ABSTRACT

Honey bee products such as honey are widely consumed as food and medicine. Many different antibiotics are used in Apiculture to keep bees away from various bacterial infections. Detection and quantitation of antibiotic contaminants is a strict requisite for honey import and export. A simple, efficient and reliable LC/MS/MS method has been developed for the simultaneous detection and identity and quantify 13 different antibiotics from two different antibiotic classes on the SCIEX 4000 QTRAP Hybrid LC/MS/MS system, providing a fast, robust, and reliable method validated for the detection of 13 antibiotics in the honey Matrix. The LC-MS/MS method is developed using the Multiple Reaction Monitoring (MRM) that detects antibiotics as per European Union regulatory guidelines with Limits of Quantitation (LOQ) for Sulfonamides and Nitroimidazoles as 0.5 μg/kg and 0.2 μg/kg respectively.

RESULTS

The data was acquired on SCIEX 4000 QTRAP® (Figure 1) and processed using Analyst® 1.6 Software with MultiQuant™ Software 2.1.1 (Figure 1) for Quantitation. Representative Extracted Ion

Table 1: MRM transitions for the method development

Table 2: Recovery and reproducibility data for Sulfonamides and Nitroimidazoles at 0.5 and 0.2 µg/kg

Method validation was performed as per EU Commission Decision 2002/657/EC, for the analysis in honey samples. The sensitivity of the method is highlighted by the Limit of quantitation (LOQ) achieved LOQ established as 0.5 μg/kg for Sulfonamides and 0.2 μg/kg for Nitroimidazoles. Calibration curves were obtained for both Sulfonamides and Nitroimidazoles. Results showed excellent linearity from 0.5 μg/kg to 5000 μg/kg for Sulfonamides and from 0.2 μg/kg to 2000 μg/kg for Nitroimidazoles. The regression coefficients of >=0.99 obtained as per EU guidelines. Figure 3 and 4 show the linearity curve for Sulfonamides and Nitroimidazoles respectively with the observed regression value of 0.99 and above.

Figure 2: Representative extracted ion Chromatogram for combination a) Extracted ion Chromatogram (XC) for Sulfonamides and b) Extracted ion Chromatogram (XC) for Nitroimidazoles at 50 µg/kg

Figure 3: Calibration curve from 0.5 to 5000 µg/kg for Sulfonamides antibiotics

Figure 4: Calibration curve from 0.2 to 2000 µg/kg for Nitroimidazoles antibiotics.

Recovery and reproducibility was investigated by spiking blank honey matrices at concentration of 0.5 μg/kg for Sulfonamides and 0.2 μg/kg for Nitroimidazoles antibiotics. All analysis showed acceptable ICV as shown in Table 2.

Figure 5: Comparison of Retention Time (min) in Standard and sample showing presence of Sulfapyridine Compound

Table 3: Sample analysis of 10 honey sample showing presence of different antibiotics

Figure 6: shows a representative example for a Standard and Sample showing retention time for Sulfapyridine and Sulfamethazine antibiotics.

REFERENCES

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