Analysis of Lamotrigine and its Glucuronide Metabolite in Water by Liquid Chromatography/ Quadrupole Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS) After Automated Solid Phase Extraction

Application Note ENV0111

This application describes the conditions necessary to automate the solid phase extraction of lamotrigine and its 2-N-glucuronide metabolite from water samples prior to analysis by LC/Q-TOF-MS using the Gilson GX-271 ASPEC™ System. Extraction recoveries ranged from 75% to 99%. The RSD for inter-day (n=5) values were between 5% and 10%, which showed good reproducibility of the methodology. The LC/Q-TOF-MS limit of detection for lamotrigine and its metabolite were 1 ng/L and 5 ng/L.

Automation of the SPE process allows one to reduce potential errors that may occur during manual extractions, increase lab efficiency, reduce solvent usage and increase sample throughput. Automation also allows one to optimize extraction conditions easily for different matrices and analytes.

Introduction

Large quantities of pharmaceuticals are consumed each year throughout the world. A variety of pharmaceuticals has been detected in low concentrations in surface water, groundwater, drinking water and soil/sediments. Pharmaceutically active compounds, including drugs and their metabolites, are an important water-quality issue (Kolpin et al., 2002; Donn, J., 2009). There is an increased interest in measuring levels of pharmaceuticals in water due to their possible impact on humans, wildlife and fish (Schultz and Furlong, 2008: Vadja et al., 2008).

Lamotrigine, also known as Lamictal® (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5- diamine), is a widely prescribed and effective drug for the treatment of epilepsy and as a mood stabilizer for the treatment of bipolar disorder. Lamotrigine is primarily metabolized by the liver to form a glucuronide conjugate (Table 1). This metabolite is primarily excreted by the kidneys. It is less toxic than the parent compound, but can undergo hydrolysis back to the parent.

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This study (Ferrer and Thurman, 2010) describes the analysis of lamotrigine and its 2-N-glucuronide metabolite in water using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC/Q-TOF-MS), as well as the automated extraction of these compounds from drinking water, groundwater, surface water and wastewater utilizing the Gilson GX-271 ASPEC System (Figure 1).

***Figure 1**. Gilson GX-271 ASPEC™ System*

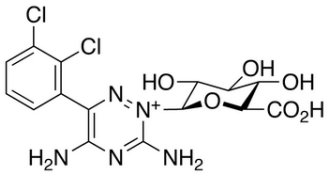
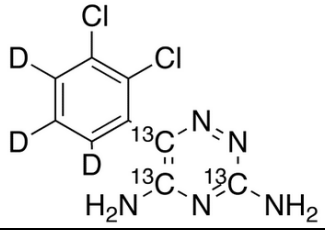
Name	Elemental Composition	Base Peak Ion	Chemical Structure
Lamotrigine	$C_3H_7Cl_2N_5$	$[M+H]^+$ $C_9H_8Cl_2N_5^+$ 256.0151	
N2-Glucuronide of Lamotrigine	$C_{15}H_{15}Cl_2N_5O_6$	$[M+H]^+$ $C_{15}H_{15}Cl_2N_5O_6^+$ 432.0472	
Lamotrigine-$13C_3$-d_3	$C_6{}^{13}C_3H_4D_3Cl_2N_5$	$[M+H]^+$ $C_6{}^{13}C_3H_4D_3Cl_2N_5^+$ 262.0440	

Table 1. Elemental Composition, Protonated Molecules, and Chemical Structures of Lamotrigine, its 2-N-Glucuronide and Lamotrigine Labeled Standard.

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Materials & Methods

Materials

All solvents used were HPLC grade or higher. All reagents were ACS grade or better. Lamotrigine and its 2-N-glucuronide were purchased from Sigma Aldrich (St. Louis, MO, USA) and from Carbosynth (Compton, UK). Lamotrigine-13C3-d3 labeled standard was purchased from Toronto Research Chemicals (North York, ON, Canada). HPLC grade water was used through-out the study. Individual stock solutions were prepared in pure methanol and stored at -18°C. Working standards were prepared from stock solutions by dilution with acetonitrile and water.

Water Sample Collection

All samples were collected in baked, glass 1L amber bottles with Teflon®-lined caps. All water samples were stored at 4°C before analysis, and sample extraction was completed within seven days for all samples. Wastewater samples were collected at the outfall of a wastewater treat-ment plant. Source water river samples were collected according to U.S. Geological Survey (USGS) protocol (USGS, 2008). Groundwater samples were collected from wells.

The SPE procedure used 500 mg/ 6 mL Waters OASIS™ HLB solid phase extraction cartridges (Milford, MA, USA). The cartridges were sealed using Gilson 6 mL sealing caps.

The solid phase extraction and liquid handling protocol is entirely automated using the Gilson GX-271 ASPEC™ system. The SPE steps are summarized with the general schematic provid-ed in the GX-271 ASPEC control software, TRILUTION® LH (Figure 2).

***Figure 2**. TRILUTION LH Basic SPE Tasks for Solid Phase Extraction of Lamotrigine and Metabolite from Water*

The summary of each step is as follows:

1. Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 3)
2. Condition the cartridge with 4 mL of methanol at 1 mL/min
3. Condition the cartridge with 6 mL HPLC grade water at 1 mL/min
4. Load 200 mL of the sample onto the SPE cartridge at 10 mL/min
5. Move the Gilson Mobile SPE Rack over the collection tubes
6. Elute the analytes with 5 mL methanol at 1 mL/min
7. Evaporate to 0.5 mL with nitrogen using a TurboVap® Concentration Worstation (Biotage, Charlottesville, VA)
8. Transfer to vial for analysis by LC/Q-TOF-MS

***Figure 3**. Gilson Mobile SPE Rack*

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LC/Q-TOF-MS Analysis

The separation of the analytes was carried out using an Agilent Series 1200 HPLC System equipped with a Zorbax Eclipse XDB-C8 column (4.6 x 150 mm, 5µm particle size). The injected sample volume was 50 µL. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A after 30 min. The flow rate was 0.6 mL/min. A 10 min post-run time was used after each analysis.

The HPLC system was connected to an Agilent 6450 ultra-high definition quadrupole time-of-flight mass spectrometer equipped with eletrospray Jet Stream Technology operating in positive ion mode. The operating parameters were as follows: capillary voltage: 4000V; neblizer pressure: 45 psig; drying gas: 10 L/min; gas temperature: 325°C; nozzle voltage: 1000V; fragmentor voltage: 190V; skimmer voltage: 60V and octapole RF at 750V. LC/MS accurate mass data were recorded across the range 50–1000 m/z at 4 GHz. The data recorded was processed with Agilent MassHunter software. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a low flow of a calibrating solution (calibrant solution A, Agilent Technologies, Santa Clara, CA, USA).

Method Validation

Method accuracy and precision were determined by recovery experiments with spiked samples. Drinking water, groundwater and surface water free of lamotrigine and its metabolite were spiked at 100 ng/L, extracted by SPE, and analyzed by LC/TOF-MS. Peak areas of the extracts were compared to peak area corresponding to a pure standard prepared in HPLC-grade water and recovery values were obtained.

Peak areas, regression parameters, and concentrations were obtained by using Agilent MassHunter software. Aliquots of standard solutions of analytes were added to water samples at seven different concentrations to obtain the standard calibration curves. All went through the SPE system and were treated like samples. To ensure accuracy, a calibration curve was developed for each type of matrix sample. An aliquot of 100 µL of surrogate labeled standard, lamotrigine-13C3-d3, was added to each calibration sample and to each environmental sample. The internal standard was used to account for recovery losses during SPE and any suppression from the matrix of the samples.

Results and Discussion

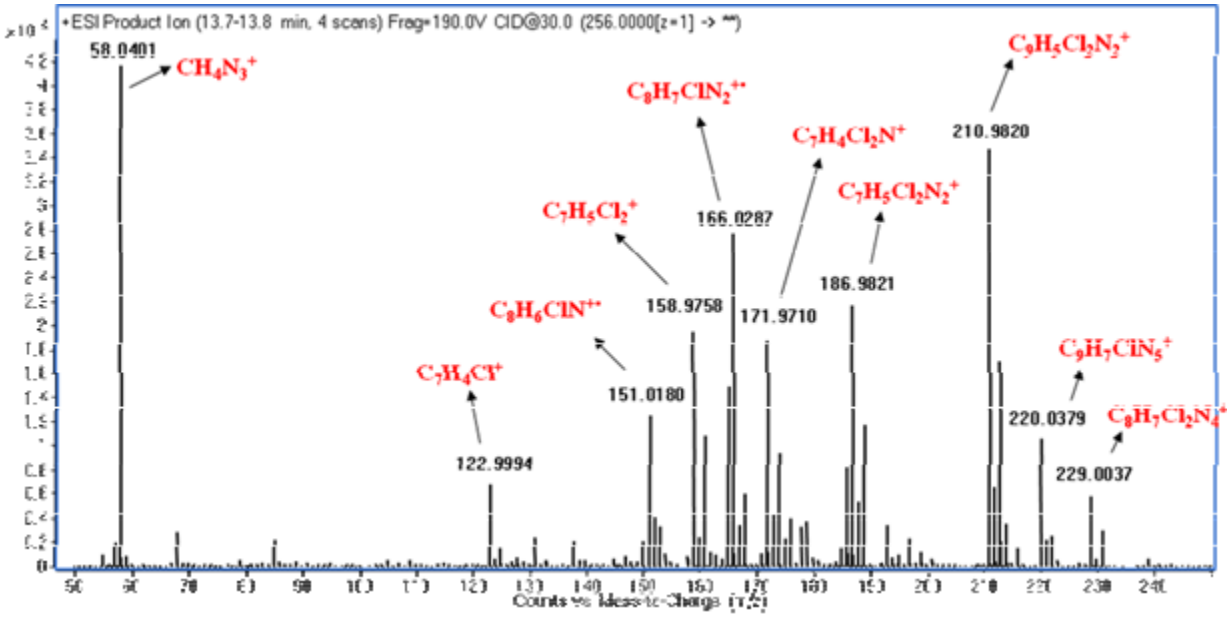

Figure 4. *LC/Q-TOF-MS Analysis of a Surface Water Sample Showing the MS-MS Spectrum of Lamotrigine.*

Analyte	Drinking Water	Groundwater	SurfaceWater	Wastewater
Lamotrigine	91 (5)	95 (6)	82 (7)	75 (10)
Lamotrigine 2-N-Glucuronide	99 (7)	98 (8)	93 (7)	77 (8)

Table 2*. Percent Recoveries and Standard Deviations (RSD) of Lamotrigine and its Glucuronide Metabolite from Drinking Water, Groundwater, Surface Water and Wastewater (N = 5).*

Samples	Lamotrigine	Lamotrigine 2-N-glucuronide
Wastewater (34 samples)		
Mean Concentration (ng/L)	488	209
Percentage Detections (%)	94	21
Groundwater (15 samples)		
Mean Concentration (ng/L)	324	17 (below LOQ)
Percentage Detections (%)	93	20
Surface water (62 samples)		
Mean Concentration (ng/L)	108	195
Percentage Detections (%)	47	13
Drinking water (7 samples)		
Mean Concentration (ng/L)	17	Not detected
Percentage Detections (%)	29	Not detected

***Table 3**. Analysis of Wastewater, Groundwater, Surface Water and Drinking Water for Different Locations in the U.S. Showing Concentrations of Lamotrigine and Its 2-N-Glucuronide*

Reference

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Summary or Conclusions

This application describes the conditions necessary to automate the solid phase extraction of lamotrigine and its 2-N-glucuronide metabolite from water samples prior to analysis by LC/Q-TOF-MS using the Gilson GX-271 ASPEC™ System. Extraction recoveries ranged from 75% to 99%. The RSD for inter-day (n=5) values were between 5% and 10%, which showed good reproducibility of the methodology. The LC/Q-TOF-MS limit of detection for lamotrigine and its metabolite were 1 ng/L and 5 ng/L.

Automation of the SPE process allows one to reduce potential errors that may occur during manual extractions, increase lab efficiency, reduce solvent usage and increase sample throughput. Automation also allows one to optimize extraction conditions easily for different matrices and analytes.

Lamotrigine was detected in 94% of the 34 wastewater effluent samples tested and 93% of the 15 alluvial groundwater samples taken from down gradient of wastewater treatment plants. Because of the widespread use of lamotrigine in the treatment of epilepsy and bipolar spectrum disorders, the wastewater plants are the principal source of lamotrigine and its glucuronide metabolite.

The 2-N-glucuronide was found in 21% of wastewater samples suggesting that certain conditions may exist in the wastewater treatment plant that prevent hydrolysis of the conjugated lamotrigine.

Lamotrigine was also detected in 47% of the surface water samples tested and 29% of the drinking water samples. These samples were collected from nine different states. The results suggest that the presence of this compound and its metabolite are widespread in environmental water samples. More studies are needed to determine the environmental impact of these compounds in the water supply.