

EVALUATION OF DIFFERENT EXTRACTION METHODS OF PESTICIDE RESIDUES IN SPICES BY LC-ToF-MS and GC-ToF-MS

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1. Aim and scope

This study is aimed to develop a multiresidue extraction method for the analysis of pesticide residues in spices, taking into account the difficulty of this matrix and the high background effect generated. The aim is the removal of volatile oils, piperine and lipid present in spice matrices to obtain the cleanest extract.

2. Short description

Homogenous samples of black pepper, cayenne and curcuma are extracted with modified QuEChERS, testing 3 different sorbents: PSA, Z-Sep, and EMR. The necessity of a freezing out step in dry ice (CO₂ at -76°C) was evaluated. Dilution factor was also studied. The obtained extracts were analyzed by both GC-ToF-MS and LC-ToF-MS. Matrix compounds were retrieved and counted using the Molecular Feature Extractor (MFE) algorithm in the MassHunter Workstation Software. The MFE creates a compound list of all the peaks in the data file that represent real molecules. At the end of the data process, a list with the mass, retention time, and intensity of all matrix components was obtained. The resulting data was evaluated to get information of the complexity of the matrices through the number and distribution of the matrix components.

3. Consumables

- Automatic pipettes, suitable for handling volumes of 10 to 200 µL and 1 to 5 mL.
- 50 ml PTFE centrifuge tubes with screw caps
- 15 ml PTFE centrifuge tubes with screw caps
- Vortex
- Automatic axial extractor

- Centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 3700 rpm
- Concentration workstation
- Injection vials, 2 ml, suitable for GC and LC auto-sampler.

4. Chemicals

- Acetonitrile ultra-gradient grade
- Trisodium citrate dihydrate
- Disodium hydrogencitrate sesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Primary secondary amine bonded silica (PSA), bulk material
- Bondesil-C18
- SupelTM QuE Z-sep, bulk material
- EMR-lipid d-SPE
- EMR-lipid polish material
- Ultra-pure water
- Pesticides standards

5. Instrumentation and analytical conditions for the LC-ToF-MS

Agilent 1290 HPLC

- Column: Agilent Eclipse Plus Rapid Resolution HD C18, 2.1 mm x 50 mm x 1.8 μ m
- Mobile phase A: Methanol 0.1% Formic Acid, 2% ultrapure water, 5mM ammonium formate
- Mobile phase B: 0.1% Formic acid in ultrapure water, 2% methanol, 5mM ammonium formate
- Flow rate: 0.3 mL/min
- Injection volume: 4 μ L

Mobile phase gradient

Time [min]	Mobile phase A	Mobile phase B
0	20%	80%
2	20%	80%
15	100%	0%
17	100%	0%

Agilent 6550 LC-QTOF-MS

- 4GHz High Resolution Mode
- ESI source gas temperature: 160°C
- Gas flow: 14 L/min
- Nebuliser gas and collision gas: nitrogen
- Nebuliser gas pressure: 30 psi
- Sheath gas flow: 12 L/min
- Sheath gas temperature: 350 °C
- Ionisation mode: positive
- Capillary voltage: 4000 V
- Octapole RF Peak: 750V
- Fragmentor 360 V

6. Instrumentation and analytical conditions for the GC-ToF-MS

Agilent 7890 A

- Column: 2 columns of HP-5MSUI, 15 m x 0.25 mm x 0.25 µm
- Column flows: 1.0 mL/min in the first column and 1.20 mL/min in the second column
- Carrier gas: helium (99.999 %) at constant pressure 14.1 psi
- Injection mode: splitless
- Ultra Inert liner with a glass wool frit
- Injection volume: 2 µL
- Injection temperature: 280 °C
- Oven temperature program:

Rate (°C/min)	Value (°C)	Hold time (min)	Runtime (min)
	60	1	1
40	120	0	2.5
5	310	0	40.5

Backflushing: at 310°C for 2 min

Agilent 7200 GC-Q-ToF

- EI ion source operating at 70 eV
- 4 GHz High Resolution Mode
- Ion source temperature: 280 °C
- Quadrupole analyzers temperature: 150 °C
- Acquisition mode: Full scan MS
- m/z range: 45-550

7. Procedure

7.1. Sample extraction

7.1.1. Clean-up sorbent study

- 2g portion of sample was weighted in a 50 mL PTFE centrifuge tube.
- 7 mL of milli-Q water were added to hydrate the spices (soaking time: 30 min)
- 10 mL of acetonitrile were added.
- The sample is shaken by an automatic axial extractor (AGYTAX®, Cirta Lab. S.L., Spain) for 7 min.
- 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogencitrate sesquihydrate were added and the samples were again shaken in the automatic axial extractor for 7 min.
- The extract was then centrifuged at 3700 rpm for 5 min.
- 9 mL supernatant were transferred to a 15 mL PTFE centrifuge tube and were placed in a polystyrene box filled with dry ice (CO₂ at -76°C) for 6 min.
- 5 mL of the extract was then separated from the frozen precipitate using a Pasteur Pipette.
- The extract was transferred to EMR-lipid tube already stabilized with 5 mL water.

- The extract was shaken in a vortex for 1 min and then centrifuged at 3700 rpm for 5 min. A 5 mL extract was transferred to an EMR-polish tube containing 1g of sodium chloride and 4g of magnesium sulfate.
- After vortex and centrifuge at 3700 rpm for 5 min, the supernatant was collected.
- For LC analysis, the extract was diluted 5 times with a mixture of methanol/water (50/50). A final dilution of 25 times is obtained (160 pg in column).
- For GC analysis, 50 μ L of the extract was evaporated and reconstituted with 50 μ L of ethyl acetate.

The same procedure was repeated using PSA. Following the freezing out step, the extract was transferred to 15 mL polystyrene tube containing 750 mg of $MgSO_4$ and 125 mg of PSA.

The same procedure was repeated using Z-sep. Following the freezing out step, the extract was transferred to 15 mL polystyrene tube containing 750 mg of $MgSO_4$ and 125 mg of Z-sep.

7.1.2. Freezing-out study

These 3 extraction methods were repeated without any freezing out step. After the first centrifugation step, 5 mL of extract were added directly to polystyrene tubes containing PSA and $MgSO_4$, Z-Sep and $MgSO_4$. In the case of EMR-lipid sorbent, 5 mL of supernatant were transferred directly to EMR-lipid tubes already stabilized with 5 mL water.

7.1.3. Dilution study

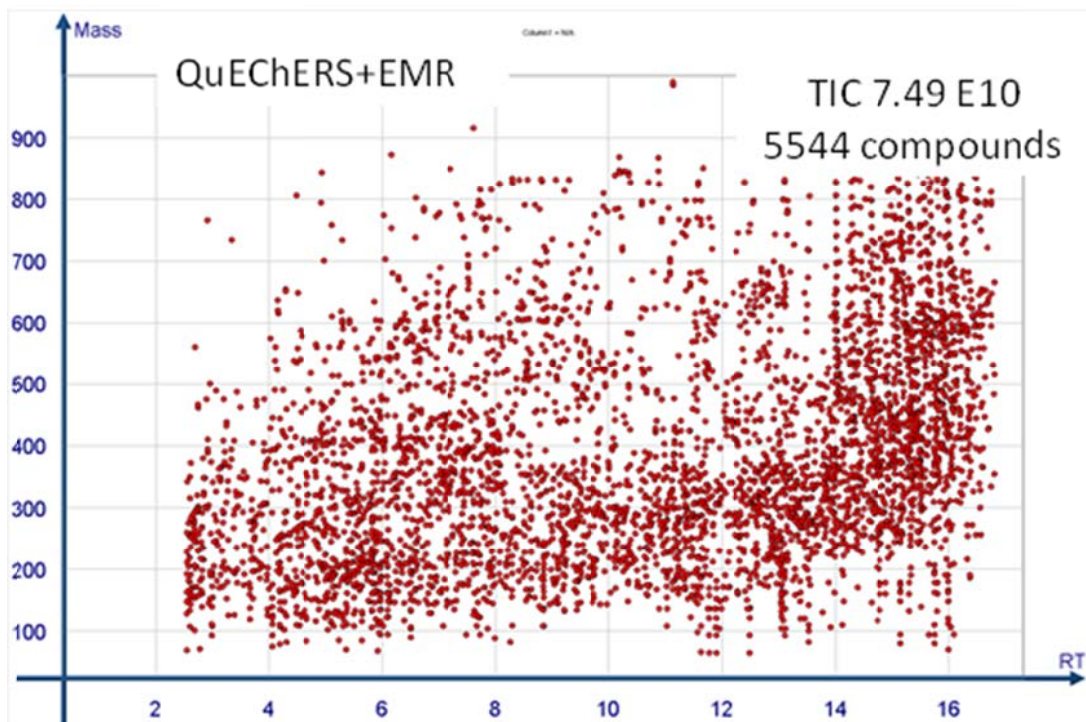
For the analysis of spices on LC, and for reducing the background signal, 2 injections were made with different dilution factors. The first injection consisted of 25 times dilution (16×10^7 pg in column) and the second injection consisted of 100 time total dilution (4×10^7 pg in column).

8. Results

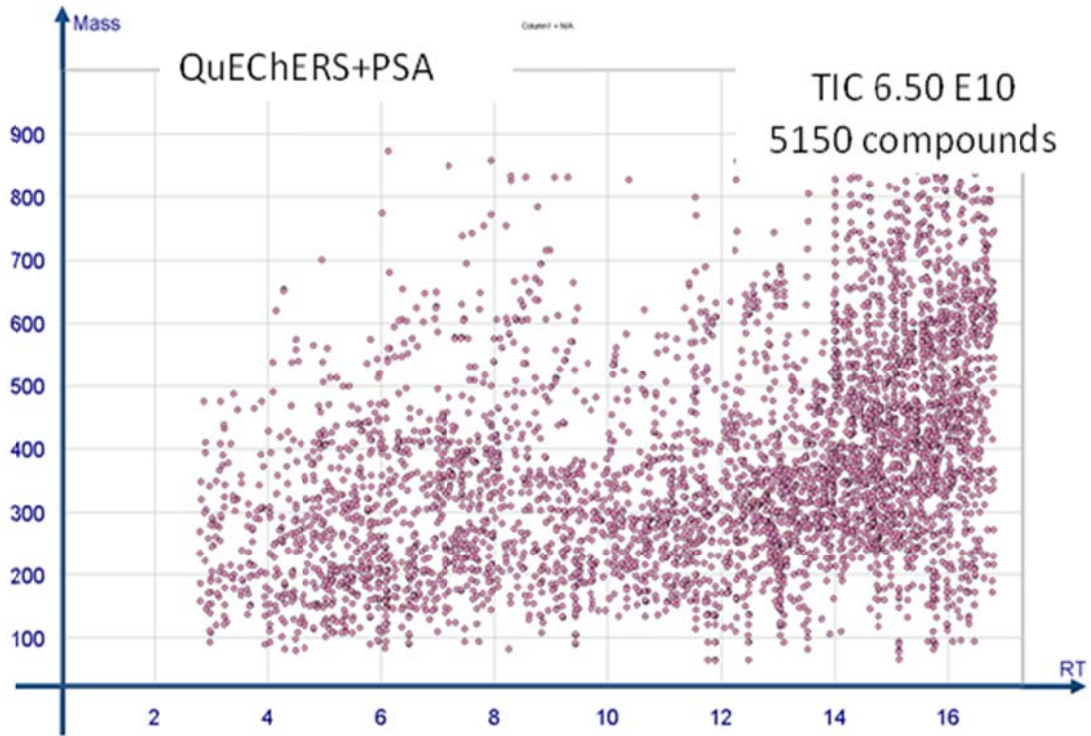
8.1. LC-ToF background distribution

8.1.1. Sorbent effect on cayenne and black pepper

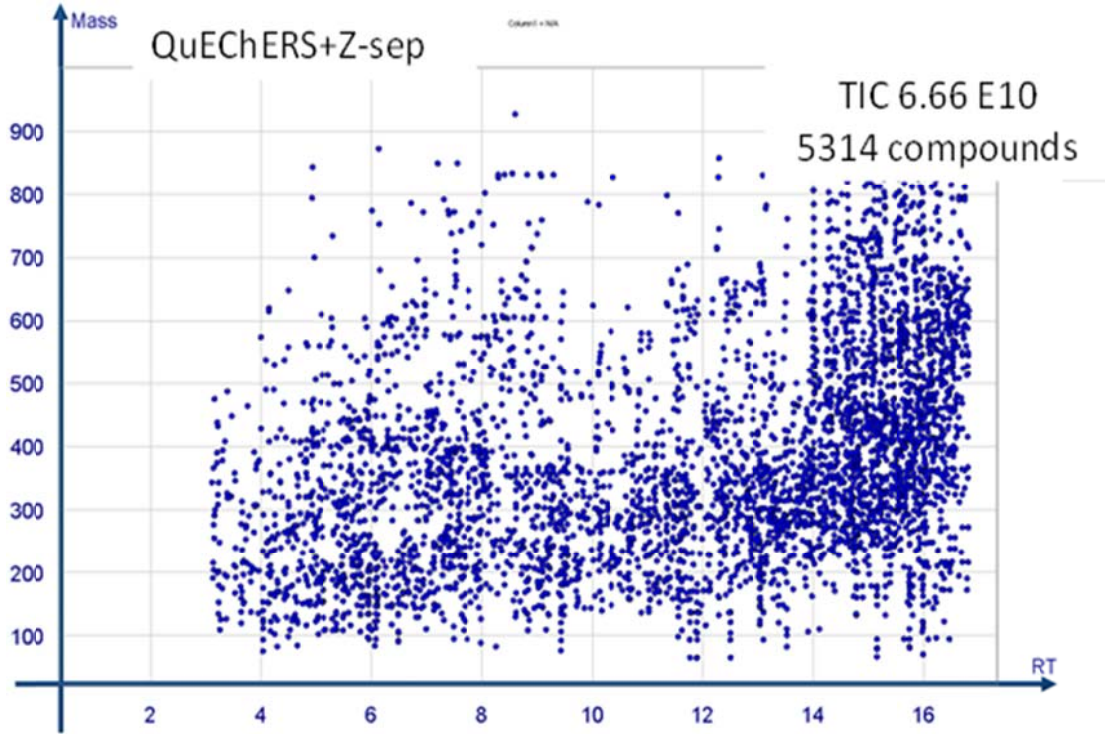
a) Number and distribution of co-extracted matrix components of Cayenne using EMR-lipid sorbent without freezing out and with 25 times dilution (16×10^7 pg in column)



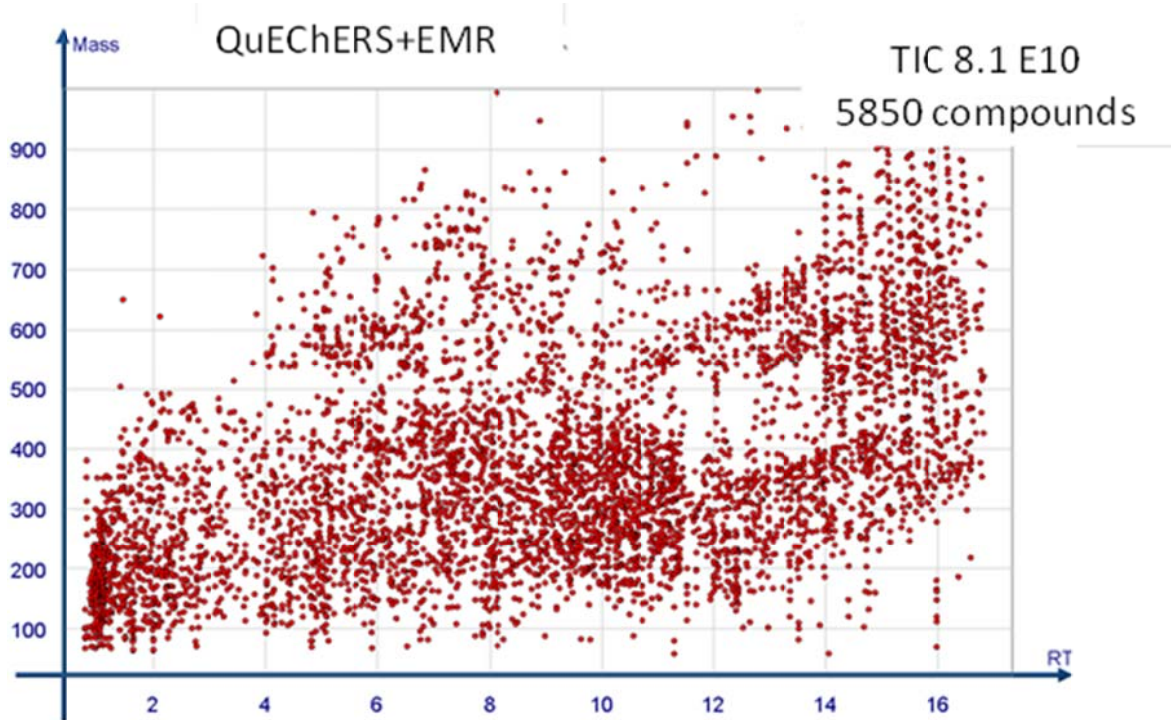
b) Number and distribution of co-extracted matrix components of Cayenne using PSA sorbent without freezing out and with 25 times dilution (16×10^7 pg in column)



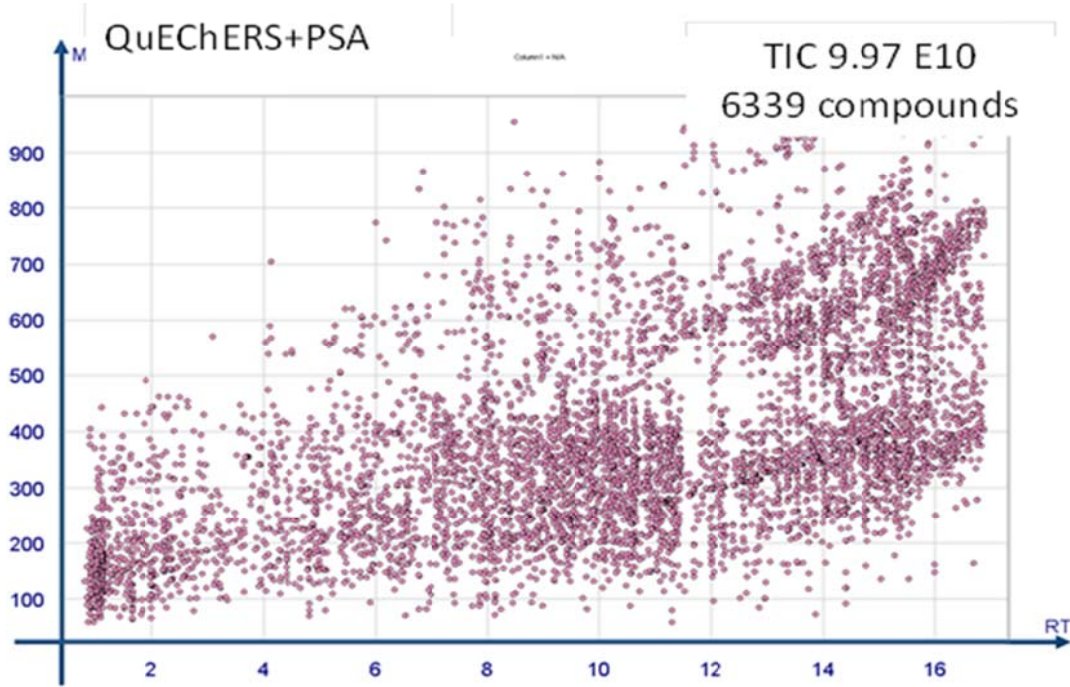
c) Number and distribution of co-extracted matrix components of Cayenne using Z-sep sorbent without freezing out and with 25 times dilution (16×10^7 pg in column)



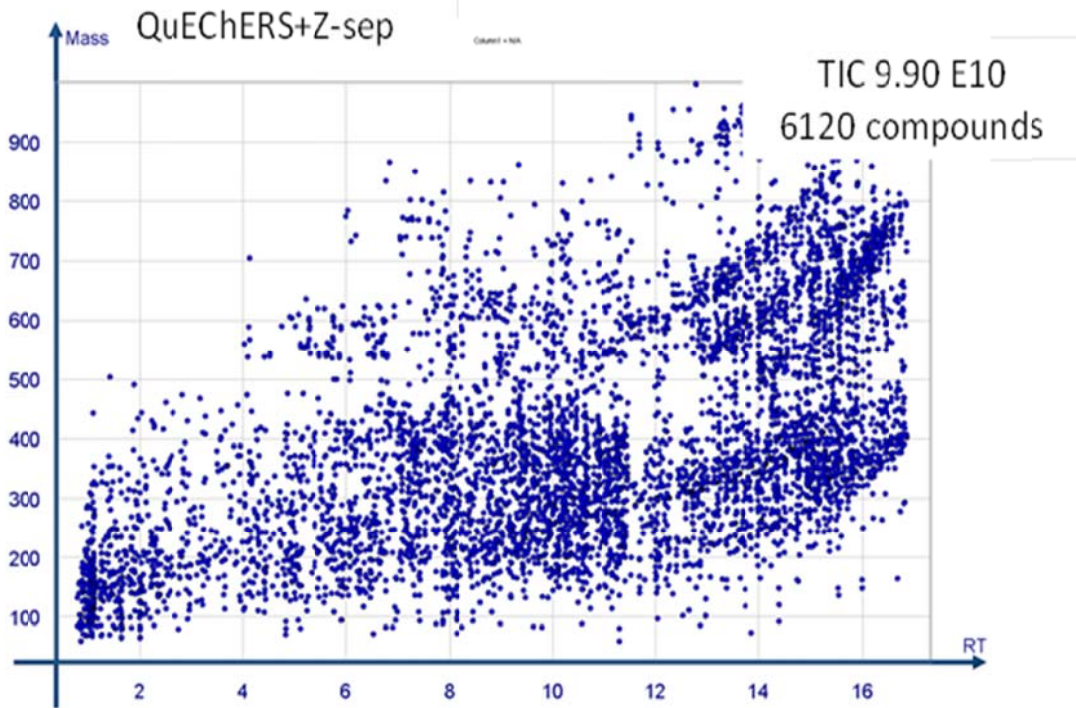
d) Number and distribution of co-extracted matrix components of black pepper using EMR-lipid sorbent without freezing out and with 25 times dilution (16×10^7 pg in column)



e) Number and distribution of co-extracted matrix components of black pepper using PSA sorbent without freezing out and with 25 times dilution (16×10^7 pg in column).

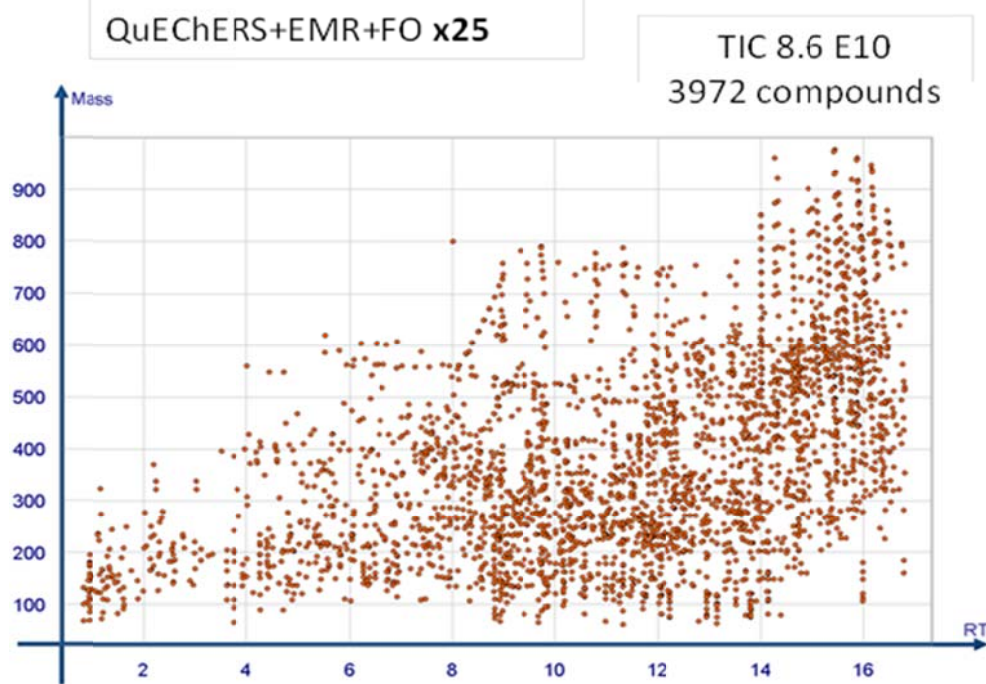


f) Number and distribution of co-extracted matrix components of black pepper using Z-sep sorbent without freezing out and with 25 times dilution (16×10^7 pg in column)

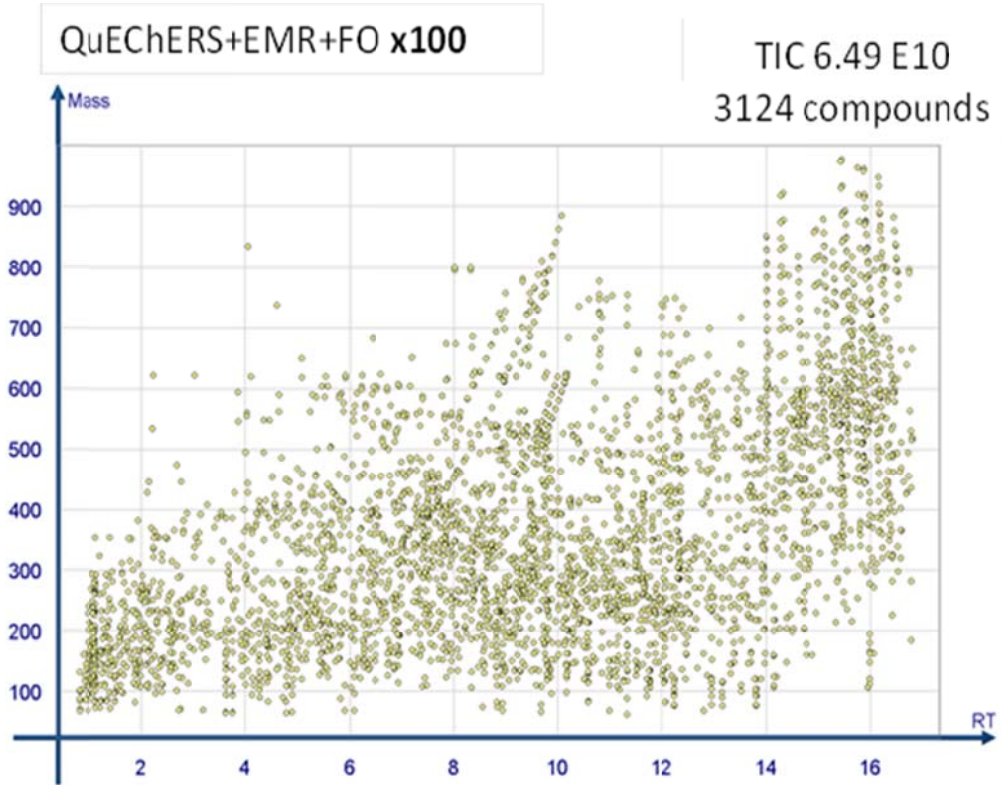


8.1.2. Freezing out and dilution effect

- a) Number and distribution of co-extracted matrix components of Curcuma using EMR sorbent with freezing out and with 25 times dilution (16×10^7 pg in column)

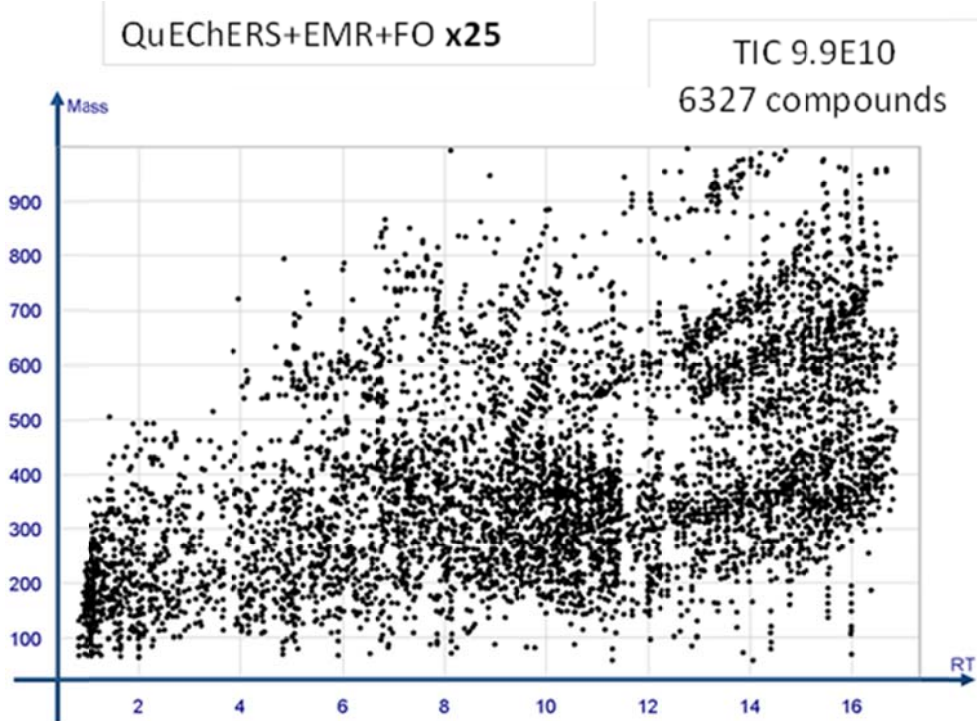


b) Number and distribution of co-extracted matrix components of Curcuma using EMR sorbent with freezing out and with 100 times dilution (4×10^7 pg in column)

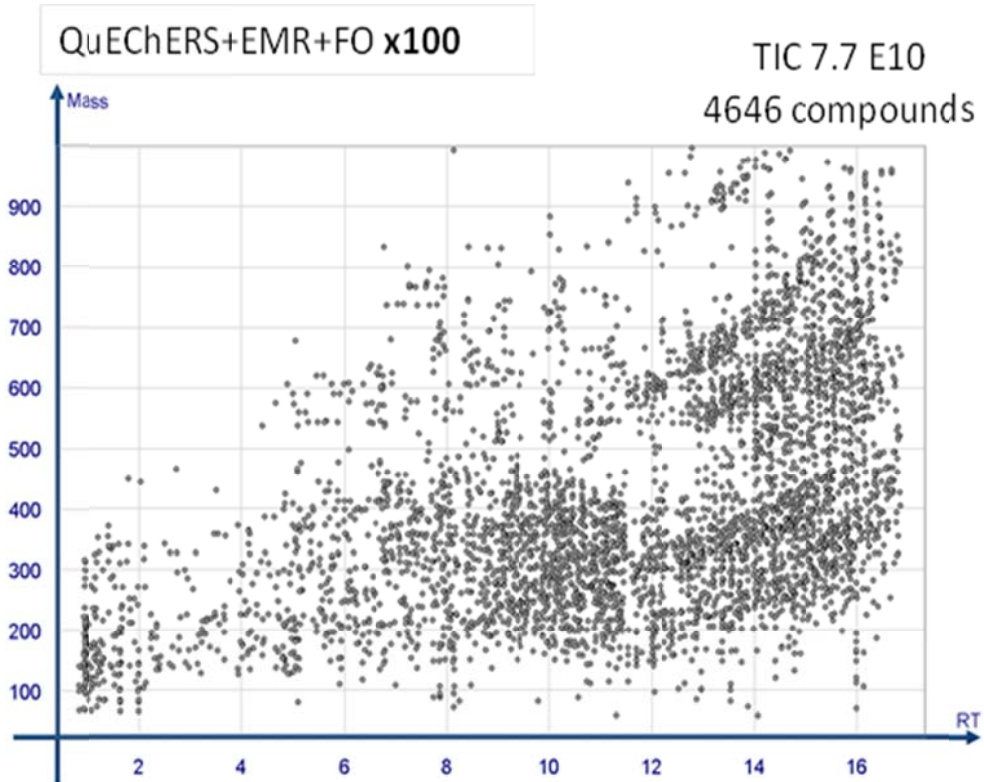


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c) Number and distribution of co-extracted matrix components of black pepper using EMR sorbent with freezing out and with 25 times dilution (16×10^7 pg in column)

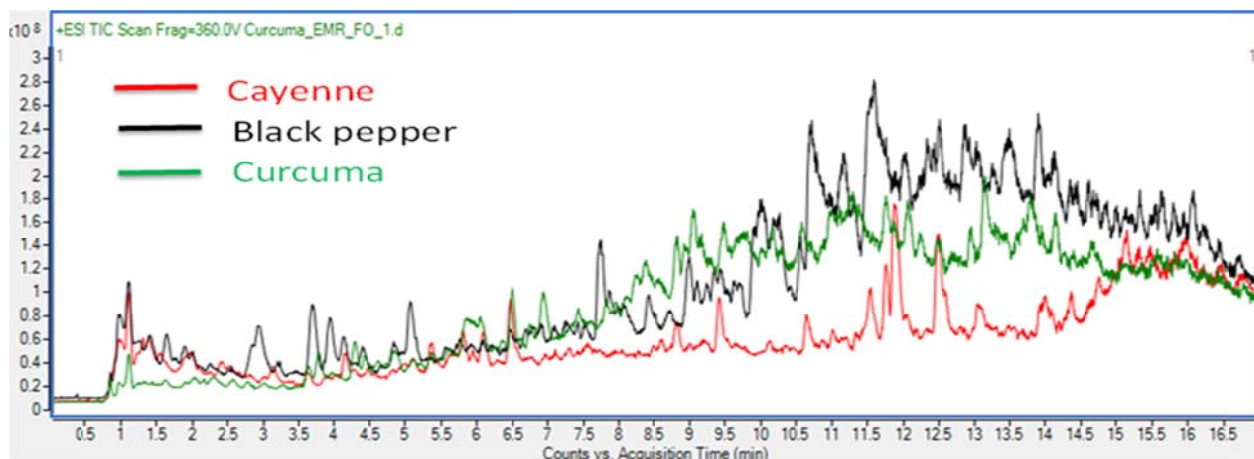


d) Number and distribution of co-extracted matrix components of black pepper using EMR sorbent with freezing out and with 100 times dilution (4×10^7 pg in column)



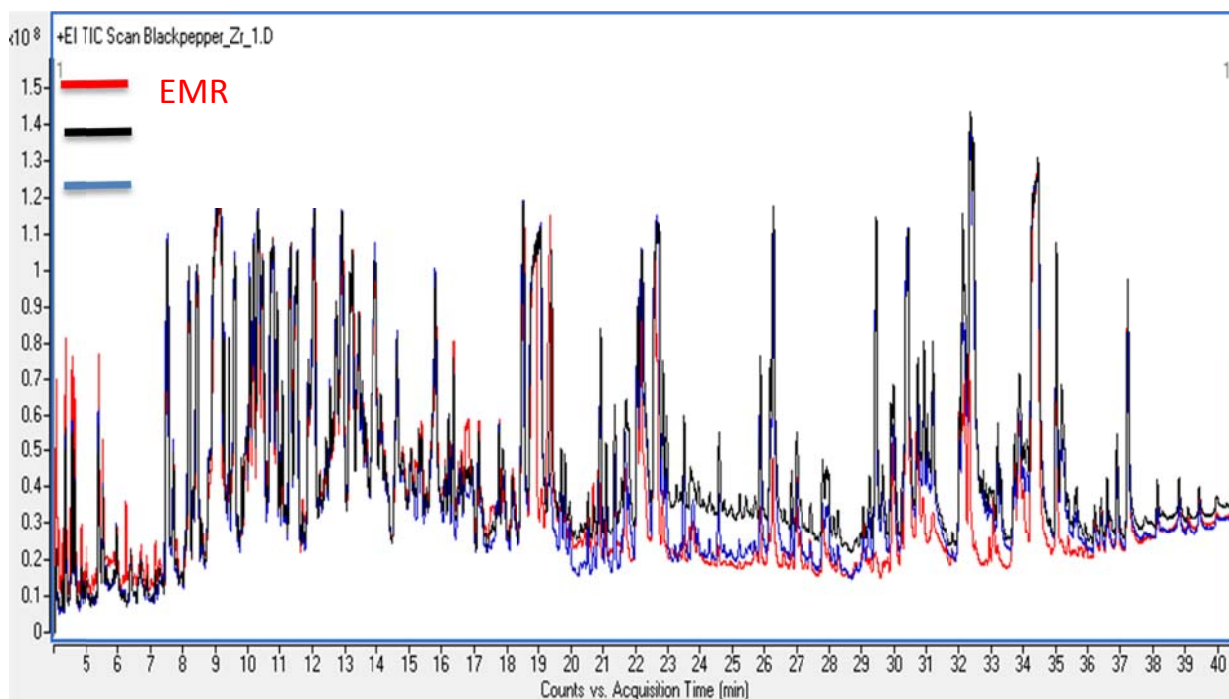
8.1.3. Spice matrices background differences.

TIC of black pepper, cayenne and curcuma using EMR-lipid sorbent with freezing out and 25 times dilution (16×10^7 pg in column).



9. GC-ToF results

a) TIC of black pepper with the 3 clean-up sorbents of EMR, Z-sep and PSA without freezing out and with 25 times total dilution (8×10^7 pg in column)



b) TIC of black pepper using EMR with 25 times total dilution (8×10^7 pg in column) and with and without freezing out.

