Determination of 4-Sulfonamidophenylhydrazine and Sulfonamide in Diarylpyrazole Derivatives by LC-MS

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Keywords: LC-MS, Sulfonamide, 4-Sulfonamidophenylhydrazine, Diarylpyrazole Derivatives.

Abstract: A sensitive and selective LC-MS method was developed for the determination of 4-sulfonamidophenylhydrazine and sulfonamide in diarylpyrazole derivatives. The analysis of two impurities was done on Inertsil Ph-3 phenyl column (100 mm×4.6 mm, 3 μm). The mobile phase was gradient elution with 0.01 mol/L ammonium acetate buffer solution (pH 4.0) and methanol at a flow rate of 0.5 mL/min. Mass spectrometry adopts electrospray ion source, monitoring in positive ion mode. The limits of quantification of 4-sulfonamidophenylhydrazine and sulfonamide were 0.4915 ng/mL and 0.5079 ng/mL, respectively. They had a good linear relationship within their respective concentration ranges, and the average recoveries were 106.96% and 106.71%, respectively. The method can be used for the determination of 4-sulfonamidophenylhydrazine and sulfonamide in diarylpyrazole derivatives.

1 INTRODUCTION

As an important intermediate in the drug market, pyrazole compounds are widely used in the synthesis and development of drug targets, and are the mainstream of current drug development (Zhong 2015, Selvam 2005, Bekhit 2004). Pyrazole compounds have many pharmacological activities, such as antibacterial, anti-inflammatory, anti-tumor, etc (Zhang 2014, Tang 2008, Liu 2007). Some pyrazole compounds have been developed into marketed drugs or undergoing clinical research, such as anti-inflammatory drug celecoxib, antibacterial drug sulfafenpyrazole, antihypertensive drug riociguat, anticancer drug anthrapyrazol, etc (Pathak 2012, Penning 1997, Ghofrani 2009). Structure-activity relationship studies have shown that the 1, 3, and 5 position substitutions of the nucleus of pyrazole compounds play a key role in the selectivity of NIAIDs, while the 4-position substitution makes the selectivity to COX-2 decrease (Wang 2014, Stauffer 2000, Katoch 2003). Synthesizers used the selective COX-2 inhibitor celecoxib as a model compound to design and synthesize multiple diaryl-substituted pyrazole derivatives. The synthetic intermediate 4-sulfonamidophenylhydrazine contains a genotoxicity warning structure, and its starting material, sulfonamides, which is relatively toxic and difficult to metabolize (Gong 2019, Baran 2011, Yu 2015), will generate azo compounds with genotoxicity warning structure in subsequent reactions. Therefore, a liquid mass spectrometry method was established to determine the content of 4-sulfonamidophenylhydrazine and sulfonamide in diarylpyrazole derivatives (Reddy 2015, Szekely 2015, Rajput 2017). The method was validated as per ICH guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, specificity, and solution stability. See Table 1 for specific information.

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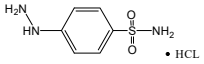
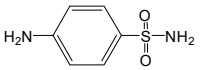
Table 1: Impurity information sheet.

Compound	Chemical Structure	Molecular Formula/ Exact Molecule Weight
4-sulfonamidophenylhydrazine		$C_6H_9N_3O_2S \cdot HCl$ / 223.68
Sulfonamide		$C_6H_8O_2N_2S$/ 172.20

2 EXPERIMENTAL

2.1 Chemical and Reagents

HPLC grade acetonitrile was obtained from Concord Technology, Tianjin, China. Analytical grade ammonium acetate and HPLC grade acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd, China. Purified water was collected through Milli-Q Plus water purification system. Diarylpyrazole derivatives API was homemade. Reference substances of 4-sulfonamidophenylhydrazine (99.5%) and sulfonamide (99.2%) were supplied by Toronto Research Chemicals, Canada.

2.2 Instrumentation

This research was performed on THERMO make Ultimate 3000 UPLC-MS system. It has dual ternary gradient pump, column oven with range of 5°C to 80°C with autosampler, diode array detector (UV) and Q-Exactive Focus Orbitrap detector.

2.3 Chromatographic Conditions

Development and validation of the method were carried on the LC-MS system. The analytical column was Inertsil Ph-3 phenyl column (100 mm×4.6 mm, 3 μm) in gradient mode using 0.01 mol/L ammonium acetate buffer solution (pH 4.0) and methanol (Table 2). The flow rate was 0.5 mL/min. The column temperature was maintained at 25℃, and the injection volume was 5 μL. The effluent did not enter the mass spectrometer after 5 minutes controlled by the switching valve.

Table 2: Gradient programme.

Time (min)	Ammonium acetate buffer (%)	Methanol (%)
0.0	60.0	40.0
4.0	60.0	40.0
5.0	20.0	80.0
15.0	20.0	80.0
15.1	60.0	40.0
20.0	60.0	40.0

2.4 Mass Spectrometer

The MS system used was an Q-Exactive Orbitrap mass spectrometer with electrospray ionization probe operated in positive polarity. Selected Ion Monitoring mode was chosen for the quantification of 4-sulfonamidophenylhydrazine and sulfonamide. 4-sulfonamidophenylhydrazine was monitored with its molecular ion [M+Na]+m/z 210.03077, and sulfonamide was monitored with its molecular ion [M+Na]+m/z 195.01987 in this method (Figure 1). Typical operating conditions were as follows: capillary temperature 320℃, aux gas heater temperature 310 ℃, sheath gas flow rate 35 arb, aux gas flow rate 10 arb, spray voltage 3.60 kV, S-lens RF level 50.0.

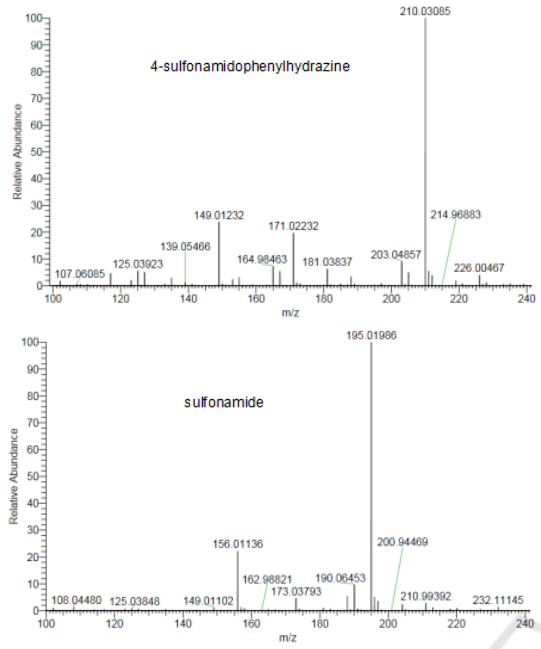


Figure 1: Representative mass spectra of 4-sulfonamidophenylhydrazine and sulfonamide.

2.5 Standard and Sample Preparation

The separate stock standard solutions were prepared by weighing an accurately amount of about 10 mg of 4-sulfonamidophenylhydrazine and sulfonamide, and transferred them into a 100 mL volumetric flask individually, the volume was made up to the mark with methanol. The mixed stock standard solution was prepared by diluting 1 mL of 4-sulfonamidophenylhydrazine standard solution and 1 mL of sulfonamide stock standard solution to 100 mL with methanol. Take an appropriate amount of the mixed stock standard solution, and gradually dilute it with methanol to make a solution containing 5 ng/mL of 4-sulfonamidophenylhydrazine and 5 ng/mL of sulfonamide, as the standard solution.

The sample solution was prepared by dissolving appropriate amount of diarylpyrazole derivatives in methanol to make a solution containing 2 mg/mL.

3 METHOD VALIDATION

3.1 Specificity

The specificity of the method was demonstrated by injecting the blank and the reference solution. The results showed that the retention time of 4-sulfonamidophenylhydrazine was 2.84 min, and the retention time of sulfonamide was 4.37 min (Figure 2). The blank chromatogram showed that no interference was observed at the retention times of 4-sulfonamidophenylhydrazine and sulfonamide.

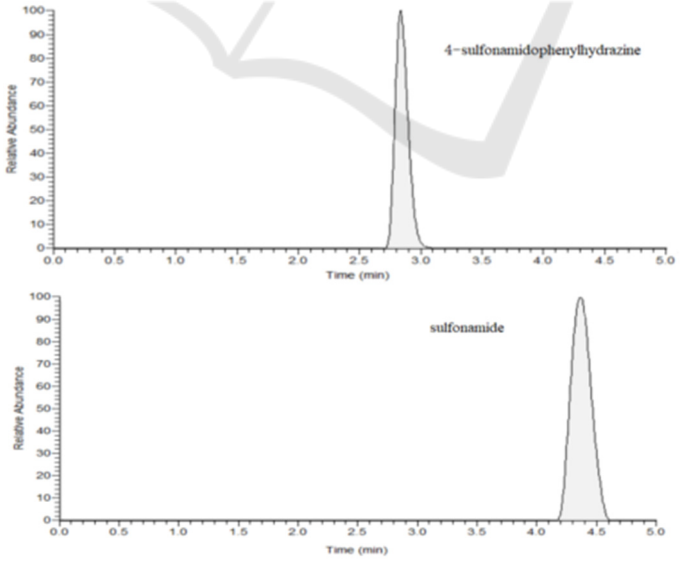


Figure 2: Specificiy of the method.

3.2 Solution Stability

The stability of 4-sulfonamidophenylhydrazine and sulfonamide was checked by keeping the standard solution in an autosampler for 12h and observing the variations in their peak areas at every 1h. The RSDs of the peak areas of 4-sulfonamidophenylhydrazine and sulfonamide were 5.28% and 3.39%, respectively. From the stability results, we found that 4-sulfonamidophenylhydrazine and sulfonamide were stable up to 12h.

3.3 Determination of LOD and LOQ

Take the standard solution and dilute it step by step with methanol until the signal-to-noise ratio S/N is close to 10 and 3 as the limit of quantification and detection of 4-sulfonamidophenylhydrazine and sulfonamide. The limits of quantification for 4-sulfonamidophenylhydrazine and sulfonamide were 0.4915 ng/mL and 0.5079 ng/mL, respectively. The detection limits of 4-sulfonamidophenylhydrazine and sulfonamide were 0.1474 ng/mL and 0.1524 ng/mL, respectively.

3.4 Linearity

The linearity stock solution was prepared by diluting 5 mL of the mixed stock standard solution to 100 mL with methanol. The linearity test solutions were prepared from the linearity stock solution at seven concentration levels from LOQ (0.25 ppm) to 300% (7.5 ppm) of the specification concentrantion (2.5 ppm). The calibration curve was obtained by drawing the graph between the peak areas and concentration of 4-sulfonamidophenylhydrazine and sulfonamide at 0.25, 0.5, 1, 1.5, 2.5, 5 and 7.5 ppm. The slope, intercept, and correlation coefficient values were derived from linear least squares regression analysis. The correlation coefficient obtained in each case was >0.9998. The corresponding linearity data is presented in Table 3. The results indicated that an excellent correlation existed between the peak areas and the concentrations of 4-sulfonamidophenylhydrazine and sulfonamide.

Table 3: Results of linearity for 4-sulfonamidophenylhydrazine and sulfonamide.

Level (ppm)	4-Sulfonamido--phenylhydrazine		Sulfonamide	
	Conc. (ng/mL)	Peak area	Conc. (ng/mL)	Peak area
LOQ	0.4915	53731.87	0.5079	108697.69
0.5	0.9829	112075.22	1.0159	208294.14
1	1.9659	249253.89	2.0318	413800.80
1.5	2.9488	367906.96	3.0477	654523.96
2.5	4.9147	633583.15	5.0795	1069378.87
5	9.8294	1238938.63	10.1590	2098273.04
7	14.7441	1878797.94	15.2384	3113619.89
Correlation	0.9999		0.9999	
Slope	127733		204377	
Intercept	6917.3		12610	

3.5 Recovery studies

A study of accuracy of 4-sulfonamidophenylhydrazine and sulfonamide from spiked samples of test preparation was conducted. Samples were prepared in triplicate at LOQ level, 100% and 150% of the specification concentrations, i.e 0.25, 2.5 and 5 ppm by spiking test preparation. The mean recovery of 4-sulfonamidophenylhydrazine and sulfonamide at mentioned concentration level was reported in the Table 4. The recoveries of 4-sulfonamidophenylhydrazine and sulfonamide at three levels were in the range of 80% to 120% and relative standard deviations were not more than 10.0%.

Table 4: Accuracy of 4-sulfonamidophenylhydrazine and sulfonamide

Sample Name	Recovery (%)	
	4-sulfonamido--phenylhydrazine	Sulfonamide
LOQ spiked sample-1	96.46	109.19
LOQ spiked sample-2	102.12	120.06
LOQ spiked sample-3	99.33	117.28
100% spiked sample-1	105.33	107.41
100% spiked sample-2	113.12	101.43
100% spiked sample-3	114.59	105.16
150% spiked sample-1	111.68	108.45
150% spiked sample-2	109.24	94.70
150% spiked sample-3	110.81	96.70
Mean recovery (%)	106.96	106.71
RSD (%)	6.03	7.94

3.6 Precision

3.6.1 System Precision

The system precision was checked by calculating the RSD of six areas of 4-sulfonamidophenylhydrazine and sulfonamide by injecting the same standard solution. The RSDs of six areas of 4-sulfonamidophenylhydrazine and sulfonamide were 2.55% and 2.03% respectively.

3.6.2 Method Precision

The precision of the method was evaluated through repeatability and intermediate precision. Repeatability was checked by calculating the RSD of six replicate determinations by injecting six freshly prepared solutions containing 2.5 ppm each of 4-sulfonamidophenylhydrazine and sulfonamide on the same day. The same experiments were done on different days by different people to evaluate the intermediate precision. As reported in Table 5, the data confirmed adequate precision of the developed method.

Table 5: Method precision of 4-sulfonamidophenylhydrazine and sulfonamide at 2.5 ppm in terms of percentage contents.

Injection	4-sulfonamido--phenylhydrazine		Sulfonamide	
	Repeata--bility	Intermediate precision	Repeata--bility	Intermediate precision
1	3.10E-04	2.95E-04	2.84E-04	2.67E-04
2	2.92E-04	2.86E-04	2.80E-04	2.64E-04
3	2.88E-04	2.85E-04	2.82E-04	2.78E-04
4	2.92E-04	2.91E-04	2.79E-04	2.58E-04
5	2.81E-04	2.70E-04	2.80E-04	2.49E-04
6	2.82E-04	2.68E-04	2.80E-04	2.49E-04
Mean (%)	2.91E-04	2.82E-04	2.81E-04	2.61E-04
RSD (%)	3.64	3.92	0.65	4.31

4 DISCUSSION

Diarylpyrazole derivatives have good solubility in methanol. Sulfonamide and 4-sulfonamidophenylhydrazine are slightly soluble in methanol. Considering the large concentration of principal components and small impurity concentration, methanol is finally determined as the solvent.

In order to control the lower limit level of genotoxic impurities, this study selected a mass spectrometer for detection. Since a higher concentration of sample solution may contaminate the MS detector, the chromatographic method that is considered to be established should be able to achieve good separation of impurities peaks and principal component peaks, so that the principal component peaks do not enter the MS detector by switching the valve. The structure of sulfonamides and 4-sulfonamidophenylhydrazine contains one benzene ring, while diarylpyrazole derivatives contain two, the test results show that the phenyl column which has a special selectivity for aromatic compounds could achieve better separation of sulfonamides, 4-sulfonamidophenylhydrazine and diarylpyrazole derivatives.

The mass detector requires the use of volatile mobile phase additives. Ammonium acetate was used to optimize the peak shape of 4-sulfonamidophenylhydrazine and sulfonamide. With adjusting the pH value to 4.0 to achieved the baseline separation of 4-sulfonamidophenylhydrazine and sulfonamide. By adjusting the organic proportion and gradient elution, the retention time of diarylpyrazole derivatives is 5 minutes later so that the switch valve could control the main peak not to flow into the mass spectrometer, while ensuring that the main peak can be completely eluted every time to avoid affecting the next sample.

5 CONCLUSIONS

A validated LC-MS analytical method has been developed for the determination of 4-sulfonamidophenylhydrazine and sulfonamide in diarylpyrazole derivatives. The proposed method was simple, accurate, precise, specific and suitable to use for the routine analysis of 4-sulfonamidophenylhydrazine and sulfonamide in diarylpyrazole derivatives.

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