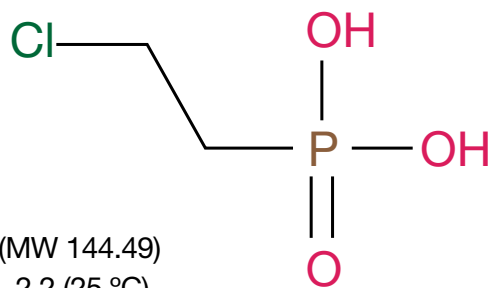


A Simple, Rapid Method For The Analysis Of Eth- ephon From Ketchup Us- ing Solid Phase Extraction And LC/MS/MS

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Figure 1. Parent Structure



Ethephon (MW 144.49)
Log K_{ow} < -2.2 (25 °C)

Materials and Methods

HPLC analysis was performed using a Shimadzu® Nexera® HPLC (Shimadzu, Columbia, Maryland 21046 U.S.A). LC/MS/MS analysis was performed using an AB SCIEX API 4000™ QTRAP® System (AB SCIEX, Framingham, MA). Solid phase extraction use of Phenomenex Strata™-X-AW (Part No. 8B-S038-ECH) followed by the use of Luna® 3µm NH₂ (Part No. 00B-4377-B0) from Phenomenex, Torrance, CA, USA. All chromatographic conditions are as specified on the individual figures.

Figure 2. Sample Preparation

Solvent Extraction:

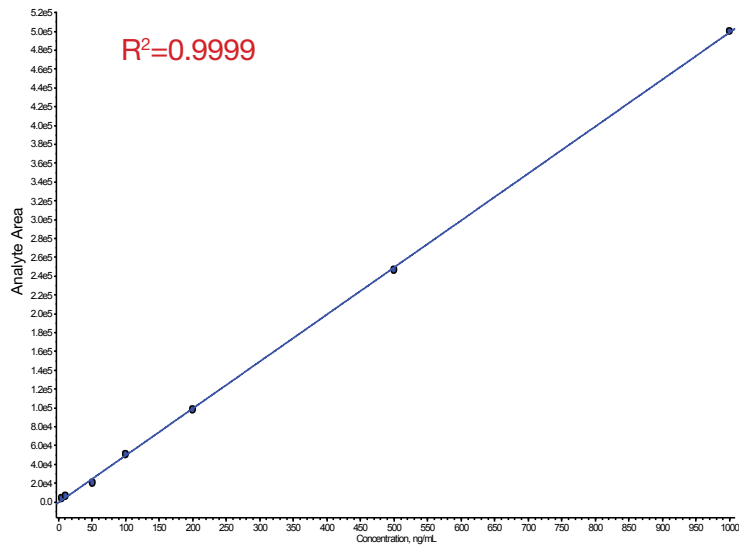
1. Weigh and transfer 2 g of ketchup into a 15 mL centrifuge tube
2. Add 2 mL of 100 mM Sodium phosphate buffer
3. Add 10 mL of Acetone and vortex until ketchup is dispersed
4. Shake using mechanical shaker for 4 min
5. Centrifuge at 6000 rpm or greater for 4 min
6. Supernatant is ready for loading onto SPE cartridge

Solid Phase Extraction:

Cartridge: Strata-X-AW 100 mg/6 mL
Part. No.: 8B-S038-ECH
Condition/Equilibrate: 3 mL of Acetone
Load: Supernatant from solvent extraction
Wash : 3 mL of Methanol
Dry: 3 min at high vacuum (>10 in. Hg)
Elute: 3 mL of Methanol with 1 % TFA
Blow down: Dry sample under nitrogen at 50 °C

Note: Reconstitute sample with 0.5 mL of Acetonitrile/ Water (9:1) and transfer to an autosampler vial for LC/MS/MS analysis

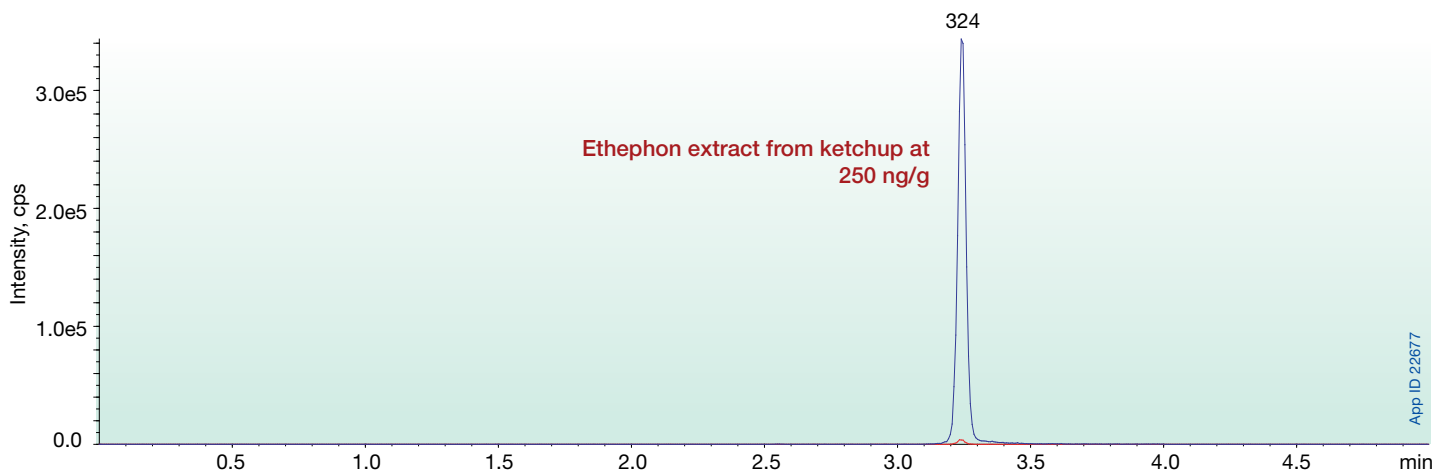
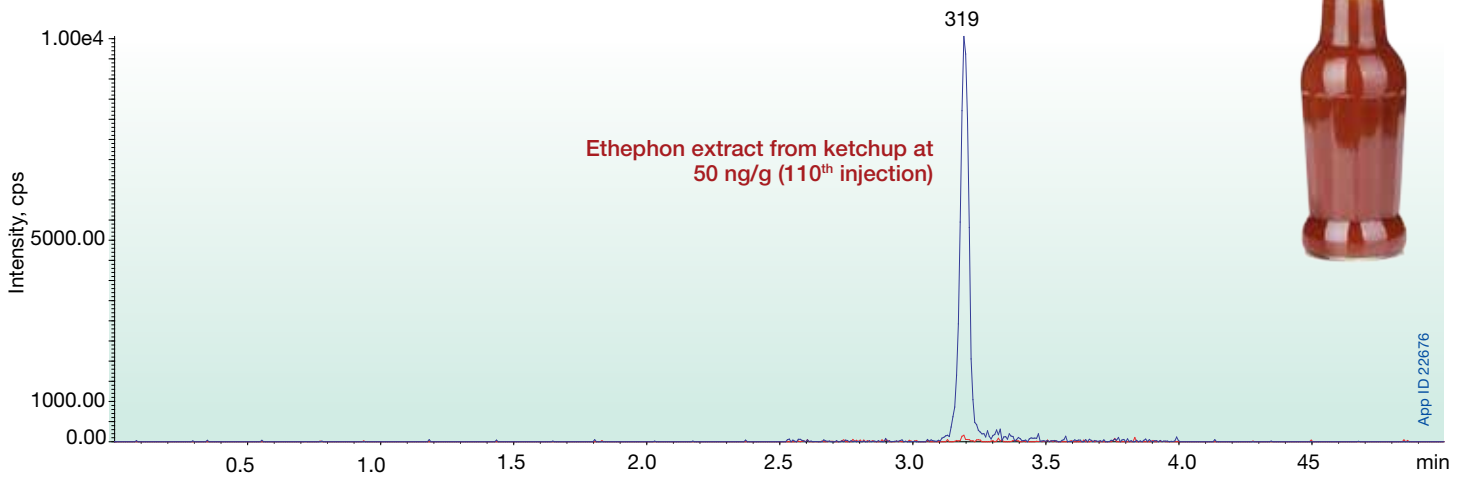
Figure 4. Calibration curve



Ethephon matrix-extracted calibration curve (5-1000 ng/g), $R^2=0.9999$

At the low end of the calibration curve (5 ng/g) the S/N ratio is 16.5. This value corresponds to a LLOQ of 3.0 ng/g and a LLOD of 0.9 ng/g (Based off of commonly accepted S/N target values for LLOQ and LLOD of 10 and 3 respectively).

Figure 3. Chromatograms



Column: Luna 3 μ m NH₂
Dimensions: 50 x 2.0 mm
Part No.: 00B-4377-B0
Mobile Phase: A: 10 mM Ammonium bicarbonate pH 10
 B: Acetonitrile
Gradient:

Time(min)	B (%)
0.0	90
5.0	10

Flow Rate: 0.4 mL/min
Inj. Volume: 10 μ L
Temperature: Ambient
Detection: API 4000 QTRAP (AB SCIEX) Tandem Mass Spec (MS/MS)
Sample: Ethephon extract from ketchup

Note:
MRM: Ethephon 142.8>106.9/35
Valco Valve:

Time (min)	Flow direction
0 \rightarrow 2.5	Waste
2.5 \rightarrow 4.0	MS
4.0 \rightarrow 8.0	Waste

TFA in the ion-source can significantly reduce MS intensity. It is recommended that a diverter valve is utilized to ensure that no residual TFA from the SPE step is transferred to the MS.

Results and Discussion

In this study, we present a fast, simple, and sensitive method for the analysis of ethephon residues in tomato ketchup.

Ethephon is first extracted from the ketchup matrix with a simple solvent extraction using acetone and sodium phosphate buffer. The extract is then cleaned up using ion-exchange SPE. As shown in **Figure 1**, the phosphate functionality of ethephon lends itself perfectly to extraction with Strata-X-AW allowing a strong 100 % methanol wash without loss of analyte. The strong wash removes a large amount of matrix contamination, resulting in a very clean extract.

The SPE extraction alone yields a high 80.1 % recovery (**Table 1**). However, the solvent extraction prior to SPE has a low extraction efficiency resulting in a 14.2 % total method recovery. In order to increase the solvent-extraction efficiency several variables were tested including extraction time, extraction solvent, pH, and solvent molarity. However, the extraction efficiency was not improved. We **think** the cause of the low solvent-extraction efficiency is related to protein-binding with the phosphate moiety of the ethephon molecule.

Following SPE, the resulting sample is then analyzed using a high-pH LC/MS/MS analytical method on an amino-type stationary phase (Luna 3 μm NH_2). The pH gradient used (**Figure 3**) focuses the analyte on the stationary phase before elution, resulting in a narrow peak-shape and a retention time away from the ion-suppression region (**Figure 3**). Due to the excellent peak shape and high SPE recovery, low limits of detection and quantitation are achieved, despite the poor solvent-extraction efficiency. The extrapolated LLOQ and LLOD are determined to be 3 ng/g and 0.9 ng/g, respectively. In addition to the low detection limits, the method achieved a calibration curve linearity value of $R^2=0.9999$ (**Figure 4**)

One drawback of the high-pH mobile phase used on the amino-type stationary phase is that the column's lifetime is shortened relative to conventional reversed phase methods. Therefore, it is recommended that the stability of the column and the resulting chromatography is monitored over time, especially as the column approaches 200 or more injections. However, as shown in **Figure 3**, after 110 injections the chromatographic performance has not degraded.

Table 1. Recovery

Ethephon concentration	Recovery %
Strata-X-AW Recovery*	80.1
Total Method Recovery**	14.2

*Strata-X-AW recovery is calculated by spiking ethephon standard into a solvent-extracted matrix blank prior to SPE

**Total method recovery is calculated by spiking ethephon standard directly into the ketchup matrix prior to solvent extraction and SPE extraction

Conclusions

Ethephon analysis using conventional analytical techniques is challenging due to the polarity of the molecule as well as the complex matrices that are being tested.

Using ion-exchange SPE effectively removes a large amount of interferences from a tomato ketchup matrix. The cleaned-up sample results in a clean LC/MS/MS baseline, allowing maxi-

mum sensitivity with limited ion suppression.

Following SPE, excellent retention and peak shape were obtained using Luna NH₂ with a high-pH mobile phase. Whereas, trials on typical reversed phase or HILIC-mode conditions resulted in either insufficient retention or severe tailing.

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References

1. Takenaka S; Journal of Agricultural and Food Chemistry. 50: 7515-7519 (2002)

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