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Note

Determination of *S*-carboxymethylcysteine in syrup formulations by high-performance liquid chromatography

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A simple, rapid, and stability indicating method for the determination of *S*-carboxymethylcysteine in cough syrup has been developed using high-performance liquid chromatography (HPLC). In contrast to many other published amino acid methods, derivatization of the compound is not required.

S-Carboxymethylcysteine is an amino acid used in cough syrups as a mucolytic to aid in cough suppression. Due to a reformulation study, a great increase of cough syrup samples were expected to be tested. As a result of this increase, a simple and rapid assay specific for *S*-carboxymethylcysteine was sought. The current gas chromatographic (GC) assay involves time consuming sample preparation since it requires derivatizing with N,O-Bis(trimethyl)-trifluoroacetamide–1% trimethylchlorosilane (BSTFA–1% TMCS). The estimated sample preparation time using the GC assay was determined to be 2–3 h while the HPLC procedure is accomplished in minutes. As a result of the shortened sample preparation time, a greater number of samples can be assayed in much less time. Other methods for amino acids involving derivatization procedures such as dansyl[1], phenylisothiocyanate[2], *o*-phthaldialdehyde[3], and phenylthiohydantoin[4,5] were considered more complicated and time consuming.

EXPERIMENTAL

S-Carboxymethylcysteine is extracted with 0.1 *M* sodium hydroxide, diluted with water and chromatographed on a 15 cm × 4 mm I.D. column packed with aminopropyl silica (NH_2, 5-μm particles), using phosphate buffer–acetonitrile as the mobile phase.

A peak retention time between 5 and 10 min allows adequate separation of degradation products and other components in cough syrup. The method is also shown to have the required accuracy, precision, and linearity necessary for stability studies and routine assays.

Instrumentation

The HPLC system used for this study was composed of the following: A single pump isocratic system (Model 590, Waters Assoc., Milford, MA, U.S.A.), an autoin-

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jection system (Model 710B, Waters Intelligent Sample Processor, Waters), a variable wavelength UV detector (Model 757, Kratos, Ramsey, NJ, U.S.A.), and integration system (Model HP-3356, Hewlett-Packard, Avondale, PA, U.S.A.). The following equipment was used for spectral examination of chromatographic peaks: HP-1040A photodiode array detector, HP-85 personal computer, HP-8290 1M flexible disc drive and HP-7470A *X–Y* plotter.

Reagents and materials

Reagent-grade monobasic potassium phosphate and sodium hydroxide were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Acetonitrile, distilled-in-glass-grade, and methanol were obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). Isopropyl alcohol and hexane were obtained from EM Science (Cherry Hill, NJ, U.S.A.).

S-Carboxymethylcysteine raw material and cough syrup formulation were obtained in-house. Spherisorb NH_2 (5 μm) bulk packing material was obtained from Phase Separations (Norwalk, CT, U.S.A.). Empty stainless-steel tubing, 316 grade, was purchased from Alltech (Deerfield, IL, U.S.A.). Columns were packed with a Model CP-V column packer (Jones Chromatography, Columbus, OH, U.S.A.).

Preparations

Diluting solvent. 0.1 *M* sodium hydroxide is prepared by weighing 4 g of sodium hydroxide into a 1-l volumetric flask then dissolving and diluting to volume with water.

Buffer solution. An amount of 13.6 g of monobasic potassium phosphate is weighed into a 2-l volumetric flask then dissolved and diluted with water. The concentration of this solution of 0.05 *M*. This solution is further diluted 5-fold with water for a resulting concentration of 0.01 *M*.

Mobile phase. A volume of 750 ml of buffer solution is mixed with 250 ml of acetonitrile and degassed under vacuum.

Standards. Amounts of 40, 50, and 60 mg of *S*-carboxymethylcysteine are accurately weighed into separated 25-ml volumetric flasks, dissolved, and diluted with 0.1 *M* sodium hydroxide. These solutions are diluted 1 to 10 with water.

Sample preparation. 5 ml of sample is pipetted into a 50-ml volumetric flask, dissolved, and diluted with 0.1 *M* sodium hydroxide. This solution is further diluted to a suitable concentration with water.

Equipment preparation. The detector is set at 220 nm and 0.1 AUFS and mobile phase is pumped through the column for approximately 30 min to equilibrate the column. The flow-rate is set to 1.0 ml/min and the injection volume is set at 50 μl.

System suitability. After a stable baseline is obtained, 50 μl of the middle strength standard is injected. The retention time of the *S*-carboxymethylcysteine peak is required to be between 5 and 10 min. If not, the flow-rate is adjusted (0.8 to 1.5 ml/min) or the molarity of the buffer is adjusted. To decrease the retention time, the molarity is increased, to increase the retention time, the molarity is decreased. Typical column efficiencies, as described in United States Pharmacopeia (USP)[6], should yield 1500 plates or greater. The column tailing factors, as described in USP, should not be more than 2.5.

RESULTS AND DISCUSSION

Specificity

The specificity of an HPLC method is an essential function if the method is to be used for stability studies. The method has been shown to resolve various known degradation products, impurities, and other components present in the sample.

One possible impurity which could be present in a sample containing *S*-carboxymethylcysteine is cysteine. When cysteine was chromatographed using the conditions described previously, there was no interference observed. A placebo syrup was also chromatographed using the same conditions with no interference observed. When a placebo syrup formulation is left in 0.1 *M* sodium hydroxide for any length of time, a degradation product forms. When this sample was chromatographed using the conditions outlined previously, the degradation product formed shows no observed interference.

In order to anticipate other degradation products which could be formed during stability studies, samples of the cough syrup formulation were exposed to the following conditions: (1) heat, 80°C for 4 days in a sealed-glass ampule; (2) light, 4000 foot-candles for 26 days in a 35-ml centrifuge tube using a General Electric Model RSM sunlamp as the light source.

The heat-stressed sample was tested at 4 days and showed 64% of the initial concentration of 49.6 mg/ml remaining. The chromatogram of this heat-stressed sample is shown in Fig. 1. The light-stressed sample was tested at 26 days and showed 98% of the initial concentration of 49.6 mg/ml. The chromatogram of the light-stressed sample is shown in Fig. 2.

To further verify the specificity of the method, the peaks generated in the stressed samples were examined on the Hewlett-Packard 1040A photodiode array detector. This instrument can store ultraviolet (UV) absorbance spectra at such a high speed that on-the-fly measurements may be taken without stopping the solvent flow through the detector. The spectra can be stored and examined closely to verify the identity of the peak. UV spectra taken from the beginning, middle, and end of

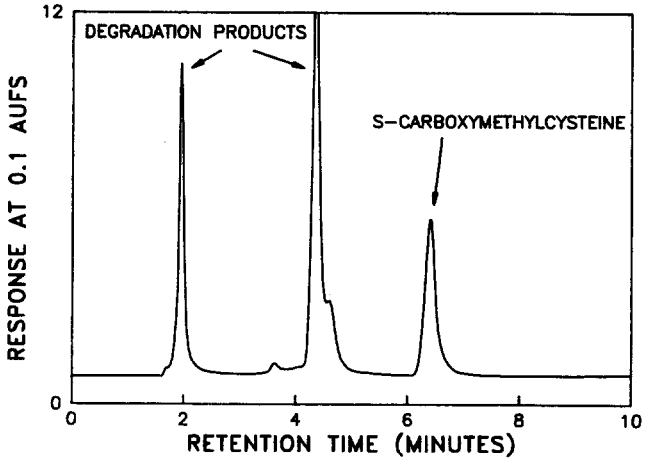

Fig. 1. Chromatogram of a heat-stressed syrup sample.

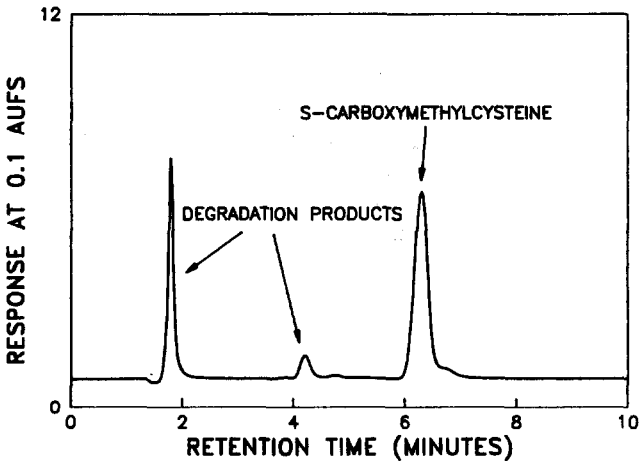

Fig. 2. Chromatogram of a light-stressed syrup sample.

each stressed sample peak were all identical which strongly supports the fact that the peaks in the sample are due to the *S*-carboxymethylcysteine and contain no significant interfering compounds.

Miscellaneous

To test for stability of standard solutions at room temperature, a standard preparation was stored for one week in a 25-ml volumetric flask on the bench top. The results of an assay on this solution indicated that 99.1% of the initial concentration remained (0.1991 mg/ml input *versus* 0.1972 mg/ml found).

CONCLUSION

This method represents a significant improvement over existing analytical procedures for the quantitative determination of *S*-carboxymethylcysteine. The procedure is rugged, stability indicating, and less time consuming since it eliminates the need for a derivatization step.

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