High Resolution Mass Spectrometry (LC/Q-TOF/MS) for the Detection of Pharmaceuticals in Water

Application Note

Environmental

Authors

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Abstract

The use of Agilent Q-TOF mass spectrometers and software tools has enabled the identification and characterization of more than 100 pharmaceuticals and their metabolites in water sources at concentrations as low as parts per trillion (ppt), including isobaric and isomeric compounds that can be difficult to distinguish by other methods.

Introduction

It has been more than two decades since the first reports of pharmaceuticals in the environment. Administered pharmaceuticals are excreted by humans, enter a sewage treatment plant where they are not entirely removed, and end up in the environment. Their presence is ubiquitous, including wastewater, surface water, and groundwater. Their concentrations are increasing, particularly for antidepressants and β-blockers [1].

There is increasing interest worldwide, by regulatory agencies, in monitoring the levels of pharmaceuticals in water, to determine the potential health risks associated with their presence. However, their concentrations are often very low (ng/L level), requiring highly selective and sensitive detection methodologies. A non-targeted approach is also required, to ensure that all pharmaceutical contaminants are detected, including degradation products and metabolites [2].

Agilent Technologies

A methodology using liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOF/MS) can provide the required sensitivity and selectivity, as well as enable non-targeted screening of pharmaceuticals in water. This application note describes the use of the Agilent 6540 Q-TOF LC/MS system and its associated software tools, including Molecular Feature Extractor, Molecular Formula Generator, and accurate mass databases to detect, identify, and quantitate very low levels of pharmaceuticals in water.

Experimental

Reagents and standards

All standard solutions (100 µg/mL) were purchased from Cerriliant (Austin, TX). The deuterated standards were obtained from Cambridge Isotopes (Cambridge, MA). HPLC grade acetonitrile and methanol were obtained from Burdick and Jackson (Muskegon, MI, USA). Formic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual stock solutions (1 µg/mL) were prepared in pure methanol, diluted from the 100 µg/mL standards and stored at –18 °C. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water to a final mixture of 10% acetonitrile and 90% water for LC/MS analysis.

Instruments

This study was conducted using an Agilent 1200 Infinity LC System coupled to a 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF/LC/MS system. The instrument conditions are shown in Table 1.

Sample preparation

Water samples (100 mL) were extracted using an automated Gilson SPE system and Oasis HLB cartridges, and eluted with 6 mL of methanol [2]. The methanol was evaporated to a final volume of 0.5 mL using a nitrogen evaporator with a water bath.

Data analysis

Several Agilent software tools were used to aid in identifying pharmaceutical compounds, including Molecular Feature Extractor, Molecular Formula Generator, Molecular Structure Correlator, and accurate mass databases. Author-constructed adaptations of the software were also used, including the chlorine filter [3].

Table 1. LC and Q-TOF/MS Conditions.

LC run conditions	
Column	Agilent ZORBAX Eclipse XDB C8, 4.6 × 150 mm, 3.5 µm particles (p/n 963967-906)
Column temperature	25 °C
Injection volume	20 µL
Mobile phase	A = 0.1% formic acid in water v/v B = acetonitrile
Linear gradient	10% B for 5 minutes, then 10% B to 100% B over 25 minutes
Flow rate	0.6 mL/min
Q-TOF MS conditions	
Ion mode	ESI, both positive and negative
Nebulizer gas	45 psi
Capillary voltage	3,500 V
Detector rate	2 GHz
Resolving power	25,000 at *m/z* 922
Accuracy	< 2 ppm

Results and Discussion

Surveying water samples for pharmaceuticals

Molecular feature extraction (MFE) can be used to group ions into compounds (features) and then plot the extracted ion chromatograms. The top 100 compounds in intensity may be extracted, the top 1,000 compounds, or more as desired. The more that are extracted, the more time is needed to investigate the sample. The time window may be adjusted as well. Figure 1 illustrates the total ion chromatogram (TIC) for a river water sample, showing the 1,498 features found in the sample.

Accurate mass databases can then be used to identify the features extracted from water samples using accurate mass and retention time. For example, an Agilent database, Forensics, was used after MFE, and it detected the compound gabapentin. The spectrum shown was found without the use of MS/MS (Figure 1). The compound dextrorphan, which is a human metabolite of the cough suppressant dextromethorphan, was also identified in water samples, based on its accurate mass after the loss of the methyl group. We have identified more than 100 pharmaceutical compounds in surface waters using these and other tools [2].

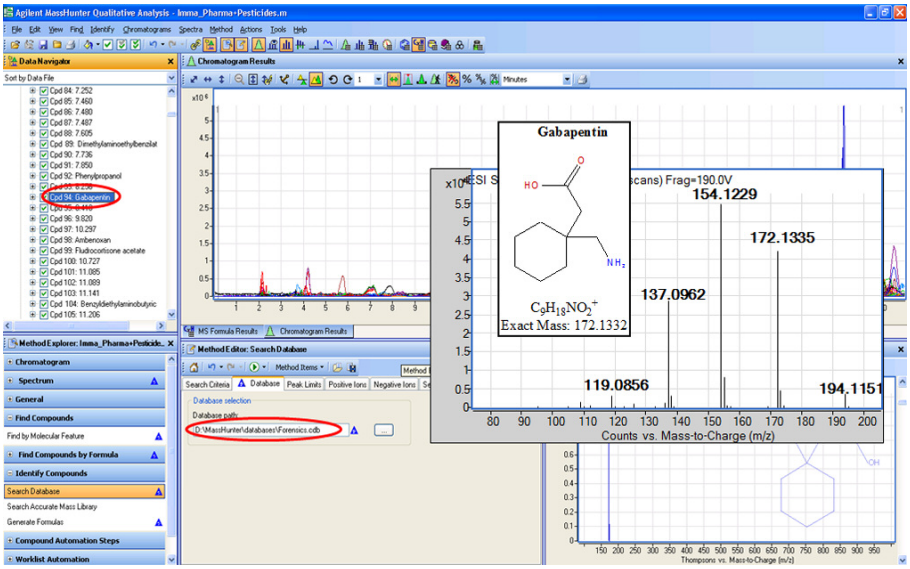

Figure 1. Molecular feature extraction from a river water sample, recognizing 1,498 features. One of these, gabapentin, was identified using the Forensics database.

Characterization of pharmaceuticals in water samples

Many software tools can be used to identify specific pharmaceuticals and their metabolites. For example, we found the antidepressant lamotrigine in a river water sample using a chlorine mass filter software tool that we adapted from MFE and Molecular Formula Generator (MFG) [3]. MFE is run first, and then MFG is set to contain at least one chlorine atom. In this example, we used two maximum chlorines and one minimum. The software then sorted all of the compounds from the MFE that contained chlorine, resulting in the identification of lamotrigine (Figure 2).

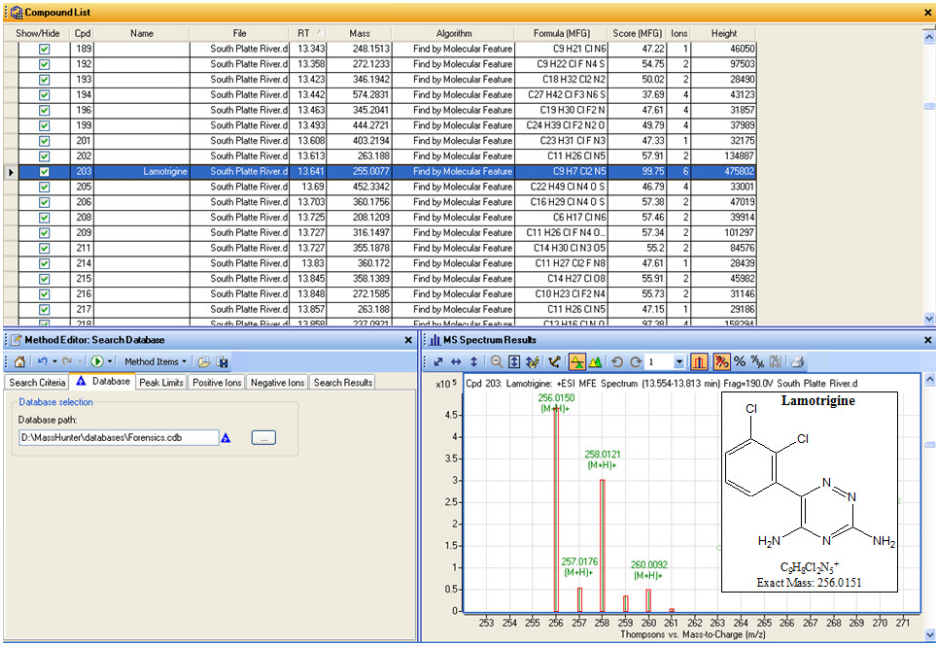

Figure 2. Using the chlorine mass filter to identify lamotrigine.

A metabolite of lamotrigine was also identified in water samples using the diagnostic ion tool and MS/MS. Upon extracting the *m/z* 256 ion, the ion chromatogram contained multiple ions at this nominal mass. One of the peaks at approximately 9 minutes had the same accurate mass as lamotrigine, which suggested either an isomer or a related metabolite. The full spectrum showed a nominal mass of *m/z* 432, which is the difference of a glucuronide, or 176 mass units (Figure 3). Mammalian metabolism often generates a glucuronide of a pharmaceutical. Two MS/MS experiments were performed to confirm this metabolite of lamotrigine. First, the *m/z* 432 ion was subjected to MS/MS to prove that the *m/z* 256 was coming from this ion (Figure 3). Next, a pseudo MS^3 experiment was conducted by increasing the fragmentation voltage and doing MS/MS of the *m/z* 256, which gave the same spectrum as the parent compound, lamotrigine (Figure 3). This finding was also verified by analyzing a pure standard of 2-N-glucuronide lamotrigine.

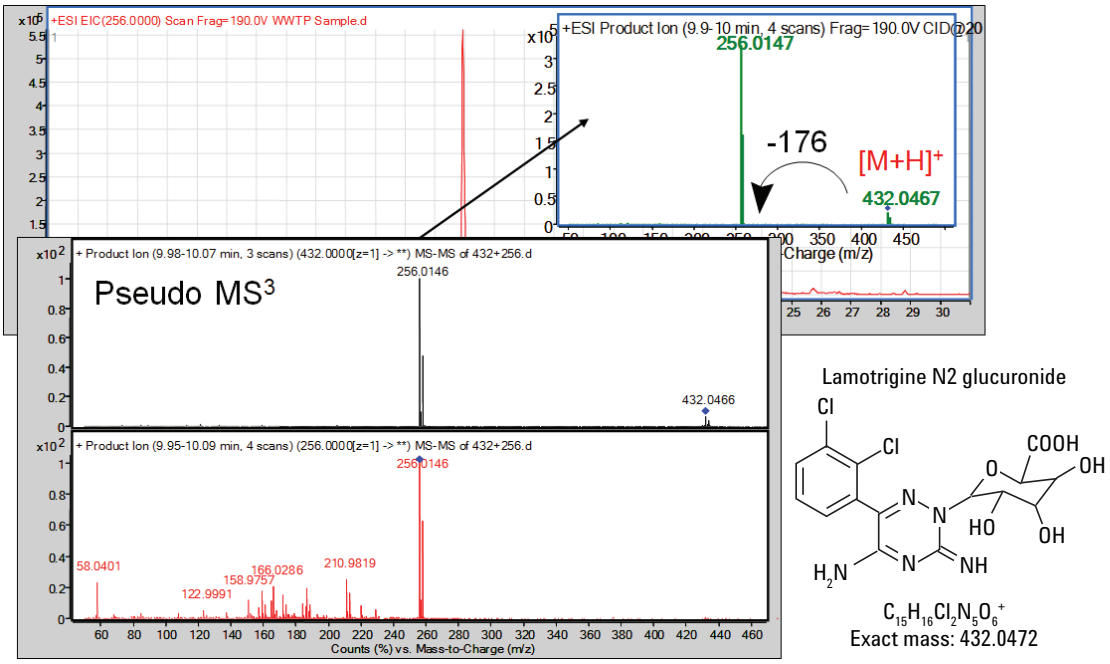

Figure 3. Diagnostic ion and MS/MS analysis to confirm the identity of a glucuronide metabolite of lamotrigine.

Distinguishing isomers and isobars

The use of Q-TOF for analysis of pharmaceuticals in water samples is essential in distinguishing and identifying isobaric and isomeric compounds. Isobars have the same nominal mass, while isomers have the same molecular formula and thus the same accurate mass. Isobars may be separated by their accurate mass, but isomers often require both MS/MS and chromatographic separation. Differences in retention time, accurate mass, fragmentation by MS/MS, and characteristic isotope signatures and isotope mass defect can then all be used for positive identification of isomers [2,4].

An isobaric pair of pharmaceuticals, lamotrigine and hydroxybupropion, occurs commonly in wastewater samples, and they differ in retention time by only 0.2 minutes. However, their accurate masses differ by 0.0948 mass units. The LC/Q-TOF/MS, operating at 25,000 resolving power, baseline separates the two masses of *m/z* 256.0149 and 256.1096. Not only are these ions separated, but also there is baseline separation of their isotopic signatures in the spectrum (Figure 4). Note that lamotrigine shows the two chlorine isotopic pattern and hydroxybupropion shows the one chlorine isotopic pattern. A single chlorine atom gives approximately 30% isotopic pattern at A+2, and two chlorine atoms give approximately a 60% isotopic signal at A+2 [3]. The mass spectra have been overlain to show the power of resolution of the mass spectrometer.

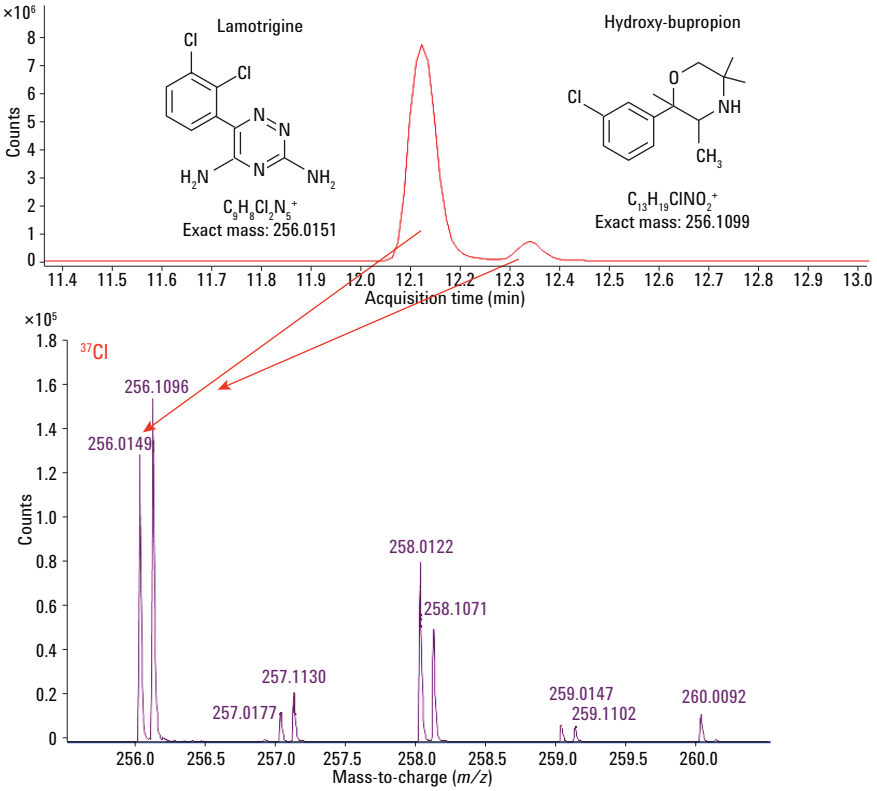

Figure 4. Distinguishing isobaric pairs by accurate mass. Lamotrigine and hydroxybupropion differ by 0.0948 mass units and 0.2 minutes in retention time. Note the baseline separation of their isotopic signatures.

Des-venlafaxine and tramadol are isomeric pharmaceutical compounds with the same formula and therefore the same accurate mass, *m/z* 264.19587. There is a slight difference in retention times, but they have two fragment ions with the same accurate mass. However, the power of MS/MS can be used to identify one different fragment ion at *m/z* 201.1274 that is only present in des-venlafaxine (Figure 5).

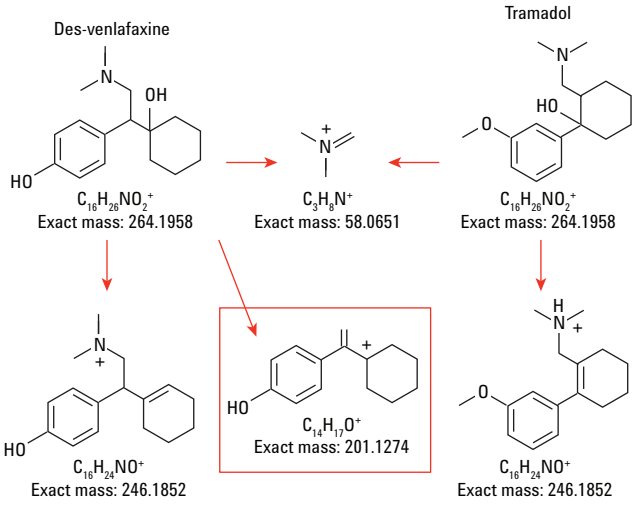

Figure 5. MS/MS transitions used to distinguish the isomers des-venlafaxine from tramadol.

Monitoring pharmaceuticals in water sources

The accurate mass capability and software tools used with the 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system have enabled the monitoring of more than 100 pharmaceuticals in various water sources. Fourteen of these compounds and their metabolites have been commonly found in surface waters from around the United States (Table 2, highlighted in yellow). Many of them are antidepressants and their metabolites, as well as some antibiotics [2]. Note that lamotrigine, for example, is widely found (97% of the surface water sources tested) at average values greater than 400 ng/L. Our studies have shown the relationships between the concentrations in wastewater effluents and those in downstream sites, as well as the presence of these compounds in groundwater [1].

Table 2. Identification of 36 pharmaceuticals found in surface waters across the US, including their average concentrations and the percentage of water samples in which they were found. Those highlighted in yellow were found most frequently.

Compound	% Detection in water samples	Average concentration (ng/L)
1,7-Dimethylxanthine	10	110
10,11-Dihydroxy-carbamazepine	45	80
10-Hydroxy-carbamazepine	85	255
Atenolol	74	166
Bupropion	68	140
Caffeine	70	220
Carbamazepine	95	350
Cetirizine	82	70
Citalopram	79	85
Clarithromycin	75	46
Cotinine	22	40
Demethyl-dextrorphan	65	10
Des-venlafaxine	78	84
Dextrorphan	75	50
Diltiazem	69	47
Diphenhydramine	80	57
Erythrohydrobupropion	78	180
Erythromycin	55	137
Erythromycin Anhydrate	35	62
Fluoxetine	25	65
Gabapentin	44	54
Gemfibrozil	74	95
Hydroxy-bupropion	75	150
Ibuprofen	20	21
Lamotrigine	97	455
Metoprolol	91	237
Metoprolol acid	85	74
2N-glucuronide lamotrigine	68	95
Naproxen	64	22
Nor-citalopram	66	74
Propranolol	88	53
Sulfamethoxazole	95	320
Thiabendazole	75	188
Triclocarban	64	96
Trimethoprim	76	264
Venlafaxine	78	310

Conclusions

Agilent LC/Q-TOF systems and software provide powerful tools for identifying and characterizing a large number of pharmaceuticals in water sources. A rapid survey can monitor more than 100 known pharmaceuticals at parts per billion (ppb) and even parts per trillion (ppt) concentrations. The Molecular Feature Extractor, Molecular Formula Generator, Molecular Structure Correlator, Fragmentation Pathways, and the chlorine mass filter can all be used to characterize the individual compounds and their metabolites. Q-TOF analysis is particularly useful for separating and identifying isobaric and isomeric compounds that can be difficult to analyze using other methods.

References

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