

Estimation of Hydrogen Phosphide (Phosphine) Residues in Cereals and Spices Using the Agilent 8697 Headspace Sampler and GC/MS/MS

Agilent 8890/7010C triple quadrupole GC/MS system



Authors

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Abstract

This application note demonstrates the use of an Agilent 8890 GC system coupled with the Agilent 8697 headspace sampler and Agilent 7010 GC/TQ to detect and quantify hydrogen phosphide (phosphine) residues in rice and red chili powder. The sample was inserted into a headspace vial and Milli-Q water was added. The vial was crimped and loaded into the headspace sampler, followed by GC/MS/MS analysis. The limit of quantification of 5 ng/g was achieved in rice and red chili powder matrices. Average recoveries ranged from 80 to 110% in various recovery experiments.

Introduction

Fumigants are gases used for controlling pests in stored food products.¹ Cereals are mainly disinfected by hydrogen phosphide (commonly known as phosphine) and liberated from stable salts such as zinc phosphide or aluminum phosphide.¹ Phosphine is a colorless gas that is used as a fumigant worldwide because of its rapid diffusion and extreme volatility.¹ Despite these desirable properties, trace amounts of phosphine may persist in foods several months after treatment and can cause severe health problems.¹

Due to its high volatility, phosphine is not amenable to club with common multiresidue methods for pesticide residue analysis in food. Thus, special single-residue methods must be applied.² Previously, methods for the analysis of phosphine have been developed that highlight the use of the Tenax-filled inlet liner along with cryogenic cooling to preconcentrate the phosphine prior to chromatographic separation. These methods require heavy analytical configurations that include a liquid nitrogen handling system. In EU legislation, maximum residue limits for phosphine in foodstuffs range from 0.01 to 0.1 mg/kg, depending on the commodity (Commission Regulation (EC) No. 149/2008).²

The method adopted in this application note demonstrates the use of the 8697 headspace sampler for sample introduction to the 8890 GC system coupled with the 7010 GC/TQ.

Column configuration

To prevent single, loose particles of the PLOT column stationary phase from entering the ion source of the mass-sensitive detector (MSD), a restriction capillary (1 m × 0.15 mm) was inserted between the PLOT column and the MSD using a purged Ultimate union. This configuration also helps in column replacement without venting the mass spectrometer.

Table 1. Headspace GC/TQ parameters.

Parameters	Values
Headspace Method Parameters	
Incubation Temperature	60 °C
Incubation Time	15 minutes
Loop Temperature	70 °C
Transfer Line Temperature	80 °C
GC Cycle Time	26 minutes
GC/MS/MS Method Parameters	
Inlet Temperature	150 °C
Analytical Column	Agilent HP-PLOT Q (p/n 19091P-Q04)
Postcolumn	Deactivated fused silica capillary (1 m × 0.15 mm)
PSD 1 Pressure	1 psi
Column Flow	Helium: 1.2 mL/min, constant flow
Injection Mode	Split (20:1)
Oven Program	Start at 50 °C (hold for 1.0 min), ramp at 4 °C/min to 70 °C, then at 50 °C/min to 260 °C, hold 10 min
MS Parameters	
Ionization Mode	EI
Ion Source Temperature	230 °C
Quadrupole Temperature (Q1 and Q2)	150 °C
SIM Mode Ions	
Quantifier Ion	34
Qualifier Ion 1	33
Qualifier Ion 2	31

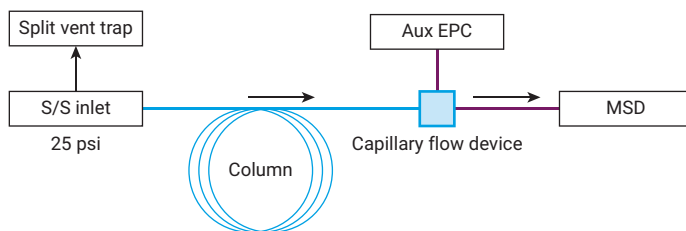


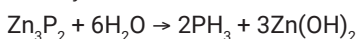
Figure 1. Schematic of column configuration with postcolumn backflush.

Experimental

Standard preparation

A hydrogen phosphide standard solution (0.2 µg/mL) was obtained from the suspension of 4 mg of zinc phosphide (Zn₃P₂ – 19% purity) powder in 1 L of distilled water. After hand-shaking the mixture, the suspension was placed into an ultrasonic bath for 15 minutes.

Zinc phosphide reacts with water to produce phosphine and zinc hydroxide:



Phosphine concentration (µg/mL) in stock solution =

$$\frac{\text{wt. of Zn}_3\text{P}_2 \text{ (mg)} \times \text{mol wt. of PH}_3 \times \text{no. of moles} \times \text{purity} \times 1,000}{\text{mol wt. of Zn}_3\text{P}_2 \times \text{final volume (mL)} \times 100}$$

$$= \frac{4 \times 34 \times 2 \times 19 \times 1,000}{258 \times 1,000 \times 100} = 0.2 \text{ µg/mL}$$

Six prespiked matrix calibration levels were prepared by adding phosphine stock solution (see Table 2) to 0.5 g of blank matrices of rice and red chili powder, respectively, taken in 20 mL headspace vials. Five milliliters of Milli-Q water was added to each calibration level and was immediately crimped, followed by running this in the headspace GC/MS system.

Table 2. Preparation of prespiked matrix calibration.

Calibration Level	Stock Solution Volume (µL)	Blank Matrix (g)	Milli-Q Water Volume (mL)	Prespiked Concentration (ng/g)
L1	12.5	0.5	5	5
L2	25	0.5	5	10
L3	50	0.5	5	20
L4	125	0.5	5	50
L5	250	0.5	5	100
L6	500	0.5	5	200

Sample preparation

Approximately 0.5 ± 0.01 g of each sample were weighed in 20 mL headspace vials. Five milliliters of Milli-Q water was added, and the vial was crimped immediately, followed by running in the headspace GC/MS system.

Results and discussion

Calibration

Figures 2 and 3 show linearity of phosphine in rice and red chili powder, respectively. The R² obtained was above 0.998.

Repeatability

A repeatable elution was obtained by injecting replicates of 20 ppb-spiked concentration of phosphine in rice and red chili powder. As shown in Table 3, %RSD data of phosphine were calculated from peak areas of four replicate injections of 20 ppb matrix-spiked standards in rice and red chili powder.

Recovery

Phosphine was spiked in samples of rice and red chili powder at concentration levels of 10 and 20 ng/g, respectively. Acceptable recoveries were obtained by quantification through prespike matrix-based calibration. Results are highlighted in Table 4.

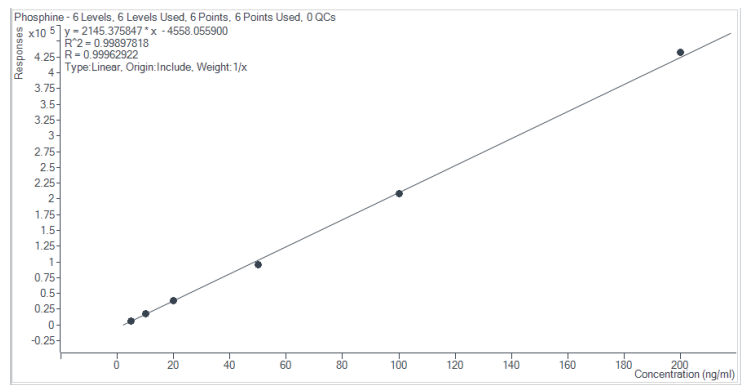
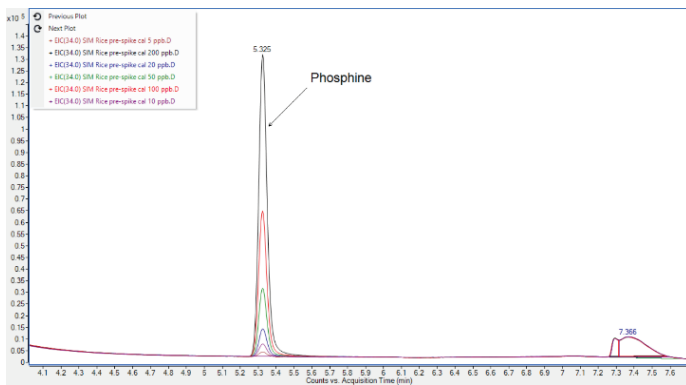


Figure 2. Overlaid chromatograms and calibration curve for prespike standards from 5 to 200 ng/g in rice.

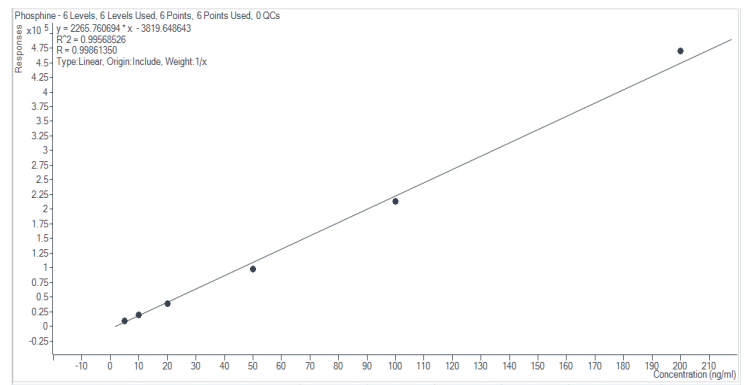
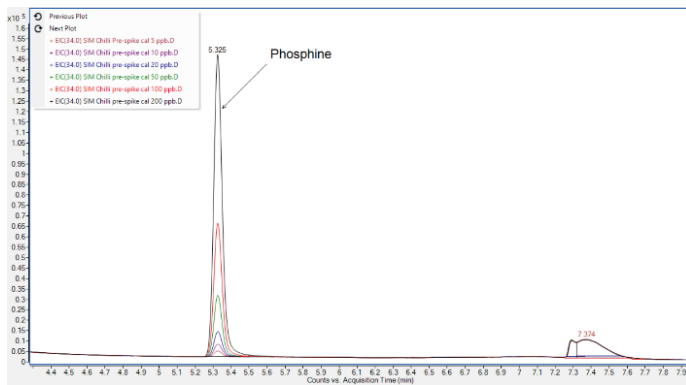


Figure 3. Overlaid chromatograms and calibration curve for prespike standards from 5 to 200 ng/g in red chili powder.

Quantifier and qualifier peaks in matrix standards

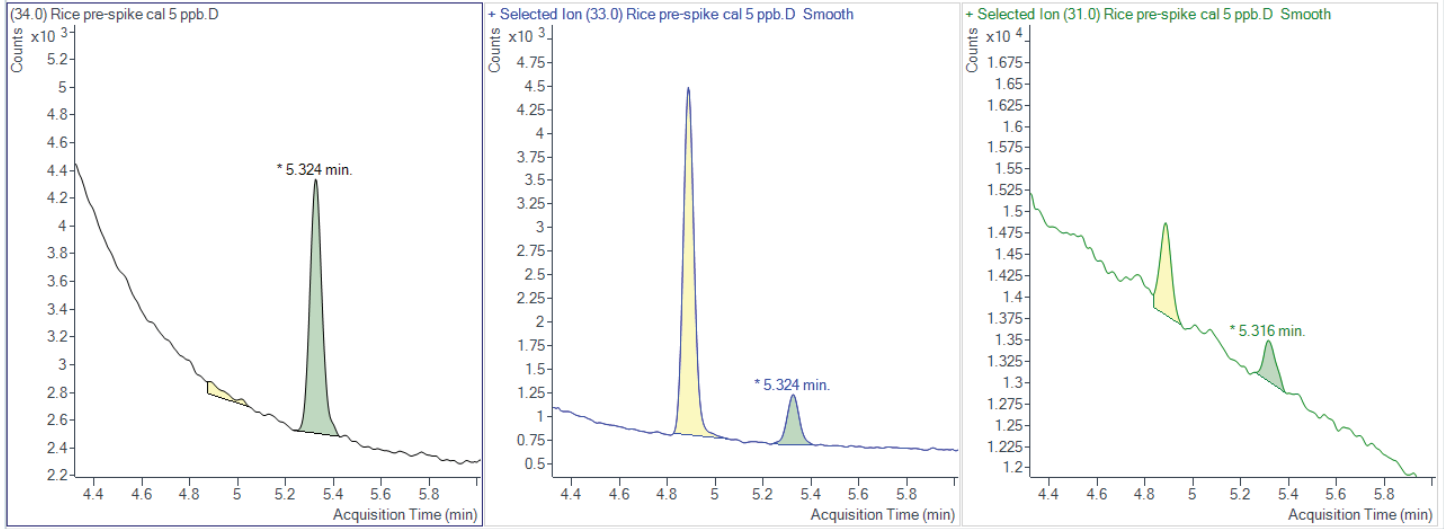


Figure 4. Quantifier and qualifier peaks in 5 ppb prespike matrix standard in rice.

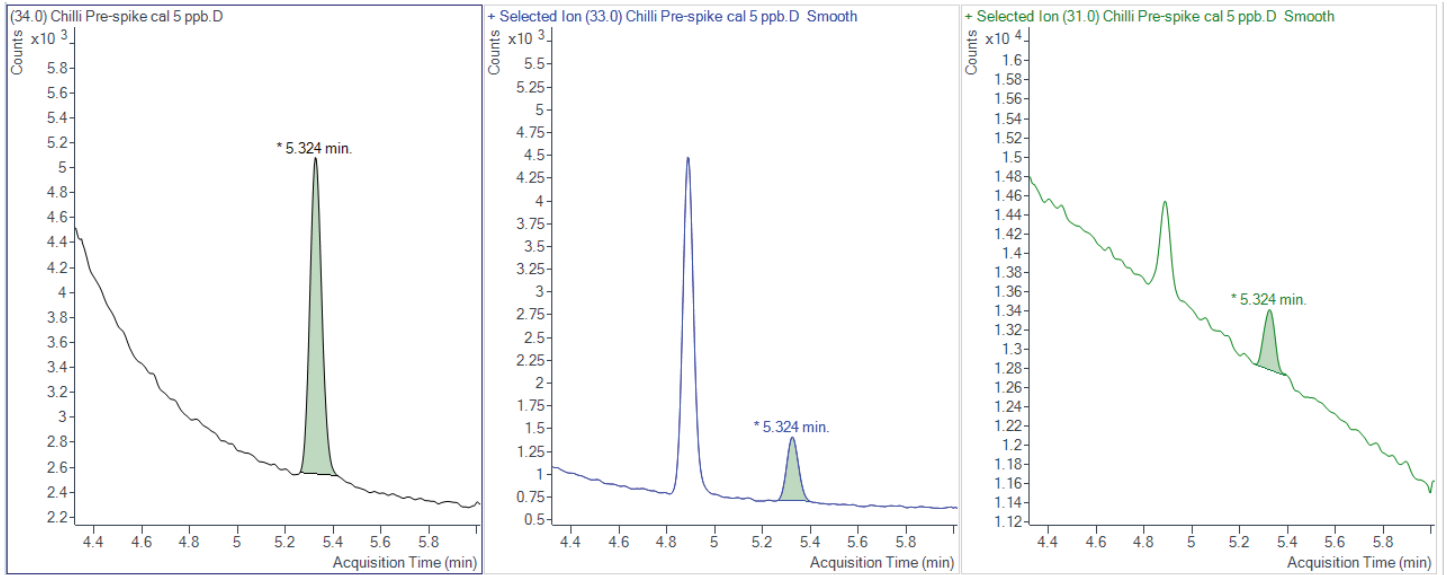


Figure 5. Quantifier and qualifier peaks in 5 ppb prespike matrix standard in red chilli powder.

Table 3. %RSD for four replicates of 20 ppb prespiked matrix standard in rice and red chili powder.

Number of Replicates	Rice		Red Chili Powder	
	RT (min)	Area Counts	RT (min)	Area Counts
20 ppb Matrix Standard Replicate 1	5.329	32,063	5.329	35,302
20 ppb Matrix Standard Replicate 2	5.324	31,285	5.329	38,722
20 ppb Matrix Standard Replicate 3	5.325	36,476	5.325	38,326
20 ppb Matrix Standard Replicate 4	5.324	33,056	5.324	33,704
Mean	5.326	33,220.000	5.327	36,513.500
SD	0.002	2,288.472	0.003	2,416.861
%RSD	0.045	6.889	0.049	6.619

Table 4. Percent recovery of phosphine in rice and red chili powder spiked at the 10 ng/g level.

Food Matrix	Phosphine		
	Spiked Concentration (ng/g)	Average Obtained Concentration (ng/g)	Recovery (%)
Rice	10	10.23	102.3
Red Chili Powder	10	10.48	104.8

Conclusion

An accurate and rugged method was developed which meets the requirements of the European Union reference laboratory (EURL) for maximum residue limits of phosphine in foodstuffs. This ranges from 0.01 to 0.1 mg/kg, depending on the commodity (Commission Regulation (EC) No 149/2008) for the analysis of phosphine in rice and red chili powder. The LOQ of the method was demonstrated at 5 ng/g for all tested matrices. Repeatable results were found for four successive replicates of matrix-based standards at 20 ng/g concentration levels. Excellent recoveries were obtained in all tested matrices at 10 ng/g spiked concentration levels. Thus, this study demonstrates the usefulness of the developed method for routine analysis of food samples for phosphine analysis at trace levels.

References

1. Wittayanan, W.; Jongmevasna, W.; Kaewklapanyacharoen, L.; Atisook, K. Method Development and Validation of Hydrogen Phosphide and Inorganic Bromide Determined as Fumigant Residues in Commercialized Rice Grains in Thailand. *Int. Food Res. J.* **2017**, *24*(3).
2. Perz, R.; Benkenstein, A.; Köbler, H.; Scherbaum, E.; Köhl, D.; Barth, A.; Anastassiades, M. Analysis of Phosphine in Dried Foodstuffs via Headspace-GC-MSD. *Aspects of Food Control and Animal Health* **2014**, (2).