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# Indirect Spectrophotometric Determination of Acetylcysteine in Pharmaceutical Sample using Fe (Ш)-Sulfosalicylic Acid System

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**Keywords:** Acetylcysteine, Fe (Ш), Sulfosalicylic acid, Discoloration spectrophotome-try.

**Abstract.** In acidic medium, Fe (Ш) can be reduced to Fe (Ⅱ) by hydrosulfuryl (-SH) in acetylcysteine molecule, and then using sulfosalicylic acid as chromogenic reagent of Fe (Ш) to determinate the acetylcysteine indirectly by discoloration spectrophotometry.An accurate fast spectrophotometric method for the determination of acetylcysteine by discoloration spectrophotometry using Fe (Ш)-sulfosalicylic acid system has been established. This proposed method had been successfully applied to determinate of acetylcysteine in real pharmaceutical, and the results agreed well with pharmacopoeial method.

## Introduction

Acetylcysteine(AC,the molecular structure of acetylcy-steine is shown in Fig.1) is known to be a powerful mucolytic agent for the treatment of chronic bronchitis and other pulmonary diseases complicated by the production of viscous mucus, and it was also used in cancer chemotherapy and as an antidote in paracetamol overdosage.It is of great importance and significance for life science.

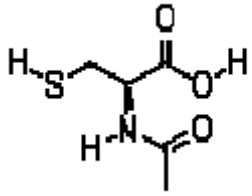


Fig.1. The molecular structure of acetylcysteine.

Up till now, spectrophotometry[1], flow injection analysis[2,3], fluorometry[4], HPLC method[5], LC method[6,7], electrochemical analysis[8,9], have been applied for the determination of acetylcysteine. However, most of the methods mentioned above need either complicated and expensive equipment or tedious procedures. This problems limit the practical application of these method. Therefore,it is essential and significant to develop a simple, accurate,rapid and sensitive method for the determination of acetylcysteine in clinical analysis and drug quality control.

In this paper,a novel method for the indirect determination of acetylcysteine by discoloration spectrophotometry using Fe(Ш)-sulfosalicylic acid system has been

established. The various effect factors on the determination of acetylcysteine were investigated in detail. The results showed that in acidic medium,Fe (Ⅲ) could be reduced to Fe (Ⅱ) by hydrosulfuryl(-SH) in acetylcysteine molecule,and then using sulfosalicylic acid as chromogenic reagent of Fe(Ⅲ), and the content of acetylcysteine was determinated indirectly through determinating the surplus content of Fe(Ⅲ) in the system.The maximum absorption wavelength of the complex of sulfosalicylic acid was 478 nm, good linear relationship was obtained between ΔA and the concentration of acetylcysteine in the range of 0.005000-0.06000 mg·mL$^{-1}$,the linear regression equation is ΔA=0.0372+5.7643C (mg·mL$^{-1}$),with a correlation coefficient of 0.9997.This proposed method had been applied to determinate of acetylcysteine in real pharmaceutical,and the results agreed well with those obtained by pharmacopoeial method.

## Experiment

### Apparatus and Reagents

A model 723S spectrophotometer (Shanghai Precision & Scientific Instrument Co,.Ltd ) was used for photometric measurements. A model UV-2401 UV–visible spectrophotometer (The Shimadzu Corporation, japan) was used for scanning the absorption spectrum.

A stock of standard solution of 0.5000 g·L$^{-1}$ acetylcysteine was prepared by dissolving 0.1000 g of acetylcysteine in 200 mL with bidistilled water and stored at 4℃ in dark place. A stock of standard solution of 0.5000 g·L$^{-1}$ $Fe^{3+}$ was prepared by dissolving 2.1586 g $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 200 mL with bidistilled water, 4.0mL 3 mol·L$^{-1}$ $H_2SO_4$ was added,and then diluting it to 500 mL with bidistilled water. Sulfosalicylic acid (Ssal) solution: 50.00 g·L$^{-1}$. Buffer solutions of different pH was prepared as references [10].

All reagents were of analytical reagent grade.Bidistilled water was used throughout.

### Method

Take two 25mL volumetric flask, a suitable amount of 0.1000 g·L$^{-1}$ acetylcysteine solution was transferred into one of the two 25 mL volumetric flask. Then 2.50 mL of 0.5000 g·L$^{-1}$ $Fe^{3+}$ solution,0.40 mL of 50.00 g·L$^{-1}$ sulfosalicylic acid solution and 10.00 mL of pH=3.0 buffer solution were added into the two 25mL volumetric flask, the solution was diluted to the mark with bidistilled water and mixed well.Aftering these mixture reacted for 30 min at 80℃ in water both and cooled back to room temperature,the absorbance(ΔA) of the blank solution ($Fe^{3+}$+ Ssal) was measured at 478 nm against the determination solution ($Fe^{3+}$+acetylcysteine+ Ssal).

## Results and Discussions

### Absorption Spectrum

The absorption spectrum of the blank solution ($Fe^{3+}$+Ssal) and the determination solution ($Fe^{3+}$+acetylcysteine+Ssal) are shown in Fig.2. It can be seen that the maximum absorption wavelength of the complex of Fe (Ⅲ) - sulfosalicylic acid is at 478 nm. Therefore, all the following measurements were carried out at 478 nm.

### Effects of Reaction Temperature and Time

The effect of temperature on absorbance (△ A) was studied. The results showed that the absorbance (△ A) reached larger and remained constant when the temperature was 80-85 ℃. Hence, 80 ℃ was selected for all further studies.

The absorbance (△ A) of different reaction time (5,10,15,20,25,30,35,40, 45,50,55,60 min) was measured at 80°C. The results showed that the absorbance (△ A) were large and does not change when the time was 30-35min.Therefore, 30 min of reaction time was chosen.

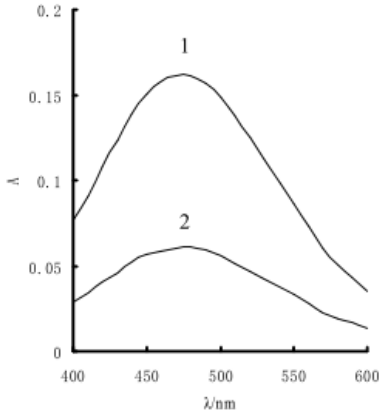


Fig. 2. Absorption spectrum 1- Fe 3+ +Ssal, 2- Fe 3+ + acetylcysteine+Ssal

### Effects of pH and the Dosage of Buffer Solution

The effects of pH on absorbance (△ A) were studied. The results showed that the absorbance (△ A) are maximal and remain almost constant when the pH was 3.0-.6. Hence, pH 3.0 buffer solutions.

The effects of the dosage of pH 3.0 buffer solution measured. The results showed that the absorbance (△ A) reached maximum and does not change when the dosage of pH3.0 buffer solution is 10.00 mL-12.50 mL. Therefore, 10.00 mL of pH3.0 buffer solution was chosen in the subsequent studies.

### Effect of the Dosage of Fe (III)

The effect of the dosage of Fe (Ⅲ) on absorbance (△ A) can be seen in Fig. 3. It could be seen from Figure 3 that absorbance (△ A) reached maximal and kept constant when the dosage of Fe (Ⅲ) was 2.25~2.50mL. Hence, 2.50 mL of the dosage of Fe (Ⅲ) was chosen.

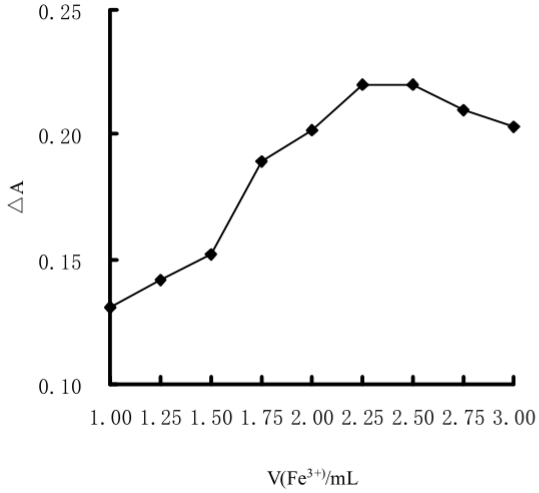


Fig. 3. Effect of the dosage of Fe (Ⅲ)

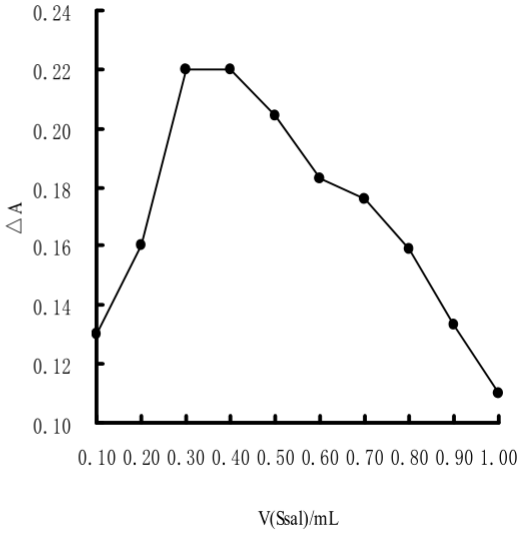


Fig. 4. Effect of the dosage of Ssal

## Effect of the Dosage of Sulfosalicylic Acid

The effect of the dosage of sulfosalicylic acid on absorbance (Δ A) were investigated (Fig. 4). As shown in Figure 4, the absorbance (Δ A) reaches its maximum and keep constant when the dosage of sulfosalicylic acid was 0.30 mL-0.40 mL. Therefore, 0.40 mL of the dosage of sulfosalicylic acid was selected.

## Interference of Coexisting Components

A systematic study of the influence of excipients, aminoacids and carbohydrate on the determination of acetylcysteine was carried out.The tolerance levels are defined with an error of the determination less than ±5%.A conclusion can be drawn from the following: 4.00 mg·mL$^{-1}$ glycine; 2.00 mg·mL$^{-1}$ glucose; 1.00 mg·mL$^{-1}$ starch, lactose,sucrose, D-fructose, L-gluta-mate and L-proline do not affect the determination. But a certain amount of L-arginine affect the determination.

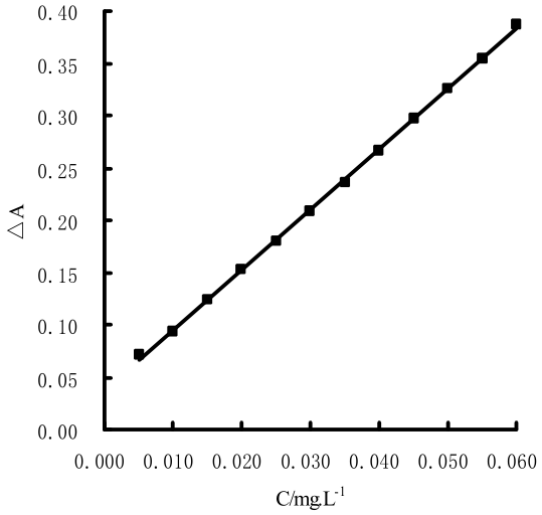


Fig. 5 Calibration curve

## Calibration Curve

Under the selected conditions, a linear relationship between absorbance (Δ A) and the concentration (C) of acetylcy-steine was obtained in the range of 0.005000-0.06000 mg ·mL$^{-1}$(Fig.5). The linear regression equation is Δ A=0.0372+5.7643C (mg ·mL$^{-1}$), with a correlation coefficient of 0.9997.

## Determination of Acetylcysteine in Pharmaceutical Sample

The proposed method was applied to the determination of acetylcysteine in acetylcysteine granules. Meanwhile, the recovery tests of standard addition were performed.The result obtained was compared with those obtained by pharmacopoeia method, as show in Table 1.

Table 1 The determination result of acetylcysteine in acetylcysteine granules n = 5

| Sample | Proposed method (mg g$^{-1}$) | RSD (%) | Pharmacopoeia Method[11] (mg g$^{-1}$) | Added (μg ·mL$^{-1}$) | Recovered (μg ·mL$^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|---|
| acetylcysteine granules | 35.78 | 0.8 | 37.46 | 20.00 | 19.36 | 96.8 |
| | | | | 30.00 | 28.52 | 95.1 |

Table 1 shows that the content of acetylcysteine in acetylcysteine granules is 35.78 mg g$^{-1}$ by this proposed method, agreed well with 37.46 mg g$^{-1}$ obtained by pharmacopoeial method. It is indicated that the content of acetylcysteine in pharmaceutical sample can be accurately determined using Fe (Ⅲ)-sulfosalicylic acid system.

## Conclusion

In this paper, a novel method for the indirect determination of acetylcysteine by discoloration spectrophotometry using Fe (Ⅲ)-sulfosalicylic acid system was reported. The proposed method has been successfully used for the determination of acetylcysteine in pharmaceutical sample,and the results agreed well with pharmacopoeia method. It is obvious that the determination of acetylcysteine by discoloration spectrophotometry using Fe (Ⅲ)-sulfosalicylic acid system has certain practical significance and foreground of application.

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