



APPLICATION NOTE

Mass Spectrometry

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Rapid Screening and Quantitation of Postharvest Fungicides on Citrus Fruits Using AxION DSA/TOF and Flexar SQ MS

Introduction

Thiabendazole and Imazalil are the major post-harvest fungicides used on surface of citrus fruits such as oranges, grapefruits and lemons to prevent mold formation and control postharvest decay by various fungal pathogens in citrus fruits¹. Figure 1 shows the structure of two fungicides. These substances have toxicity in higher doses, with effects

such as liver and intestinal disorders and carcinogenic effects. The MRL (maximum residue limits) of imazalil and thiabendazole for conventional citrus fruits are 10 ppm and 5 ppm in the U.S. and Europe, respectively^{2,3}. The use of these fungicides on organic citrus fruits is prohibited. The maximum residue limits for these compounds on conventional citrus fruits and possible toxic effects require that these compounds should be monitored before consumption of fruit to ensure food safety. Previously, these fungicides on citrus fruits have been measured by different LC/MS techniques with varying sample preparation techniques such as liquid-liquid extraction^{4,5} and QUECHERS^{6,7}.

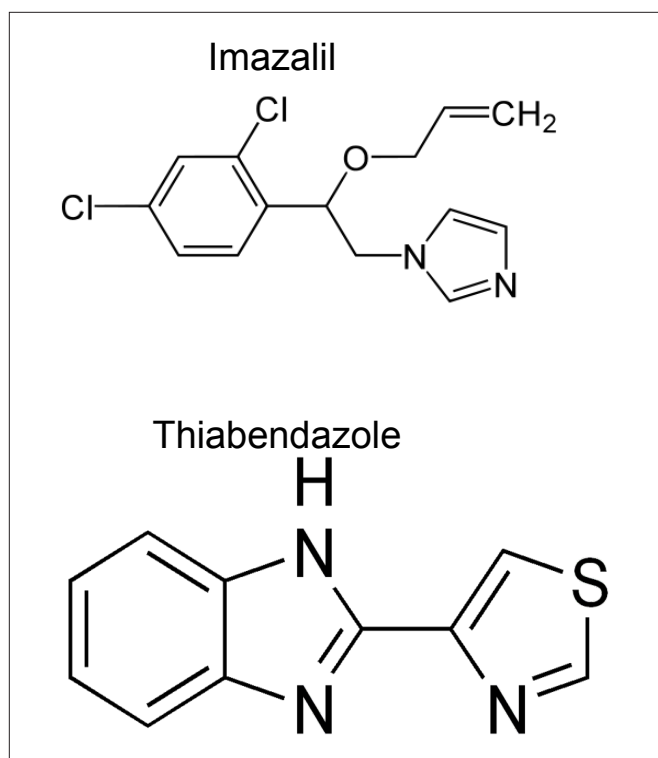


Figure 1. Structure of two fungicides.

The main drawback of these methods is that a great deal of time and money is consumed in the method development, sample preparation and analysis time. In this work, we demonstrated that the AxION® Direct Sample Analysis (DSA™) system integrated with the AxION 2 Time-of-Flight (TOF) mass spectrometer can be used for rapid screening of fungicides on conventional and organic citrus fruit samples with minimal sample preparation. After initial rapid screening, only the citrus fruits samples showing potential presence of fungicides were selected for quantitation using the Flexar™ FX-10 coupled with the Flexar SQ 300 MS (LC/SQ) with Ultraspray™ ESI Source. This work shows an improved and productive work flow by screening samples using DSA/TOF in 10 seconds per sample followed by LC/SQ for only those samples that tested positive. This reduction in number of total samples analyzed using chromatography decreases the amount of total time spent for sample analysis as well as decreases lab overhead costs with reduction in solvent usage and disposal.

Method

Sample Preparation

Five conventional citrus fruits and two organic citrus fruits were obtained from local supermarket. These citrus fruits were peeled and 10 gm of the peel was extracted in 30 ml of acetonitrile. After extraction, 5 µL of extracted sample was pipetted onto stainless mesh for rapid screening measurement with DSA/TOF. After that, acetonitrile extracted samples were cleaned with modified one step QUECHERS method before running them with LC/MS. 1.5 ml of ACN extract was added to tube with 25 mg of PSA adsorbent and 150 mg MgSO₄

and centrifuged at 7800 rpm for 10 minute. After centrifuging, 0.6 ml of supernatant was dried under nitrogen for 30 min and reconstituted in 0.6 ml of 35/65 methanol/water and was used for analysis with LC/MS.

DSA/TOF Parameters

Five µl of each acetonitrile extracted sample was pipetted directly onto the stainless mesh of the AxION DSA system for ionization and analysis. The DSA/TOF experimental parameters were as follows: corona current of 5 µA, heater temperature of 300 °C. The AxION 2 TOF MS was run in positive ionization mode with flight voltage of -8000 V. The capillary exit voltage was set to 100 V for the analysis. Mass spectra were acquired in a range of m/z 100-700 at an acquisition rate of 5 spectra/s. All samples were analyzed within 10 s. To obtain high mass accuracy, the AxION 2 TOF instrument was calibrated before each analysis by infusing a calibrant solution into the DSA source at 10 µl/min.

LC/SQ MS Parameters

After rapid screening of samples with DSA/TOF, the samples showing potential presence of fungicides were further analyzed for quantitation using the LC/SQ MS. The separation of fungicides was carried out using a PerkinElmer Brownlee™ Supra C18 column (2.1x100 mm, 3 µm) at flow rate of 0.5 ml/min and using a linear gradient of 35% methanol in water to 95 % methanol in water with 0.05% acetic acid in 5 minutes. The column temperature was maintained at 30 °C and injection volume was 3 µL. The ESI source temperature and drying gas flow rate were 350 °C and 15 L/min. The SIM dwell time was 100 msec. Both molecular and fragment ions of fungicides, imazalil and thiabendazole, were monitored to provide further confirmation of their presence in citrus fruits by LC/MS. The optimum capillary exit voltages for measurement of thiabendazole molecular and fragment ion were 90 and 160 V, respectively. The optimum capillary exit voltages for measurement of imazalil molecular and fragment ion were 80 and 140 V, respectively.

Results

Fungicide Screening by DSA/TOF

Figure 2 and Figure 3 show extracted ion chromatogram (EIC) for thiabendazole and imazalil for two organic citrus fruits and five conventional citrus fruits. The data shows that thiabendazole was present in all five conventional citrus fruits, whereas imazalil was present in 3 out of 5 conventional citrus fruits. Organic citrus fruits should be absent of fungicides as they are prohibited from use. The potential presence of fungicides shown using DSA/TOF suggests that these samples should be further investigated using LC/SQ MS. The data in Figures 2-4 suggests that these fungicides were present at lower level in organic orange as compared to other conventional citrus fruits. The zoomed in EIC data for organic grapefruit showed that these fungicides were completely absent. Figure 5 and Figure 6 showed the mass spectra of

conventional and organic orange, respectively. The presence of fungicides in these citrus fruit samples was further confirmed by below 5 ppm mass accuracy of measurements with DSA/TOF. The data with DSA/TOF showed that one or both fungicides was present in all conventional citrus fruits, whereas both fungicides show potential presence in 1 out of 2 organic citrus fruits. This work showed that DSA/TOF can also be used to perform initial screening for the presence of fungicides on fruits that can then be further analyzed using LC/SQ for traditional quantitation.

Fungicide Quantitation by LC/SQ MS

Figure 7 shows SIM for molecular ion and fragment ion for 1 ppm standard of 2 fungicides using the LC/SQ. The on column detection limit for thiabendazole and imazalil was 0.3 ppb and 0.45 ppb, respectively. Figures 8 and 9 demonstrated that the response for 2 fungicides was linear from 1 ppb to 1000 ppb with correlation coefficient R^2 better than 0.9933. Figure 10 displayed SIM for molecular ion and fragment ion for 2 fungicides in conventional orange brand #1. Further confirmation of the presence of fungicides in citrus fruits was obtained using in source CID (collision induced decomposition) and monitoring their fragment ion at higher capillary exit voltage and retention time matching with the standard. Table 1 showed the level of fungicides measured in five conventional citrus fruit samples and one contaminated organic citrus fruit sample. The data showed the amount of fungicides on conventional citrus fruit samples was below MRL value of 5ppm. Also, it confirmed that one of organic citrus fruit sample was contaminated with low levels of fungicide as suggested by the DSA/TOF screening method.

Conclusions

This method shows the first work for rapid screening of fungicides in conventional and organic citrus fruits using DSA/TOF in 10 seconds. The data showed that fungicides were present in all five conventional citrus fruits and one out of two organic citrus fruits. The mass accuracy of all measurements with DSA/TOF was less than 5 ppm with external calibration. All samples were screened, with minimal sample preparation, in 10 sec. per sample. After rapid screening of citrus fruit samples for fungicides, the samples with fungicides were selected for quantitation with LC/SQ MS. A five minute LC/MS method was developed for quantitation of fungicides in citrus fruits. Both molecular and fragments ions were monitored in SIM mode for both imazalil and thiabendazole to confirm their presence in citrus fruit samples. All tested conventional citrus fruit samples had total fungicide amount (0.5 to 1.5 ppm) which was less than MRL of 5 ppm. The organic grapefruit sample showed absence of fungicides, whereas organic orange showed presence of total fungicide amount of about 0.02 ppm.

Table 1. Fungicide levels measured on one organic and five conventional citrus fruits bought at a local grocery store using LC/SQ MS.

Sample	Thiabendazole (ppm)	Imazalil (ppm)
Organic orange	0.013	0.009
Orange Brand # 1	0.490	0.726
Orange Brand # 2	0.290	Not Detected
Orange Brand # 3	0.649	0.578
Grapefruit	0.509	Not Detected
Lemon	0.250	1.390

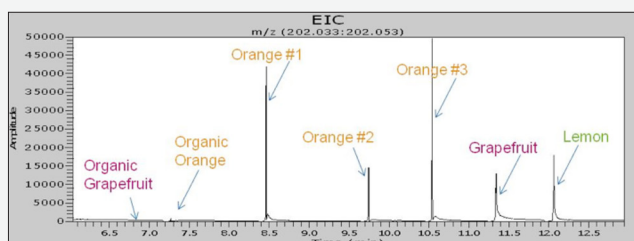


Figure 2. Extracted ion chromatogram for thiabendazole in two organic and five conventional citrus fruits using DSA/TOF.

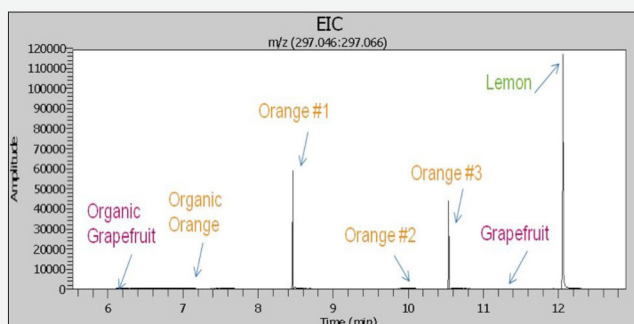


Figure 3. Extracted ion chromatogram for imazalil in two organic and five conventional citrus fruits using DSA/TOF.

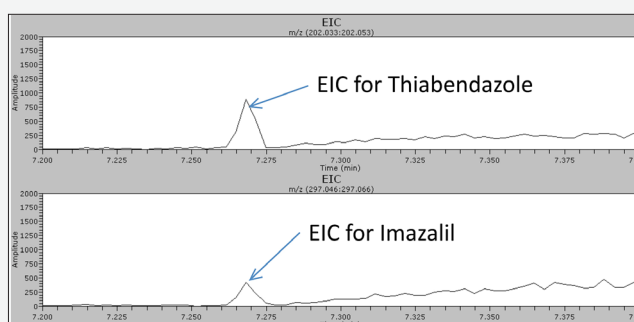


Figure 4. Zoomed in extracted ion chromatogram for imazalil and thiabendazole in organic orange using DSA/TOF.

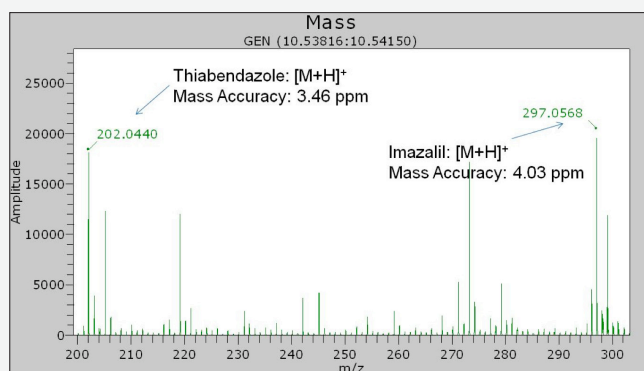


Figure 5. Mass spectra of conventional orange #3 obtained with DSA/TOF.

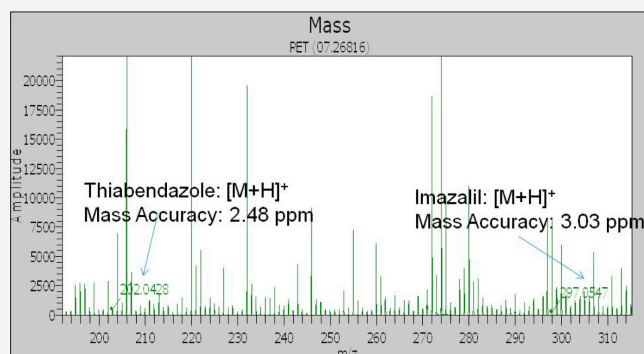


Figure 6. Mass spectra of organic orange obtained with DSA/TOF.

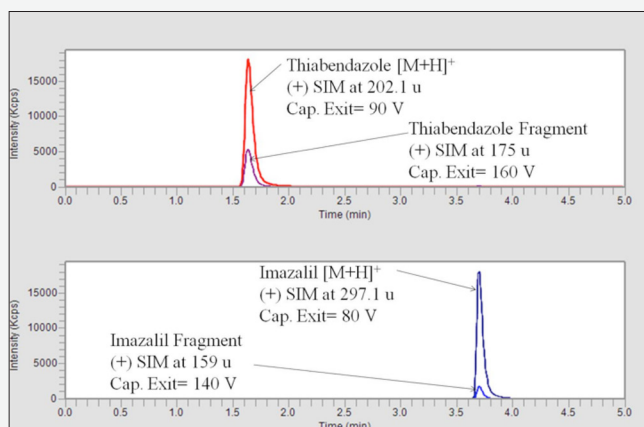


Figure 7. SIM of 1 ppm standard of two fungicides using LC/SQ-MS.

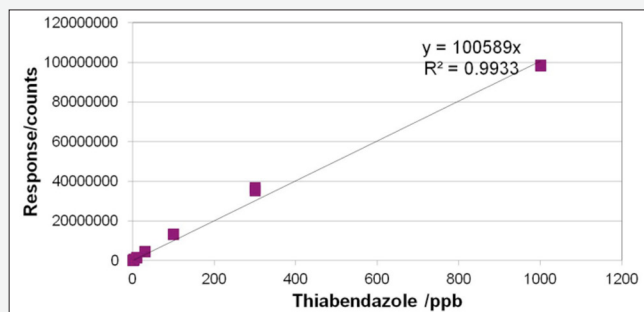


Figure 8. Calibration curve for thiabendazole over concentration range from 1 to 1000 ppb obtained with LC/SQ-MS.

With this workflow, the samples can be screened rapidly with DSA/TOF and can be divided into two categories of positive and negative samples. After initial screening, only positive samples need to be run for quantitation with LC/MS. In comparison to other established workflows such as LC/MS and GC/MS, the workflow, which couples DSA/TOF with LC/MS, will reduce total analysis time and reduce solvent usage which decreases laboratory overhead costs and improves overall productivity.

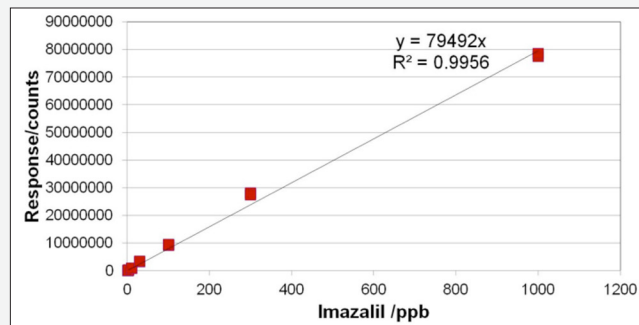


Figure 9. Calibration curve for imazalil over concentration range from 1 to 1000 ppb obtained with LC/SQ-MS.

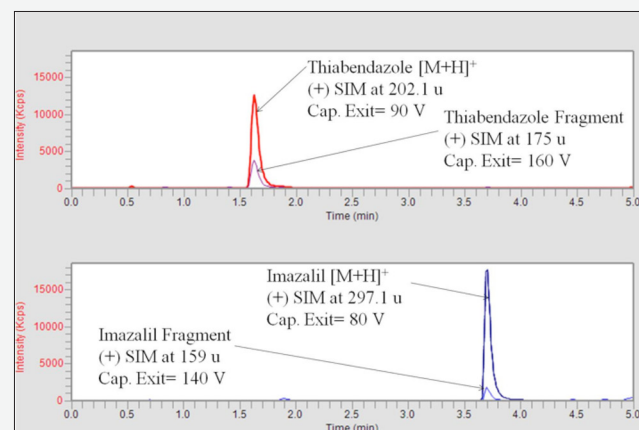


Figure 10. SIM of two fungicides in peel extract of conventional orange #1 using LC/SQ-MS

References

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