Modified QuEChERS for HILIC LC/MS/MS Analysis of Nicotine and Its Metabolites in Fish

Application Note

Food Testing & Agriculture

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Abstract

Nicotine is one of the most widely used botanical insecticides. Although it is banned in the EU and there are recent initiatives, including the announcement of banning nicotine as an insecticide from the US Environmental Protection Agency (EPA) [1], nicotine contamination in fish can still be present and kill aquatic organisms [2]. Nicotine goes through the typical pesticide lifecycle and is a common chemical found in tobacco products.

Importing and exporting foods between countries are everyday trade activities. Food safety testing has become an important task for many organizations such as food testing laboratories and governmental agencies. Screening many food samples in laboratories in a timely manner with appropriate throughput and reliable data is one of the essential features that food scientists look for. This application note demonstrates an easy-to-use Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation method for fish samples for the analysis of nicotine and its metabolites with hydrophilic interaction chromatography (HILIC).

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Introduction

QuEChERS has become a popular sample preparation method for a variety of food samples. The QuEChERS method is mainly composed of two steps. The first step is extraction and the second step is dispersive-SPE cleanup. The first extraction step takes approximately 10 minutes, which includes 1 minute of hand shaking 50 mL size plastic tubes. The dispersive-SPE cleanup step takes another 10 minutes. During this 20 minute sample preparation process, a maximum of 42 samples can be preparted when four 50 mL extraction tubes are hand shaken simultaneously.

Experimental

Methanol and ACN were LC/MS grade. Water was filtered Milli-Q or LC/MS grade. Ammonium formate and NaOH were ACS grade. Nicotine, anabasine, cotinine, and matrine were purchased from Sigma-Aldrich Co. Wild-caught cod fillet was purchased from a local grocery store.

Conditions

Column:	Agilent Poroshell 120 HILIC, 2.1 × 100 mm, 2.7 µm (p/n 695775-901)	
Sample prep:	Agilent QuEChERS AOAC extraction kit (p/n 5982-5755CH), Agilent QuEChERS dispersive SPE kit (p/n 5982-5022)	
Eluent:	A, 10 mM ammonium formate, pH 3.0; B, ACN	
Injection volume:	20 µL	
Flow rate:	0.7 mL/min	
Gradient:	Time (min)	% B
	0	90
	4.0	70
	4.5	70
	4.6	90
	6	90
LC/MS/MS:	Agilent 1290 Infinity LC System and an Agilent 6460 Triple Quadrupole LC/MS with JetStream	
Drying gas:	300 °C, 5 L/min	
Sheath gas:	350 °C, 11 L/min	
Nebulizer:	45 psi	
Capillary:	3,500 V (positive)	
Nozzle voltage:	500 V (positive)	

Table 1. Analyte characteristics

	Nicotine	Cotinine	Anabasine	Matrine (ISTD)
log P	1.20	0.07	1.25	1.71
pKa	8.02	8.80	11.00	7.72
MRM	163.1 → 132.1	177.1 → 80.1	163.1 → 118.1	249.2 → 148.1
Collision energy	82	112	92	32

Sample preparation

The wild-caught cod fillet was cut into small pieces (less than 1 cm) and comminuted in a grinder with dry ice. The comminuted fish was kept at -70 °C until analysis. For the extraction step, 2 g of the comminuted fish was weighed in a 50 mL AOAC extraction tube, followed by spiking the standard mix solution and adding 13 mL of water to make the total loading amount 15 g. This was adjusted to pH 11 with NaOH. Two ceramic homogenizers, 15 mL ACN, and the AOAC extraction salt packet, which included 6 g $MgSO_4$ and 1.5 g NaAcetate, were added. The tube was shaken vigorously by hand for 1 minute and centrifuged at 5,000 rpm for 5 minutes. Clear phase separation was observed, forming four layers from the bottom of the extraction tubes in the order salt layer, aqueous layer, fish matrix layer, and ACN layer. From the top ACN layer, 1 mL was transferred to a 2 mL dispersive-SPE tube for cleanup. The dispersive-SPE tube was vortex mixed for 10 seconds and microcentrifuged for 10 minutes at 10,000 rpm. Supernatant (0.4 mL) was transferred to an autosampler vial with a built-in filter, and the internal standard was spiked prior to HILIC LC/MS/MS analysis. Figure 1 summarizes the procedure.

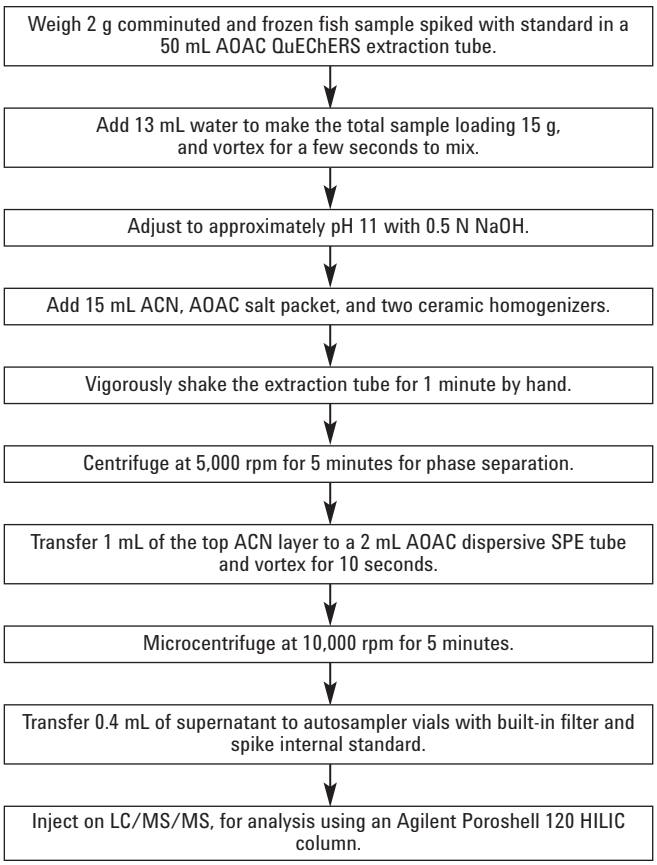

Figure 1. Modified QuEChERS sample preparation workflow for analysis of nicotine and its metabolites in fish.

Results and Discussion

Chromatographically, all compounds were baseline separated. Matrine was retained close to nicotine with the Poroshell 120 HILIC column (Figure 2). Analytes of interest showed excellent linearity ($R^2 \geq 0.998$) within the concentration range of 1 to 500 ng/g in fish (Figure 3). Typical maximum residue limits (MRLs) of nicotine for most commodities were 10 ng/g. For all compounds, the limits of detection (LOD) and limits of quantitation (LOQ) for all compounds were 1 ng/g and 5 ng/g in fish, respectively. These are below the MRL levels and are acceptable for food testing. Very good recoveries and %RSD values were found for n = 8 and three different levels of concentration at low (5 ng/g), mid (50 ng/g), and high (100 ng/g). Table 2 summarizes the results.

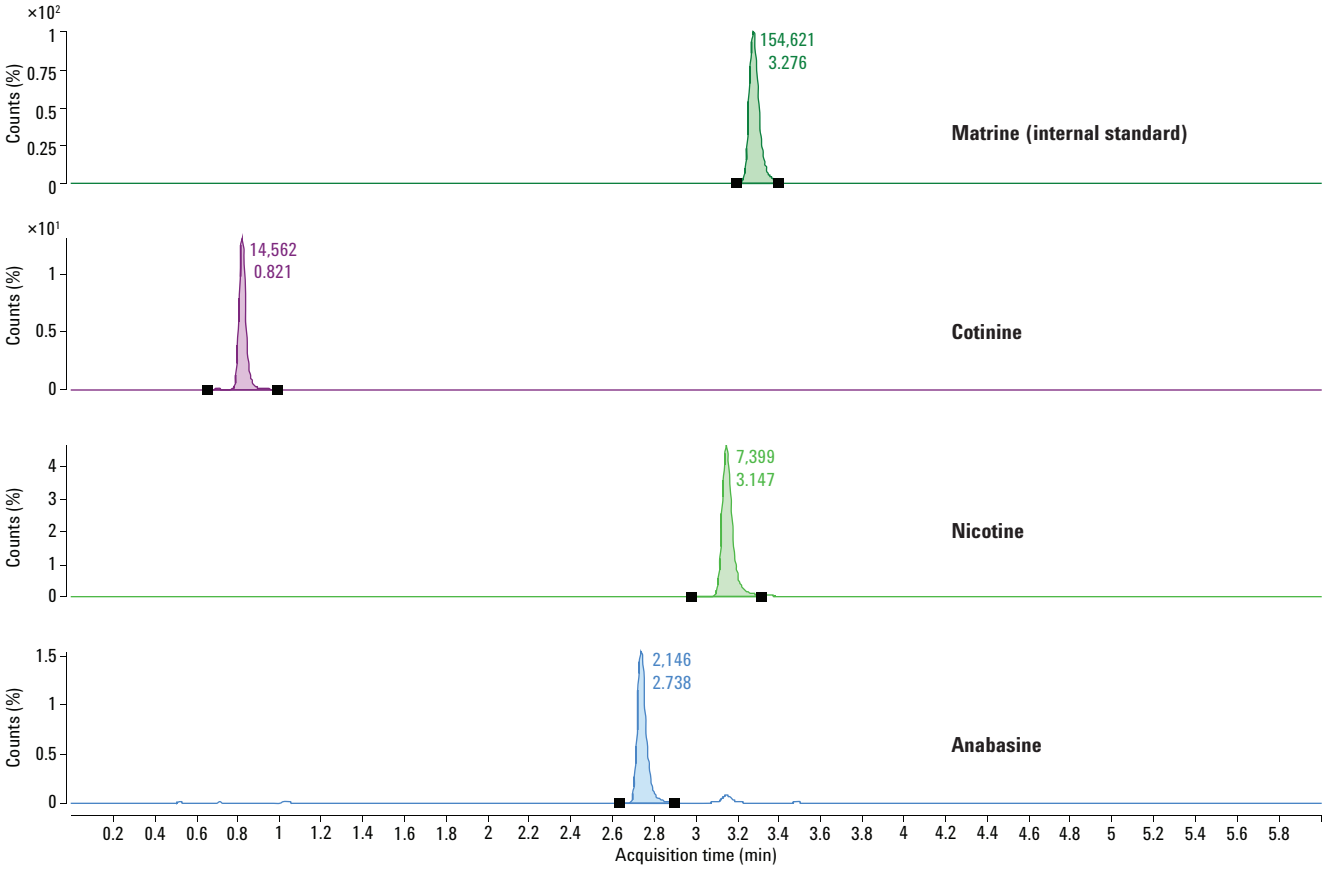

Figure 2. MS chromatogram of nicotine, its metabolites, and internal standard at 5 ng/g of fish.

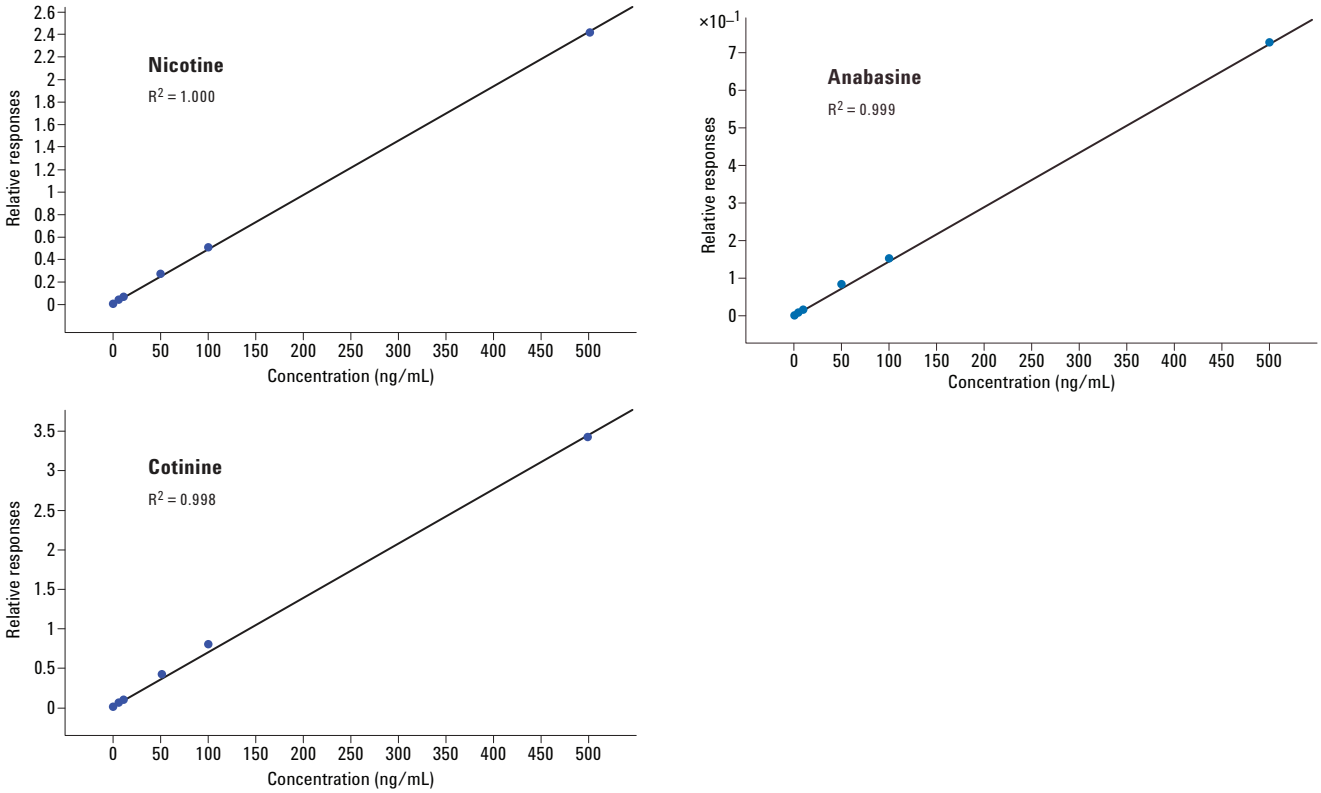

Figure 3. Calibration curves for nicotine and its metabolites in fish (1 to 500 ng/g at six different concentrations).

Table 2. Summary of results of LOD, LOQ, calibration curve linearity (R^2), recovery, and %RSD (n = 8 for each concentration).

				5 ng/mL (low)		50 ng/mL (mid)		100 ng/mL (high)	
	LOD (ng/mL)	LOQ (ng/mL)	Linearity, R^2	% Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Nicotine	1	5	1.000	113.7	6.4	97.7	1.6	90.0	2.1
Cotinine	1	5	0.998	127.9	5.4	117.0	2.2	93.4	2.1
Anabasine	1	5	0.999	91.8	6.2	86.4	1.6	77.8	2.5

Conclusions

A modified QuEChERS method provided a quick and easy sample preparation tool for the analysis of nicotine and metabolites in fish. Without requiring special tools or accessories, and just by shaking, vortex mixing, and centrifugation, up to 48 fish samples were produced in 20 minutes or less. Accompanied by a superficially porous column, an Agilent Poroshell 120 HILIC, good chromatographic separation was achieved for all polar compounds, with excellent linearity in calibration and recovery data.

References

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2. E. Slaughter, R. Gersberg, K. Watanabe, J. Rudolph, C. Stansky, T.E. Novotny. *Tob. Control* **20** (Supp. 1), 25 (2011).
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