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Original Article

DETERMINATION OF S-METHYL L-CYSTEINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

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ABSTRACT

Objective: A simple, reproducible and sensitive high-performance liquid chromatography method has been developed for determination of S-Methyl L-Cysteine. S-Methyl L-Cysteine is widely observed and most common amino acid in plants, including many edible vegetables, which is responsible for reducing blood cholesterol level in the body.

Methods: S-Methyl L-Cysteine was chromatographed using Phosphate buffer of pH 6.5: Acetonitrile in the ratio of 97:3. The liquid chromatogram was equipped with a variable wavelength UV detector, an injector and a data processor. Inertsustain GL-Science Column C-18 (150 mm x 4.6 mm; 5µ) was used as a stationary phase.

Results: The retention time of S-Methyl L-Cysteine was observed as 2.261±0.0016 min. The linearity value for S-Methyl L-Cysteine was found to be 100-2000 µg/ml with Correlation of Determination (R^2) value as 0.9992. LOD and LOQ values obtained are 29.51µg/ml and 89.74 µg/ml, respectively.

Conclusion: The method was developed and validated successfully as per ICH guidelines for analytical method validation.

Keywords: Amino acid determination, Analytical method validation, High performance liquid chromatography, S-Methyl L-Cysteine, 3-methylthioalanine

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INTRODUCTION

S-Methyl cysteine is the amino acid with the chemical formula $C_4H_9NO_2S$. It is a white colour solid powder with molecular mass of 135.18 $g*mol^{-1}$. It is the S-methylated derivative of cysteine. This amino acid occurs widely in plants, including many edible vegetables [1]. S-Methyl L-Cysteine, is chemically known as 2-amino-3-(methylthio) propionic acid. The other name of S-Methyl L-Cysteine is 3-methylthioalanine. The structure of S-Methyl L-Cysteine is shown in fig. 1. Plants of the Crucifereae family contain S-methyl cysteine sulfoxide, which is principally responsible for reducing blood cholesterol levels in the body.

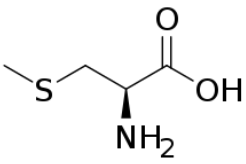

Fig. 1: Structure of S-methyl L-cysteine

Dietary sulphur compounds are of potential value in protecting humans against chronic diseases [2-4]. The literature is particularly rich in studies supporting the anticancer properties of cruciferous vegetables. These are uniquely characterised by high levels of sulfur-delivering compounds such as isothiocyanate precursors [5, 6].

As per literature only a few reports were observed for analysis and determination of S-Methyl L-Cysteine. One LC-MS/MS method has been reported for the measurement of S-Methyl L-Cysteine with the help of a reference standard [7]. Few studies are also reported to show the potential use of S-Methyl L-Cysteine as bioactive material [8-11], which is mainly found in *Allium* species (family: Liliaceae) [12-14]. HPLC method is the best method of choice for determination of various active pharmaceutical ingredients and formulations with high sensitivity and best reproducibility [15, 16]. As per the literature survey, not a single method is developed till now for determination of this highly useful amino acid. Therefore, successful attempts were made to develop a sensitive and precise HPLC method for the determination of S-Methyl L-Cysteinein its pure form. The developed method was successfully validated as per ICH guidelines of analytical method validation [17].

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical grade and HPLC-grade water was used throughout. Pure analytical grade Potassium dihydrogen phosphate and NaOH were obtained from Merck India Pvt Ltd., Mumbai, India. HPLC-grade water and acetonitrile were used during the study (RANKEM™, RFCL Ltd., New Delhi, India).

Apparatus

The major instrumentation comprises of a Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with a UV-Visible detector, Inertsustain GL-Science Column C-18 (150 mm x 4.6 mm; 5µ, GL Sciences Cat No.5020-07345) and an Equitron-ultrasonic sonicator (Labnet scientific services, Chennai, India)were used for method development.

Optimized chromatographic conditions

Chromatographic estimation of S-Methyl L-Cysteine were performed under the following conditions:

The liquid chromatogram is equipped with a variable wavelength UV detector, an injector and a data processor. Inertsustain GL-Science Column C-18 (150 mm x 4.6 mm; 5µ, GL Sciences Cat No.5020-07345) was used as a stationary phase at ambient temperature. For the preparation of buffer, dissolve 2.5 gm of potassium dihydrogen orthophosphate in 1000 ml distilled water and adjust the pH to 6.5 with 10% w/v sodium hydroxide solution. Filter the solution with

0.45µ membrane filter. Sonicate to degas. The mobile phase comprised Buffer: acetonitrile (97:3, v/v), was pumped at a flow rate of 0.8 ml min^{-1}. The source of radiation was D2 lamp emitting a continuous ultraviolet radiation between 180 nm to 400 nm. The injection volume was set as 10 µl for each injection and detection wavelength was selected as 220 nm using UV detection. Column temperature was set as 50 °C and the auto-sampler temperature was set as 25 °C for each kind of sample measurement.

Preparation of the standard solutions

All reagents were tested for stability in solution and during the actual analysis; the behaviour of the analytes remained unchanged during each type of working conditions. The solutions of S-Methyl L-Cysteine were found to be stable during each kind of experimental measurement. Each and every kind of sample preparation and preparation of the mobile phase were done at ambient temperature. Standard solution of S-Methyl L-Cysteine was prepared by taking 100 mg of S-Methyl L-Cysteine and it was dissolved in 100 ml of HPLC grade water to get 1000 ppm of S-methyl-L-Cysteine. It was filtered through 0.45-micron Nylon filter paper. From these stock solutions, various dilutions were made for S-Methyl L-Cysteine for calibration curves. Evaluation was done by measuring peak areas.

Validation parameters [15-17]

Linearity and range

A calibration curve was plotted over a concentration range of 100-2000µg/ml for S-Methyl L-Cysteine. Stock solutions of S-Methyl L-Cysteine, having concentration 5000µg/ml were prepared separately and further diluted for calibration curves of S-Methyl L-Cysteine. Accurately measured standard stock solution S-Methyl L-Cysteine was used for further preparations of standard solutions. 10 µl of each solution was injected under the operating chromatographic conditions described above.

Precision

Intraday precision and inter-day precision for the developed method was measured in terms of % RSD. The experiments were repeated six times a day for intraday precision and on six different days for interday precision. The concentration values for both intraday precision and interday precision were calculated six times separately and percent relative standard deviation were calculated. Finally, the mean of % RSD (% RSD = [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) was calculated. The precision of the instrument was checked by repeated scanning of the same spot of both drugs six times without changing the condition of the instrument.

Recovery studies (Accuracy)

Accuracy of proposed method and interference from excipients was determined by recovery experiments. Recovery experiments were carried out by the standard addition method (spiking method). This study was performed by addition of known amounts of S-Methyl L-Cysteine (80%, 100% and 120%) to that of known concentration of S-Methyl L-Cysteine, which was pre-analyzed and % of pure S-Methyl L-Cysteine recovered, were calculated.

Limit of detection (LOD)

Limit of detection was calculated using the following equation as per ICH guidelines.

LOD = 3.3 × S/m where S is the standard deviation of the peak areas of the drug and m is the slope of the corresponding calibration curve. It is expressed as signal to noise ratio of 3:1.

Limit of quantitation (LOQ)

Limit of quantification (LOQ) was calculated using the following equation as per ICH guidelines. LOQ = 10 × S/m where S is the standard deviation of the peak areas of the drug and m is the slope of the corresponding calibration curve. It is expressed as a signal to noise ratio of 10:1.

System suitability

Number of theoretical plates was determined by employing the formula, $n = 16(t/w)^2$ where t=retention time and w = width of the peak. Tailing factor was derived from the formula t = w/2t where w = half of the width, t = retention time**.** The retention time was also observed as a system suitability factor.

RESULTS AND DISCUSSION

RP-HPLC method optimization

S-Methyl L-Cysteine was freely soluble in water; therefore, water was selected as a solvent for further preparation of working standards of S-Methyl L-Cysteine. Various mobile phases were tried with buffers of a variety of pH and retention time as well as peak area and peak symmetry was observed. Best results were obtained using Buffer: acetonitrile in the ratio of 97:3 v/v as mobile phase using C-18 column. The retention time (RT) ofS-Methyl L-Cysteine was found to be2.261±0. 0016. The selected mobile phase gives the best result in terms of faster retention as well as each kind of chromatographic measurement throughout the development of the entire method. The chromatogram of S-Methyl L-Cysteine is shown in fig. 2.

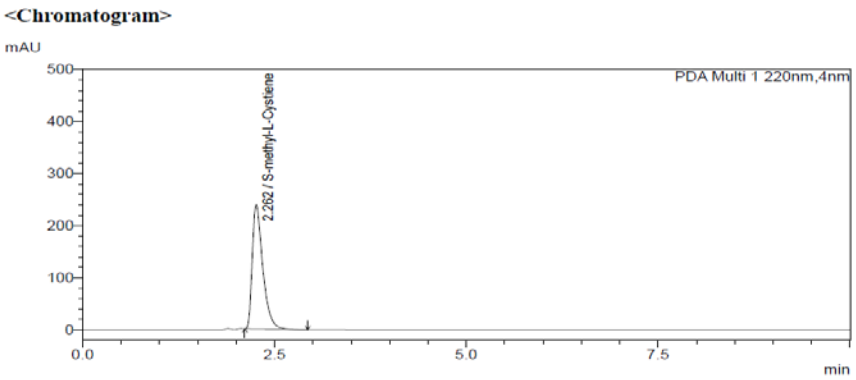

Fig. 2: Chromatogram of S-methyl L-cysteine

Method validation

Linearity and range

The linearity value for S-Methyl L-Cysteine was found to be 100-2000 µg/ml with a Correlation of Determination (R^2) value as 0.9992. Therefore, the method is found to be linear for estimation of S-methyl L-Cysteine in Range of 100-2000 µg/ml. The average linear regression equation was represented as Y = 2173.5x+52225, where X is the concentration of drug Peak areas and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficient. The Linearity curve of S-methyl L-Cysteine is represented in fig. 3.

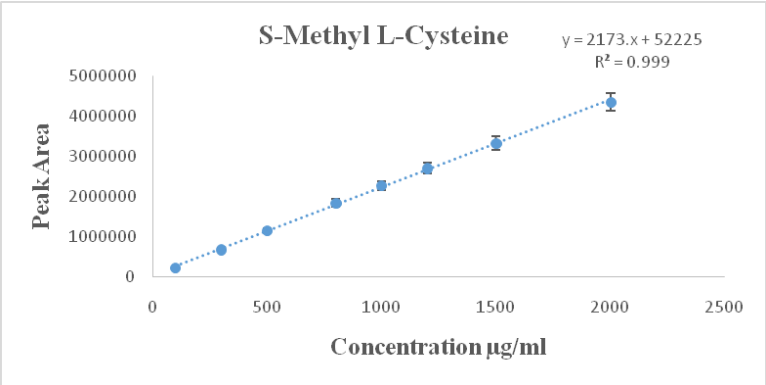

Fig. 3: Linearity curve of S-methyl L-Cysteine, various validation parameters for S-methyl L-Cysteine are summarized in table 1

Table 1: Validation parameters of developed HPLC methods for S-methyl L-cysteine

Validation parameters	S-methyl L-Cysteine
Linearity range (µg/ml)	100-2000
Scanning wavelength	220 nm
Regression equation	Y = 2173.5x+52225
Correlation coefficient (R^2)	0.9992
Accuracy (Recovery study)	100.15±0.5607
LOD (µg/ml) (n = 6)	29.51±0.4791
LOQ (µg/ml) (n = 6)	89.74±0.7849
Precision–Intraday (%RSD)	0.9471
Interday (%RSD)	1.2141
Repeatability (%RSD)	0.0941

(RSD-Relative Standard Deviation)

Precision

The intra-day and inter-day precision for S-methyl L-Cysteine was found to be 0.9471 and 1.2141 in terms of %RSD. These values indicate that the developed novel method is precise. The %RSD for the precision of the instrument by measuring the peak area was found to be 0.0941 for six repetitions. The %RSD for measuring the peak area (less than 2%), ensured the proper functioning of the HPLC system. The results are depicted in table 1

Recovery studies (Accuracy)

When the recovery study was carried out in preanalyzed pure sample of S-methyl L-Cysteine after spiking with 80%, 100% and 120% of the pure drugs, percentage recovery was found to be 99.91±0.9441 for S-methyl L-Cysteine (table 2).

Table 2: Results of recovery studies of S-methyl L-Cysteine by spiking of a known amount of standard method

Pure S-methyl L-cysteine added with preanalysed sample (%-of standard preanalysed sample)	Added amount of pure S-methyl L-Cysteine sample (µg/ml)	Estimated content (µg/ml)	% Recovery[a]
0	0	499	99.8±0.1473
80	399.2	898.2	99.71±0.2791
100	499	998	100.97±0.7142
120	598.8	1097.8	100.14±1.1024
Mean recovery±Standard deviation			100.15±0.5607

[a]mean value of three determinations

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

The Values of LOD and LOQ for S-methyl L-Cysteine were found to be 29.51 µg/ml and 89.74 µg/ml, respectively (table 1).

System suitability

The number of theoretical plates, tailing factors and retention times were represented in table 3.

Table 3: Results of system suitability of S-methyl L-cysteine by the developed HPLC method

System suitability parameters	S-methyl L-Cysteine[a]
Retention Time	2.261±0.0016
Tailing Factor	1.247±0.9471
Theoretical Plates	3941±1.247

[a]mean value of six determinations

CONCLUSION

The newly developed HPLC method for the estimation of S-methyl L-Cysteine was found to be sensitive, accurate, precise and reproducible as well. The developed method can be used as a method of choice in the absence of official monographs for determination of S-methyl L-Cysteine. The developed method will be widely useful as a standard method for the determination of S-methyl L-Cysteine containing commercial formulations as well.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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