Determination of isothiazolinone fungicides in cigarette accessories

Using LC-MS/MS on the QTRAP 4500 system

Liu Qing, Yang Zong, Liu Bingjie, Guo Lihai
SCIEX, China

Isothiazolinones are a class of high-efficiency, broad-spectrum fungicides that are widely used in the pulp and paper industry to prevent pulp corruption and deterioration during production processes. Among them, 2-methyl-4-isothiazolin-3-one (MI), 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) and 1,2-benzisothiazolin-3-Ketone (BIT) are the most used.[1] These compounds are a class of sensitizers or chemicals with the potential to become allergens and may cause allergies or dermatitis if they come into contact with skin. Excessive contact with isothiazolinones may even cause skin burns. Among them, CMI and MI are the most sensitizing and irritating. Strict restrictions on the use of isothiazolinone fungicides in food contact materials, toys, cosmetics and other daily necessities exist in China and globally.[1] This application note presents the analysis of isothiazolinone fungicides in products such as smokeless paper and water-based glues used in cigarettes.

In this method, ultrasonic solvent extraction was used for sample preparation and LC-MS/MS technology was used for data acquisition. Multiple reaction monitoring (MRM) scan mode and isotope internal standards were then used for accurate quantification of isothiazolinone in cigarette paper (Figure 1).

Key features for isothiazolinone analysis with the QTRAP 4500 system

- Common fungicides were detected and measured in complex glue and paper samples.
- Calibration curves ranged from 0.1 to 100 µg/L, with good linear correlation and excellent method %CV values for triplicate injections.
- A short chromatographic run time and simple, transferrable settings suggest that this method can be effectively implemented for routine analysis.

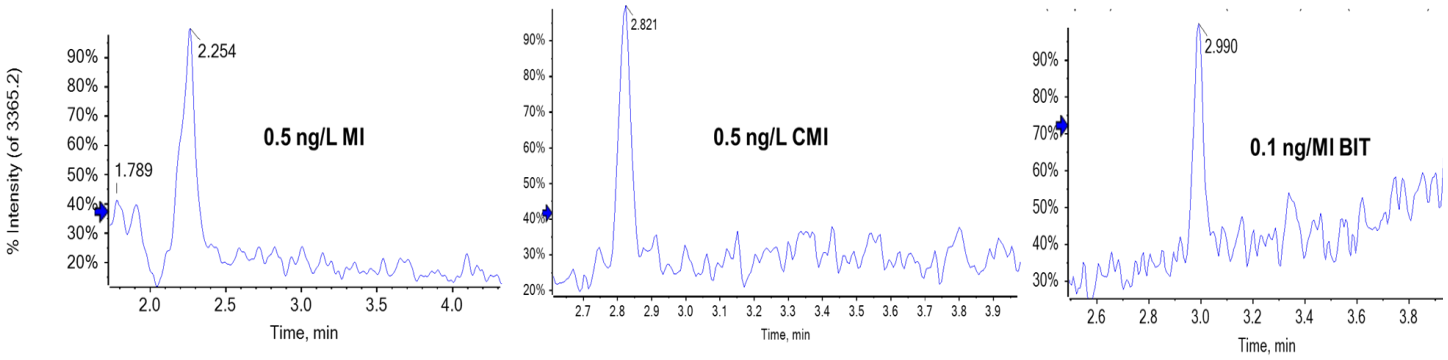

Figure 1. Chromatographic peaks are shown at the lowest quantifiable levels of each of the three analytes. From left to right they are 0.5 ng/L for MI, 0.5 ng/L for CMI and 0.1 ng/L for BIT.

Methods

Sample preparation: The water-based glue samples were prepared by weighing 0.3 g of tobacco water-based gum into a 50 mL conical flask. After dispersing with 5 mL of water, 20 mL of methanol was added, and the ultrasonic extraction was performed at 350 W for 30 min. A 5 mL aliquot of the extract was centrifuged for 10 min at a speed of 10,000 r/min. A 1 mL aliquot of supernatant was transferred to a 10 mL volumetric flask. A 100 µL aliquot of mixed internal standard stock solution was then added. The sample was diluted to a final volume of 10 µL and mixed. Finally, the extract was passed through a 0.22 µm organic phase filter membrane for testing.

The paper samples were prepared by cutting the paper into 0.5 cm × 0.5 cm pieces, mixing them evenly and sealing them for later use. Then, 0.50 g of the paper sample was weighed into a 50 mL centrifuge tube, and 200 µL of internal standard working solution was added. Next, 20 mL of methanol was added, and ultrasonic extraction was performed for 30 min. The samples were centrifuged at 5,000 r/min for 5 min and filtered with 0.22 µm organic phase membrane filtration for LC-MS/MS analysis.

Chromatography: The LC system used was an ExionLC AD system from SCIEX. The analytical column used was a Waters ACQUITY UPLC HSS T3 column (100Å, 1.8 µm, 2.1 mm × 100 mm). Mobile phase A was an aqueous solution containing 0.1% formic acid, and mobile phase B was an acetonitrile solution containing 0.1% formic acid. A flow rate of 0.4 mL/min and an injection volume of 5 µL was used. The gradient run time was 7 min, with starting conditions of 3% B for 3 min followed by a ramp to 80% B over 2 min, which was held for 0.1 min, then a drop back to 3% B over 1.9 min.

Mass spectrometry: The mass spectrometer used was a QTRAP 4500 system from SCIEX. MRM scan mode was used and operated in positive ion mode. Ionization was conducted in electrospray ionization (ESI) mode. Ion source parameters include an ionization voltage (IS) of 5500 V, ion source temperature of 550°C, curtain gas (CUR) of 20 psi, collision gas (CAD) of 9 psi, atomizing gas (GS1) of 550 psi and auxiliary atomizing gas (GS2) of 55 psi.

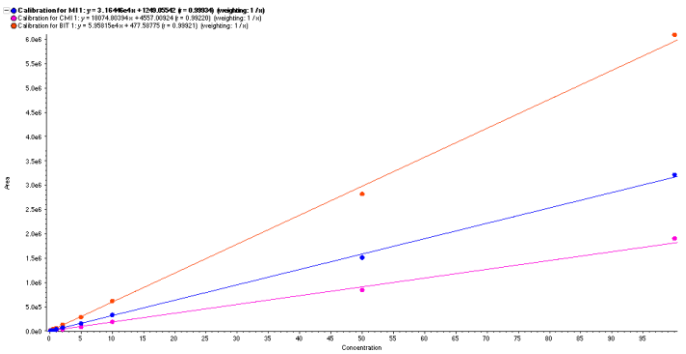

Figure 2. Calibration curves for selected analytes. Shown are the linear calibration curves for MI (blue, 0.5–100 ng/mL), CMI (red, 0.5–100 ng/mL) and BIT (pink, 0.1–100 ng/mL). Good linearity was observed with r > 0.99.

Results

The detection limits of MI, CMI and BIT with this method are 0.5, 0.5 and 0.1 ng/mL, respectively (Figure 1). The calibration curves of MI (0.5–100 ng/mL), CMI (0.5–100 ng/mL) and BIT (0.1–100 ng/mL) all have acceptable linear correlation with r > 0.99 for all (Figure 2).

To investigate method reproducibility and extraction recovery, water-based glue samples and paper samples were each spiked with MI, CMI and BIT at concentrations of 0.5, 0.5 and 0.1 ng/mL, respectively. A continuous series of 6 injections resulted in a relative standard deviation (RSD) of <3.5% on the analyte measurements (Table 1). Additionally, the extraction recovery rates were 80%–110%, with an RSD of <3.5% (Table 2).

Table 1. Matrix spike experiments for MI, CMI and BIT at 0.5, 0.5 and 0.1 ng/mL.

Compound	Spiking conc. (ng/mL)	Number of replicates	Glue sample, RSD%	Paper sample, RSD%
MI	*0.5*	*6*	*3.2*	*2.1*
CMI	*0.5*	*6*	*2.8*	*1.6*
BIT	*0.1*	*6*	*2.6*	*2.3*

Conclusions

In this experiment, high performance liquid chromatography tandem mass spectrometry was used to establish the MI, CMI and BIT methods for water-based glue samples and paper samples. The linearity of MI, CMI (0.5–500 ng/mL) and BIT (0.1–500 ng/mL) had a correlation coefficient r > 0.99. The spiked MI, CMI and BIT samples at concentrations of 0.5, 0.5 and 0.1 had RSDs of <3.5% and recoveries of 80%–120%. The detection limits of MI, CMI and BIT for water-based glue samples and paper samples using this method were 0.5, 0.5 and 0.1 ng/mL, respectively. As the use of isothiazolinone fungicides in cigarette accessories continues, sensitive and reproducible methods like the one demonstrated here will remain necessary to ensure public safety.

Table 2. Recovery experiments at low-level spike concentration in glue and paper samples.

Compound name	Concentration (n/mL)	Number of replicates	Glue sample, recovery %	Glue sample, RSD%	Paper sample, recovery %	Paper sample, RSD%
MI	0.5	*6*	*89*	*3.5*	*96*	*3.1*
CMI	0.5	*6*	*94*	*3.2*	*95*	*2.6*
BIT	*0.1*	*6*	*102*	*2.9*	*101*	*2.5*

References

1. Zhou et al. Determination of 3 isothiazolinone biocides in paper for cigarette by liquid chromatography-tandem mass spectrometry. Tobacco Science and Technology 2016 Aug, 49(8).

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices