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Stability and compatibility of an aerosol mixture including *N*-acetylcysteine, netilmicin and betamethasone

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Abstract

The physicochemical stability and the compatibility between *N*-acetylcysteine (1 g/5 ml), betamethasone (4 mg/1 ml) and netilmicin (100 mg/1 ml) were studied at room temperature (25 ± 2°C) over 1 h. During this study, drug concentrations were measured using three separate HPLC methods with UV detection at $t = 0$, 5, 10, 20, 30, and 60 min. The pH of the mixture was determined. Degradation products of the drugs were assayed using HPLC. This study demonstrates the stability and compatibility of the mixture over 1 h at room temperature. The pinkish non-remnant coloration observed when pouring *N*-acetylcysteine into a recipient has no effect on the stability of the drug.

Keywords: *N*-Acetylcysteine; Netilmicin; Betamethasone; Compatibility; Stability

1. Introduction

N-Acetylcysteine is a mucolytic agent that reduces the viscosity of secretions probably by the splitting of disulphide bonds in mucoproteins and therefore increasing expectorations (Ziment, 1978). It is widely used in the treatment of chronic pulmonary disease such as cystic fibrosis and is administered by nebulisation of a 20% solution. A survey carried out in France showed that in 50% of the prescriptions *N*-acetylcysteine was prescribed by general physicians with other agents such as antibiotics and/or corticosteroids for nebulisation (Anonymous, 1994). In particular, this avoids the numerous day-long nebulisations for the patient but raises compatibility problems between drug mixtures.

The most frequent drug combination prescribed was *N*-acetylcysteine, netilmicin and betamethasone phosphate. Literature regarding *N*-acetylcysteine stability and compatibility with other drugs is very poor. The *N*-acetylcysteine (Mucomyst®) monograph in the French drug reference book reports that ''owing to physicochemical incompatibilities, concomitant aerosolization of other antibiotics in the same solution must be avoided'' (Vidal, 1998). The same information is

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Table 1
Chromatographic conditions for *N*-acetylcysteine (NA), netilmicin (N) and betamethasone (B) measurements[a]

Method	Column	Mobile phase	Wavelength (nm)	Sample volume (μl)	Expected drug concentration*	Retention time (min)
NA	Lichrosorb RP 10 μm; 250 × 4 mm (Merck, Nogent, France)	0.05 M KH_2PO_4; FR = 1.5 ml/min	210	20	0.3 mg/ml	NA: 3.2; NNDA: 5.0; IS_1: 8.7
N	Hypersil ODS $C_{18;}$ 125 × 4 mm (Shandon, Eragny, France)	ACN/0.1 M KH_2PO_4 (30:70), pH 6.5; FR = 1.0 ml/min	254	20	0.15 mg/ml	N: 3.5; IS_2: 5.1
B	Novapack C_{18} 300 × 3.9 mm (Waters, St Quentin, France)	Methanol/0.05 M KH_2PO_4 (47:53), pH 6; FR = 1.0 ml/min	254	100	10 μg/ml	B: 7

[a] NA, *N*-acetylcysteine; NNDA, *NN′*diacetylcystine; N, netilmicin; B, betamethasone; IS_1, DL-phenylalanine; IS_2, gentamicin.
* Expected drug concentration of sample B injected onto column.

Table 2
Drug concentrations of sample B expressed as percentage remaining in the mixture at 25°C[a]

Time (min)	*N*-Acetylcysteine (% remaining)	Netilmicin (% remaining)	Betamethasone (% remaining)
0	99.5 ± 5.4	99.7 ± 1.8	98.6 ± 2.0
5	96.0 ± 1.8	100.8 ± 0.8	98.9 ± 1.1
10	101.2 ± 4.6	99.5 ± 2.1	96.4 ± 3.6
20	99.9 ± 2.2	101.4 ± 1.9	95.9 ± 1.5
30	99.8 ± 0.4	98.1 ± 1.3	96.4 ± 1.9
60	98.5 ± 2.5	98.6 ± 2.4	97.4 ± 2.1

[a] Values are mean ± S.D. of triplicate samples of the mixture.

detailed in *The Extra Pharmacopoeia*: "antibiotics including amphotericin, ampicillin sodium, erythromycin lactobionate and some tetracyclines are either incompatible or may be inactivated on mixture with acetylcysteine" (Martindale, 1996). Further data in the literature are related to the stability only of *N*-acetylcysteine ophthalmic solution (Fawcett et al., 1993; Anaizi et al., 1997). Data suggest that 10% *N*-acetylcysteine eyedrop solutions are stable from 90 to 120 days depending on the manufacturing process. Eventually, a change in colour of solutions of acetylcysteine to light purple can be observed, but this does not indicate significant impairment of safety or efficacy (Martindale, 1996). In addition, a stability study of gentamicin, another aminoglycoside antibiotic whose structure is close to netilmicin, showed that gentamicin is degraded within 48 h in the presence of dextrose (Graham et al., 1997).

The aim of this study was to determine the stability of a nebulisation solution containing *N*-acetylcysteine (1 g/5 ml), netilmicin (100 mg/1 ml) and betamethasone (4 mg/1 ml) at room temperature (25 ± 2°C).

2. Materials and methods

2.1. Reagents

N-Acetylcysteine (Mucomyst®) 1 g/5 ml was purchased from Bristol-Myers Squibb (Paris, France), Bétamethasone (Celestene®) 8 mg/2 ml, and netilmicin (Netromicine®) 100 mg/1 ml were purchased from Schering-Plough (Levallois-Perret, France) respectively. All other reagents were

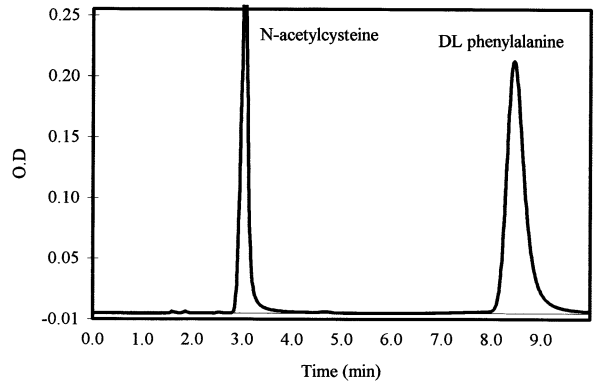

Fig. 1. HPLC chromatogram of the mixture (Sample B) after 60 min with respect to *N*-acetylcysteine measurements according to chromatographic conditions described in Table 1, i.e. method NA for acetylcysteine.

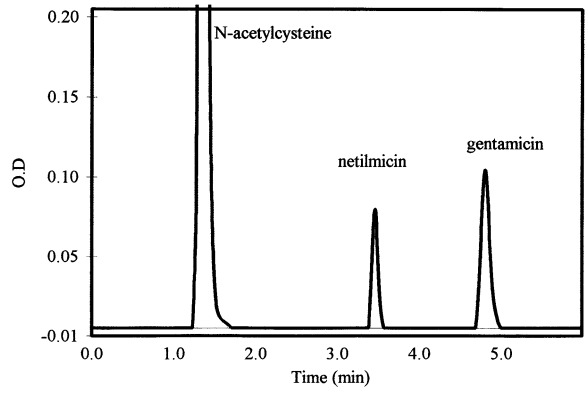

Fig. 2. HPLC chromatogram of the mixture (Sample B) after 60 min with respect to netilmicin measurements according to chromatographic conditions described in Table 1, i.e. method N for netilmicin.

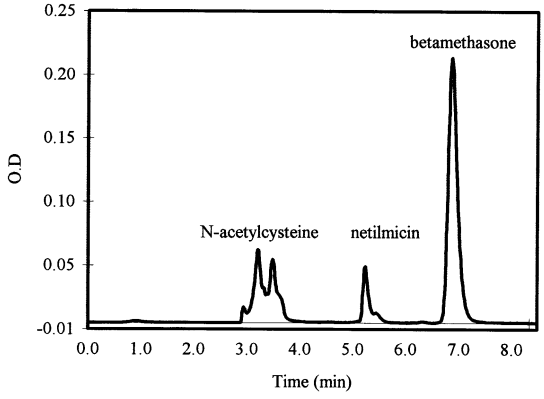

Fig. 3. HPLC chromatogram of the mixture (Sample B) after 60 min with respect to betamethasone measurements according to chromatographic conditions described in Table 1, i.e. method B for betamethasone.

of analytical grade and were purchased from Merck (Nogent/Marne, France).

Stock solutions for HPLC assays were prepared from pure substances and kept frozen (-20°C) until dilution prior to use.

2.2. Apparatus

The chromatography system consisted of the following components: a 125 Solvent Module, a 166 Detector (Beckman, Gagny, France) and a Rheodyne 7725 injection valve (Rheodyne, Cotati, USA) equipped with either a 20- or 100-μl loop. The detector output was connected to system Gold software 8.1 (Beckman).

Details of HPLC experimental conditions are given in Table 1. Three different stability-indicating HPLC assays that accurately and specifically measure *N*-acetylcysteine, netilmicin and betamethasone were used to determine the amount of each drug in the mixture. Degradation products were also identified for each drug using these HPLC methods. Regarding the betamethasone assay, no internal standard was employed as both *N*-acetylcysteine and netilmicin were detected when chromatographic conditions for measuring betamethasone were used.

The linearity and range were determined from a six-point calibration curve with the concentrations ranging from 0.1 to 1 mg/ml for *N*-acetylcysteine, from 0.05 to 0.4 mg/ml for netilmicin and from 2 to 20 mg/l for betamethasone. For intraday and interday repeatability, the coefficients of variation (%) were ≤ 2.7, 4.0 and 4.0 for HPLC assays of *N*-acetylcysteine, netilmicin and betamethasone respectively.

The drug content of the sample solution was determined using the following equation:

$$C_D\ (\%) = (C_B/C_A) \times 100$$

where C_D is the concentration of drug (i.e. *N*-acetylcysteine, netilmicin or betamethasone) expressed as a percentage in the sample solution remaining after time t, C_B is the concentration of drug (i.e. *N*-acetylcysteine, netilmicin or betamethasone) expressed as mg/l in the sample solution after t (min) and C_A is the concentration of drug (i.e. *N*-acetylcysteine, netilmicin or betamethasone) expressed as mg/l in the control solution after t (min). Sample A (the control) contained only one of the three drugs diluted with sterile water for injection up to 7 ml.

Standard and control solutions were freshly prepared on each analysis day and run three times. Experiments were repeated three times. Analyses were performed at $t = 0$, 5, 10, 20, 30, and 60 min.

To demonstrate the stability-indicating nature of the assay, forced degradation studies were conducted for the three drugs by adding either 3 ml of HCl or 3 N NaOH. The three different samples containing *N*-acetylcysteine, netilmicin and betamethasone were placed in a water bath set at 90°C for 12 h. Injection of the samples onto the chromatograph at the end of the degradation process showed several peaks: (1) at 5 min for *N*,*N*′diacetylcystine, a degradation product of *N*-acetylcysteine, (2) at 1.5 and 2 min, two unidentified degradation products of netilmicin, (3) at 3; 3.5 and 5.5 min three unidentified degradation products of betamethasone.

3. Results

Throughout the study period (1 h), the mean *N*-acetylcysteine, netilmicin, and betamethasone concentrations remained above 95% in any exper-

iment. The pH was similar at both the beginning and the end of the study: pH values were 6.7 and 6.8 at $t = 0$ and 60 min respectively. The assayed concentrations, expressed as percentage remaining, are presented in Table 2.

A slight pinkish transient coloration was observed when pouring *N*-acetylcysteine into a beaker. Thereafter, there was no colour development, evidence of turbidity or gas formation during the 1-h period.

No peaks were eluted at the retention time of the different degradation products identified for the three drugs. Moreover, the three HPLC methods were highly specific as no degradation product peaks were eluted at the retention time of any of the three parent drugs. Figs. 1–3 show chromatograms of the samples B after 60 min for measurements of *N*-acetylcysteine, netilmicin and betamethasone respectively.

There were no significant differences in the loss of each drug between the controls (A) and the test tubes (B) stored at room temperature.

4. Discussion and conclusions

This is the first report in the literature regarding *N*-acetylcysteine compatibility with other drugs in solutions. The study demonstrates that the mixture including *N*-acetylcysteine, betamethasone and netilmicin is stable for 1 h at room temperature. The following applied:

- no variation of mixture solution pH;
- no significant degradation of each drug (determination using highly specific methods). The slight differences observed in the percentage remaining, for betamethasone especially, are randomly distributed and not likely to reflect any drug degradation.

The light purple coloration is a well described phenomenon (Kensler, pers. commun.). When exposed to air and being oxidised, iron traces form an unstable coloured chelate with *N*-acetylcysteine and EDTA.

Aerosolization of *N*-acetylcysteine, betamethasone and netilmicin are often prescribed for patients suffering from chronic pulmonary diseases. The lack of knowledge with respect to the physico-chemical stability of the mixture makes administration of a triple drug combination therapy difficult. Administration of each drug separately means that the patient could experience an average of nine or more aerosolizations per day. This makes compliance to therapy a challenge. The stability of the mixture for a 1-h period should prove to be sufficient time for the nurse or the patient to prepare and administer the mixture. Simultaneous aerosolization of the three drugs should be possible.

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