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DETERMINATION OF ISOSORBIDE MONONITRATE (ISMN) AND ITS TWO RELATED SUBSTANCES IN ISOSORBIDE MONONITRATE AND SODIUM CHLORIDE INJECTION

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ABSTRACT: A high-performance liquid chromatography (HPLC) method has been developed for the determination of isosorbide mononitrate and its related substances in isosorbide mononitrate and sodium chloride injection. An C18 column (5μm, 250 ×4.6 mm) was used for the determination, with methanol and water (25:75,v/v)as the mobile phase at the flow rate of 1 mL min^{-1}. The detection wavelength was 210 nm. The related substances were quantitated versus an external standard. The method was capable of resolving all of the two known related substances. The two related substances were isosorbide dinitrate (ISDN) and 2- isosorbide mononitrate (2-ISMN), respectively. The method was successfully applied to quantify related substances. The limits of detection of isosorbide mononitrate, isosorbide dinitrate and 2-isosorbide mononitrate (2-ISMN) were all 0.3ng. And the limits of quantitation of isosorbide mononitrate, isosorbide dinitrate and 2-isosorbide mononitrate (2-ISMN) were all 0.8ng. The method was found to be accurate, precise, specific, linear and sensitive, for the determination.

INTRODUCTION: Nowadays, with the development of science and technology, people's quality of life improved a lot and people's life style became colourful. However, disease spectra have significantly changed at the same time. Cardiovascular disease has become the main disease harming human health and quality of life and its morbidity rate presents a rising trend.

Additionally, its disability and mortality rates have increased [1]. In the early 1980s, the company of Biovail first developed the isosorbide mononitrate which is a drug used principally in the treatment of angina pectoris and acts by dilating the blood vessels so as to reduce the blood pressure [2].

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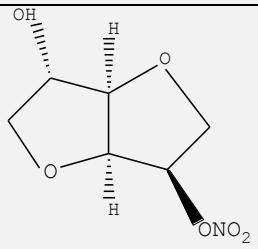
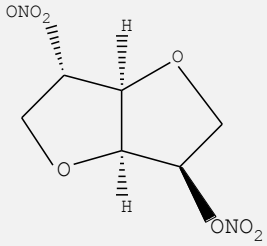
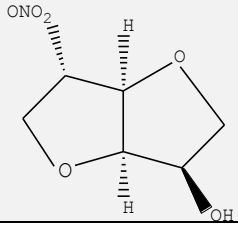
Isosorbide mononitrate is a nitrate-class drug used for the prophylactic treatment of angina pectoris; that is, it is taken in order to prevent or at least reduce the occurrence of angina [3].

There are several process impurities/related substances associated with the manufacture of Isosorbide mononitrate drug substance. Different process related impurities are observed with various synthetic routes and/or manufacturing processes.

In this paper, we used isosorbide mononitrate and sodium chloride injection as samples for sample-destructive testing, according to the testing standards of isosorbide mononitrate and sodium chloride injection in the Chinese Pharmacopoeia [4].

Two of the known isosorbide mononitrate related substances studied here are isosorbide dinitrate and 2- isosorbide mononitrate [5]. Structures of these related substances and their chemical names are provided in **Table 1**.

TABLE 1: CHEMICAL NAMES AND STRUCTURES FOR ISOSORBIDE MONONITRATE AND ITS RELATED SUBSTANCES

Substance	Structure
5-ISMN	
ISDN	
2-ISMN	

MATERIAL AND METHODS: HPLC grade methanol was purchased from Kemiou Industrial Co., Ltd, Tianjin, China. Isosorbide mononitrate, isosorbide dinitrate and 2- isosorbide mononitrate reference standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. The isosorbide mononitrate and sodium chloride injection were supplied from Shijiangzhuang NBP Pharmaceutical Co. Ltd.

Chromatography: The HPLC system consisted of a quaternary LC-20AT pump, a SPD-20A UV detector and the chromatographic column was a C_{18} (5µm, 250×4.6mm). The mobile phase composed of methanol and water (25:75). The detection wavelength for the determination was 210 nm. The injection volume was 20µL. The temperature of the column was room temperature.

Standard solutions [6]: Isosorbide mononitrate standard solution: Took isosorbide mononitrate reference standards, accurately weighed, dissolved in mobile phase and quantitatively diluted, equivalent to 80µg/mL of isosorbide mononitrate approximately, as the standard solution.

Isosorbide dinitrate and 2-isosorbide mononitrate mixture standard solution: Took isosorbide dinitrate and 2- isosorbide mononitrate reference standards, accurately weighed, dissolved in mobile phase and quantitatively diluted, equivalent to 40µg/mL of isosorbide dinitrate and 2-isosorbide mononitrate respectively, as the standard solution.

Sample preparation: Took isosorbide mononitrate and sodium chloride injection, accurately measured, dissolved in mobile phase and quantitatively diluted, equivalent to 80µg/mL of isosorbide mononitrate approximately.

Placebo: A placebo (blank formulation solution) was prepared consisting of purified water, sodium chloride solution.

System suitability: The system was deemed suitable if the following acceptance criteria were satisfied. The tailing factor for the isosorbide mononitrate peak in the resolution solution was not more than 1.6. The resolution between the two substances peaks was not less than 1.5.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ for analytes were estimated by injecting a series of diluted solutions with known concentrations. Solutions of isosorbide mononitrate and two of its related substances were prepared in duplicate. The signal-to-noise ratios for isosorbide mononitrate and the two related substances were calculated. The LOD was evaluated as the concentration, which produced a peak with a signal-to-noise ratio of about 3. The LOQ was evaluated as the concentration that produced a peak with a signal-to-noise ratio of about 10^7.

Specificity:

Force degradation studies: Solutions of isosorbide mononitrate formulation were stressed with acidic, basic, oxidative, thermal, and photolytic conditions. Prior to analysis, the acid stressed samples were neutralized with base, and the base stressed samples were neutralized with acid. The force-degraded samples were analyzed using a HPLC system. The conditions of the force degradation studies were listed in **Table 2**.

TABLE 2: THE CONDITIONS OF THE FORCE DEGRADATION STUDIES

	Conditions
Peroxide	2ml of 10% hydrogen peroxide, boiling water bath for 1h
Acid	5mL of 0.1 mol / L hydrochloric acid, boiling water bath for 1h
Base	5 mL of 0.1 mol / L sodium hydroxide solution, boiling water bath for 1 h
Heat	Boiling water bath for 3 h
Light	Strong light for 10 days

Accuracy and linearity for isosorbide mononitrate [8]:

- **Recovery**: Accuracy of the method was calculated by recovery studies at three levels by standard addition method. Samples of product placebo were spiked with isosorbide mononitrate drug substance at 80, 100, and 120% of the product label claim.

- **Linearity**: Samples of product placebo were spiked with different concentration isosorbide mononitrate stock solution. The spiked samples were assayed for isosorbide mononitrate content and the determined peak areas were plotted versus the spiked concentrations and a linear regression analysis was performed.

Accuracy and linearity for related substances:

- **Recovery**: Take isosorbide dinitrate and 2-isosorbide mononitrate standard stock solution, accurately weighed, added to the placebo, dissolved in mobile phase and quantitatively diluted. A composite standard solution containing each of the two related substances was prepared and injected in triplicate.

- **Linearity**: Solutions of two related substances containing at different concentrations were prepared in duplicate and chromatographed. The signal-to-noise ratios for each component were determined.

Ruggedness: Six replicate samples of the injection solution from the same bulk formulation were prepared and assayed using two different laboratories, analysts, instruments and in different days. The individual assay results for isosorbide mononitrate and for related substances were calculated.

The RSD for the six assays were determined for each laboratory and agreement between the mean results was calculated.

Stability of standard and sample solutions: The stability of isosorbide mononitrate in prepared sample solutions was evaluated for 12h under room condition. The assay values obtained at the end of the storage period were compared to the initial concentrations to evaluate the stability of solutions.

Precision of standard solutions: The standard solutions were injected in triplicate.

RESULTS AND DISCUSSIONS: Various mobile phases and columns were used to arrive at a method that achieved an optimal separation for all the components. The chromatographic method described here separated all the related substances of isosorbide mononitrate.

Limits of detection (LOD) and quantitation (LOQ): Table 4 provides the determined LOD and LOQ values for isosorbide mononitrate and the related substances. The limits of detection of isosorbide mononitrate, isosorbide dinitrate and 2-isosorbide mononitrate were all 0.3ng. And the limits of quantitation of isosorbide mononitrate, isosorbide dinitrate and 2- isosorbide mononitrate were all 0.8ng.

Specificity:

Chromatographic profiles: The specificity of the method was determined by individually chromatographing isosorbide mononitrate and two of its related substances (see **Table 3**). The chromatograms show that the method was specific and no interferences from the blank. The typical retention times and relative retention times for each component were presented in Table 3. **Fig. 1** showed typical chromatograms of isosorbide mononitrate and the two related substances.

TABLE 3: TYPICAL RETENTION TIME, LOD AND LOQ CONCENTIONS OF COMPONENTS

Peak ID	Component	Typical retention time (min)	LOD concentration (µg/mL)	LOQ concentration (µg/mL)
1	2-isosorbide mononitrate	5.61	0.015	0.04
2	isosorbide mononitrate	6.48	0.015	0.04
3	isosorbide dinitrate	24.85	0.015	0.04

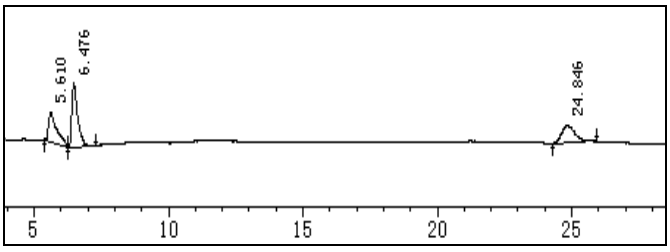


FIG. 1: CHROMATOGRAPHIC PROFILES OF ISOSORBIDE MONONITRATE AND ITS RELATED SUBSTANCES

Force-degradation studies: The results of the Force-degradation studies were as **Table 4**.

The application of stress conditions did not generate any degradation products that interfered with the determination of isosorbide mononitrate. The results indicated the isosorbide mononitrate peak in all the stressed samples was single pure peak. For the drug product samples that were stressed with thermal condition, no appreciable degradation was observed. However, for the base, acidic and oxidative stressed condition, the unknown degradation peaks observed in the samples.

TABLE 4: FORCED DEGRADATION OF STUDIES OF ISOSORBIDE MONONITRATE

	Time(h)	Mass balance (%)	RRT of degradation products
Peroxide	1	99.48	9.742
Acid	1	99.32	17.80
Base	1	99.47	4.04,6.74,15.16
Heat	3	100.00	None detected
Light	240	99.44	10.60

Accuracy/recovery/linearity for isosorbide mononitrate: The results of the recovery studies show that the method was accurate for the determination of isosorbide mononitrate. The individual isosorbide mononitrate recoveries for placebo samples spiked at 80–120% of label claim ranged from 99.91 to 100.79%.

The overall mean recovery was 100.67%. All recovery results were presented in **Table 5**. The method was found to be linear for isosorbide mononitrate in the 80-120% of label claim. The correlation coefficient (R^2) was 0.9998 and the linear regression equation was presented in **Table 8**.

TABLE 5: ACCURACY AND RECOVERY FOR ISOSORBIDE MONONITRATE

	Spiked amount (µg)	Amount determined (µg)	Recovery (%)	Mean recovery (%)	RSD (%)	Mean RSD (%)
	633.60	642.03	101.33			
80%	633.60	642.24	101.36	101.31	0.06	
	633.60	641.36	101.22			
	792.00	790.91	99.86			
100%	792.00	792.21	100.03	99.91	0.08	100.67
	792.00	790.79	99.85			
	950.40	957.60	100.76			
120%	950.40	958.68	100.87	100.79	0.06	
	950.40	957.47	100.74			

Accuracy/recovery/linearity for related substances: The recovery results indicate that the method was also accurate for the determination of the two related substances. The results were shown in **table 6 and 7**. The relative standard deviation (RSD) for isosorbide dinitrate and 2- isosorbide mononitrate were 0.56% and 0.82% respectively, within the limits. The method was found to be linear with correlation coefficients (R^2) of 0.993 and 0.992 for the two related substances, respectively, within the limits. The linear regression data for the two related substances were presented in **Table 8**.

TABLE 6: ACCURACY AND RECOVERY FOR ISOSORBIDE DINITRATE

Spiked amount (µg)	Amount determined (µg)	Recovery (%)	Mean recovery (%)	RSD (%)	Mean RSD (%)
24.19	24.05	99.41			
24.19	24.29	100.41	99.99	0.51	
24.19	24.23	100.14			
20.16	20.14	99.90			
20.16	20.31	100.75	100.45	0.48	0.56
20.16	20.30	100.72			
16.13	16.07	99.62			
16.13	16.26	100.83	100.05	0.68	
16.13	16.08	99.69			

TABLE 7: ACCURACY AND RECOVERY FOR 2-ISOSORBIDE MONONITRATE

Spiked amount (µg)	Amount determined (µg)	Recovery (%)	Mean recovery (%)	RSD (%)	Mean RSD (%)
23.81	23.84	100.15			
23.81	23.86	100.21	100.28	0.17	
23.81	23.92	100.47			
19.84	19.84	100.00			
19.84	19.62	98.89	99.95	1.04	0.82
19.84	20.03	100.97			
15.87	15.75	99.21			
15.87	16.08	101.28	100.65	1.24	
15.87	16.10	101.45			

TABLE 8: LINEAR RANGE, COEFFICIENT OF CORRELATION FOR ISOSORBIDE MONONITRATE AND TWO OF ITS RELATED SUBSTANCES

Component	Linear range (µg/ml)	R^2	Linear equation
Isosorbide Mononitrate	18-160	0.9998	y = 16950x - 22465
Isosorbide dinitrate	0.04-0.9	0.9993	y = 24094 x-101.8
2-isosorbide mononitrate	0.03-0.8	0.9992	y = 18141 x+ 84.59

Stability of sample solutions: The stability of isosorbide mononitrate and two relative substances in standard solutions was evaluated. Prepared samples and standards had been shown to be stable at ambient conditions for at least 12 hours. No other degradation products were observed for any of the solutions tested (**table 9**).

TABLE 9: STABILITY OF ISOSORBIDE MONONITRATE AND TWO RELATIVE SUBSTANCES

Time	Isosorbide dinitrate (Peak area)	2- isosorbide mononitrate (Peak area)	isosorbide mononitrate (Peak area)
0	9815	7128	1311591
2	9951	7131	1313019
4	9884	7150	1316718
6	9842	7160	1314457
12	9866	7144	1309125
RSD%	0.52	0.19	0.21

CONCLUSION: The proposed method was found to be accurate, precise, specific, sensitive and linear for the determination of isosorbide mononitrate and two of its related substances in the injection. The method is therefore suitable for the determination of isosorbide mononitrate and its related substances in isosorbide mononitrate and sodium chloride injection.

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