Compatibility and osmolality of inhaled *N*-acetylcysteine nebulizing solution with fenoterol and ipratropium

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Purpose. The compatibility, pH, and osmolality of *N*-acetylcysteine (NAC) nebulizing solution in the presence of ipratropium bromide or fenoterol hydrobromide were studied.

Methods. Portions (400 μL) of each mixture were sampled immediately upon mixing and one, two, three, four, five, six, and seven hours after mixing and assayed by high-performance liquid chromatography. Osmolality was measured by sampling 100 μL from the filling cup at a five-minute interval during nebulization and by the freezing-point-depression method.

Results. Adding NAC solution to fenoterol solution raised the pH from 3.20 to 7.90 and the osmolality to a mean ± S.D. of 1400.67 ± 4.51 mOsm/kg. Fenoterol concentrations decreased to 93.71% and NAC concentrations to 92.54% of initial concentrations after seven hours. Mixing ipratropium with NAC solution raised the pH from 3.74 to 7.95 and the osmolality to a mean ± S.D. of 1413 ± 11.79 mOsm/kg. The initial ipratropium concentration declined 7.39% and 10.91% one and two hours after mixing with NAC solution, respectively.

Conclusion. NAC and ipratropium were stable in nebulizing solution within one hour of mixing. NAC and fenoterol were compatible for at least seven hours.

Index terms: Acetylcysteine; Concentration; Fenoterol hydrobromide; Hydrogen ion concentration; Incompatibilities; Ipratropium bromide; Mucolytic agents; Nebulizers; Osmolality; Parasympatholytic agents; Stability; Storage; Sympathomimetic agents

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Optimal drug delivery is the key to successful management of lung diseases. Inhalation therapy delivers medications directly to the site of action and allows a very small amount of drug into the circulation, thus minimizing adverse effects.[1-3] Adrenergic β_2-receptor agonists, anticholinergics, corticosteroids, and mucolytics have been formulated as inhalation therapy to treat respiratory illnesses. Treatment of pulmonary diseases often requires multiple doses of several medications. Mixing solutions for administration together has advantages of less frequent nebulization and better patient compliance.[4] However, mixing may change the stability and compatibility of the active ingredients, as well as the pharmaceutical characteristics of formulations.[5] Ingredients should be measured to ensure that solutions are chemically and physically compatible. To prevent tissue irritation, the pH of inhaled drug solutions is recommended to be between 2.6 and 10.0.[6] Hypertonic solutions (>1200 mOsm/kg) and hypotonic solutions (<150 mOsm/kg) may cause coughing or bronchoconstriction. The compatibility and properties of admixtures should be addressed to optimize inhalation therapy.

N-acetylcysteine (NAC) is a commonly used mucolytic agent delivered by nebulization or direct tracheobronchial instillation through a tracheostomy. It acts by splitting the disulfide bonds of mucoproteins, liquefying the mucus, and reducing the viscosity of sputum.[7] As a typical thiol-containing compound, NAC is readily oxidized to its inactive disulfide dimer, *N*,*N*′-diacetylcysteine (DiNAC).[8] An opened vial of NAC should be stored at 2–8 °C to retard

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oxidation and be used within 48 hours. The mucolytic activity of NAC requires its pH to remain between 7 and 9.[7] Commercially available NAC solution is prepared as its sodium salt from sodium hydroxide and has an alkaline pH. With the chemical properties of easy oxidation and alkaline solution, NAC has been reported to be incompatible with several antibiotics, including ampicillin, tetracycline, amphotericin B, and erythromycin lactobionate.[9]

One of the adverse effects of inhaled NAC is bronchospasm, which can be prevented by the concomitant use of a bronchodilator, without loss of mucolytic effectiveness.[7,10,11] In addition to the unpleasant odor and taste of hydrogen sulfide associated with NAC nebulizing solution, its hyperosmolality is a major cause of bronchospasm. The hypertonicity-induced bronchospasm has been reported to be prevented by concurrent use of salbutamol.[12] However, the compatibility of NAC and bronchodilators in admixtures should be investigated to ensure safety and efficacy. Air oxidation of phenolic sympathomimetic amines, including epinephrine, isoproterenol, terbutaline, and fenoterol, to inactive quinines is more rapid in alkaline solutions than in a neutral or acidic environment.[13] Ipratropium bromide, an anticholinergic bronchodilator, is an *N*-isopropyl derivative of atropine containing an ester linkage.[14] Its hydrolysis is also pH dependent, with maximum stability generally observed at pH 3–4, and it is catalyzed by either hydronium or hydroxyl ions.[15] The alkaline pH of NAC nebulizing solution may affect the stability of bronchodilators.

Given the therapeutic benefit of using NAC in combination with a bronchodilator, the compatibility and safety of extemporaneously compounded admixtures should be determined. The purpose of this study was to determine the compatibility, pH, and osmolality of NAC nebulizing solution mixed with ipratropium bromide or fenoterol hydrobromide at 25 °C.

Methods

Commercial nebulizing solutions were used containing NAC sodium 352.4 mg per 2 mL per ampul (20%, weight to volume),[a] ipratropium bromide 500 µg per 2 mL per vial,[b] and fenoterol hydrobromide 1.25 mg per 2 mL per vial.[c] All other chemicals and solvents were analytical grade commercial products, except DiNAC standard, which was prepared from acetylation of L-cysteine[d] with acetic anhydride.[16] Mass spectrometry and thin-layer chromatography confirmed the validity of the prepared DiNAC.

Six mixtures were designed to measure the osmolality of the solution in the filling cup of a jet-type nebulizer.[e] Three mixed solutions were nebulized for 15 minutes, including (1) 2 mL of NAC solution and 2 mL of fenoterol solution, (2) 2 mL of NAC solution and 2 mL of ipratropium solution, and (3) 2 mL of ipratropium solution and 2 mL of fenoterol solution. Four 100-µL samples were collected from each mixture 1, 5, 10, and 15 minutes after nebulization. Another three mixed solutions were nebulized for 30 minutes by adding 4 mL of 0.45% sodium chloride solution to the three solutions described previously. Seven 100-µL samples were collected from each solution 0, 5, 10, 15, 20, 25, and 30 minutes after nebulization. Each sample was withdrawn directly from the filling cup for measurement of osmolality with an advanced microosmometer.[f] Osmolality was calculated by the freezing-point-depression method with reproducibility of ±0.5% for solutions with low viscosity.

To test drug concentration, NAC sodium nebulizing solution 2 mL per ampul[a] was emptied into a glass tube with a film-sealed glass cap. Ipratropium bromide nebulizing solution 2 mL per vial or fenoterol hydrobromide inhalation solution 2 mL per vial was added to NAC nebulizing solution. Each solution of 2 mL per vial was also mixed with 2 mL of distilled water to serve as a control solution. The mixture was gently mixed on a tube rotator at 25 rpm throughout the study period at room temperature (25 °C). Portions (400 µL) of each admixture were removed immediately and one, two, three, four, five, six, and seven hours after mixing. The samples of NAC and DiNAC solutions were diluted 150-fold and ipratropium and fenoterol solutions 2-fold and 4-fold, respectively, with purified water. Immediately after withdrawal, the samples were injected into a high-performance liquid chromatography (HPLC) column for analysis.

The HPLC equipment consisted of a pump,[g] an autosampler,[h] an ultraviolet (UV) light detector,[i] and an integrator.[j] For the analysis of NAC and DiNAC concentrations, the HPLC column used an HPLC cartridge (250 × 4 mm).[k] The mobile phase consisted of a mixture of acetonitrile[l] and 0.2% (by volume) acetic acid with a pH of 3 (3:97 by volume). The flow rate was set at 1 mL/min. The UV detector was operated at a wavelength of 230 nm. Under these conditions, NAC and DiNAC eluted at 5.9 and 12.29 minutes, respectively. Ipratropium was analyzed by using the same HPLC column as for NAC and DiNAC; the mobile phase was acetonitrile-diluted acetic acid (0.0375% by volume) with a pH of 3.5 (20:80 by volume), containing 8.47 mM sodium methanesulfonate[m] as an ion-exchange reagent. The retention time was 10.9 minutes at a flow rate of 1 mL/min and a wavelength of 220 nm. The analysis of fenoterol was performed by using a C_{18} column.[n] The mobile phase was a mixture of methanol[o] and 0.0375% (by volume) acetic acid with a pH of 3.5 (40:60 by volume). UV detection was achieved at 280 nm. The retention time was 5.79 minutes at a flow rate of 1 mL/min.

NAC 10-mg/mL standards diluted with distilled water to five concentrations (40, 100, 400, 600, and 800 µg/mL) were prepared (three solutions at each concentration) to generate the standard curve; intraday coefficients of variation (CVs) were 0.75%, 0.40%, 0.21%, 2.98%, and 0.13%, respectively, and interday CVs were 0.60%, 2.15%, 1.67%, 2.58%, and 2.65%, respectively. Diluted DiNAC 1-mg/mL standards were prepared at 5, 10, 50, 100, 200, and 300 µg/mL for the standard curve; intraday CVs were 3.36%, 4.22%, 6.09%, 4.08%, 0.88%, and 0.78%, respectively, and interday CVs were 2.62%, 4.64%, 4.63%, 0.55%, 1.63%, and 0.77%, respectively. Ipratropium 0.4-mg/mL standard was diluted to 20, 30, 40, 60, and 80 µg/mL; intraday CVs were 2.32%, 2.28%, 1.30%, 1.73%, and 1.19%, respectively, and interday CVs were 0.27%, 1.80%, 2.64%, 0.48%, and 1.10%, respectively. Fenoterol 1-mg/mL standard was diluted to 20, 40, 60, 100, and 200 µg/mL; intraday CVs were 0.66%, 1.13%, 0.36%, 0.04%, and 0.33%, respectively, and interday CVs were 1.38%, 0.77%, 0.82%, 0.45%, and 0.27%, respectively. Least-squares linear regression showed excellent linearity ($r^2 \geq 0.999$) for the four assays.

The percentage of each drug remaining at each time point was calculated; stability was defined as the retention of ≥90% of the initial concentration. All measurements were done in triplicate, and results are reported as mean ± S.D. Statistical analysis was performed with Student's *t* test or analysis of variance (ANOVA) by using the Statistical Package for the Social Sciences, version 11.0, software program (SPSS Inc., Chicago, IL). Significance for all analyses was defined as a p value of <0.05.

Results and discussion

Oxidation of NAC to DiNAC. Concentrations of NAC in mixed solutions and control solutions are shown in Table 1. NAC was easy to oxidize to DiNAC at 25 °C when examined by thin-layer chromatography. HPLC showed a mass balance during the study period. The concentration of NAC in molarity plus the molarity of DiNAC multiplied by 2 did not differ at each sampling time ($p = 0.218$, ANOVA), which was indicative of DiNAC being the major degradation product during NAC oxidation. If degradation products or impurities other than DiNAC were present, the quantities were not detectable.

Figure 1 compares the concentration profiles of DiNAC in each solution. The fractional decomposition of NAC in both admixtures was less than 0.15 over seven hours. The DiNAC concentration in the mixture of NAC and fenoterol solutions was significantly lower than that in NAC control solution from two hours to seven hours. The significantly lower DiNAC concentration in the NAC–fenoterol inhalant mixture indicates that mixing fenoterol inhalant solution decreased the NAC oxidation rate. Since hydroxyl ions accelerated the oxidation of substituted phenols to quinones, the alkaline mixture of NAC nebulizing solution and fenoterol inhalant solution contributed to increased fenoterol oxidation to sympathomimetically inactive quinones.[17]

pH of nebulizing solution. Throughout the study period, there were no visible changes in color and no occurrence of precipitation in the mixed solutions. All solutions were clear and colorless. However, the initial pH before mixing was 7.58 ± 0.08 for NAC nebulizing solution but 3.74 ± 0.07 for ipratropium solution and 3.20 ± 0.04 for fenoterol solution. The baseline pH values strongly indicated incompatibility. Admixing NAC with ipratropium or fenoterol solution yielded mixtures with a pH of 7.95 ± 0.09 and 7.90 ± 0.03, respectively. After mixing, the pH of both mixtures did not change significantly from time zero to seven hours.

Compatibility of NAC and ipratropium in nebulizing solution. The concentrations of NAC and ipratropium in NAC–ipratropium mixtures and control solutions are shown in Tables 1 and 2. Adding NAC to the ipratropium nebulizing solution abruptly raised the pH and consequently caused ipratropium hydrolysis to tropic acid and a tropine derivative. Compounds containing an ester linkage, such as ipratropium, were susceptible to hydrolysis, and hydroxyl ions had a greater influence on the rate of hydrolysis.[15]

Table 1.
***N*-acetylcysteine (NAC) Concentration in Mixed Solutions and Control Solutions over Time ($n = 3$)**

Time (hr)	Mean ± S.D. NAC Conc., mg/mL (% of Control upon Mixing)		
	In NAC Nebulizing Solution plus Distilled Water	In NAC Nebulizing Solution plus Ipratropium Nebulizing Solution	In NAC Nebulizing Solution plus Fenoterol Inhalant Solution
0	89.15 ± 0.64 (100.01 ± 0.71)	89.81 ± 1.82 (100.74 ± 2.04)	88.47 ± 0.99 (99.24 ± 1.11)
1	89.39 ± 1.60 (100.27 ± 1.79)	87.31 ± 2.05 (97.94 ± 2.30)	87.91 ± 0.33 (98.60 ± 0.37)
2	88.16 ± 1.81 (98.89 ± 2.03)	87.40 ± 1.15 (98.03 ± 1.29)	86.70 ± 0.44 (97.25 ± 0.49)
3	87.17 ± 2.62 (97.78 ± 2.94)	87.43 ± 1.11 (98.07 ± 1.24)	85.76 ± 0.08 (96.20 ± 0.09)
4	84.85 ± 0.38 (95.18 ± 0.43)	85.23 ± 0.84 (95.61 ± 0.94)	86.12 ± 0.93 (96.60 ± 1.04)
5	85.25 ± 1.11 (95.62 ± 1.24)	83.82 ± 1.00 (94.02 ± 1.13)	83.41 ± 0.90 (93.56 ± 1.01)
6	82.70 ± 1.74 (92.77 ± 1.95)	80.38 ± 5.01 (90.17 ± 5.62)	83.78 ± 0.52 (93.97 ± 0.59)
7	80.53 ± 1.34 (90.33 ± 1.50)	80.66 ± 1.30 (90.47 ± 1.46)	82.50 ± 0.58 (92.54 ± 0.65)

Figure 1. Concentration of *N,N'*-diacetylcysteine (DiNAC) in test mixtures and control solutions for seven hours. Filled diamonds = DiNAC concentration in *N*-acetylcysteine sodium (NAC) solution 2 mL plus distilled water 2 mL, open diamonds = DiNAC concentration in NAC solution 2 mL plus ipratropium bromide solution 2 mL, filled triangles = DiNAC concentration in NAC solution 2 mL plus fenoterol hydrobromide 2 mL, asterisks = DiNAC concentration is significantly less than that in NAC plus distilled water ($p < 0.05$, one-way analysis of variance).

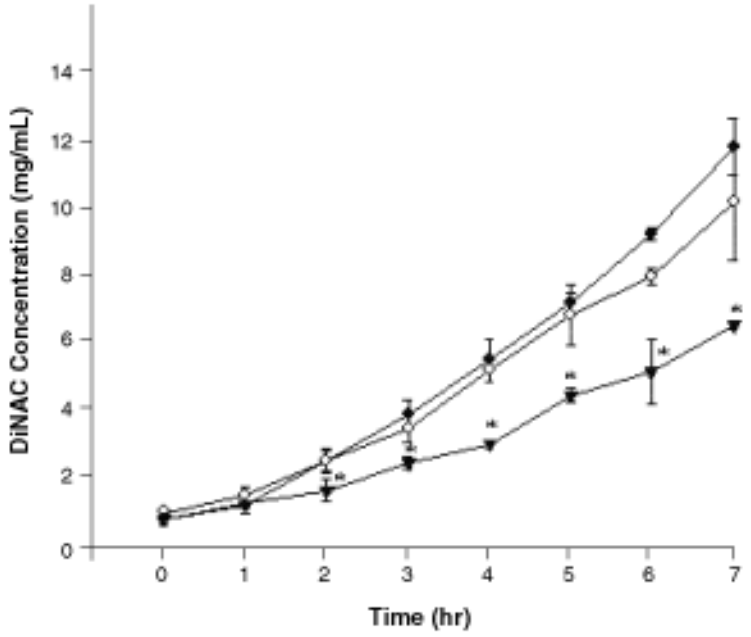


Table 2.
Ipratropium Concentration in Mixture with *N*-acetylcysteine (NAC) Solution over Time ($n = 3$)

	Mean ± S.D. Ipratropium Conc., µg/mL (% of Control upon Mixing)	
Time (hr)	In Ipratropium Nebulizing Solution plus Distilled Water	In Ipratropium Nebulizing Solution plus NAC Nebulizing Solution
0	112.61 ± 3.54 (100.00 ± 3.15)	104.42 ± 2.85 (92.73 ± 2.53)
1	106.25 ± 2.60 (94.35 ± 2.31)	104.29 ± 2.52 (92.61 ± 2.24)
2	110.16 ± 5.80 (97.82 ± 5.15)	100.33 ± 0.67 (89.09 ± 0.59)
3	103.60 ± 2.61 (92.00 ± 2.32)	102.96 ± 2.60 (91.43 ± 2.31)
4	108.09 ± 2.12 (95.98 ± 1.88)	99.22 ± 1.16 (88.11 ± 1.03)
5	103.46 ± 1.17 (91.88 ± 1.04)	100.24 ± 3.34 (89.02 ± 2.97)
6	105.02 ± 5.67 (93.26 ± 5.03)	97.51 ± 3.42 (86.59 ± 3.04)
7	105.88 ± 5.61 (94.03 ± 4.98)	99.02 ± 4.84 (87.93 ± 4.30)

At a pH above 4, a unit increase in pH caused a 10-fold increase in the apparent first-order rate constant for the ester hydrolysis of atropine at 30 °C[18] and resulted in a mean 7.27% loss of ipratropium immediately after mixing and a more than 10% loss after two hours. A more than 10% loss of ipratropium is clinically significant; the NAC–ipratropium nebulizing solution should be used within one hour.

Compatibility of NAC and fenoterol in nebulizing solution. The concentrations of NAC and fenoterol in NAC–fenoterol mixtures and control solutions are shown in Tables 1 and 3. Adding fenoterol solution to NAC nebulizing solution abruptly raised the pH from acid to alkaline. The percentage of drug remaining immediately after mixing was 95.60% ± 0.37% for fenoterol and 99.24% ± 1.11% for NAC. Retention of both fenoterol and NAC concentrations remained above 90% after seven hours (Table 3). Since the oxidation of phenols was catalyzed by hydroxyl ions, mixing NAC nebulizing solution with fenoterol inhalant solution contributed to the increase in the rate of fenoterol oxidation.[17] Despite the observed oxidative reactions in the NAC–fenoterol inhalant mixture, the drugs were chemically compatible for at least seven hours.

Osmolality changes. NAC nebulizing solution was extremely hyperosmolal (2258.67 ± 51.59 mOsm/kg). Diluting NAC nebulizing solution with 0.45% sodium chloride solution decreased the osmolality to 875.67 ± 10.97 mOsm/kg and may reduce irritation of the airways. Simultaneous administration of fenoterol or ipratropium inhalant solution with NAC nebulizing solution could also prevent high-osmolality-induced bronchospasm. The osmolality of undiluted ipratropium nebulizing solution was 276.33 ± 1.53 mOsm/kg, and fenoterol inhalant solution had an osmolality of 274.67 ± 1.15 mOsm/kg. However, adding NAC to ipratropium or fenoterol inhalant solution raised the osmolality to 1413 ± 11.79 and 1400.67 ± 4.51 mOsm/kg, respectively.

It has been reported that paradoxical bronchoconstriction occurred instead of bronchodilation in wheezy infants during administration by nebulization of a β_2-receptor agonist.[19] The increased osmolality of the nebulized solution appeared to be the cause of the bronchoconstriction. In both study mixtures, the increase in osmolality in the final five minutes was about three times that in the first five minutes. The continuous increase in osmolality during

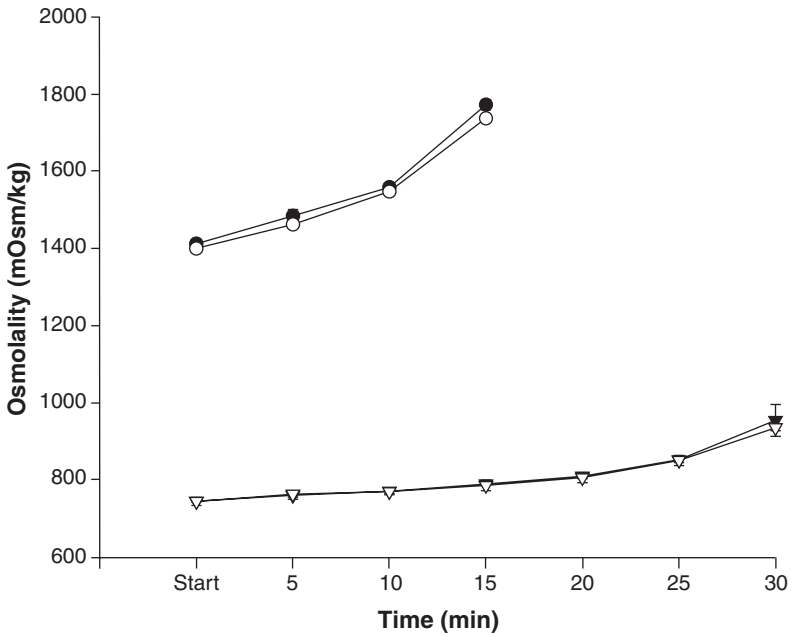
nebulization agrees with the findings of Schöni et al.[20] and can be explained by the theory of aerosol production. As compressed air was blasted through a small hole in a capillary tube at which the lateral pressure fell, the liquid was drawn up by the Bernoulli effect and mixed with the airflow to produce the droplets. Evaporation of nebulized droplets raised the concentration, consequently increasing osmolality.[21] The large increase in osmolality was observed near the end of a nebulization period, when the total amount of liquid left in the filling cup became small.

After dilution with 0.45% sodium chloride solution to a starting volume of 8 mL, osmolality increased at a lower rate than for undiluted mixtures with a starting volume of 4 mL, as observed from the steeper curves for undiluted solutions than for diluted ones (Figure 2). The osmolality of both of the mixtures diluted with 4 mL of 0.45% sodium chloride solution was less than 1200 mOsm/kg during the 15 minutes of nebulization. The percent increase in osmolality at the end of nebulization was not different between the undiluted and diluted mixtures: 25.48% ± 0.49% versus 28.13% ± 5.84% for the NAC–ipratropium mixture ($p = 0.515$) and 24.08% ± 0.74% versus 25.64% ± 2.17% for the NAC–fenoterol mixture ($p = 0.304$).

Table 3.
Fenoterol Concentration over Time ($n = 3$)

	Mean ± S.D. Fenoterol Conc., µg/mL (% of Control upon Mixing)	
Time (hr)	In Fenoterol Inhalant Solution plus Distilled Water	In Fenoterol Inhalant Solution plus *N*-acetylcysteine Nebulizing Solution
0	255.15 ± 3.46 (100.00 ± 1.36)	243.91 ± 0.95 (95.60 ± 0.37)
1	253.88 ± 1.51 (99.50 ± 0.59)	241.32 ± 0.55 (94.58 ± 0.22)
2	256.52 ± 2.19 (100.54 ± 0.86)	240.29 ± 1.93 (94.17 ± 0.76)
3	254.97 ± 2.02 (99.93 ± 0.79)	240.82 ± 1.14 (94.38 ± 0.45)
4	256.81 ± 1.72 (100.65 ± 0.67)	241.99 ± 1.11 (94.84 ± 0.44)
5	258.13 ± 1.39 (101.17 ± 0.55)	241.07 ± 1.64 (94.48 ± 0.64)
6	258.38 ± 2.47 (101.27 ± 0.97)	242.49 ± 1.28 (95.04 ± 0.50)
7	258.51 ± 2.10 (101.32 ± 0.82)	239.10 ± 2.16 (93.71 ± 0.85)

Figure 2. Osmolality of *N*-acetylcysteine sodium (NAC) nebulizer solution 2 mL plus ipratropium nebulizer solution 2 mL (●), NAC nebulizer solution 2 mL plus fenoterol inhalant solution 2 mL (○), NAC nebulizer solution 2 mL plus ipratropium nebulizer solution 2 mL plus 0.45% sodium chloride injection 4 mL (▼), and NAC nebulizer solution 2 mL plus fenoterol inhalant solution 2 mL plus 0.45% sodium chloride injection 4 mL (▽).



Conclusion

NAC and ipratropium were stable in nebulizing solution within one hour of mixing. NAC and fenoterol were compatible for at least seven hours. The NAC solution, either alone or in combination with fenoterol or ipratropium solution, should be diluted with 0.45% sodium chloride solution before nebulization to decrease the osmolality.

[a]Acetein, Senju Pharmaceutical Co. Ltd., Osaka, Japan, lot A121.
[b]Atrovent, Boehringer Ingelheim Ltd., Bracknell, England, lot 138396.
[c]Berotec, Boehringer Ingelheim, lot 139259.
[d]L-cysteine, donated by the Department of Health, National Laboratories of Foods and Drugs, Executive Yuan, Taipei, Taiwan.
[e]Jet-type nebulizer, model 5650, airflow 9 L/min, Pulmo-Aide, DeVilbiss, PA.
[f]Microosmometer, Hermann Roebling, Berlin, Germany.
[g]HPLC pump, model L-7100, Hitachi Ltd., Tokyo, Japan.
[h]HPLC autosampler, model L-7200, Hitachi.
[i]HPLC UV detector, model L-4000H, Hitachi.
[j]HPLC integrator, model D-7500, Hitachi.
[k]LiChroCART HPLC cartridge, 250 × 4 mm, LiChrospher 100 RP-18e packing (5-µm particle size), Merck & Co, Darmstadt, Germany.
[l]Acetonitrile, BDH Laboratory Supplies, Poole, England.
[m]Sodium methanesulfonate, Aldrich Chemical Co., Milwaukee, WI.
[n]µBondapak C_{18} column, 300 × 3.9 mm, 10-µm particle size, Waters Co., Milford, MA.
[o]Methanol, Labscan Asia Co. Ltd., Bangkok, Thailand.

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