Doxofylline and methylprednisolone sodium succinate are stable and compatible under normal injection conditions

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Abstract: To assess the physical compatibility and chemical stability of doxofylline with methylprednisolone sodium succinate in 0.9% sodium chloride or 5% dextrose injection for intravenous infusion. Twenty mL doxofylline solution (0.74 mg/mL) and 1 mL methylprednisolone sodium succinate solution (0.15 mg/mL) were added into 250 mL polyolefin bags containing 5% dextrose injection or 0.9% sodium chloride injection, and stored for 24 h at 20-25°C. Chemical compatibility was measured with high-performance liquid chromatography (HPLC), and physical compatibility was determined visually. The results showed that samples were clear and colorless when viewed in normal fluorescent room light. The pH value exhibited little change. The particulate content of ≥ 25 μm was low and within the specification limit. The particulate content of ≥ 10 μm decreased over time and was similar to the control solution. Analysis of chemical stability revealed that doxofylline is stable with methylprednisolone sodium succinate for up to 24 h, and the degradation of methylprednisolone sodium succinate is unrelated to doxofylline, but is closely related to the pH value of the solution. Doxofylline and methylprednisolone sodium succinate did not affect the stability of each other.

Keywords: Doxofylline, methylprednisolone sodium succinate, sodium chloride injection, dextrose injection, compatibility, stability.

INTRODUCTION

Doxofylline is a novel bronchodilator that differs from theophylline by the presence of a dioxolane group in position 7. The bronchodilator activities of doxofylline have been documented in animal studies and in clinical trials involving patients with either bronchial asthma or chronic obstructive pulmonary disease (Bagnato, 1999). Methylprednisolone sodium succinate, a synthetic glucocorticoid and a soluble prodrug of methyl-prednisolone, is widely used clinically and experimentally as an acute anti-inflammatory treatment (Tyrell, 1995). Theophylline and methylprednisolone sodium succinate have been shown to exhibit therapeutic synergy for acute airway obstruction and acute asthma (Cosio *et al*., 2004; Jónsson *et al*., 1988; Nassif *et al*., 1981). Due to the fact that theophylline is a potentially toxic drug with a narrow therapeutic ratio, even when used properly, it may cause side-effects such as sleeping difficulties, irritability, mild headaches, loss of appetite, nausea, vomiting, and stomach ache. Doxofylline has similar efficacy but better tolerability and safety than theophylline (Bagnato, 1999). To the best of our knowledge, no studies have addressed the compatibility of doxofylline and methylprednisolone sodium succinate.

In this study, we examined the physical and chemical compatibility of doxofylline and methylprednisolone sodium succinate mixed in 250 mL polyolefin bags of 5% dextrose injection and 0.9% sodium chloride injection stored at 20-25°C. Our results suggest that doxofylline and methylprednisolone sodium succinate are stable for only up to 4 h when mixed in 0.9% sodium chloride injection, but stable for up to 16 h when mixed with 5% dextrose injection.

MATERIALS AND METHODS

Doxofylline (99.5%) and methylprednisolone (>98%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methylprednisolone sodium succinate (>97%) was obtained from Sigma (Beijing, China). Phosphoric acid and potassium dihydrogen phosphate of analytical grade were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Methanol of HPLC-grade was obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared by a MilliQ apparatus (Millipore, Milford, NH, USA). The drugs studied were commercial products suitable for clinical use, and were provided in injectable forms. Doxofylline injection (doxofylline 100 mg in 10 mL, lot #081106) was obtained from Fuhehuaxing Pharmaceutical (Heilongjiang, China). Methylprednisolone sodium succinate for injection (methylprednisolone sodium succinate 40 mg and 1 mL solution, lot #R05471) was purchased from Pfizer (Puurs, Belgium), and 0.9% sodium chloride injection (250 mL, lot #W2080607H) and 5% dextrose injection (250 mL, lot # W2080611 H) were obtained from Kelun Pharmaceutical (Sichuan, China).

Materials and chromatographic conditions

A HPLC method, pH-meter, and an injection microparticle analyzer were used to conduct the analyses.

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Chromatographic analysis was performed using a Waters 2695 separation module, and a Waters 2487 dual wavelength detector (Waters, Milford, MA, USA). Detection and quantification were performed using Empower software. Separation was achieved with a Waters symmetry C_{18} reversed-phase column (150 mm×4.6 mm, 5 µm) at 35°C. The mobile phase consisted of 60:40 (v/v) HPLC-grade methanol and 0.02 M phosphate buffer with the pH adjusted to 3.2. The flow rate was set at 1.0 mL/min, and the injection volume was 10 µL. A dual wavelength detection strategy was used; elution was performed at a wavelength of 244 nm. Typical chromatograms of each drug mixture are shown in fig. 1.

The pH value was measured with a precision pH meter (Model pHS-3C, Leici, Shanghai, China). Laser injection microparticle analyzer (Model ZWF-J6, Tianhe Medical Instrument, Tianjin, China) was used in accordance with the specifications of Pharmacopoeia of People's Republic of China (Pan and Yu, 2005) for large-volume injections, and compatibility criteria included ≤25 particles of ≥10µm in size per milliliter and ≤3 particles of ≥25µm per milliliter.

Preparation of solutions and storage conditions

Two vials of injectable doxofylline (100 mg in 10 mL) were injected into a 250 mL polypropylene bag containing 0.9% sodium chloride or 5% dextrose, and mixed well to represent the control solution of doxfylline. A vial of injectable methylprednisolone sodium succinate (40 mg in 1 mL) was injected into a 250 mL polypropylene bag containing 0.9% sodium chloride or 5% dextrose, and mixed well to represent the control solution of methylprednisolone sodium succinate. Two vials of injectable doxofylline (100 mg in 10 mL) and a vial of injectable methylprednisolone sodium succinate (40 mg in 1 mL) were injected into a 250 mL polypropylene bag containing 0.9% sodium chloride or 5% dextrose, and mixed well to represent the test compatible solution. Bags were stored for 24 h at room temperature (20-25°C) without protection from the light.

Compatibility test

All mixtures were prepared in triplicate. Immediately after sample preparation, and at specific time intervals after storage (0, 2, 4, 8, 12, 16, and 24 h), the physical stability of the mixture was assessed by visual observation for clarity, precipitation, color change, pH changes, and

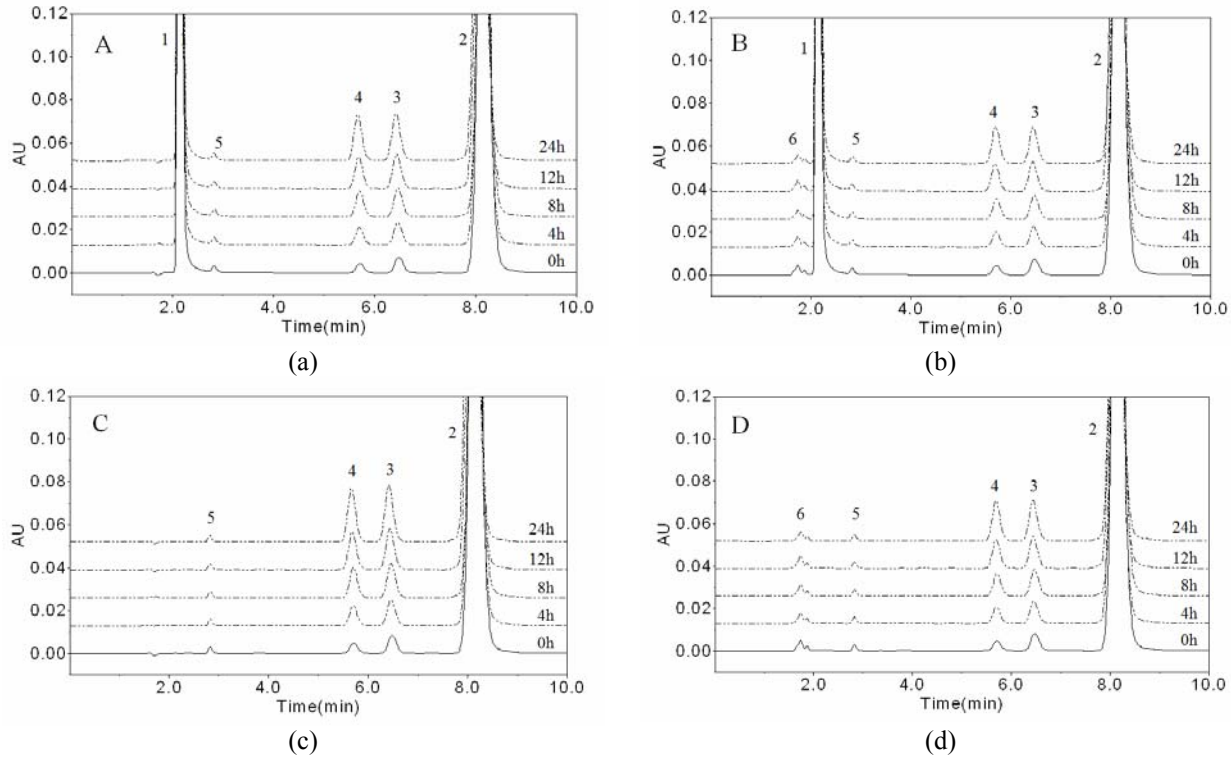

Fig. 1: Chromatograms of: (A) doxofylline and methylprednisolone sodium succinate mixed in 0.9% sodium chloride injection at 0, 4, 8, 12 and 24 h; (B) doxofylline and methylprednisolone sodium succinate mixed in 5% dextrose injection at 0, 4, 8, 12 and 24 h; (C) methylprednisolone sodium succinate with 0.9% sodium chloride at 0, 4, 8, 12 and 24 h; (D) methylprednisolone sodium succinate with 5% dextrose chloride at 0, 4, 8, 12 and 24 h. Approximate retention times: doxofylline = 2.30 min; methylprednisolone sodium succinate = 8.23 min. Peaks labeled 1, 2, 3, 4 ,5 and 6 correspond to doxofylline, methylprednisolone sodium succinate, methylprednisolone, methylprednisolone analogs, benzyl alcohol, and dextrose, respectively.

the presence of particulate matter. Chemical stability was simultaneously assessed by the quantification of doxofylline and methylprednisolone sodium succinate in the mixtures. In order to identify possible gross immediate incompatibilities, and to validate the accuracy of solution preparation, the drug concentration of the mixture with the greatest nominal drug concentration was compared immediately upon preparation to that in the corresponding control solution. For all mixtures, recovery at 24 h was compared to the initial drug concentration. Chemical compatibility was defined as the retention of 95-105% of the initial concentration. An impurity was defined as any chromatographic peak not attributable to the control solutions (on the basis of relative retention times) and having an area > 0.10% of that for the total response.

RESULTS

Chromatography analysis

The samples analyzed were indicated by the 3D plot of the DAD detector signal. The maximal and second strong absorption wave band of doxofylline was at 210 nm and 273 nm, respectively. The maximal absorption wave band of methylprednisolone sodium succinate was at 244 nm. Since the concentration and the absorption intensity of doxofylline were much higher than methylprednisolone sodium succinate in the compatible mixtures, and doxofylline had a relatively stable absorption around 244 nm (fig. 2), detection was performed at 244 nm.

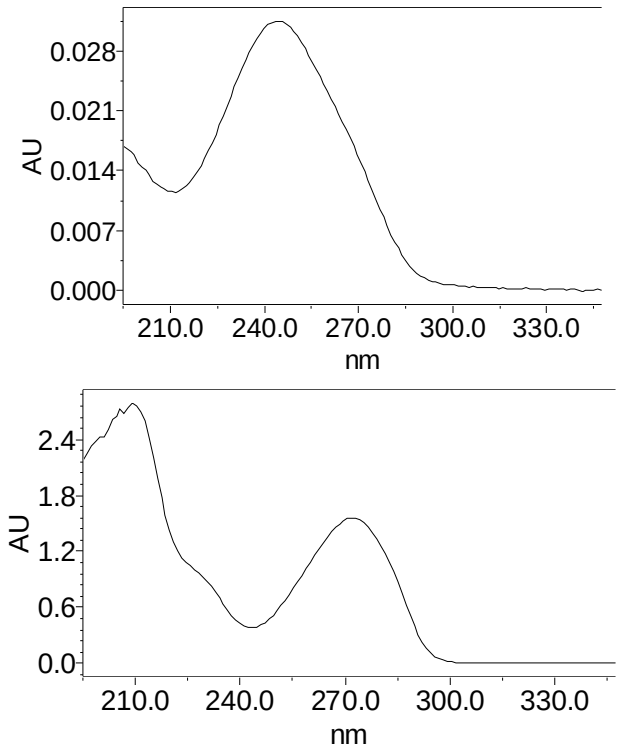

Fig. 2: UV spectrum of (A) doxofylline and (B) methylprednisolone sodium succinate.

Next, we employed HPLC to detect the degradation of doxofylline (500 µg/mL) and methylprednisolone sodium succinate (400 µg/mL) under stressed conditions. Each drug was exposed to 1 *N* hydrochloric acid, 1 *N* sodium hydroxide, 3% hydrogen peroxide, 0.9% sodium chloride solution, and 5% dextrose injection at 60°C overnight. The results demonstrated that doxofylline and methylprednisolone sodium succinate degraded substantially when exposed to 1 *N* hydrochloric acid, 1 *N* sodium hydroxide, or 3% hydrogen peroxide at 60°C, but none of the degradation products interfered with the peaks of the intact drugs, as judged from their respective retention time. No precipitation was observed.

Calibration curves of doxofylline and methylprednisolone sodium succinate were constructed at concentrations of 200-1000 and 60-240 µg/mL, respectively. The amount of drugs was plotted versus the concentrations of drugs. The calibration curves of doxofylline and methylprednisolone sodium succinate were linear with a regression coefficient of 0.9999. The calibration equations of doxofylline and methylprednisolone sodium succinate were: $y = 7300x + 11400$ (n=5) and $y = 25600x + 15300$ (n=5), respectively, with y as the peak-area of drugs, and x as the corresponding concentration of drugs. Intra-day and inter-day precision and accuracy were determined at low, medium, and high concentrations of doxofylline (300, 500, and 800 µg/mL) and phentolamine mesilate (90, 150 and 210 µg/mL) by replicate analyses, respectively. The intra-day and inter-day precisions were measured to be within 0.53-1.45% for doxofylline and 0.51-1.82% for methylprednisolone sodium succinate, respectively.

Stability analysis

All mixtures appeared clear, and no color change or precipitation was observed over 24 h at 20-25°C. Compatibility data of the presence of particulate matter, pH changes, and the quantification of doxofylline and methylprednisolone sodium succinate in control solutions containing only doxofylline or methylprednisolone sodium succinate and test compatible solutions were investigated and are summarized in tables 1 and 2.

DISCUSSION

In the physical compatibility test, the pH of the test solutions was near neutral and did not change significantly. The number of particles of ≥ 25 µm per milliliter was low and within the specification, but the number of particles of ≥ 10 µm per milliliter was out of the specification. It decreased with time and was similar to the control solution of only methylprednisolone sodium succinate added in 5% dextrose injection or in 0.9% sodium chloride injection. These results indicate that methylprednisolone sodium succinate is a sterile powder for injection which is difficult to dissolve completely in a short time. Doxofylline and methylprednisolone sodium

Table 1: Particulate matter of doxofylline and methylprednisolone sodium succinate mixed in 0.9% sodium chloride injection and in 5% dextrose injection

Time (h)	0.9% sodium chloride injection						5% dextrose injection					
	A		B		A + B		A		B		A + B	
	≥10μm	≥25μm	≥10μm	≥25μm	≥10μm	≥25μm	≥10μm	≥25μm	≥10μm	≥25μm	≥10μm	≥25μm
0	6.4	1.0	70.9	0.1	67.0	0.7	9.3	1.7	85.6	0.2	80.9	1.4
2	2.4	0.2	63.6	0.0	56.7	0.0	4.4	0.6	73.6	0.0	65.6	0.0
4	1.7	0.1	59.9	0.2	56.2	0.2	2.6	0.1	69.3	0.0	65.0	0.0
8	1.8	0.0	49.1	0.0	53.1	0.1	1.7	0.0	55.2	0.0	59.7	0.0
12	0.5	0.0	26.7	0.0	34.8	0.0	0.9	0.0	34.1	0.0	44.4	0.0
16	2.1	0.0	10.3	0.2	24.4	0.4	1.0	0.0	21.6	0.0	51.2	0.0
24	4.1	0.0	4.8	0.1	12.0	0.3	0.5	0.0	11.9	0.0	29.8	0.0

A: doxofylline B: methylprednisolone sodium succinate

Table 2: PH values and the ratio (%) of the amount of doxofylline and methylprednisolone sodium succinate in the mixture, to the original amount

Time (h)	0.9% sodium chloride injection							5% dextrose injection						
	A		B		A + B			A		B		A + B		
	pH	A%	pH	B%	pH	A%	B%	pH	A%	pH	B%	pH	A%	B%
0	5.2	100.00	7.5	100.00	7.5	100.00	100.00	3.7	100.00	7.1	100.00	7.1	100.00	100.00
2	5.4	99.17	7.4	97.82	7.4	100.33	99.11	3.7	100.20	7.1	100.33	7.1	99.45	99.75
4	5.4	100.27	7.4	96.73	7.4	99.96	97.71	3.8	99.69	7.1	98.63	7.1	100.30	100.71
8	5.5	100.21	7.4	96.84	7.4	98.96	93.97	3.7	98.83	7.1	98.98	7.1	98.45	97.91
12	5.6	99.78	7.3	95.66	7.3	99.02	92.58	3.8	99.09	7.0	97.81	7.1	102.61	97.01
16	5.6	99.29	7.1	94.12	7.3	98.78	90.87	3.9	99.67	7.0	97.48	7.2	100.14	96.87
24	5.5	99.39	7.3	89.42	7.3	98.89	88.67	3.8	99.03	7.1	93.42	7.1	98.19	93.47

A: doxofylline B: methylprednisolone sodium succinate

succinate did not increase the number of particles of solution when mixed together.

In the chemical compatibility test, the concentration of doxofylline showed no significant change, while that of methylprednisolone sodium succinate decreased significantly with the ratio being 88.67% and 91.47% in 0.9% sodium chloride and 5% dextrose injection with doxofylline, respectively. In addition, methylprednisolone (a pharmacologically active form of methylprednisolone sodium succinate), and methylprednisolone analogs were detected with increased concentrations (fig. 1). The concentration of doxofylline in the control solutions of doxfylline showed no significant change within 24 h. However, we found that the concentration of methylprednisolone sodium in the control solutions of methylprednisolone sodium succinate decreased significantly with the ratio being 89.42% and 93.42% in 0.9% sodium chloride and 5% dextrose injection within 24 h, respectively. Methylprednisolone and analogs were also detected with increased concentrations. These data indicate that the degradation of methylprednisolone sodium succinate is not related to doxofylline, but is closely related to the pH value of the solution.

According to the stability analysis results, doxofylline and methylprednisolone sodium succinate were compatible in 5% dextrose injection or in 0.9% sodium chloride injection. The stability of the mixtures depends more on the stability of the components in the mixture that are most unstable.

CONCLUSION

Based on several indexes shown above, we found that doxofylline and methylprednisolone sodium succinate did not affect the stability of each other in 5% dextrose injection or in 0.9% sodium chloride injection.

Doxofylline and methylprednisolone sodium succinate were stable for only up to 4 h when mixed in 0.9% sodium chloride injection, but were stable for up to 16 h when mixed in 5% dextrose injection at 20-25°C.

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