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Content Detection of Acetylcysteine Mixed with Combivent® (Containing Salbutamol and Ipratropium Bromide) by Hplc

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Abstract

Clinically, Combivent® (Compound ipratropium bromide solution which contains salbutamol and ipratropium bromide) and acetylcysteine inhalant are often mixed and administered at the same time, so as to achieve the effects of antiasthmatic and expectorant. After mixing, its content and inhalation performance shall be investigated by High Performance Liquid Chromatography (HPLC). The specificity, linearity, recovery, precision and stability of salbutamol and ipratropium bromide and acetylcysteine were tested to prove the reliability of developed HPLC method. The developed HPLC method had high specificity, with linear R2≥0.999, recovery RSD, precision RSD and stability RSD less than 2.0% at 8 time points. The HPLC methodology developed in this study can be used for the determination of salbutamol and ipratropium bromide mixed with acetylcysteine. To provide reference for the determination of its content after mixing and provide data support for its clinical medication.

Keywords: Inhalant; Salbutamol; Ipratropium Bromide; Acetylcysteine; HPLC

Introduction

Inhalant refers to the preparation that delivers drugs to the affected area through gas or aerosol for treatment [1-3]. Due to its characteristics, it's often used in the treatment of respiratory diseases. Inhalant can provide high local concentration in the respiratory system. Moreover, it can deliver the drug into the microcirculation through large surface areas such as throat mucosa and alveoli, so that the bioavailability of the drug is higher, and there is no first-pass effect [4]. At the same time, taking advantage of the characteristic that the absorption site of the inhalant is in the throat mucosa and alveolar, the inhalant has a faster onset time than the oral preparation in the treatment of respiratory diseases such as respiratory infection or asthma [5]. Compound ipratropium bromide solution is a compound preparation composed of salbutamol and ipratropium bromide. Salbutamol is a short acting β2 adrenoceptor agonist can inhibit the release of histamine and alleviate bronchospasm. Ipratropium bromide is smooth muscle M receptor blocker, which can reduce bronchoconstriction and dilate bronchus. Studies have shown that the anti-asthma effect of double target is more effective [6,7]. Acetylcysteine is often used as an expectorant. It reduces the viscosity of sputum through sulfhydryl group and facilitates sputum excretion. It has been reported that inhaled acetylcysteine is used as an adjuvant for resolving phlegm in patients with moderate and severe COPD [8]. Clinically, to alleviate spasm symptoms and reduce sputum, compound ipratropium bromide solution and acetylcysteine are commonly mixed in a nebulizer for atomization [9-12]. However, after the two drugs are mixed, their content changes will affect the drug inhalation effect [13-15]. Therefore, this study developed a HPLC method for the determination of salbutamol and ipratropium bromide mixed with acetylcysteine, which provides a methodological basis for the determination of the content of the two inhalants after mixing and provides a reference for their clinical use.

Materials

Methanol (Thermo Fisher Scientific Co., Ltd., lot No.: 203195, purity 99.9%, chromatographic grade); Acetonitrile (Thermo Fisher Scientific Co., Ltd., lot No.: 197164, purity 99.95%, chromatographic grade); Potassium dihydrogen phosphate

(Shanghai Aladdin Biochemical Technology Co., Ltd., lot No.: b1912076, purity 99.5%, chromatographic grade); Ipratropium bromide (National Institute of Control of Pharmaceutical and Biological Products, lot No: 100522-200601, content 100%); Salbutamol sulfate (National Institute of Control of Pharmaceutical and Biological Products, lot No.: 100328-200703, content 99.3%); Budesonide (National Institute of Control of Pharmaceutical and Biological Products, lot No.: 100989-201502, content 98.9%); Beclomethasone propionate (National Institute of Control of Pharmaceutical and Biological Products, lot No.: 10019-201504, content 99.0%); N-acetyl-L-cysteine (Shanghai Yuanye Biotechnology Co., Ltd., lot No.: l22j8x40528, content: 99%).

Method

HPLC conditions

HPLC: waters e2695, Chromatographic column: Agilent TC-C18(150 × 4.6 mm, 5mm), wavelength: 210 nm, Flow rate: 1.0 ml/min, Injection volume: 20 µl, Column temperature: 25°c, Mobile phase: take potassium dihydrogen phosphate solution (2.50 g of potassium dihydrogen phosphate was dissolved in 800 ml water, adjust the pH value to 3.20 ± 0.05 with phosphoric acid solution and made to a constant volume of 1 L) as mobile phase A and acetonitrile as mobile phase B. The gradient conditions are shown in (Table 1.1).

Table 1: Gradient conditions.

Time (min)	Flow Rate(ml/min)	A(%)	B(%)
0	1	95	5
10	1	80	20
15	1	80	20
16	1	95	5
25	1	95	5

Solution preparation

10 mg of ipratropium bromide was weighed accurately into a 10 ml volumetric flask and diluted with 50% methanol (methanol: water=1:1) to 10 ml, shake well, made a solution containing 1 mg of ipratropium hydrobromide in each ml of solvent as the stock solution; Salbutamol and N-acetyl-L-cysteine were prepared as the stock solution by the same method.

Method validation results

Specificity

Took N-acetyl-L-cysteine (NAC, 200 µg/ml), salbutamol (SAL, 10µg/ml), ipratropium bromide (IPRA, 10 µg/ml) and blank solvent each 20 µl for HPLC analysis. Record the chromatogram (Figure 1). The results show that the above components are completely separated and do not affect each other's detection. The retention times were 2.668, 4.951 and 11.739 respectively.

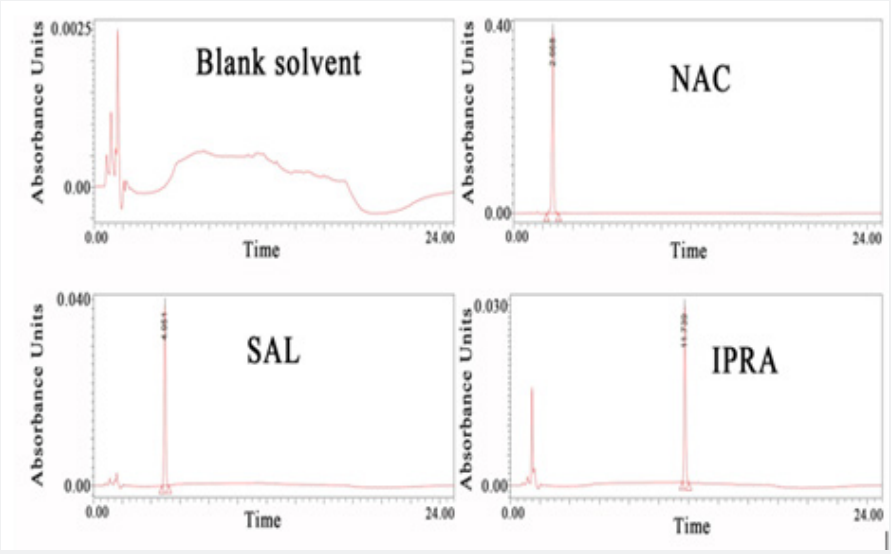

Figure 1: Results of specificity test.

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Linear range

Take each sample stock solution, IPRA, Sal and NAC mixed with methanol: potassium dihydrogen phosphate buffer (1:1) and diluted to contain IPRA, Sal: 40, 30, 25, 20, 15, 10, 5, 1 and 0.5 μg/ml□NAC: 800, 600, 500, 400, 300, 200, 100, 20, 10 μg/ml mixed solutions of different concentrations. 20 μl at each concentration level was took for HPLC analysis. Taking the Concentration(C) as the Abscissa (x) and the corresponding peak Area(A) as the Ordinate (y), the concentration peak area linear regression equations of the three samples were obtained. The results show that the peak area of each sample has a good linear relationship with the concentration in the above concentration range (Table 2). 4.3 recovery. Take each sample stock solution, IPRA, Sal and NAC mixed with methanol: potassium dihydrogen phosphate buffer (1:1) and diluted to contain IPRA and sal: 16.0 μg/ml (80%) 20.0 μg/ ml (100%) and 24.0 μg/ml (120%) NAC: 320.0 μg/ml (80%) □400.0 μg/ml(100%) and 480.0 μg/ml(120%) of mixed solutions of different concentrations, 20 μl at each concentration level was took for HPLC analysis. The concentration of each sample was calculated by linear equation, and the recovery was calculated by the ratio of theoretical amount to the actual amount (Table 3-5). The RSD of the three concentration levels is less than 2%, indicating that the method has good accuracy. 4.4 precision Take each sample stock solution, IPRA, Sal and NAC mixed with methanol: potassium dihydrogen phosphate buffer (1:1) and diluted to contain IPRA and sal: 10.0 μg/ml, NAC: 200.0 μg/ml mixed solution. 20 μl of the mixed solution was took for HPLC analysis, repeated injection 6 times, and calculated the RSD of peak area (Table 6). According to the data in the table, the RSD of each sample is less than 2.0%, indicating that the precision of this method makes the grade

Table 2: Linear equation of Samples.

C(μg/ml)oncentration	Peak Area of NAC	Peak Area of SAL	Peak Area of IPRA
0.5(10)	157271	10154	13821
1(20)	313209	20393	27702
5(100)	1538190	100743	138547
10(200)	3018224	200091	275865
15(300)	4430690	297874	409760
20(400)	5804927	394565	542626
25(500)	7170467	491655	676194
30(600)	8555105	591991	813046
40(800)	11174706	785275	1076695
linear equation	y=13992x+ 129433	y=19620x+ 2046.8	y=26923x+ 3340.2
R2	0.9994	1	1

Table 3: NAC recovery results.

No.	Concentration level	Theoretical value(μg)	Measured value(μg)	Recovery (%)	X±SD (%)
1	80%	320	324.21	101.32%	324.75±0.85
2			325.72	101.79%	
3			324.3	101.34%	
1	100%	400	406.23	101.56%	405.44±1.12
2			404.15	101.04%	
3			405.93	101.48%	
1	120%	480	486.28	101.31%	486.33±0.05
2			486.34	101.32%	
3			486.36	101.33%	

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Table 4: SAL recovery results.

No.	Concentration level	Theoretical value(μg)	Measured value(μg)	Recovery (%)	X ±SD (%)
1	80%	16	16.15	100.91%	16.18±0.03
2			16.2	101.27%	
3			16.19	101.18%	
1	100%	20	20.23	101.14%	20.29±0.06
2			20.33	101.64%	
3			20.32	101.61%	
1	120%	24	24.18	100.74%	24.19±0.01
2			24.19	100.80%	
3			24.2	100.81%	

Table 5: IPRA recovery results.

No.	Concentration level	Theoretical value(μg)	Measured value(μg)	Recovery (%)	X ±SD (%)
1	80%	16	16.08	100.52%	16.11±0.03
2			16.13	100.79%	
3			16.13	100.82%	
1	100%	20	20.18	100.89%	20.23±0.05
2			20.26	101.31%	
3			20.25	101.26%	
1	120%	24	24.13	100.53%	24.13±0.02
2			24.12	100.50%	
3			24.15	100.62%	

Table 6: Results of precision test.

Number of Injection Times	1	2	3	4	5	6	Mean	RSD
NAC peak area	2972954	2976784	2972195	2974730	2972135	2968872	2972945	0.09%
SALpeak area	285942	285937	285832	286102	285705	285849	285894.5	0.05%
IPRA peak area	202778	202896	202906	202887	202844	202824	202855.83	0.02%

Stability

Take each sample stock solution, IPRA, Sal and NAC mixed with methanol: potassium dihydrogen phosphate buffer (1:1) and diluted to contain IPRA and sal: 10.0 μg/ml, NAC: 100.0 μg/ml mixed solution. 20 μl of the mixed solution was took for HPLC analysis, and detected again at 2, 4, 6, 8, 10, 12 and 14 h after the first injection, and calculate the RSD of the peak area. Within 14 h, the RSD of each sample was less than 2.0% (Table 7). It shows that this method has good stability.

Table 7: Results of Solution stability test.

Time(h)	0	2	4	6	8	10	12	14	RSD
NAC peak area	2975173	2974476	2975786	2980196	2979971	2972195	2970768	2967141	0.15%
SALpeak area	285604	284502	285240	285836	286039	285832	286058	286147	0.19%
IPRA peak area	203824	202365	202349	202867	203097	202906	203052	203136	0.23%

Conclusion

Through the HPLC methodology test, it is verified that the specificity, linearity, recovery, precision, and stability of the liquid phase method meet the requirements [1] and can be used for the content determination of compound ipratropium bromide mixed with acetylcysteine. At the same time, this study also provides data reference for the clinical use of the above two drugs.

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