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A reversed-phase high-performance liquid chromatographic assay for the determination of *N*-acetylcysteine in aqueous formulations

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Abstract: A stability-indicating assay is described for the determination of *N*-acetylcysteine in aqueous pharmaceutical formulations. The sample is diluted to an appropriate concentration with dilute aqueous orthophosphoric acid. An aliquot of the solution, containing added L-tyrosine as an internal standard, is chromatographed using a 10-μm C_{18} stationary phase with dilute orthophosphoric acid (pH 2.0) containing 0.5% w/v of sodium perchlorate as the mobile phase. The assay, which has a relative standard deviation of about 0.8%, can also be used as a test for related impurities in *N*-acetylcysteine. It is also suitable for determining the *N*-acetylcysteine content of the drug substance.

Keywords: N-*Acetyl*-l-*cysteine; reversed-phase high-performance liquid chromatography; degradation studies; impurities.*

Introduction

N-Acetylcysteine (Formula **I** of Fig. 1) is a mucolytic agent that is commonly used to reduce sputum viscosity in chronic asthma and bronchitis and to reduce the viscosity of ophthalmic secretions [1–2]. It is also used as an antidote for paracetamol poisoning [3].

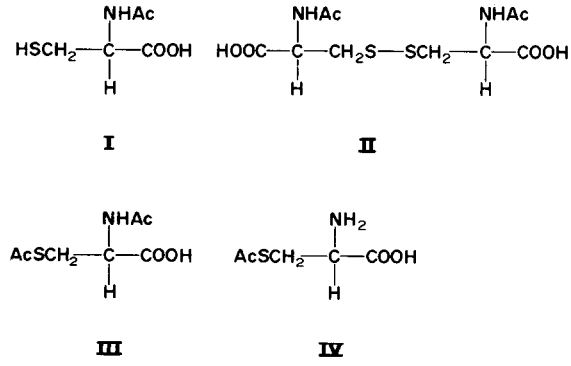

Figure 1
Structures of compounds.

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The assay of the *British Pharmacopoeia* (1980), Addendum 1982, is a direct titrimetric method with 0.05M iodine as the titrant. This method is not specific and cannot distinguish *N*-acetylcysteine from potential manufacturing impurities and degradation products (e.g. L-cysteine). In addition, the reaction product (*N,N'*-diacetylcystine (**II**)) is the principal degradation product of **I** in neutral aqueous solution exposed to molecular oxygen.

Various other titrimetric methods have been reported [4–6]. Liquid chromatographic methods for determining **I** in biological samples have also been developed [7–10]; these methods require derivatization of the amino acid prior to injection of the sample solution on to the chromatograph.

The present report describes a simple reversed-phase high-performance liquid chromatographic (HPLC) method which is specific for *N*-acetylcysteine and has adequate sensitivity for the determination of impurities in concentrations of 0.1% or less.

Experimental

Materials

N-Acetylcysteine (BDH Chemicals, Poole, UK), *N-N'*-diacetylcystine (K.W.R. Chemicals, London, UK), *N*-acetylserine (Sigma Chemical Co., St Louis, USA), *N*-acetylalanine (Sigma Chemical Co., St Louis, USA) and L-tyrosine (Hopkin & Williams, Chadwell Heath, Essex, UK) were obtained in pure form from commercial sources. *N,S*-Diacetylcysteine (**III**) was prepared in the laboratory by the method of Smith and Gorin [11]. Solvents and reagents were of commercial analytical grade.

Chromatographic conditions

The system comprised a high pressure pump (ConstaMetric Model III, Laboratory Data Control, Stone), a valve injector (Rheodyne Model 7125), a column oven (Column Oven Unit CTO-2A, Shimadzu Corp., Kyoto, Japan), a variable wavelength detector (SpectroMonitor D, Laboratory Data Control) and a computer (Hewlett Packard Model 3356). Columns (200 × 5 mm i.d.) packed with 10-μm C_{18} Spherisorb ODS (Phase Separations, Queensferry, UK) at 60°C were used. The mobile phase was a 0.5% m/v aqueous solution of sodium perchlorate; the pH was adjusted to 2.0 with orthophosphoric acid and the solution was then filtered and de-aerated. The flow-rate was adjusted to 2.0 ml/min; UV detection at 215 nm was employed since *N*-acetylcysteine shows end absorption only.

Internal standard solutions

A solution containing about 0.4 mg/ml of L-tyrosine in dilute orthophosphoric acid (pH 2.0) was used.

Standard preparation

About 25 mg of *N*-acetylcysteine reference material was weighed accurately, dissolved in 10.0 ml of internal standard solution, and the volume adjusted to 50.0 ml with dilute orthophosphoric acid (pH 2.0).

Sample preparation

(i) *Aqueous solutions.* The samples were diluted with dilute orthophosphoric acid (pH 2.0) to obtain a solution containing a nominal 5 mg/ml of *N*-acetylcysteine based on the

label claim. An aliquot of 5.0 ml of this solution and 10.0 ml of internal standard solution were transferred to a 50 ml volumetric flask, and diluted to volume with dilute orthophosphoric acid (pH 2.0).

(ii) *Drug substance.* Solutions of the samples were prepared as described for the reference standard.

Procedure

Aliquots (20-μl) of the standard and sample preparations were chromatographed. Reference standards were chromatographed in duplicate at the start and singly at the end of the run, and after every fourth sample. Each standard was freshly prepared prior to injection. The detector attenuation was set at 0.1–0.2 a.u.f.s. To determine related impurities, the attenuation was adjusted to 0.05 a.u.f.s. with the aid of a 10mV recorder. The content of *N*-acetylcysteine was calculated from the ratio of the peak areas of the analyte and the internal standard in the sample and reference solutions.

Recovery studies

Recovery studies were carried out by adding known amounts of *N*-acetylcysteine to appropriately constituted base blanks. The samples were then treated as described under Sample preparation.

Results and Discussion

Chromatography and selectivity

In preliminary experiments the composition of the mobile phase was adjusted to give good separation between **I**, **II** and the internal standard L-tyrosine. The chromatogram (Fig. 2) of **I**, **II** and L-tyrosine illustrates that the method is free from interference and gives symmetrical peak shapes. A base blank was prepared and chromatographed; no peaks other than that at the solvent front were observed. An aqueous formulation containing 5.5 m/v of **I** was stored for 6 months at 22°C exposed to air, and subsequently tested by the method; the resultant chromatogram showed impurity peaks at 2.0 and 8.3 min (Fig. 3).

Several related compounds were chromatographed to test the chromatographic selectivity. The results in Table 1 show that most of the compounds were sufficiently well resolved to allow quantitation of the minor components; no impurity was found which might interfere with the assay at the levels of occurrence of these impurities.

Linearity experiments

The linearity of detector response was tested by chromatographing solutions containing various amounts of **I**; the concentration of internal standard was kept constant. A plot of peak area ratio (Y) versus *N*-acetylcysteine concentration (X mg/ml) ($n = 12$), over the concentration range 0–1.1 mg/ml of *N*-acetylcysteine, gave the equation $Y = 0.05895X - 0.0968$; the standard error (S.E.) of the gradient was 0.01353. The linearity of the detector response to **I** and **II** in samples was tested by chromatographing solutions containing various volumes of an aqueous formulation of **I**; a plot of peak area ratio (Y) versus volume of sample (X ml) gave the equation $Y = 0.06503X + 0.00212$ ($n = 4$) for **I**, and $Y = 0.09441X + 0.01784$ ($n = 4$) for **II**.

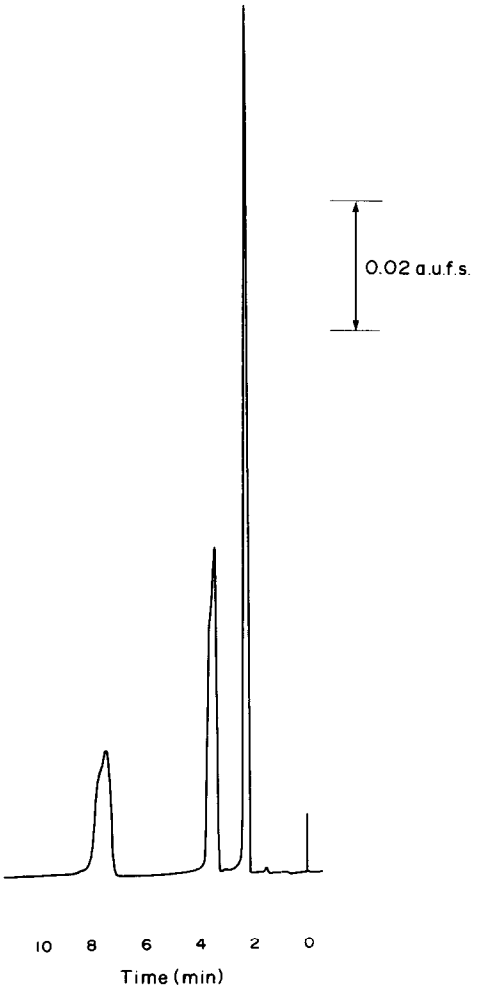

Figure 2
Chromatogram of *N*-acetylcysteine, N,N′-diacetylcystine and L-tyrosine.

Table 1
System selectivity

Compound	Retention time* (min)	Relative retention (α)†
L-Cystine	1.3	Elutes with solvent front
L-Cysteine	1.4	0.07
IV	2.4	0.79
I	2.7	1.00
III	7.6	4.5
II	8.3	5.0

* Solvent front appears at 1.3 min.
† Relative to *N*-acetylcysteine.

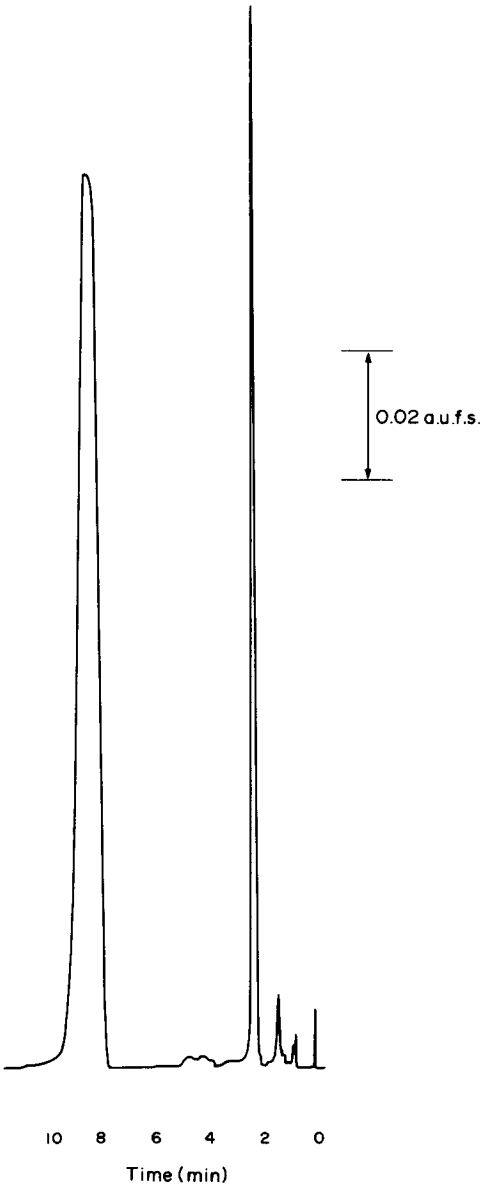

Figure 3
Chromatogram of a degraded aqueous formulation.

Recovery experiments

Known weights of **I** were added to a base blank solution and analysed by the HPLC method (Table 2). A plot of the amount of **I** recovered (Y mg) versus the amount of **I** added (X mg) gave the equation $Y = 0.98468X + 3.0453$; the S.E. was 0.01366.

Precision of assay

The *N*-acetylcysteine content of two batches of an aqueous formulation was determined ten times. The mean content was 5.35 m/v for both batches; relative standard deviations were 1.0% and 0.6%, respectively.

Table 2
Recovery of *N*-acetylcysteine

Weight added (mg)	Weight recovered (mg)	Recovery* (%m/m)
196.2	196.4	100.1
244.1	244.5	100.2
281.0	276.5	98.5
298.3	298.2	100.0
336.8	334.9	99.4
365.4	363.1	99.4

*Mean recovery = 99.6%.
Relative Standard Deviation = 0.6%.
Correlation coefficient = 0.9996.

Comparison of assay with BP method

Five batches of solutions containing **I** were assayed by the HPLC method and also by the method of the *BP* (1980), Addendum 1982; an appropriate volume of sample was used. Results are given in Table 3.

Using the Wilcoxon signed rank test to compare the results obtained, there was no significant difference between the two methods ($p = 0.05$). Student's *t*-test cannot be applied as the data are not normally distributed.

Degradation studies

The ease with which **I** is converted into **II** in the presence of atmospheric oxygen is well documented [12]. The data in Table 4 confirm that **II** is the principal degradation product of **I** in aqueous solutions where oxygen is not totally excluded and the pH of the solution is between pH 5 and 8.

Where exclusion of oxygen is total, **III** is formed as a major degradation product. The formation of **III**, **IV** and L-cysteine from **I** will be discussed in a subsequent paper, but it is worthy of note here that the rate of conversion of **I** to **IV** at pH 2.0 is approximately 0.15%/h at 4°C and 0.3%/h at ambient temperature. However, at pH 5–8 only traces of **IV** are found and the short-term stability of the principal degradation product **II** is unaffected by changes in pH. Consideration of these facts influenced the selection of dilute orthophosphoric acid (pH 2.0) as the solvent for preparation of the sample solution.

Table 3
Comparison of assay methods

Batch	No. of determinations	*N*-Acetylcysteine content (%m/v)	
		Assay	BP method
A	10	5.35	5.36
B	10	5.35	5.36
C	4	5.44	5.42
D	2	5.07	5.16
E	2	5.24	5.25

Table 4
Stability of *N*-acetylcysteine in an aqueous formulation (pH 6.0) in contact with atmospheric oxygen

Storage	**N*-acetylcysteine content (%m/v)	*N,N'*diacetylcystine content (%m/v)
12 months at 4°C	5.07	0.24
12 months at 20°C	5.00	0.45
12 months at 30°C	4.64	0.73
12 months at 37°C	4.50	0.84

* Initial assay = 5.26%m/v by the method of the BP.

Conclusion

The assay described in this paper is stability-indicating and is capable of resolving **I** from its likely manufacturing impurities and potential degradation products. It also provides for the simultaneous determination of **II** in aqueous formulations of **I**, which is of importance since **II** is readily formed where solutions are exposed to molecular oxygen. The method is both robust and accurate.

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