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Liquid Chromatography Mass Spectrometry LCMS-2050

Application News

Simultaneous Determination of Five Genotoxic Aryl Sulfonate Impurities in Pharmaceuticals by LCMS-2050

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User Benefits

- A novel method for determining the content of five genotoxic aromatic sulfonate impurities in drugs was established by using LCMS-2050 single quadrupole mass spectrometer.
- The pretreatment of this method was simple, and it had a wide linear range and a high accuracy rate.

■ Introduction

Genotoxic impurities (GTIs) refer to substances that directly or indirectly damage cellular DNA, generating gene mutations or in vivo mutagenesis. GITs may can cause damage to human genetic material at extremely low concentrations, which in turn leads to gene mutations and promotes the occurrence of tumors. Because of the potential risks of GITs, it is essential to monitor any potentially existing genotoxic impurities in active pharmaceutical ingredients or medicinal products.

In the pharmaceutical industry, when drug active ingredients exist in the forms of alkyl sulfonates, benzenesulfonates, p-toluenesulfonates, and hydroxyethyl sulfonates or sulfonic acid reagents are utilized in the drug synthesis process, sulfonate esters are regarded as potential GTIs. GTIs of sulfonate esters can be classified as alkyl sulfonate esters and aryl sulfonate esters based on different substituents.

Previously, relevant literature has reported quantitative detection methods for some GTIs of sulfonate esters, such as GC-MS, GC-FID, LC-MS/MS, and complex derivatization treatment of samples might be necessary. In this study, LC-MS was mainly utilized for the quantitative analysis of five GTIs of aryl sulfonate esters. This method features simple pretreatment and high accuracy, which can offer a reference for the detection of genotoxic impurities of aryl sulfonate esters in drugs.

■ Sample Preparation

45 mg of a certain drug powder was precisely weighed and placed in a 1 mL volumetric flask. Methanol was used to adjust the volume to the mark, followed by thorough shaking and ultrasonication for 10 minutes. The volumetric flask was taken out and cooled to room temperature. Methanol was added to make up to the mark. The mixture was homogenized and passed through a 0.22 µm nylon filter membrane before instrumental analysis.

The mixed standard stock solution (100 mg/L) was taken and serially diluted with methanol to obtain standard working solutions at concentrations of 5, 10, 20, 50, 100, 200, and 500 µg/L for subsequent determination.

■ Analysis Conditions

The analytical conditions are shown in Table 1. A Nexera XR UHPLC system with a photodiode array detector (PDA) was coupled to the single quadrupole mass spectrometer (LCMS-2050). The LCMS-2050 is equipped with a heated DUIS™ ion source for ionization, which combines the advantages of both ESI and APCI sources.

Table 1 Analysis Conditions

System	: Nexera™ XR
Column	: Shim-pack™ GISS C18 (100 mm × 2.1 mm I.D., 1.9 µm)*1
Detection	: PDA at 190 to 800 nm
Temperature	: 40 °C
Injection volume	: 2 µL
Mobile phases	: A-5 mM ammonium formate in Water B-methanol
Flow rate	: 0.3 mL/min
Mode	: Gradient elution
Time program (%B)	: 35% (0 min) → 50% (3.5 min) →90% (4.5-7 min) → 35% (7.01-9 min)
FCV Valve Position	: 0 (0 min) → 1 (2.8 min) →0 (7min)*2
System	: LCMS-2050 (DUIS Positive)
Mode	: SIM
Interface Voltage	: 2.0 kV
Nebulizing gas	: 3 L/min
Drying gas	: 7 L/min
Heating gas	: 5 L/min
DL temp	: 100 °C
Desolvation temp	: 150 °C

Table 2 SIM Conditions

ID	Compound	Polarity	*m/z*
1	Methyl benzenesulfonate	+	190.04
2	Ethyl benzenesulfonate	+	204.06
3	Methyl p-toluenesulfonate	+	204.06
4	Ethyl p-toluenesulfonate	+	218.08
5	Isopropyl toluene-4-sulphonate	+	232.09

*1 P/N：227-30048-02
*2: "1" indicates to mass spectrum, and "0" indicates that the flow path is switched to waste liquid

■ Chromatographic Condition Optimization

In this report, during the optimization of liquid phase conditions, different mobile phases are compared. it was found that the target substances had a relatively high response under the gradient elution of 5 mmol/L ammonium formate aqueous solution and methanol. At the same time, because the content of genotoxic impurities in the main drug is extremely low, in order to analyze the content of genetic impurities and ensure sensitivity, and to avoid high concentrations of the main drug and excipients entering the mass spectrometer and causing contamination, it is necessary to switch the main drug and excipients to the waste liquid through the flow path switching valve and only allow the impurities to be tested to enter the mass spectrometer for detection.

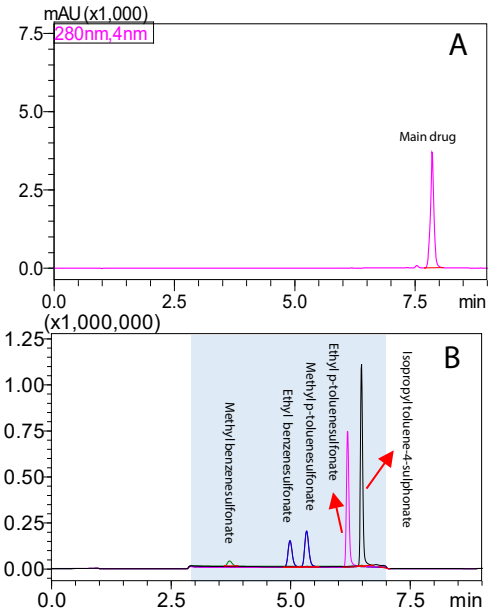

Fig.1 PDA Chromatogram of Sample (A, Main Drug Concentration 0.8 mg/mL) and SIM Chromatogram of Mixed Standard Solution (B, Concentration 100 μg/L)

■ Specificity

Fig. 2 shows the SIM chromatogram of the blank and the standard solution (10 μg/L). There is no obvious interference at the target peak, so the method has good specificity.

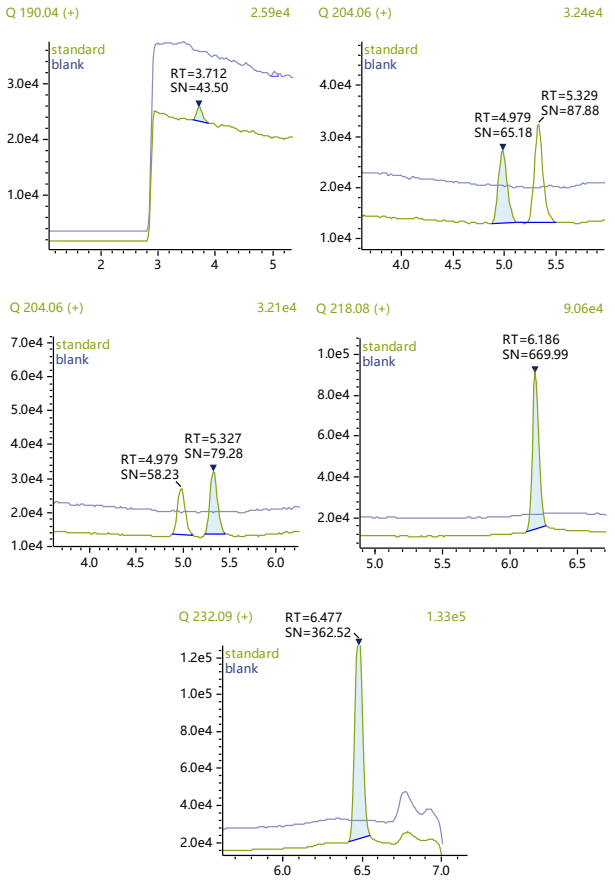

Fig. 2 SIM chromatogram of blank and standard solution

■ Calibration Curve

The calibration curve (external standard method) prepared using the standard sample showed good linearity in a wide dynamic range of 100-fold for all compounds. Fig. 3 shows the calibration curve.

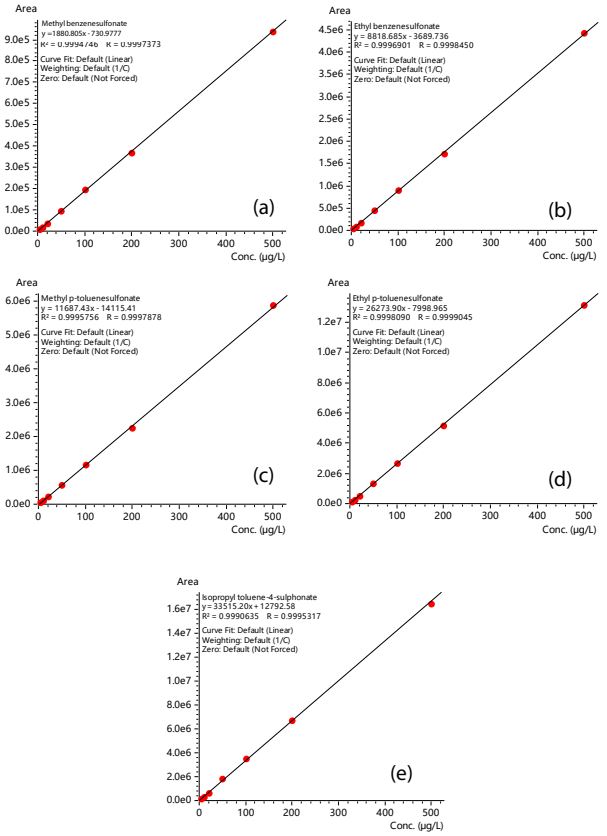

Fig. 3 Calibration curve(5-500 μg/L)
(a) Methyl benzenesulfonate, (b) Ethyl benzenesulfonate
(c) Methyl p-toluenesulfonate, (d) Ethyl p-toluenesulfonate
(e) Isopropyl toluene-4-sulphonate

■ Repeatability

Table3 shows the repeatability for the mix standard solution which concentration of 10, 50 and 500 μg/L (n=6).

Table 3 RSD% of R.T. and Area

Compound	10 μg/L		50 μg/L		500 μg/L	
	R.T	Area	R.T	Area	R.T	Area
Methyl benzenesulfonate	0.10	5.26	0.12	2.74	0.12	0.75
Ethyl benzenesulfonate	0.08	1.53	0.10	1.34	0.10	2.19
Methyl p-toluenesulfonate	0.08	1.66	0.09	0.73	0.10	1.80
Ethyl p-toluenesulfonate	0.03	0.67	0.05	1.07	0.06	1.19
Isopropyl toluene-4-sulphonate	1.22	4.29	0.06	0.61	0.06	2.38

Table 4 The mean recovery rate (%) of the spiked sample

Compound	0.22 μg/g	0.44 μg/g	2.2 μg/g
	Mean recovery (%)	Mean recovery (%)	Mean recovery (%)
Methyl benzenesulfonate	111.1	105.7	106.6
Ethyl benzenesulfonate	112.6	108.4	110.5
Methyl p-toluenesulfonate	115.3	112.6	108.2
Ethyl p-toluenesulfonate	104.1	100.5	106.3
Isopropyl toluene-4sulphonate	110.9	115.0	108.7

■ Recovery

None of the five genotoxic impurities of aryl sulfonate esters were detected in a certain drug. Standard solutions of various concentrations were spiked to the drug for the recovery experiments, and each addition amount was determined in triplicate. The recovery rates are presented in Table 4, and the results indicate that the recovery rates comply with the requirements of the Pharmacopoeia of the People's Republic of China 2020 Edition.

■ Conclusion

A novel method for determining the content of five genotoxic impurity aryl sulfonates in drugs was established by using the LCMS-2050. Within the concentration range of 5 - 500 µg/L, the correlation coefficients of the five genotoxic impurity aryl sulfonates were all greater than 0.994. When a mixed standard solution of three concentrations was added to the drugs, the recovery rates of each component ranged from 110.5% to 115.3%. This method features simple pretreatment, excellent specificity and high sensitivity, and can provide a reference for the determination of the content of five genotoxic impurities aryl sulfonates in drugs.

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