow rates and an initial column temperature of column 110°C

Column temperature	Flow rate (mL/min)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
110°C	0.8	3.412	50311	30283.17	0.994	0.99
		3.386	50875	26149.43	1.002	1.15
		3.396	50656	24017.07	0.856	1.25
	1.0	2.734	50240	17840.12	0.822	1.68
		2.722	50037	207320.2	0.932	0.14
		2.722	50008	20763.31	0.976	1.44
	1.2	2.296	51349	8779.278	0.828	3.42
		2.308	49178	11011.42	0.654	2.72
		2.306	51359	13757.95	0.903	2.18

HETP: Height equivalent to a theoretical plate

Table 6: The relationship between retention time, peak area, number of theoretical plates, column efficiency, resolution, and tailing factor for methylsulfonylmethane at various flow rates and an initial column temperature of column 120°C

Column temperature	Flow rate (mL/min)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)
120°C	0.8	3.077	55413	20548.17	0.957	3.41
		3.048	56478	18564.26	1.203	2.72
		3.092	52336	22654.84	0.941	1.46
	1.0	2.524	58412	23154.15	1.025	1.50
		2.486	55648	26465.26	1.335	3.00
		2.598	54852	24515.21	1.051	1.80
	1.2	2.054	53215	26648.04	1.326	1.90
		1.984	52156	19354.51	1.512	2.98
		2.012	50123	23645.35	1.320	2.87

HETP: Height equivalent to a theoretical plate

Table 7: Conformity test results for the hyaluronic acid system

Concentration (µL/mL)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)	Average	Standard deviation	%KV
20	6.463	1643597	2583	0.993	58.08	1669780	18605.52	1.11
	6.459	1683699	2477	0.976	60.55			
	6.432	1685824	2418	0.872	62.03			
	6.409	1651481	2317	0.838	64.73			
	6.391	1668269	2164	0.902	69.32			
	6.423	1685811	2302	0.895	65.77			

HETP: Height equivalent to a theoretical plate

Table 8: Conformity test results for the methylsulfonylmethane system

Concentration (µL/mL)	Retention time (min)	Peak area (µV/s)	Theoretical plates (N)	Tailing factor (Tf)	HETP (cm)	Average	Standard deviation	%KV
4000	3.327	54266	22161.562	0.993	0.14	54285.17	57.1084	0.11
	3.336	54231	21209.208	1.294	0.14			
	3.347	54271	13836.078	0.976	0.22			
	3.338	54325	15080.732	1.32	0.2			
	3.338	54380	22954.957	1.296	0.13			
	3.347	54238	17840.273	1.193	0.17			

HETP: Height equivalent to a theoretical plate

of the sample and should fall within 98–120% to be acceptable [9]. In this study, this was conducted by producing simulated samples by from mixtures of the two analytes with appropriate pharmaceutical excipients. The mixtures were first prepared, derivatized (for hyaluronic acid) and then analyzed using high-performance fluorescence detector liquid chromatography. Methylsulfonylmethane was prepared then analyzed using by GC. The simulated samples were produced at three different concentrations, namely, 80%, 100%, and 120%, which corresponded to hyaluronic acid concentrations of 16 µg/mL, 20 µg/mL, and 24 µg/mL, while the simulated methylsulfonylmethane samples contained of 3200 µg/mL, 4000 µg/mL, and 4800 µg/mL, respectively. Recovery of methylsulfonylmethane ranged between 100.37% and 101.50%, while those for hyaluronic acid ranged between 98.59% and 100.42%, both of which were considered excellent. Result shown in Tables 11 and 12.

Table 9: Calibration curve data, LOD, and LOQ for methylsulfonylmethane

Concentration (µL/mL)	Peak area (µV/s)	yi=a+bx	$(y-yi)^2$	$S_{(y/x)}$	LOD (µg/mL)	LOQ (µg/mL)
2000	20793	22518.5	2977350.25	1730.6455	332.9	1109.67
4000	54105	53710.5	155630.25			
8000	118345	116094.5	5064750.25			
10,000	148495	147286.5	1460472.25			
12,000	177244	178478.5	1523990.25			
15,000	224373	225266.5	798342.25			
N=6			∑=11980535.5			

LOD: Limit of detection, LOQ: Limit of quantification

Table 10: Calibration curve data, LOD, and LOQ for hyaluronic acid

Concentration (µL/mL)	Peak area (µV/s)	yi=a+bx	$(y-yi)^2$	$S_{(y/x)}$	LOD (µg/mL)	LOQ (µg/mL)
5	339912	237976	0390948096	85086.4131	3.55	11.84
10	603443	616546	71688609			
20	245385	373686	2209808004			
30	74688	130826	151475044			
40	925521	887966	410378025			
50	685411	645106	624493025			
N=6			∑=28958790803			

LOD: Limit of detection , LOQ: Limit of quantification

Table 11: Accuracy and precision data for hyaluronic acid

Concentration (µg/mL)	Peak area (µV/s)	Measured concentration (µg/mL)	% Recovery	Average	Standard deviation	KV (%)
8	2456552	7.886864	98.59	99.03	0.7858	0.79
	2464738	7.994981	99.94			
	2456437	7.885345	98.57			
10	2619691	10.04154	100.42	100.34	0.105	0.1
	2618220	10.02211	100.22			
	2619478	10.03872	100.39			
12	2770009	12.02688	100.22	99.51	0.6614	0.66
	2758155	11.87931	98.92			
	2762367	11.92595	99.38			

Table 12: Accuracy and precision data for methylsulfonylmethane

Concentration (µg/mL)	Peak area (µV/s)	Measured concentration (µg/mL)	% Recovery	Average	Standard deviation	KV (%)
3200	41736	3232.207	101.01	100.96	0.5696	0.56
	41416	3211.689	100.37			
	41983	3248.044	101.5			
4000	54208	4031.899	100.8	100.65	0.1302	0.13
	54136	4027.283	100.68			
	54107	4025.423	100.64			
	54031	4020.55	100.51			
	54010	4019.204	100.48			
	54191	4030.809	100.77			
4800	66776	4837.747	100.79	100.86	0.0689	0.07
	66879	4844.351	100.92			
	66832	4841.338	100.86			

Table 13: Quantification data for hyaluronic acid levels in supplements

Concentration (µg/mL)	Peak area (µV/s)	Measured concentration (µg/mL)	% Recovery	Average	Standard deviation	KV (%)
10	2609213	9.9031	99.03	98.63	0.3488	0.35
	2604953	9.8469	98.47			
	2604378	9.8393	98.39			

Table 14: Quantification data for methylsulfonylmethane levels in supplements

Concentration (µg/mL)	Peak area (µV/s)	Measured concentration (µg/mL)	% Recovery	Average	Standard deviation	KV (%)
4000	53592	3992.402	99.81	99.35	0.4010	0.40
	53141	3963.484	99.09			
	53179	3965.921	99.15			

Precision reflects the reproducibility of results from multiple analyzes of the same material and is measured by % KV. In general, % KV values below 2% are considered acceptable [9]. Here, the % KV was 0.07%–0.56% for methylsulfonylmethane and 0.10%–0.79% for hyaluronic acid, both of which were considered acceptable.

To determine the levels of the two compounds, the samples were prepared containing both. Hyaluronic acid was derivatized using FMOC-Cl first and then analyzed HPLC to yield a sample content of 98.63%. Meanwhile, GC indicated a methylsulfonylmethane content of 99.35%. Result shown in Tables 13 and 14.

CONCLUSION

The optimum condition for analysis of hyaluronic acid by HPLC was with an excitation wavelength 255 nm and emission wavelength of 330 nm and with a mobile phase of acetonitrile-acetate pH 4.2 (1:4) at a flow rate of 1.0 mL/min. The optimum conditions for the analysis of methylsulfonylmethane by GC were with an initial temperature of column 110°C and a subsequent increase of 1°C/min to a temperature of 200°C, with an injector temperature of 250°C, a detector temperature of 250°C, and a nitrogen carrier gas flow rate of 0.80 mL/min.

The validation conducted here consisted of selectivity, linearity, detection and quantitation limit, and accuracy and precision tests. Both methods fulfilled the applicable criteria with respect to these analyses. For hyaluronic acid, the correlation coefficient was 0.9983 in a concentration range of 5–50 µg/mL and the LOD and LOQ values were 3.55 µg/mL and 11.84 µg/mL, respectively. Accuracy values as assessed by recover were 98.59–100.42% and precision as expressed by % KV was below 2%. For methylsulfonylmethane, the correlation coefficient was 0.9998 in the range of 2000–15,000 µg/mL and the LOD and LOQ were 332.90 µg/mL and 1109.67 µg/mL, respectively. Accuracy ranged between 100.37% and 101.50% and precision was below 2%.

A simulated sample containing both compounds was assessed to contained 98.63% hyaluronic acid and 99.35% methylsulfonylmethane.

For further research, hydrolysis of hyaluronic acid compounds is needed to obtain a more sensitive method.

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