Stability of Mg IG/GSH combo injection

Mary Gilbert[1, a, *], Benjamin Smith[1, b],

[1]Shionogi & Co, Ltd,1-8, Doshomachi 3-chome, Chuo-Ku, Osaka, 541-0045, Japan
a.gilbert0045@sina.com, b.b_smith_120102@163.com
***Corresponding Author**

Abstract: Objective: To investigate the stability of magnesium isoglycyrrhizinate (Mg IG) injection solution when mixed with reduced glutathione (GSH) injection solution at room temperature. Method: High-performance liquid chromatography was used to determine the concentration changes of Mg IG injection solution mixed with GSH injection solution in 5% and 10% glucose solutions. Results: No significant changes in concentration were observed within 0, 0.5, 1, 1.5, 2, and 4 hours after mixing the Mg IG injection solution with the GSH injection solution. Conclusion: Mg IG injection solution can be mixed with GSH injection solution in 5% and 10% glucose solutions and should be used within 4 hours.

Keywords: Magnesium Isoglycyrrhizinate, Glutathione, Traditional Chinese Medicine Compatibility, Drug Stability

1. Introduction

Magnesium isoglycyrrhizinate (Mg IG) is a magnesium salt of the stereoisomer of natural glycyrrhizic acid, with the structure of alpha-form glycyrrhizic acid, and is a fourth-generation glycyrrhizic acid preparation. It is mainly used in the clinic for acute and chronic hepatitis, drug-induced liver injury, and as an auxiliary liver-protective drug for chemotherapy. Reduced glutathione (GSH) is a tripeptide composed of glutamic acid, cysteine, and glycine, which participates in the tricarboxylic acid cycle and sugar metabolism in the body, providing high energy. The liver is the main site for GSH synthesis, hence liver diseases are an important factor in the decrease of GSH. Therefore, exogenous supplementation of GSH is beneficial for the liver to smoothly carry out material metabolism. It is mainly used in the clinic for acute and chronic viral hepatitis, drug-induced liver injury, and alcoholic liver disease. Clinical trials have proven that the combination of Mg IG and GSH, which have different mechanisms of action, show good efficacy in the treatment of liver diseases. For patients with limited solvent volume, the two drugs need to be used in combination, but there have been no reports on the stability of clinical combination. This study uses high-performance liquid chromatography to determine the concentration of Mg IG injection solution mixed with GSH injection solution, and studies the stability of the combination of the two drugs, thereby providing a reference for clinical medication.

2. Materials

2.1. Instruments

A Waters E2695 high-performance liquid chromatography system and a Mettler S20 pH meter were used.

2.2. Test drugs and reagents

Mg IG Injection (Jiangsu Zheng da Tian qing Co., Ltd., specification: 50mg/10ml); Reduced Glutathione for Injection (Shanghai Fudan Fu Chun Pharmaceutical Co., Ltd., specification: 0.6g); Mg IG Reference Standard (China Institute for Drug Control, specification: 100mg, batch number: 201302, concentration: 90.8%); GSH Reference Standard (China Institute for Drug Control, specification: 200mg, batch number: 200702, no concentration indicated); 5% Glucose Injection (Anhui Shuang he Pharmaceutical Co., Ltd., specification: 250ml); 10% Glucose Injection (Anhui Shuang he Pharmaceutical Co., Ltd., specification: 100ml); Other reagents are of analytical purity; Water is deionized water.

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3. Solution preparation

3.1. Sample solution 1

At room temperature (25°C), Mg IG injection 0.1g and reduced glutathione (GSH) 1.2g are mixed with 250ml of 10% glucose solution according to the dosage specified in the instructions.

3.2. Sample solution 2

At room temperature (25°C), Mg IG injection 0.1g and reduced glutathione (GSH) 1.2g are mixed with 250ml of 5% glucose solution according to the dosage specified in the instructions.

3.3. Sample solution 3

At room temperature (25°C), Mg IG injection 0.2g and reduced glutathione (GSH) 1.2g are mixed with 250ml of 10% glucose solution according to the dosage specified in the instructions.

3.4. Sample solution 4

At room temperature (25°C), Mg IG injection 0.2g and reduced glutathione (GSH) 1.2g are mixed with 250ml of 5% glucose solution according to the dosage specified in the instructions.

3.5. Reference solution 1

Accurately weigh an appropriate amount of injectable GSH and dissolve it in a sodium dihydrogen phosphate heptanesulfonate solution (prepared by adding 6.8g of sodium dihydrogen phosphate and 2.2g of heptanesulfonic sodium to 1000ml of water, then adjusting the pH to 3.0 with phosphoric acid solution) to prepare a stock solution of injectable GSH with concentrations of 10, 50, 100, 500, and 5000μg/ml.

3.6. Reference solution 2

Accurately measure an appropriate amount of Mg IG reference standard into a 25ml volumetric flask, add the mobile phase up to the mark, and mix well to obtain a Mg IG stock solution with concentrations of 10, 50, 100, 500, and 1000μg/ml.

4. High-Performance liquid chromatography (HPLC) for drug concentration determination

4.1. Determination of GSH concentration in injections

4.1.1. Detection wavelength and chromatographic conditions

Column: Discover C18 (250mm × 4.6mm, 5μm); Mobile phase: Phosphate buffer solution (dissolve 6.80g of sodium dihydrogen phosphate and 2.20g of 1-heptanesulfonic acid sodium salt in water to make 1000ml, adjust the pH to 3.0 with phosphoric acid) - Methanol (96:4); Flow rate: 1ml/min, Detection wavelength: 210nm. The theoretical plate count calculated based on the GSH peak should not be less than 2000.

4.1.2. Interference test

Prepare 10% and 5% glucose solutions containing Mg IG injection at concentrations of 0.4 and 0.8mg/ml according to the dosage specified in the instructions. Under the aforementioned chromatographic conditions, inject 5μl and record the chromatogram. The results indicate that the analysis of GSH will not be affected under these system conditions.

4.1.3. Degradation test

Take an appropriate amount of the reference solution for acid, base, heat, light, and oxidation degradation, and check the separation of degradation products from the main peak under the aforementioned chromatographic conditions.

Take the GSH reference solution and subject it to the following degradation methods: (1) Acid degradation test: Adjust the pH to 2.0 with 10% hydrochloric acid solution; (2) Base degradation test:

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Adjust the pH to 12.0 with 10% sodium hydroxide solution; (3) High-temperature degradation test: Boil in water at 100°C; (4) Light degradation test: Expose the sample to a light intensity of (4500±500)lx; (5) Oxidation degradation test: Add 30% hydrogen peroxide solution. All degradation tests are maintained for 30 minutes.

Test according to the chromatographic conditions in 3.1.1 to investigate the changes in the reference solution under acid, base, heat, light, and oxidation degradation conditions. The results show that the system conditions can achieve good separation between GSH and degradation products under severe conditions.

4.1.4. Limit of detection and quantitation test

Take a segment of the blank baseline and measure the height of the noise peak. Dilute the test solution to a concentration of 2.0μg/ml, take 5μl for injection, and if the main peak height is three times the noise peak height, this concentration is considered the limit of detection, with a minimum detectable amount of 10ng. Dilute the test solution to a concentration of 5.0μg/ml, take 5μl for injection, and if the main peak height is ten times the noise peak height, this concentration is considered the limit of quantitation, thus the limit of quantitation is 25.0ng.

4.1.5. Linearity test

Draw the reference solution 1 and inject it into the liquid chromatograph according to the chromatographic conditions in 4.1.1, recording the peak area. Plot the peak area against the amount of injection (μ g) using least squares method for linear regression, obtaining the regression equation: y=2729.5x+12891, r=0.9999. The experimental results indicate that GSH has a good linear relationship in the range of 0.05~25μg.

4.1.6. Precision test

Take the reference solution 1 of GSH injection with a concentration of 100μg/ml, inject it six times consecutively, and measure the peak area. Calculate the relative standard deviation (RSD) to be 0.29%, indicating good precision.

4.1.7. Stability test

Take the reference solution 1 of GSH injection with a concentration of 100μg/ml, and inject it at 0, 0.5, 1, 1.5, 2, and 4 hours, measuring the peak area each time. Calculate the RSD to be 0.36%, indicating that the sample is essentially stable within 4 hours.

4.1.8. Reproducibility and recovery test

Accurately weigh 25.8mg of injectable GSH, dissolve it in the mobile phase and make up to volume in a 25ml volumetric flask to obtain a solution with a concentration of about 5000μg/ml. Take 1ml and dilute it to a 10ml volumetric flask to obtain the injection sample solution. According to the chromatographic conditions in 4.1.1, take 5μl for injection into the liquid chromatograph and measure the precise concentration to be 89.2μg/ml. Accurately take nine portions of the injectable GSH sample solution, each 1ml, and separately and precisely add 0.1, 0.2, and 0.3ml of the GSH standard stock solution to prepare low, medium, and high concentration solutions, each in triplicate, make up to volume in a 10ml volumetric flask with the mobile phase, and mix well. Under the aforementioned chromatographic conditions, the average recovery rate is determined to be 101.58%, with an RSD of 0.53%.

4.2. Determination conditions for Mg IG injection concentration

4.2.1. Detection wavelength and chromatographic conditions

Column: Symmetry C18 (150mm × 4.6mm, 5μm); Mobile phase: Phosphate buffer (pH 7.4): Acetonitrile = 78:22; Flow rate: 1ml/min, Detection wavelength: 250nm. The theoretical plate count calculated based on the Mg IG peak should not be less than 2000.

4.3. Determination conditions for Mg IG injection concentration

4.3.1. Detection wavelength and chromatographic conditions

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4.3.2. Interference test

According to the dosage specified in the instructions, prepare 10% and 5% glucose solutions containing injectable GSH at a concentration of 4.8mg/ml. Under the chromatographic conditions in 4.3.1, inject 20μl and record the chromatogram. The results indicate that the analysis of Mg IG will not be affected under these system conditions.

4.3.3. Degradation test

Take an appropriate amount of the reference solution for acid, base, heat, light, and oxidation degradation, and check the separation of degradation products from the main peak under the aforementioned chromatographic conditions.

Take the Mg IG reference solution and subject it to the same degradation methods as the GSH reference solution.

Test according to the chromatographic conditions in 4.1.1 to investigate the changes in the reference solution under acid, base, heat, light, and oxidation degradation conditions. The results indicate that the system conditions can achieve good separation between Mg IG and its degradation products under severe conditions.

4.3.4. Limit of detection and quantitation test

Take a segment of the blank baseline and measure the height of the noise peak. Dilute the test solution to a concentration of 0.16μg/ml, take 20μl for injection, and if the main peak height is three times the noise peak height, this concentration is considered the limit of detection, with a minimum detectable amount of 3.2ng. Dilute the test solution to a concentration of 0.53μg/ml, take 20μl for injection, and if the main peak height is ten times the noise peak height, this concentration is considered the limit of quantitation, thus the limit of quantitation is 10.6ng.

4.3.5. Linearity test

Draw the reference solution 2 and inject it into the liquid chromatograph according to the chromatographic conditions in 4.3.1, recording the peak area. Plot the peak area against the amount of injection (μ g) using least squares method for linear regression, obtaining the regression equation: y=14832x+41291, r=0.9999. The experimental results indicate that Mg IG has a good linear relationship in the range of 0.2~20μg.

4.3.6. Precision test

Take the reference solution 2 of Mg IG injection with a concentration of 100μg/ml, inject it six times consecutively, and measure the peak area. Calculate the relative standard deviation (RSD) to be 0.77%, indicating good precision.

4.3.7. Stability test

Take the reference solution 2 of Mg IG injection with a concentration of 100μg/ml, and inject it at 0, 0.5, 1, 1.5, 2, 4, and 6 hours, measuring the peak area each time. Calculate the RSD to be 0.80%, indicating that the sample is essentially stable within 6 hours.

4.3.8. Reproducibility and recovery test

Accurately measure 0.2ml of Mg IG injection and dilute it to a 10ml volumetric flask to obtain the injection sample solution. According to the chromatographic conditions in 4.3.1, take 20μl for injection into the liquid chromatograph and measure the precise concentration to be 113.59μg/ml. Accurately measure nine portions of the Mg IG injection, each 0.2ml, and separately and precisely add 0.5, 1.0, and 1.5ml of the Mg IG standard stock solution to prepare low, medium, and high concentration solutions, each in triplicate, make up to volume in a 10ml volumetric flask with the mobile phase, and mix well. Under the aforementioned chromatographic conditions, the average recovery rate is determined to be 101.41%, with an RSD of 0.94%.

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5. Drug concentration determination

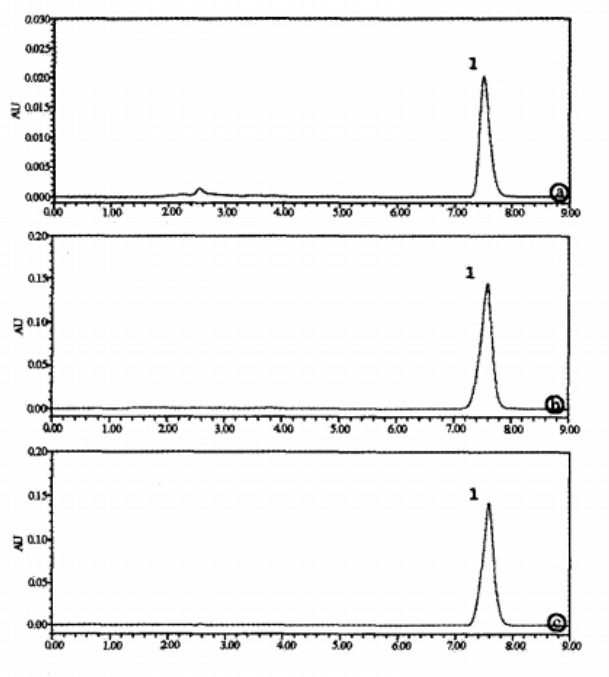
According to the chromatographic conditions in 4.1.1 and 4.2.1, take samples 1 to 4, inject, record the peak area, and calculate the content of Mg IG injection and injectable GSH in samples 1 to 4, respectively.

5.1. Determination of GSH concentration in injections

After mixing Mg IG injection with injectable GSH in 10% and 5% glucose injections, the concentration of GSH shows no significant change (Table 1, Figure 1).

Table 1 Determination of GSH concentration in Mg IG injection combined with 10% and 5% glucose injection solutions (μ g /ml)

Injection Time	Sample Solution 1	Sample Solution 2	Sample Solution 3	Sample Solution 4
0 h	4374.14	4305.23	4056.06	215.90
0.5 h	4398.33	4281.02	4026.84	4240.76
1 h	4446.64	4270.09	4097.65	4216.36
1.5 h	4456.53	4266.43	3992.41	4254.00
2 h	4390.20	4300.62	4066.21	4248.80
4 h	4440.42	4309.44	4009.12	4242.04
Peak Area (μ g /ml)	4417.71	4288.80	4041.38	4236.31
RSD (%)	0.78	0.44	0.97	0.39



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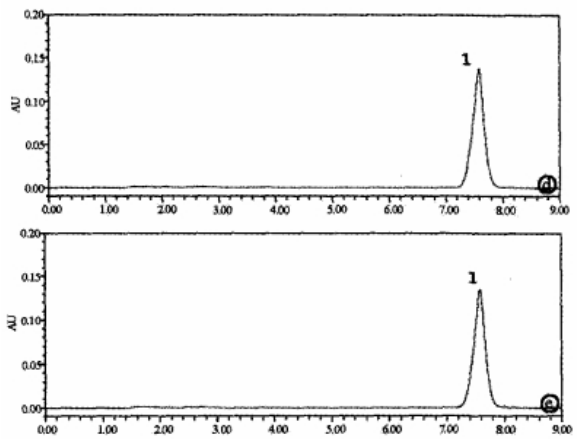


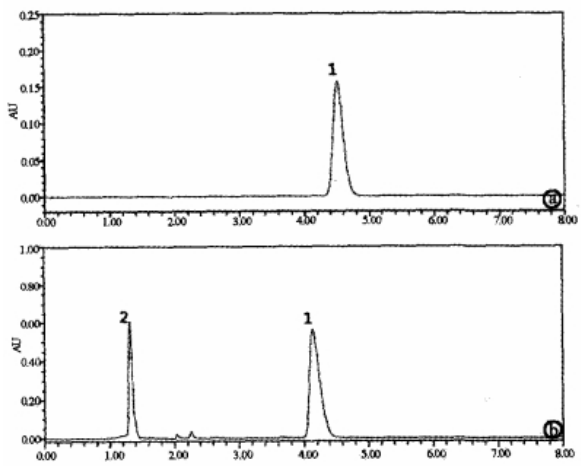
Figure 1 High-performance liquid chromatography (HPLC) chart of GSH concentration determination conditions for injectable GSH (Peak 1 is the GSH peak): a: Injectable GSH; b: Sample solution 1; c: Sample solution 2; d: Sample solution 3; e: Sample solution 4

5.2. Determination of Mg IG injection concentration

After mixing Mg IG injection with injectable GSH in 10% and 5% glucose injections, the concentration of Mg IG injection showed no significant change (Table 2, Figure 2).

Table 2 Determination results of Mg IG concentration after mixing Mg IG injection with injectable GSH in 10% and 5% glucose injection solutions (μ g/ml)

Injection Time	Sample Solution 1	Sample Solution 2	Sample Solution 3	Sample Solution 4
0h	416.26	427.97	800.65	798.89
0.5 h	424.81	427.91	799.47	796.00
1 h	423.84	427.56	796.50	793.07
1.5 h	423.39	426.41	797.95	797.10
2 h	423.45	426.36	791.53	794.01
4 h	425.17	427.55	790.54	794.24
Peak Area (μ g /ml)	422.82	427.30	796.11	795.55
RSD (%)	0.78	0.17	0.53	0.28



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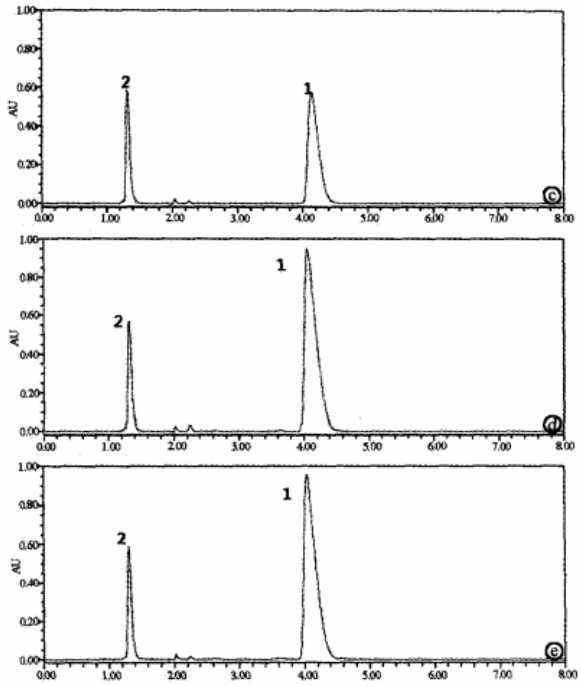

Figure 2 High-performance liquid chromatography (HPLC) chart of Mg IG concentration determination conditions (Peak 1 is the Mg IG peak, Peak 2 is the GSH peak): a: Mg IG injection; b: Sample solution 1; c: Sample solution 2; d: Sample solution 3; e: Sample solution 4.

6. Discussion

Mg IG injection solution exerts its effects by acting on hormone receptors, affecting ion channels (inhibiting calcium ion influx), activating or inhibiting the activity of enzymes, and regulating the metabolism of substances. It has a corticosteroid-like action and strong anti-inflammatory and immune-modulating activities [5-7]. The a-form of glycyrrhizic acid has good lipophilicity, strong anti-inflammatory activity, and high liver targeting, and existing studies [8] have shown that it has better efficacy and safety than previous glycyrrhizic acid preparations. Injectable GSH can protect against the destruction of antioxidants to sulfhydryl groups, protect proteins and enzymes containing sulfhydryl groups in cell membranes from destruction, and also combat the damage of free radicals to vital organs [9]. Both Mg IG injection solution and injectable GSH are commonly used drugs for patients with liver disease. Due to the difference in their mechanisms of action, the two drugs are often used in combination in clinical practice to improve the therapeutic effect of liver protection and enzyme reduction for patients with liver disease. In clinical practice, for patients with liver disease complicated by ascites and poor cardiopulmonary and renal function, the two drugs are often mixed and used in combination to reduce the volume of fluid supplements. However, the instruction manuals for both do not mention whether there are any compatibility contraindications between the two.

Wang Yan Xia et al. [10] demonstrated that Mg IG injection solution, when mixed with 10% and 5% glucose injection solutions, showed no significant changes in appearance, pH value, concentration, related substances, visible, and insoluble particle counts within 24 hours. This indicates that Mg IG injection solution is stable when mixed with these two injection solutions for 24 hours and is compatible. Zhou Xueqin et al. [11] showed that injectable GSH mixed with 5% and 10% glucose injection solutions remains stable within 2 hours at temperatures of 25°C and 37°C. Experimental data prove that Mg IG injection solution and injectable GSH are stable when mixed with glucose, and considering the particularities of liver disease, glucose is commonly used as a solvent in clinical fluid therapy. Therefore, this study uses high-performance liquid chromatography to determine the stability of Mg IG injection solution mixed with injectable GSH in 5% and 10% glucose, providing practical guidance for clinical medication use.

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Considering that medications are prepared in intravenous admixture centers and take a certain time to be administered to patients, this experiment examines the stability within 4 hours of mixing. Upon mixing the two drugs, within 4 hours, visual observation shows that the solution is colorless and clear. The experimental results indicate that after mixing Mg IG injection solution with injectable GSH, there are no significant changes in the concentration indicators of either substance within 0, 0.5, 1, 1.5, 2, and 4 hours. It is recommended that if the two drugs are used in combination in clinical practice, they should be used up within 4 hours after mixing. To provide more definitive evidence of the stability of the combination of the two drugs for clinical use, further research will be conducted in the future to monitor changes in pH and the concentration of insoluble particles.

7. Conclusion

This study conducted a comprehensive analysis of the compatibility and stability of magnesium isoglycyrrhizinate (Mg IG) injection solution and reduced glutathione (GSH) for injection in different concentrations of glucose solution using high-performance liquid chromatography (HPLC). The experimental results indicate that at room temperature, the drug concentrations of both medications remained stable and showed no significant changes within 4 hours after being mixed in 5% and 10% glucose solutions. This finding is of significant importance for guiding the rational use of medication in clinical practice, especially for patients who require limited solvent volumes. The combination use of the two drugs is not only safe and feasible but also ensures therapeutic efficacy while reducing the volume of fluid supplements, thereby alleviating the burden on patients. However, to ensure the efficacy of the drugs and the safety of patients, it is recommended that the mixture be used up within 4 hours after preparation. Future research will further explore the stability of the drug combination over a longer time scale and under different environmental conditions, providing more comprehensive information on drug compatibility for clinical use.

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