An Automated Extraction Solution for Imidazolinone Herbicides

By Tyler Trent

Application Note

Abstract

Imidazolinone herbicides are a family of five compounds including: Imazapyr, Imazapic, Imazethapyr, Imazamox, and Imazaquin. These herbicides are used to control a wide range of broadleaf weeds, by inhibiting the Acetohydroxy Acid Synthesis (AHAS), which is the first common enzyme in the biosynthesis of branched chain amino acids.

In this study, the performance and versatility of the AutoMate-Q40, QuEChERS workflow solution, was evaluated for the extraction of Imidazolinone herbicides. A Liquid Chromatograph (LC) coupled to a Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of these herbicides in agricultural commodities. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. By using the AutoMate-Q40 to streamline this extraction, it provides us with appropriate analytical results, falling in the established method guidelines (recovery range of 70-120% and an RSD <20%) for the target compounds.

Introduction

Imidazolinone herbicides effectively control a wide-range of weed species. This group of herbicides is comprised of five main chemical compounds: ***Imazamox***, ***Imazapic***, ***Imazapyr***, ***Imazaquin***, and ***Imazethapyr***.[1] This group forms a unique class of synthetic compounds (Figure 1). Each compound has an identical Imidazolinone ring structure with an attached carboxylic acid group. They only differ by the functional group attached to the Imidazolinone ring structure.[2]

Imidazolinones are broad-spectrum herbicides that prevent and rid in-season weed formation. These herbicides hinder the activity of the enzyme Acetolactate Synthesis (ALS), also called Acetohydroxy Acid Synthesis (AHAS).[3,4] ALS and AHAS are necessary for the biosynthesis of branched chain amino acids which will eventually cause death to treated plants.

This project will evaluate the performance and versatility of the AutoMate-Q40 for the extraction of Imidazolinone herbicides. Liquid Chromatography coupled to a Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was employed for the detection of these herbicides in agricultural commodities. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. By using the AutoMate-Q40 to streamline this extraction, it provides us with appropriate analytical results falling in the established method guidelines (recovery range of 70-120% and an RSD <20%) for the target compounds.

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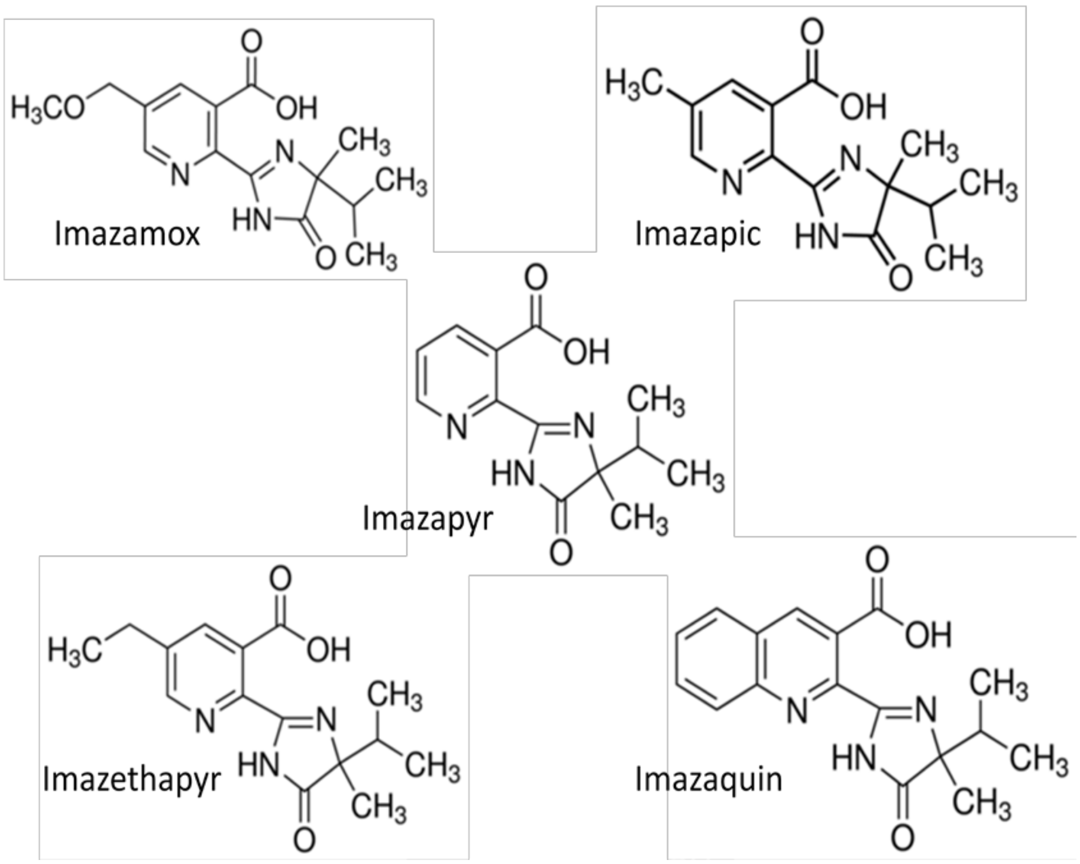
Experimental Instrument Conditions

Chemical Structures

See Figure 1 for the Imidazolinone herbicides used in this study

Figure 1 Imidazolinone herbicides Structures[1]



Solutions and Standards

A 0.5 M buffered extraction solution was prepared by adding 19.28 g of Ammonium Acetate into 0.5 L H_2O/MeOH (90:10), then it was adjusted to a pH 8 with Ammonium Hydroxide.

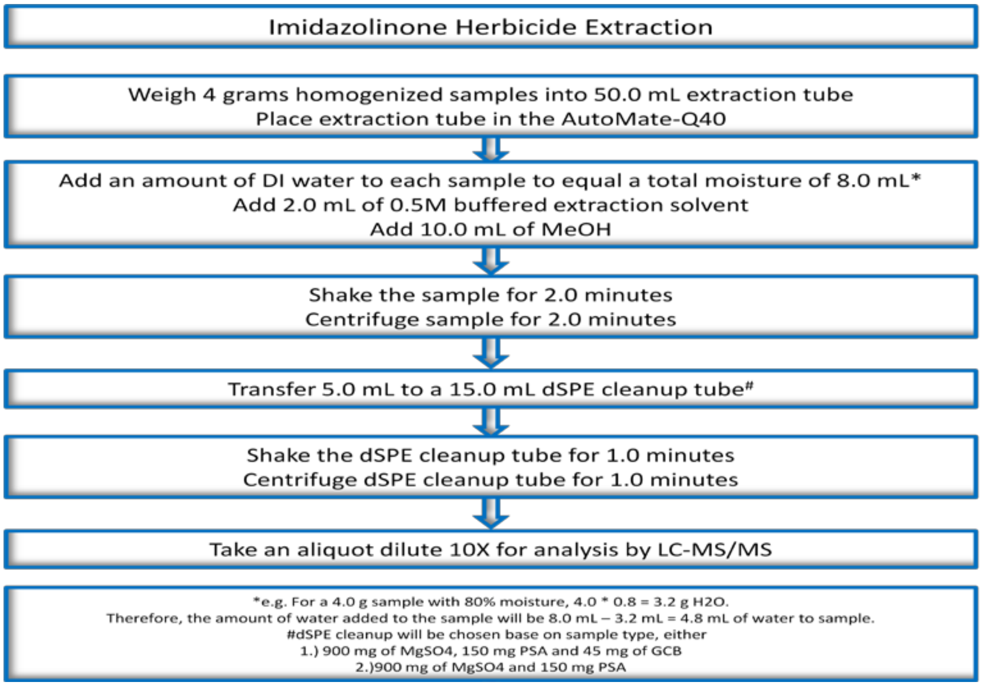
A stock standard solution (1000.0 mg/mL) of each Imidazolinone was prepared in Methanol. From these five stock standard solutions a 2.0 µL/mL QC standard solution was prepared fresh daily in Methanol.

Sample Preparation/Extraction

Whole carrots with leafy stems and lettuce were purchased from a local grocery store. The body of the carrot was separated from the leafy stem as we wanted to run the body and stem separate during analysis. The samples were milled to reduce the particle size and to improve homogeneity. The samples were stored in the freezer until the samples were extracted.

Figure 2 shows the sample preparation and extraction steps that are needed to extract Imidazolinone herbicides from the carrot body, the carrot leafy stem, and lettuce.

Figure 2 Imidazolinone Herbicide Extraction



Instrumentation and Analytical Conditions

Sample analysis was conducted using a Shimadzu Nexera LC interfaced to an AB Sciex 4500 QTrap Triple-Quad Mass Spectrometer (LC-MS/MS). For separation of the compounds of interest, a Phenomenex Kinetex 2.6u Biphenyl 100 Å (50 x 2.1mm) column was used. Table I and Table II demonstrates the optimized LC-MS/MS analysis parameters for both the chromatographic separation and optimal analyte transitions. Figure 3 shows the scheduled MRM chromatogram spiked at 400.0 µL/L.

Table I Critical LC-MS/MS SRM Transitions and Parameters for AB Sciex 4500 QTrap			
Curtain Gas (CUR)			20
Ion Spray Voltage (IS)			5500
Temperature (TEM)			350
Collision Gas (CAD)			Medium
Analyte Transitions			
Compounds	RT (min)	Precursor Ion (m/z)	Quantization product Ion (m/z)
Imazapyr	5.35	262.0	216.9
Imazapyr	5.35	262.0	202.0
Imazamox	6.86	306.0	260.9
Imazamox	6.86	306.0	192.9
Imazapic	7.00	276.0	230.9
Imazapic	7.00	276.0	163.0
Imazethapyr	8.17	290.0	244.9
Imazethapyr	8.17	290.0	176.9
Imazaquin	9.32	312.0	266.9
Imazaquin	9.32	312.0	198.8

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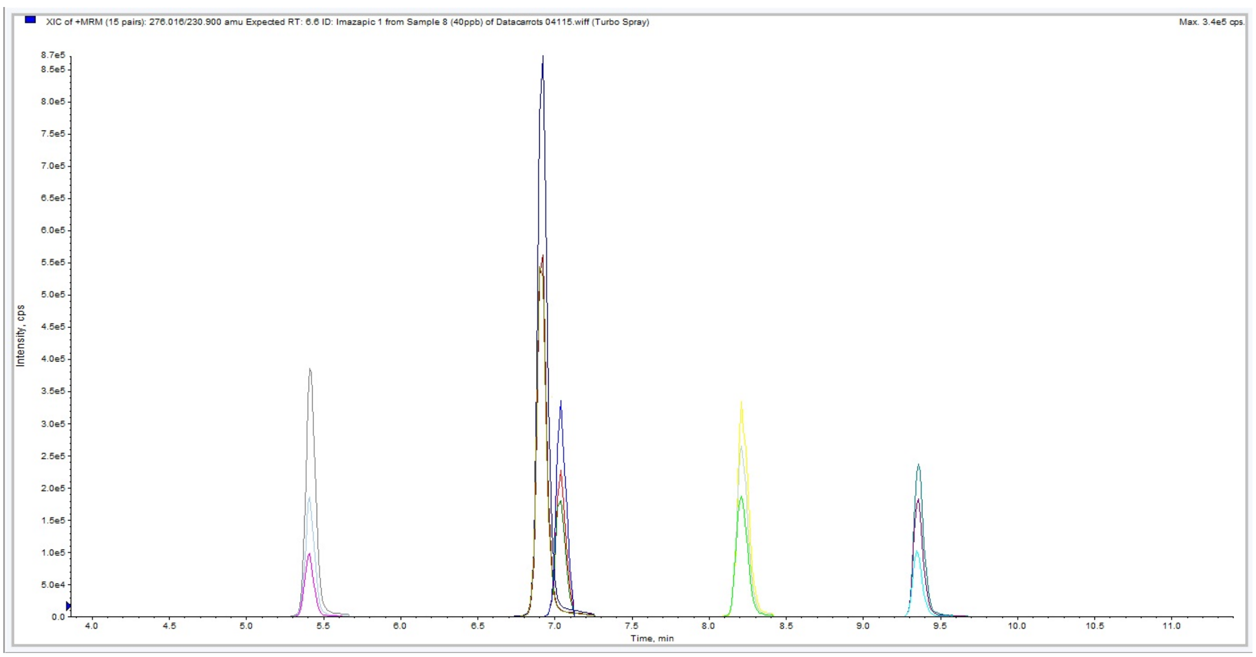
Table II Shimadzu Nexera LC Parameters		
Column	Phenomenex Kinetex 2.6u Biphenyl 100 Å	
Dimensions	50 X 2.00 mm	
Mobile Phase	A:5mm Ammonium Formate w/0.1% Formic in H_2O:MeOH (90:10)	
	B:5mm Ammonium Formate w/0.1% Formic in MeOH: H_2O(90:10)	
Gradient	Time	%B
	0.0	0%
	1.0	0%
	15.0	100%
	18.0	100%
	18.5	0%
	20.0	STOP
Flow Rate (mL/min)	0.400	
Column Temperature (°C)	40	

Figure 3 Scheduled MRM chromatogram spiked at 400.0 µL/L of Imidazolinone Herbicides



Results

Automating the imidazolinone herbicide extraction enables fast, easy, reliable and highly reproducible extractions. The AutoMate-Q40 offers labor savings and improves the repeatability and consistency between the extracted samples.

A precision and accuracy study was performed using the AutoMate-Q40. The system was able to fortify the carrots, leafy stems, and lettuce samples at 12.5ng/g and 25.0 ng/g. This is accomplished through the systems automated standard addition feature. All control samples used for this study showed no significant residue of Imidazolinone herbicides.

Table III Imidazolinone Herbicides Carrot Results								
Compound	Body of Carrot#				Leafy Stem of Carrot *			
	12.5ng/g Spike		25.0ng/g Spike		12.5ng/g Spike		25.0ng/g Spike	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Imazapic	105.0	4.8	88.7	7.0	98.6	10.4	93.0	2.4
Imazapyr	86.5	4.8	87.1	9.0	100.0	9.1	89.7	3.9
Imazaquin	105.2	5.9	100.5	2.7	101.0	8.0	89.7	7.6
Imazethapyr	94.7	8.6	89.2	9.2	97.5	6.2	92.7	4.5
Imazamox	101.4	7.2	99.6	7.4	98.8	5.7	92.8	2.7
#cleanup contained $MgSO_4$ and PSA, *cleanup contained $MgSO_4$, PSA, and GCB								

Table IV Imidazolinone Herbicides Lettuce Results				
Compound	Lettuce#			
	12.5ng/g Spike		25.0ng/g Spike	
	%Recovery	%RSD	%Recovery	%RSD
Imazapic	99.0	10.8	104.5	2.4
Imazapyr	95.3	10.0	103.2	4.5
Imazaquin	92.4	5.8	101.6	1.7
Imazethapyr	93.4	6.5	119.7	5.5
Imazamox	89.9	8.4	80.0	0.6
#cleanup contained $MgSO_4$ and PSA				

Table III and Table IV shows that when using the AutoMate-Q40 to extract Imidazolinone herbicide residues from carrots and lettuce recoveries ranging from 80.0% to 119.7%. Table III and Table IV also shows that the results have excellent precision ranging from 0.6% to 10.8%.

Conclusion

Automation of this imidazolinone herbicide extraction method produced reliable results for the spiked samples. Automating this extraction shows the versatility of the AutoMate-Q40 and how it can be adapted to other extractions. The AutoMate-Q40 led to improved repeatability, a reduction in the likelihood of human error and the potential for significant labor savings.

Precision and accuracy were assessed for the two commodities analyzed. Results for the automated procedure were well within the criteria set forth in this study: Average recoveries for the range of commodities were between 80.0% and 119.7% with good precision (ca. 6% RSD).

References

1. www.sigmaaldrich.com for the structures of the compounds
2. Krieger, Robert; Frederick G. Hess; Jane E. Harris; Kimberly Pendino; Kathryn Ponnock (2001). "Handbook of Pesticide Toxicology". *Imidazolinones* **1**: 1641–1642.
3. Lamberth, Clemens (2012). *Bioactive Heterocyclic Compound Classes: Agrochemicals*. John Wiley & Sons. pp. 47–49.
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Acknowledgements

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