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Direct determination of s-carboxymethyl-l-cysteine in syrups by reversed-phase high-performance liquid chromatography

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Abstract

A simple reversed-phase high-performance liquid chromatography method was developed for the determination of s-carboxymethyl-l-cysteine in syrup preparations. The experiments were performed without specific sample pre-treatment. The LC conditions used were acetonitrile-10 mM sodium dihydrogenphosphate buffer, pH 2.0 (1:99, v/v) on a C_{18} Inersil column with a flow rate of 1.5 ml/min. Ultraviolet detection was carried out at 240 nm. The method showed excellent linearity ($r^2 > 0.9998$) over the concentration range tested (0.8–25.6 mg/ml) with good precision and accuracy (%R.S.D. 0.7%). Recoveries were good (> 99%) with a limit of detection and limit of quantitation of 0.1 and 0.8 mg/ml. Other compositions in the syrup vehicle did not interfere the analysis of s-carboxymethyl-l-cysteine.

Keywords: s-Carboxymethyl-l-cysteine; Reversed-phase chromatography

1. Introduction

S-carboxymethyl-l-cysteine or carbocysteine has been currently used as a mucolytic agent in the adjunctive therapy of respiratory tract disorders characterized by excessive and/or viscous mucus [1]. It is a derivative of acetylcysteine in which the sulhydryl group is blocked by a carboxylic acid residue. Commercially available dosage forms of s-carboxymethyl-l-cysteine are tablets and syrups. Determination of s-carboxymethyl-l-cysteine in syrups is complicate due to inactive ingredients such as preservatives, coloring and flavoring agents in the preparations. A number of analytical methods such as high performance liquid chromatography (HPLC) [2–5], capillary electrophoresis [6,7], spectrophotometric method [8] and electrochemical method [9] have been employed for the determination of s-carboxymethyl-l-cysteine in preparations and biological fluids. Among these methods, HPLC has been the most widely used method for the analysis of this compound. Some of the described methods are expensive since they require the use of specific detectors for example a mass-spectrometer [2] and a fluorescent detector [4,5]. Other methods require deriva-

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tizations of the sample or sample pre-treatments [5,8,9], which can be laborious and time-consuming.

The objective of this study was to optimize and validate a method for the determination of s-carboxymethyl-l-cysteine in syrup preparations. Unlike other studies, the present work proposed a simple isocratic reversed-phase HPLC method using a UV detector for determination of s-carboxymethyl-l-cysteine in syrup. Derivatizations of the sample or sample pre-treatments are not required by this method. The method is inexpensive, rapid, reliable and reproducible with quantitation over a calibration curve range 0.8–25.6 mg/ml. The sample analysis time is 5 min per sample, allowing as many as 12 injections per hour.

2. Experimental

2.1. Chemicals

S-carboxymethyl-l-cysteine (99% purity) was purchased from Aldrich (Milwaukee, USA). S-carboxymethyl-l-cysteine syrups were supplied by local pharmaceutical manufacturers. All solvents used were of HPLC grade obtained from Merck (Darmstadt, Germany) and Milli Q system water was used.

2.2. Chromatography

The chromatographic system was the Shimadzu HPLC LC-10 AVP series (Tokyo, Japan) containing a LC-10 ATVP pump, a DGU-14A degasser, a 7725 manual injector with automatic sensing switch, a SPD-10 AVP detector, and a SCL-10 AVP system workstation. Separation was carried out at room temperature on a reversed-phase GL Science C_{18} Inersil ODS-3V column (250×4.6 mm 5 μm particle size) equipped with a guard column. The mobile phase consisted of acetonitrile-10 mM sodium dihydrogenphosphate buffer, pH 2.0 (1:99, v/v). These conditions were modified from the method for determination of N-acetylcysteine [10]. The flow rate was 1.5 ml/min, the detection wavelength was 240 nm and the injection volume was 20 μl.

2.3. Sample and placebo preparations

The syrup sample consists of carbocyteine as an active ingredient and other inactive components, which are methyl paraben, propyl paraben, citric acid, propylene glycol, glycerine, sorbitol and syrup. Sample solutions were prepared by diluting 5 ml of syrup (equivalent to 100 mg of s-carboxymethyl-l-cysteine) with water to give a final concentration of 2.0 mg/ml. A synthetic placebo was prepared by mixing all ingredients in the syrup except s-carboxymethyl-l-cysteine. The placebo solution was prepared as described in sample solution.

2.4. Standard solution preparations

Stock standard solution of s-carboxymethyl-l-cysteine was prepared by dissolving 100.0 mg of s-carboxymethyl-l-cysteine in a few drops of 1 N NaOH and water to give the final concentration of 4.0 mg/ml. To perform system linearity for the standards, standard solutions of s-carboxymethyl-l-cysteine in the range 0.8–25.6 mg/ml were prepared from the stock standard solution. The linear regression line was plotted between the peak areas and the known amount of s-carboxymethyl-l-cysteine using Microsoft Excel®. The regression equation and the regression coefficient (r^2) values were obtained.

Stability of the standard solution was investigated by determination of s-carboxymethyl-l-cysteine in the freshly prepared solution comparing to the solution stored in a refrigerator set to maintain at 4°C for 30 days.

2.5. Precision, accuracy, method linearity, recovery, reproducibility and robustness

Precision was determined at three points of the calibration curve (0.8, 6.4 and 25.6 mg/ml). Six injections were made for each standard solution. Precision was expressed as % relative standard deviation (%R.S.D.). Accuracy and recovery were determined in the following manner: standard solutions of s-carboxymethyl-l-cysteine ranging from 50 to 150% of the percent label amount (100 mg/5 ml) were added into the syrup placebo, the

measured amount of the standards were calculated using the calibration curve (five injections were made for each standard). The method linear regression line was plotted between the amount of standard recovered and the amount of standard added. The regression equation and the regression coefficient (r^2) values for the linearity of the method was obtained using Microsoft Excel®. Inter-day variability was determined by analyzing one batch of s-carboxymethyl-l-cysteine syrup on days 1 and 5 (six injections were made). The reproducibility was express as %R.S.D. Robustness was examined by varying the analysts using the optimized conditions and %R.S.D. was calculated.

2.6. Limit of detection (LOD) and quantitation (LOQ)

The amount of standard, which could be detected with a signal to noise ratio ≥ 3 was considered to be limit of detection. The lowest amount of standard, which could be quantified with reasonable precision and accuracy (%R.S.D. $< 3\%$) was defined as the limit of quantitation.

2.7. Determination of s-carboxymethyl-l-cysteine in syrups

Six batches of s-carboxymethyl-l-cysteine syrups from two local manufacturers were determined using the HPLC conditions described earlier. Sample and placebo preparations from each manufacturer were prepared as previously described (five injections were made for each preparation).

3. Results and discussion

3.1. Optimization of reversed-phase HPLC chromatographic conditions

Optimization experiments were performed by varying the flow rate (1.5, 1.8 and 2.0 ml/min), amount of acetonitrile (1, 3 and 5%) and pH of the buffer (1.5, 2.0 and 2.5). No significant differences were found when the pH of the buffer was varied. Flow rate and amount of acetonitrile greatly affected the tailing factor of the separation. Using the flow rate of 1.5 ml/min, the mobile phase containing 1% acetonitrile provided the best peak shape with the smallest tailing factor (1.02). Increasing the flow rate also increased the tailing factor, thus worsen the peak shape. The optimized conditions found were acetonitrile-10 mM sodium dihydrogenphosphate buffer pH 2.0 (1:99, v/v) running at a flow rate of 1.5 ml/min. The representative chromatograms showing the analysis of s-carboxymethyl-l-cysteine in syrup of is shown in Fig. 1. The retention time (t_R) and the capacity factor (k') were 2.7 min and 0.5, respectively. Chromatogram of placebo is shown in Fig. 1(C). The chromatograms show no interference from the placebo.

3.2. Stability

The stock standard solution of s-carboxymethyl-l-cysteine remained stable for at least 30 days when stored in a refrigerator set to maintain at 4°C. This conclusion is based on comparison of a stock solution that had been stored for 30 days to that of the freshly prepared stock solution.

3.3. Method validation

Calibration curve parameters and statistics for s-carboxymethyl-l-cysteine is in Table 1. Results were calculated using peak area. Calibration curves for s-carboxymethyl-l-cysteine were linear using linear regression in the concentration range from 0.8 to 25.6 mg/ml, with correlation coefficients ≥ 0.9998 for all curves. The method was precise at three concentration of standards analyzed and the %R.S.Ds of retention time and peak area were 0.2 and 0.7%, respectively. Precision and accuracy data is shown in Table 2 and the percent biases were within 0.7%. The linearity of the method for s-carboxymethyl-l-cysteine was performed by calculating the recovery of the amount of s-carboxymethyl-l-cysteine added

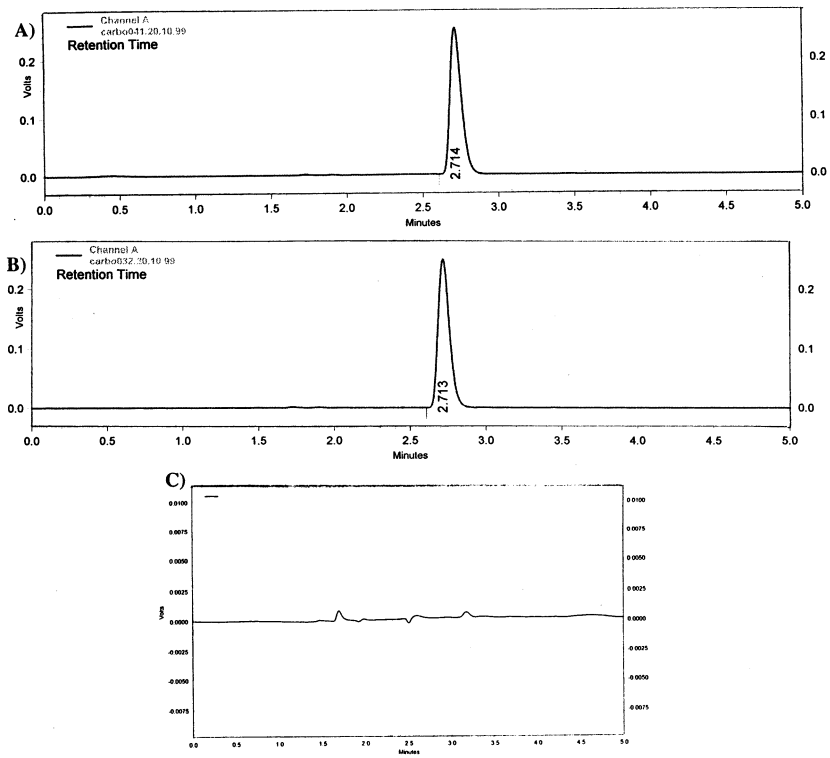

Fig. 1. A representative chromatogram of: (A) standard s-carboxymethyl-l-cysteine; (B) s-carboxymethyl-l-cysteine in syrup; and (C) syrup placebo. Chromatography conditions as described in Section 2.

and the amount found. Triplicate injections were made at each concentration with the following results: $y = 1.0013x - 2.45$ ($r^2 = 0.9999$; $n = 7$). Recoveries of s-carboxymethyl-l-cysteine from their matrices were efficient as shown in Table 2. The mean recovery was 99.9% and the mean %R.S.D. was 0.5%, assuming the label amount to be 100 mg/5 ml. Inter-day variability was determined by analyzing s-carboxymethyl-l-cysteine syrup on days 1 and 5 (six injections were made) and the %R.S.D.s were 0.6 and 1.1%, respectively. Robustness of the method was performed by two analysts (six determinations) using the proposed method and the same instrumentation. The results showed no significant differences: 95.9% (found) and 1.0 (R.S.D.%). The LOD of s-carboxymethyl-l-cysteine, based on a signal-to-noise ratio of 3, was determined to be 0.1 mg/ml. The LOQ based on a signal-to-noise ratio of 10 was found to be 0.8 mg/ml.

3.4. Determination of s-carboxymethyl-l-cysteine in syrups

Data from assay of commercially available s-carboxymethyl-l-cysteine syrup from two different manufacturers is shown in Table 3 and the percent label amount was within 95.3–98.3%. Other ingredients in the syrups from both manufacturers

Table 1
Calibration curve parameters and statistics of carbocysteine

Curve coefficient	Slope	y-Intercept	Correlation
1	683.38	10154	0.9999
2	681.66	11025	0.9998
3	684.14	6989	0.9999
Mean ($n = 3$)	683.06	9389	0.9999
S.D.	1.27	2123.43	
R.S.D. (%)	0.19	22.61	

Table 2
Precision, accuracy and recoveries for s-carboxymethyl-l-cysteine ($n = 5$)

Amount added (as % of theoretical)	Recovery (%)	Bias (%)	R.S.D. (%)
50	99.3	0.7	0.1
80	100.5	0.5	0.6
90	100.3	0.3	0.7
100	100.3	0.3	0.2
110	100.0	0.0	0.6
120	99.8	0.2	0.4
150	99.5	0.5	0.5

did not interfere the assay of s-carboxymethyl-l-cysteine (data not shown).

Experiments of using the proposed method as a stability-indicating assay are under investigation. Stability profiles of carbocysteine are performing at 10, 30 and 60°C for 1, 3, 5, 8 and 12 months. A loss of 10% of the initial concentration will be considered to indicate instability of the compound.

4. Conclusions

An isocratic HPLC method was developed and validated for the direct analysis of s-carboxymethyl-l-cysteine in syrups. With this method, analysis of s-carboxymethyl-l-cysteine can be performed within 5 min per sample since it does not require extraction or sample pre-treatment prior HPLC analysis. The recoveries obtained were > 99% with a LOD and LOQ of 0.1 and 0.8 mg/ml, respectively. S-carboxymethyl-l-cysteine is not official in any pharmacopoeias, and a reference method for the analysis of this compound is not available. To my knowledge, this is the first report on the isocratic HPLC condition for determination of s-carboxymethyl-l-cysteine in syrups using direct UV measurement. The developed method provided several advantages in term of speed, cost, simplicity and reliability. Additionally, the method is simple to be reproduced by analysts.

Table 3
Assay of commercially available s-carboxymethyl-l-cysteine syrups ($n = 5$)

Batch number	Found % (R.S.D.%)	
	Manufacturer A	Manufacturer B
1	95.5 (0.6)	95.6 (1.1)
2	95.3 (0.7)	95.5 (0.9)
3	95.8 (0.4)	98.3 (0.5)
4	96.3 (1.2)	95.6 (0.3)
5	95.2 (0.8)	96.4 (1.0)
6	94.6 (0.9)	96.6 (0.8)

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