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Improving reproducibility and recovery by reducing ionization suppression of LC-MS/MS for quantitation of pesticide residues in chickpea powder

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Introduction

Chickpea is one of the earliest cultivated legumes and is high in protein. It is an important cuisine in India, the Middle East, and Mediterranean countries. For food safety, pesticide testing needs to be sensitive and selective. Described here is a LC-MS/MS method is a LC-MS/MS method to quantitate pesticide residues in chickpea.

Even though tandem mass spectrometry is highly selective and sensitive, matrix co-extractives can change the ionization efficiency of pesticides and thereby cause signal suppression or enhancement. QuEChERS based extraction followed by dispersive solid-phase clean up reduces the concentration of matrix in the final extract. Dilution of this sample extract further reduces the matrix effect result in improved recovery and reproducible results. Matrix effect can be further compensated by either strategy like matrix-matched or matrix-based calibration.

Figure 1. 1290 Infinity II coupled to 6470 TQ.

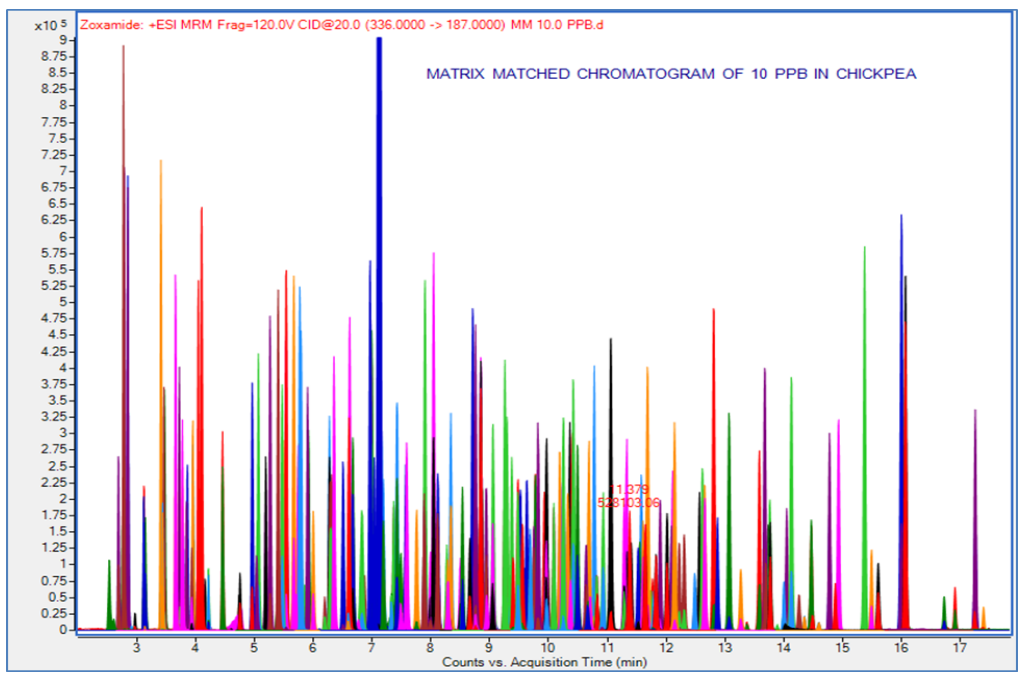


Figure 2. 253 pesticides in chickpea matrix at 10 ppb.

Experimental

Sample Preparation

Approximately two-gram chickpea powder is accurately weighed into a 50mL centrifuge tube. Sample soaked in water was extracted using the AOAC QuEChERS extraction kit and ten mL of acetonitrile. The mixture is then centrifuged, and the supernatant is taken for clean-up. MgSO4, PSA, and C-18 were ingredients in the cleanup kit. Cleaned up extract is further diluted five times before instrumental analysis. Agilent 6470 TQ was used for the analysis. Multiple reaction monitoring (MRM)-based method with specified retention time is employed to analyze pesticides in chickpea powder. Extracted chickpea samples are spiked at different concentration levels of pesticides to prepare the matrix-matched calibration curves.

Chromatographic conditions

Mobile Phase A
0.5mM Ammonium Fluoride and 4.5mM ammonium formate + 0.1% Formic acid in water
Mobile phase B
0.5mM Ammonium Fluoride and 4.5mM ammonium formate + 0.1% Formic acid in water: Methanol (5:95, V/V)
Column
Agilent Zorbax Eclipse plus RP C18,
(150 mm X 3.0 mm, 1.8 um)

The data acquisition with specified retention time provided a greater number of data points across the chromatographic peak.

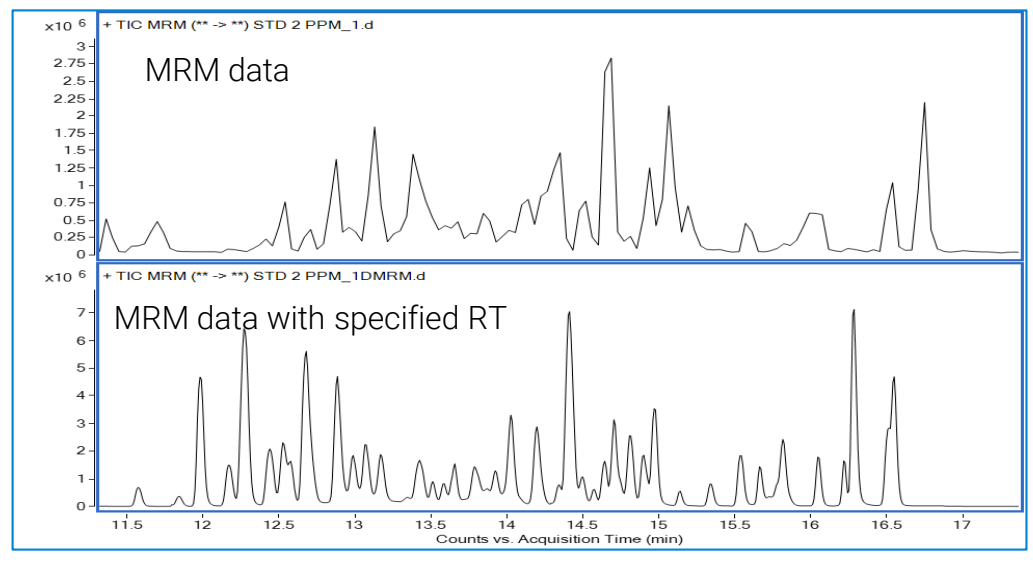


Figure 3: MRM acquisition data vs MRM acquisition with specified retention time (RT) data.

Data generated as a part of method development

Pesticide method is developed by using Agilent MassHunter Pesticide personal compound triggered MRM database. tMRM database and library for more than 700 pesticides that includes compound names, up to 10 MRM transitions, MRM method parameters such as Fragmentor voltages for parent *m/z* and collision energies for each of the fragments. This also enables pesticide screening with tMRM library verification and thereby avoid any false positive result.

Comprehensive pesticide mix used containing 253 LC-MS amenable pesticides is used for generating the calibration curves. Extracted chickpea samples are spiked at different concentration levels of pesticides to prepare the matrix matched calibration curves.

The developed method is partially validated as per SANTE/11813/2017. Matrix matched calibration curves were made between 0.1 to 50 ppb. The overall limit of detection (LOD) and limit of quantification (LOQ) for the method by considering all analytes were 2 ppb and 10 ppb, respectively. Regression coefficients for the majority of the analytes were found to be more than 0.9950. For each analyte, 2 MRM transitions are selected which satisfy the requirement of 4 identification points for the confirmation of analytes in the sample.

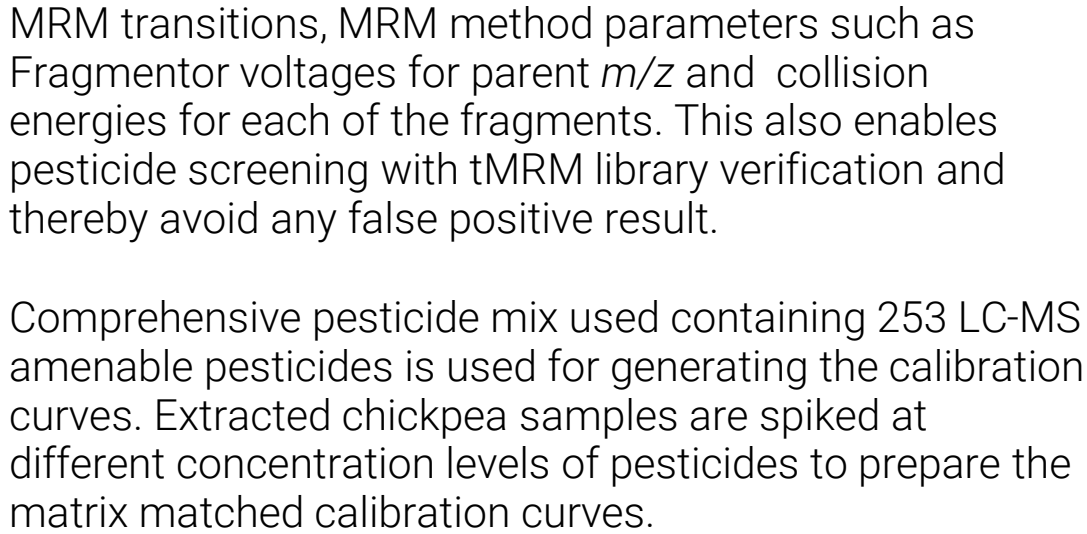
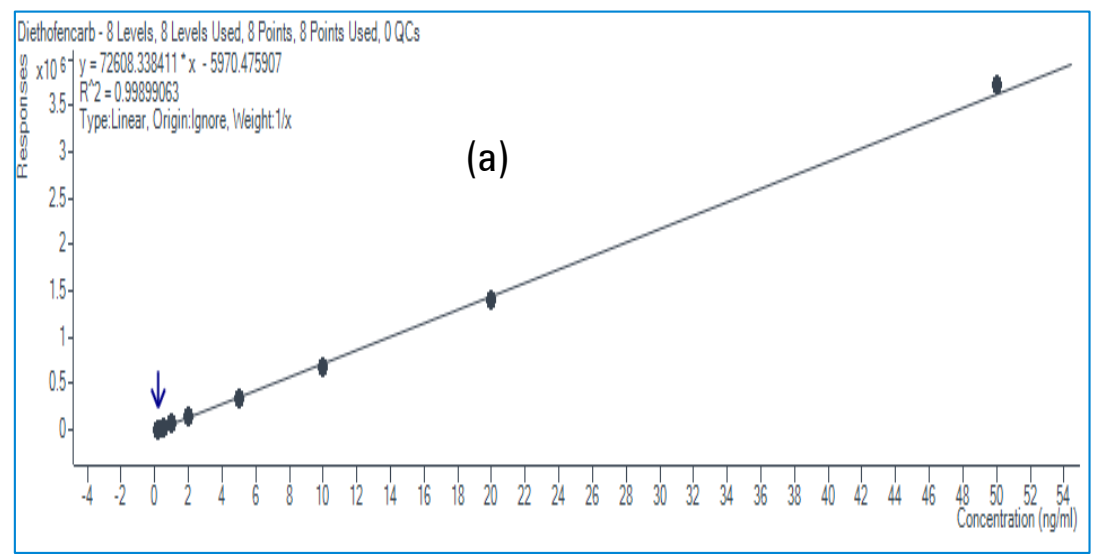


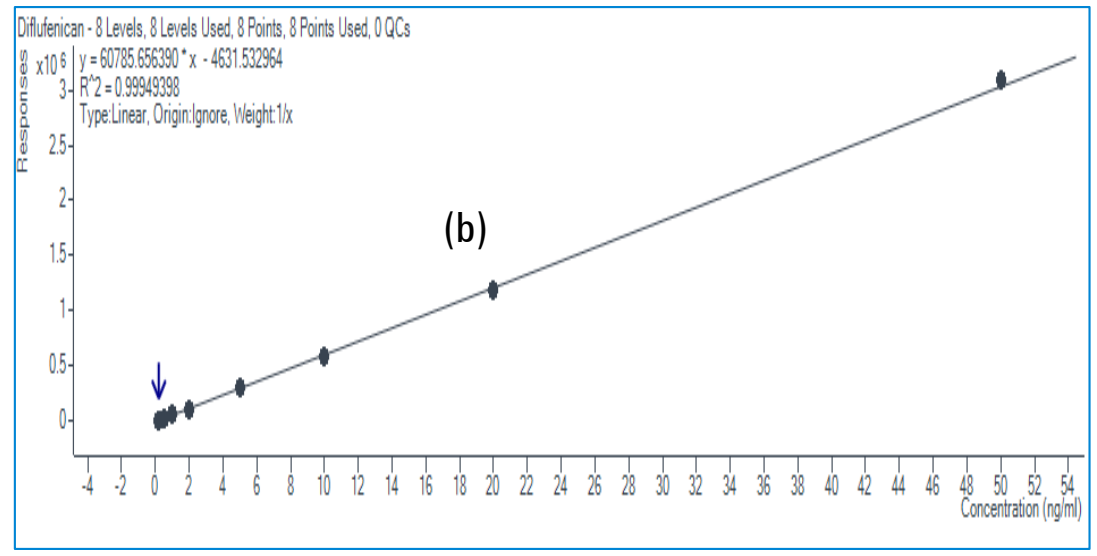
Figure 4: Ion transition ratio of Amidosulfuron calculated by MassHunter quant software

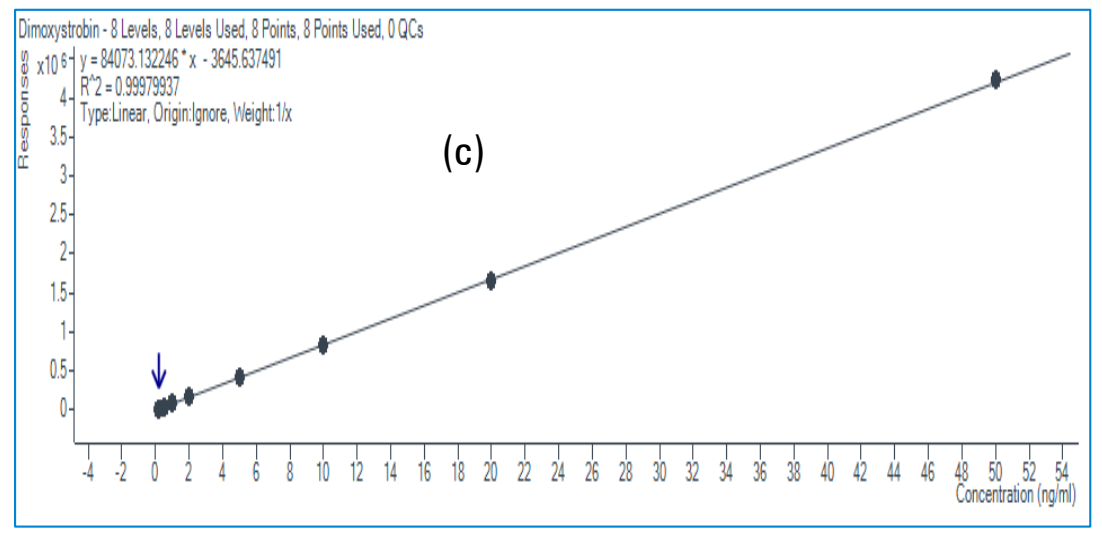
Identification of parent ion give 1 point and identification of each fragment ions in the sample provide 1.5 points.

MassHunter Quant software automatically identifies the quantifier and qualifier ions and calculates the ion transition ratio of standards and samples. It also shows how much the sample ratio is varying from the standard MRM ratio.

Representative matrix matched calibration curves







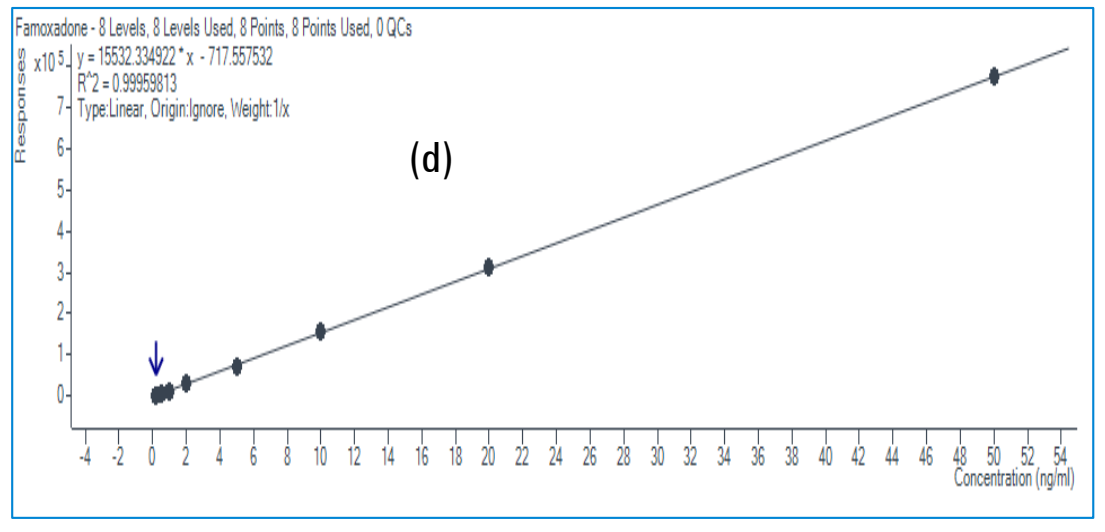


Figure 5. Matrix matched calibration curves (0.1 ng/ml to 50 ng/ml) of a) dithiofencarb, b) Diflufenican, c) dimethoxystrobin & d) Famoxadone.

Results and Discussion

Recovery study result

Recovery study was also performed at the LOQ level by spiking pesticide standards in blank extracted samples. 10 ppb spiking level in sample gives an absolute quantity of 0.4 ppb in the final extract after dilutions. 20 µL of 1 ppm (ug/mL) pesticide standard mix is spiked to 2 g Chickpea Powder. Absolute quantity of pesticides presents in 20 ul of 1 ppm pesticide mix= 20.0 ng. 20 ng is spiked to 2 g Chickpea powder. Concentration spiked is 20ng/2g= 10 ppb. After following the sample preparation protocol for the spiked samples, effective final concentration injected to the system would become 0.4 ppb for Spike level 1, 10 ppb.

More than 80% of the pesticides in this study showed a recovery above 75%. For most of the compounds, recovery improved after dilution of extract.

For example, Trimethacarb showed a recovery of 36% before dilution. Recovery improved to 79% after dilution. For low recovery analytes, matrix-based calibration curves are used, and recovery losses are compensated. Chickpea samples purchased from local grocery shops were analyzed and quantified against the prepared calibration curves. Results obtained from the calibration table was multiplied with a dilution factor of 25.

None of the analyzed samples were detected with pesticides above the LOQ level. Software used to provide the qualifier to quantifier ratio (ion ratio) for both standard and sample. Activation of the feature of simultaneous collection of additional fragment ions found to be very useful for spectral library matching and thereby confirmation in case of any positive result.

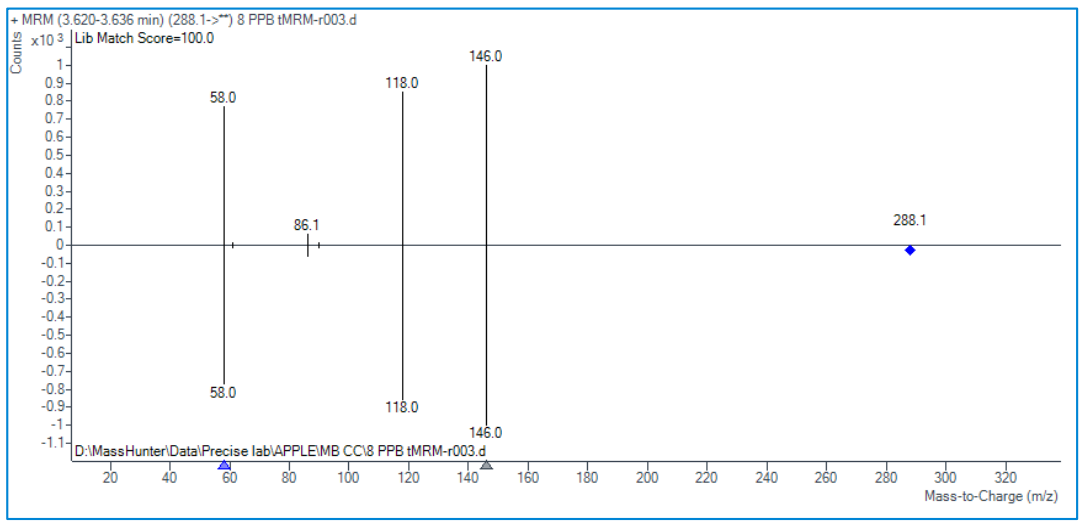


Figure 6: Simultaneous collection of additional fragment ions for Vamidation

The sensitivity of the LC-MS/MS system used in this study allowed the user to dilute the extract. Dilution reduces the matrix effect which results in, improved recovery, consistent performance of the instrument and thereby achieves reproducible results over long batches of sample analysis.

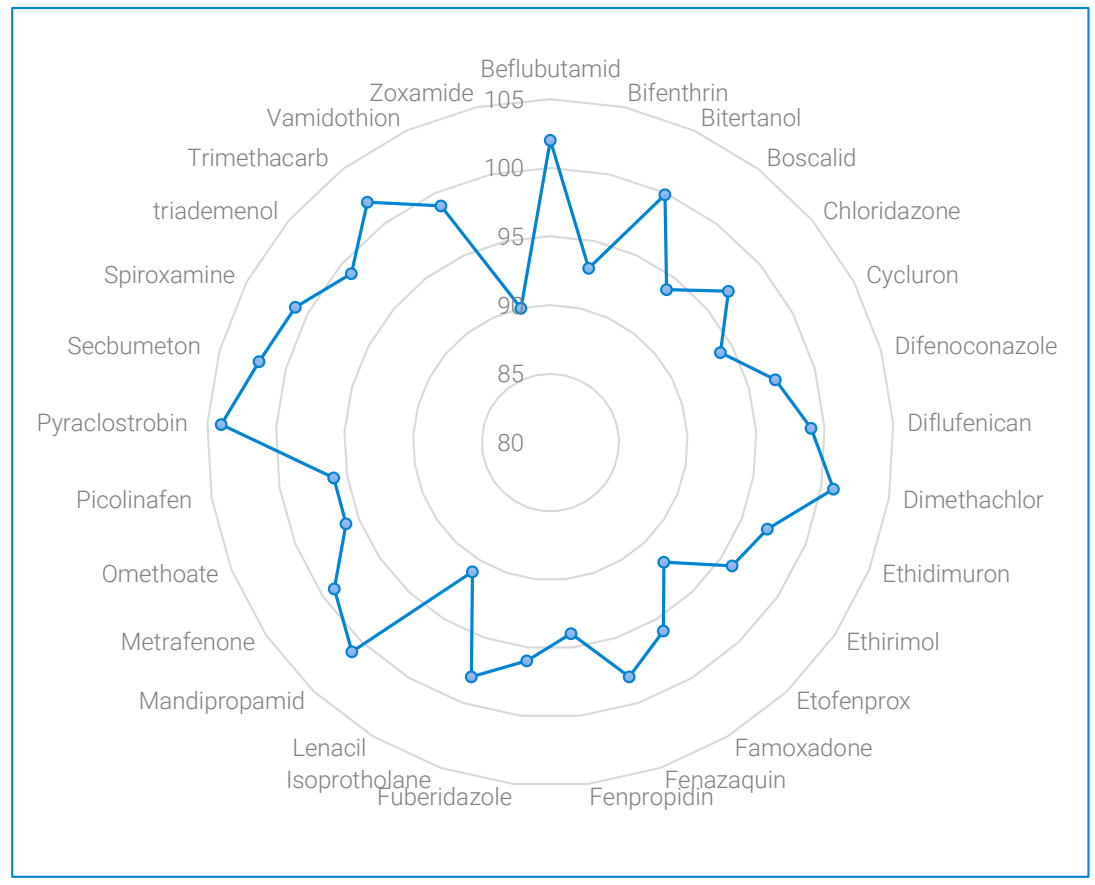


Figure 7: RADAR plot of recovery values of various pesticides in the study.

Conclusions

- LC-MS/MS method is developed for the analysis of pesticides in Chickpea powder samples.
- Retention time based acquisition provide more number of data points across the chromatographic peak compared to MRM in case of multi residue method.
- Recoveries of most of the analytes are above 80% with respect to matrix matched calibration curve. In case of low recovery analytes, matrix-based calibration curves are used, and recovery losses are compensated.
- Clean up followed by dilution of chickpea extract reduce ionization suppression, provide improved recovery and reproducible results for long batches.

References

1. Agilent Application note 5991-8154EN
2. Agilent Application note 5991-6357EN

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